Milk: the Mammary Gland and Its Secretion

Edited by

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CHAPTER 13

Nutrition of the Lactating Woman

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I. Introduction

In this Chapter, we are concerned with the metabolic cost of lactating, and with the role of diet in sustaining lactation. It is necessary to begin by saying something about the general background, an understanding of which is essential to the proper interpretation of experimental results and estimates of nutritional requirements.

A. CONCEPTS AND DEFINITIONS

Every mother may be presumed to have a maximum capacity for lactation, a potential which is genetically determined. In this respect, women are not all alike, some having a much higher potential than others. There is reason to

believe that the capacity for lactation is more variable in man than in animals, because successful lactation is not necessary for human survival. Substitute mothers or breast-milk substitutes are, and always have been, available when a mother cannot or will not breast-feed; therefore low-yielding strains do not die out. This is undoubtedly true in modern civilization. Natural selection may be more influential in primitive races, but information is lacking.

The actual capacity for lactation is not necessarily as great as the genetic potential. In first lactations, capacity declines progressively as maternal age increases; possibly some process akin to "disuse atrophy" takes place when the mammary glands remain unused for long periods (Baird *et al.*, 1958). Second and later lactations are often more successful than the first, in conformity with the fact that many reproductive functions require a "practice run" to attain full efficiency. Capacity for lactation at a given time may be diminished by disease, or even by the mother's emotional state through inhibition of the milk-ejection reflex.

Capacity for lactation, a physiological attribute, should be clearly distinguished from breast feeding. The capacity for lactation, even if the mother feeds her baby solely from the breast, may not be used to the full. Generally, the supply adjusts itself to the demand, within the limits of capacity; but in special circumstances women of high capacity, such as donors to a breast-milk "bank," may continue to produce much more milk than the baby requires. This is a point of some importance, since wet nurses and donors of milk were the subjects used in some commonly quoted investigations of human lactation. The results obtained are therefore not representative of lactating women in general.

B. Sources of Information and their Limitations

Information on human lactation is derived, inevitably, from women who are lactating. This is a statement of the obvious, but its implications are much more troublesome than is commonly realized. Many mothers decide not to breast feed, or suppress lactation for one reason or another after a relatively short trial. In Britain more than half of all mothers have stopped lactating within 3 months of parturition (Hytten & Thomson, 1955), and according to Meyer (1958) three out of four American mothers have stopped lactating when they leave the lying-in hospital. The mothers who stop lactating are not a representative sample of all mothers with babies of similar age. For example, studies in Aberdeen, Scotland (Hytten, 1954f; Hytten & Thomson, 1955) showed that the incidence of artificial feeding at the time of discharge from a maternity hospital, during the 2nd week after parturition, was higher than average among primiparae in the older age-groups, women of poor physique, and women who had been delivered by Caesarean section, or who had had

pre-eclamptic toxaemia. At 3 months after delivery, 39 % of tall women in the upper social classes had stopped breast feeding, and 61 % of short women in the unskilled manual working classes. Under these circumstances, data collected 3 months after parturition represent only about one-third of all mothers with babies of that age, with considerable selection by physique and social class. Obviously, it is impossible to measure capacity to lactate, as opposed to breast-feeding performance, in a truly representative sample of women, even if the technical difficulties can be overcome. The most we can hope is to measure and analyse the milk produced by certain specified kinds of mother, those who do lactate for a specified period after parturition. The involuntary selection which this limitation entails presents many traps for the unwary. For example, the data of Hytten & Thomson (1955), showing that breast feeding at 3 months post-partum is especially common in women of superior physique living in favourable social conditions, might be taken to support indirectly an argument that superior nutrition favours the capacity to lactate satisfactorily. But this is to equate breast feeding with capacity for lactation, which is unjustifiable. The fact is that the women in poorer circumstances were less keen to breast feed, and only a minority used their capacity for lactation for more than a short time (Hytten *et al.*, 1958).

C. Some Technical Difficulties

1. *Measurement of the Milk Yield*

In clinical practice, it is usual to estimate the volume of milk produced during a given feed by weighing the baby before and after it is put to the breast. Provided the weighing is carefully done, and there are no losses of urine, faeces or vomit, or changes of clothing between weighings, this method should give a reasonably accurate estimate of the quantity of milk taken by the baby; the insensible weight loss of the baby during nursing is probably immaterial. If the baby has emptied the breasts, the yield corresponds to the milk production. If some milk is left in the breasts, it may be expressed and measured.

Another method of estimating the yield is to express all the milk manually (Davies, 1945) or to extract it by pump. Such methods may not remove all the milk available; few mothers are completely conditioned to manual or mechanical milking, so that there may be some inhibition of the milk-ejection reflex. Hytten (1954a) estimated the 24-hour milk production on the 6th day of lactation by test-weighing followed by manual expression of residual milk, and on the 7th day of lactation by manual expression alone. The mean 6th day yield was about 40 ml greater than that for the 7th day. Since the true milk production on the 7th day may reasonably be presumed to have been

greater than that on the 6th day, this result suggested that manual removal gave an underestimate. In a further trial, test-weighing on the 6th day was compared with the yield obtained on the 7th day with an electric pump, the "Humalactor," which mimics the action of the baby's jaws on the nipple; the 7th day yield was 25 ml greater than the 6th day yield. It is well known that a progressive rise in fat content occurs during a feeding, so that milk from completely emptied breasts would be expected to have a higher average fat content than milk from breasts that have been only partially emptied. There was no significant difference between the fat contents of the 7th day milk in the two series. This suggests that both methods of milking can probably empty a given breast at any time in most subjects, but that manual expression, either from its discomfort or because of its lack of natural stimulation, at the time or in anticipation, causes a decreased amount of milk to be secreted.

2. *Measurement of the Composition of Milk*

It is easy to obtain some milk for chemical analysis. Many of the older data relate to "spot" samples obtained by expression before, during, or after a feed, but it soon came to be realized that the composition of milk changes considerably during the course of a single feed. This source of variation can be eliminated by extracting the whole of the milk in the breast, taking a sample for analysis, and feeding the remainder to the baby. However, diurnal variations from feed to feed also occur, and some workers allow for this by collecting milk at a specified feed, e.g., the 10 a.m. feed. But even this degree of standardization is insufficient. Kon & Mawson (1950) showed that the mean fat content of milks removed between 10 a.m. and 12 noon was high when the interval since the last feed was short, and vice versa; this alone appears to account for differences of mean fat content found by Kon & Mawson in milk samples drawn from two areas in England, which might otherwise have been ascribed to socio-economic or dietary differences.

Some workers have tried to overcome the practical difficulties of obtaining representative samples of breast milk by making the assumption that both breasts produce milk of similar volume and quality. Milk is removed from one breast for analysis, while the baby feeds from the other. Hytten (1956) has shown that this assumption is ill-founded, since quite substantial differences may exist between milks produced by the two breasts.

Morrison (1952) has reviewed the factors that influence breast-milk composition, and Hytten (1954b,c,d) has given more recent data. In Hytten's view, there is no escape from the necessity to obtain complete 24-hour samples, if misleading measurements are to be avoided. This is seldom done, so that comparisons between milk analyses reported in the literature must be made with the utmost caution.

3. *Assessment of the Adequacy of Lactation*

It cannot be assumed that because a mother is breast feeding she is lactating adequately. Adequate lactation implies that the milk supply permits the baby to thrive. It is not entirely easy to define a "thriving" baby, and much depends on the standard expected. The weight gained is a convenient criterion, and a gain of about one ounce (about 30 g) daily is commonly regarded as satisfactory in clinical practice. But many healthy babies gain much more and some apparently healthy babies gain less. A fixed standard of growth in weight is, at best, an arbitrary index of health and development, which may be misleading in individual instances.

In the absence of disease, it is reasonable to assume that if a breast-fed baby is not thriving the lactation that sustains it is inadequate. On the other hand, problems of excess milk production may arise in early lactation, before the supply has become adjusted to demand; or, more rarely, if the mothers studied are wet nurses or donors to a breast-milk "bank." This is of importance in studies used to estimate requirements in lactation, since the requirements will be greater than those that would suffice for the satisfactory nourishment of the average baby.

II. Diet Surveys and Feeding Experiments

A. Diets taken by Lactating Women

Surprisingly few investigators have published information on the nutritive values, expressed in absolute terms, of diets taken by lactating women. Table I summarizes those that are readily accessible, including unpublished results obtained by us for eleven primiparae. It is obvious that the means fall into two groups with average daily calorie values of about 2600 kcal or less, and of about 3000 kcal or more

The three subjects of Shukers *et al.* (1932) who were living at home, appear to have been fairly active, and were taking self-chosen diets consisting of foods "which they believed to favor milk flow." The same subjects are described by Macy *et al.* (1930) as having earned considerable sums by selling excess milk or by wet-nursing. All three had remarkably high milk yields, the average daily yield of one subject during a lactation of at least 450 days being no less than 3·13 litres. The twelve multiparae studied by Kaucher *et al.* (1946) were chosen during pregnancy "on the bases of their medical histories, physical examinations, and records of having successfully nursed their other children "; during lactation they were the subjects of metabolic investigation and were under strict dietary control, being given diets of high nutritive value (Kaucher *et al.*, 1945). Deem (1931) studied five subjects who lived in a home for unmarried mothers; they were selected during the early puerperium as being trustworthy, keen and intelligent, and "appeared to have excessive

TABLE I

MEAN NUTRITIVE VALUES (STANDARD DEVIATIONS IN PARENTHESES) OF DIETS TAKEN BY LACTATING WOMEN

Subjects	Place	Year	Calories (kcal)	Protein (g)	Calcium (g)	Iron (mg)	Vitamin A (i.u.)	Thiamine (mg)	Riboflavin (mg)	Nicotinic acid (mg)	Ascorbic acid (mg)	Source
3 very high-yielding wet nurses[1]	Detroit, U.S.A.	Before 1931	4233	158	2·87							Shukers *et al.* (1932)
12 multiparae[3] (30–31 estimations)	Detroit, U.S.A.	Before 1944	2928 (359)	109 (12)			(3·3 mg)	1·2 (0·21)	3·1 (0·33)	16 (2·6)	125	Kaucher *et al.* (1946)
5 ?primiparae[1]	Auckland, New Zealand	Before 1931	3132	87								Deem (1931)
36 women[2]	Shoreditch, London, U.K.	1942	2410 (401)	88 (17·7)	0·8 (0·27)	17 (0·43)	2175	1·37 (0·30)			39 (17·7)	Bransby *et al.* (1950b)
23 women[1]		1943	2448 (574)	84 (20·5)	1·0 (0·28)	15 (0·46)	2300	1·67 (0·49)	2·0 (0·72)		32 (12·9)	
50 women[2]		1944–45	2403 (587)	89 (23)	1·2 (0·33)	12 (0·37)	2645	1·35 (0·47)			63 (39)	

11 primiparae[1]	Aberdeen, U.K.	1955	2579 (270)	82·6 (14·8)	1·02 (0·25)	—	6896 (2330)	1·42 (0·22)	2·12 (0·47)	13·67 (2·97)	72·6 (19·6)	Unpublished data of the present authors
70 multiparae[4]	South India	Before 1957	1858	42·7	0·3	22·5						Pasricha (1958)
"Recommended allowances"	U.S.A.		3300	98	2·0	15	8000	1·7	2·5	19	150	N.R.C.[5] (1958)
	U.K.		3000	111	2·0	15	8000	1·4	2·1	14	50	B.M.A.[6] (1950)

[1]Weighing-inventory surveys. [2]"Homely measures" inventory. [3]Chemical analysis of samples. [4]Questionnaire.
[5](U.S.A.) National Research Council: Food and Nutrition Board. [6]British Medical Association: Committee on Nutrition.

appetites, a fact which is not surprising as they did all the manual labour of the home, such as milking cows, washing clothes, scrubbing floors and gardening."

All the studies cited so far concern specially selected or unusually active women, or women who were under close dietary supervision. This may account for the high calorie values recorded. All the remaining surveys dealt with women living at home on self-chosen diets.

The only known selection exercised in our study of eleven Aberdeen primiparae was that all their babies were thriving, and all mothers were sufficiently intelligent and co-operative to take part for one week in a weighing-inventory diet survey, according to the procedure described by Thomson (1958). Little is known about subjects studied by Bransby *et al.* (1950b) but they appear to have been representative lactating women of relatively low socio-economic status, living at home in Shoreditch, London, during the 1939–45 war. Presumably they included some who were not lactating adequately. The subjects of Pasricha (1958) were Indian multiparae of poor socio-economic status, almost certainly of poor physique, and low body weight (see Narasinga Rao *et al.*, 1958; Gopalan, 1958). Nothing is said about their lactation performance or activity.

The New Zealand and American results, which are not truly diet-survey data, may be atypically high. The British results may give a more reliable indication of the average diets of typical lactating women in Western society. The Indian results may be typical of diets in the poorer socio-economic strata of an underdeveloped community.

Only the data of Shukers *et al.* (1932), Deem (1931) and Kaucher *et al.* (1946) include values for milk yield and composition as well as for dietary intakes of individual subjects. There is no obvious relationship between the nutritive values of the diets and the lactational performances.

The dietary-intake data in Table I are compared with the "recommended allowances" for lactation of the (U.S.A.) National Research Council: Food and Nutrition Board (1958) and of the British Medical Association: Committee on Nutrition (1950). The allowances approximate most closely to the diets of the specially fed subjects studied by Kaucher *et al.* (1946). The significance of the discrepancies between some of the allowances and the observed diets of all the other groups should be interpreted in the light of discussion below (Section III).

B. Poverty and Famine

It has been noted above that the—admittedly inadequate—recorded nutritive values of diets taken by lactating women are not correlated in any obvious way with the yields and composition of breast milk.

This negative finding may indeed reflect the general situation. We have not found any unequivocal evidence that lactation is seriously impaired in countries where diets are in general poor. The reports are contradictory. For example, good yields of milk of satisfactory quality have been reported in the Bantu (Walker *et al.*, 1954) and in Indians of the poorer classes (Gopalan, 1956). On the other hand, Dos Santos Reis (1953) said that breast milk in the Mozambique is scanty and of poor quality (but breast-fed infants are said to gain an average of 24 g daily, which does not indicate any gross insufficiency); and Holemans & Martin (1954) reported that women of the Kwango, in the Belgian Congo, produced milk of low protein content while subsisting on diets poor in protein (but the protein content given, 1·1 g per 100 ml, would usually be regarded as quite normal).

The whole subject is obscured by the difficulty of comparing milks obtained by different sampling methods and at different stages of lactation from women of different ages and parities. Whatever the truth may be, it is difficult to reconcile the common belief that easy breast feeding is almost universal in primitive communities with a belief that milk yield and composition are readily influenced by the quantity and quality of the maternal diet taken during lactation. It may be that, within very wide limits, lactation can be subsidized from the maternal tissues, as long as reserves are available.

Evidence based on wartime conditions is no more satisfactory and is, of course, liable to be complicated by special circumstances, such as anxiety, which may influence the milk flow. The literature has been reviewed by Gunther & Stanier (1951) and only some of the more striking reports will be mentioned here. von Sydow (1945) studied ten undernourished Polish women 11 to 15 days after they had been released from Ravensbrück concentration camp. The fat contents were found to be high, but this may possibly be attributed in part to the sampling method used. Smith (1947) says that during the Dutch famine of 1944–45 as high a proportion of women breast-fed their babies as during the same months in the year before the famine. Smith also quotes Jonxis as finding no significant deviation in composition from normal breast milk during the famine. On the other hand Antonov (1947) found, during the siege of Leningrad, that although milk continued to be secreted, the quantity was often reduced and the length of lactation shortened.

It seems fair to conclude, from a study of the literature, that under-nutrition has by no means necessarily a bad effect upon lactation so long as nutrients can be made available from the maternal tissues. We are not aware of any systematic attempts to study the effect on lactation of experimentally restricted diets, but Gunther & Stanier (1951) recount an interesting case-history from Keller (1910): "A doctor's wife wishing to regain her figure undertook a Banting cure. From the time when her child was 5 weeks old

she lived for 16 days on 400 g of meat with two rolls and a little vegetable each day. In this time she lost 2·3 kg of body weight, and the volume of milk secreted rose slightly, but remained of the order of one pint a day."

C. Feeding Experiments and Therapeutic Trials

1. *Protein, Fat and Carbohydrate*

More than forty years ago, Hoobler (1917) introduced his feeding experiment as follows: "The problem of constructing a diet for a mother with a failing or deficient milk supply is one of the most difficult which is presented to the pediatrist. The laity have any number of suggestions as to foods necessary; the physician has his favorite galactogogues; the nostrum vender advertises his wares as infallible milk producers, but in spite of all of these many mothers who begin nursing their babies soon find that their babies do not thrive on the milk which they are able to produce. . . . The literature is practically barren of any extended researches on the effect of diet on human milk production."

Setting out to remedy this situation, Hoobler enlisted the co-operation of a few lactating mothers (wet nurses, and therefore presumably already lactating well) who were given diets of varying calorie values and protein contents. His findings are not easy to interpret, but there is no obvious relationship between the calorie values of the diets given and of the milk yield, or between dietary protein and milk protein. Hoobler's advocacy of animal protein, and especially cow's milk protein, in the diet appears to rest upon slender evidence.

Fifteen years later, Deem (1931) studied five healthy lactating women, living in a home for unmarried mothers, who were given for a week at a time each of seven diets in succession. The results are summarized in Table II. Deem claimed that the high-fat diet increased the fat content of milk and that the high-protein diets increased milk yield. Inspection of the data, however, makes at least the latter claim somewhat unconvincing, since the changes induced were much less than variability between subjects, and the data for individual subjects by no means all showed the pattern of the means in Table II. The changes in fat content in response to a high-fat diet may be accepted provisionally. The high-fat diet had an exceptionally high calorie value and followed a high-sugar diet also with a high calorie value. Morrison (1952) quotes Polonovski (1933) as showing that the fat content of milk could be raised by adding glucose to the maternal diet and notes that Deem's results may be due to "carry-over" effects. Morrison also quotes Ružičič (1934) as having found that the fat content of milk (provided by wet nurses) rose to a maximum when a diet consisting entirely of butter and meat was

given, and fell to a minimum when a diet of meat and bread or of bread alone was given. Ružičič's feeding experiments, however, lasted only 1 to 3 days.

TABLE II

EFFECT OF DIET ON MILK YIELD AND COMPOSITION (DEEM, 1931)

Milk	Diet* A	B	A	C	D	E	F
Yield (ml)	927	1037	1002	1091	1064	1076	1017
Protein (g/100 ml)	1·16	1·26	1·08	1·19	1·11	1·09	1·08
Fat (g/100 ml)	3·8	3·4	3·5	3·7	3·6	4·3	4·2
Lactose (g/100 ml)	7·67	7·41	7·53	7·40	7·41	7·28	7·36

*Diet	Calories (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)
A "Institution"	3132	87	96	458
B "High protein"	3203	147	88	388
C "High protein + vitamin B" (3 g Marmite)	2899	144	85	370
D "High sugar"	3905	90	98	641
E "High fat"	4315	88	251	399
F "Low protein"	2826	61	123	350

Escudero & Pierangeli (1940–41) studied the effects of dietary supervision and supplementation of women contributing to a milk "bank." Their results are discussed by Morrison (1952), from whom Table III is taken. The concentration of milk fat rose with increase of dietary protein, fat or carbohydrate; the effect appears to have been especially marked with increase of dietary fat. Increase of dietary protein raised the level of milk protein as well

as of milk fat. The method of milk sampling used by Escudero & Pierangeli is not clear; Morrison says that correspondence with Dr Pierangeli led him to believe that the samples represented after-milk, drawn after the baby had been fed. The method of determining food intakes is also not entirely clear.

Gunther & Stanier (1951) found that a supplement of 150 g of fat, or the calorie equivalent as bread, given to mothers during the puerperium did not alter significantly the fat and protein contents of milk secreted on the 8th day post-partum.

Ebbs & Kelley (1942) reported that breast feeding was better maintained among poorly-fed women who received a dietary supplement during pregnancy. The nutritive values of the diets used are not stated in absolute terms, and there are no data on milk yield and composition.

It is not easy to draw conclusions with confidence. Many of the subjects of these feeding experiments were wet-nurses or women chosen because they were breast feeding satisfactorily and none seems to have been chosen because lactation was unsatisfactory. The experiments therefore suggest, rather vaguely, that an adequate milk supply can be "improved" by making a good diet better. It would indeed be interesting to know if a failing lactation can be restored by improving the maternal diet; this is the important question from a clinical point of view. Evidence is lacking.

Human milk fat usually contains a higher concentration of unsaturated fatty acids than the fat of cow's milk (see Table III, Chapter 18). According to Pasquali (1951) and Söderhjelm (1953), women whose infants suffered from eczema were secreting milk with an unusually low content of unsaturated fatty acids; the latter author also claims that administration of refined sesame oil or cod-liver oil to nursing mothers rapidly increased the amounts of poly-unsaturated fatty acids in milk fat. In one out of two cases treated with sesame oil, where the baby had eczema, clinical improvement was noted.

A recent paper by Insull *et al.* (1959) indicates that the fatty acid composition of human milk can be radically altered without affecting either milk yield or overall fat content. Their experiments showed that milk fat closely resembled dietary fat during energy equilibrium but on a diet deficient in energy milk fat approached the composition of human depot fat. When excess non-fat calories were fed to the mother the milk showed a striking increase in lauric and myristic acids and a marked decline of all polyenoic acids.

2. *Water*

The investigations of Lelong *et al.* (1949), Duckman & Hubbard (1950) and of Illingworth & Kilpatrick (1953) failed to confirm the common belief that milk output can be increased by drinking more water, or diminished by restricting the intake of fluid.

TABLE III

EFFECT OF DIET ON MILK COMPOSITION
(Calculated by Morrison (1952) from Escudero & Pierangeli (1940–41); number of samples in parentheses)

Milk component	Protein level in diet (g per kg body weight) 1·0–1·5	1·5–2·0	2·0–2·5
Protein (g/100 ml)	(48) 1·095 ± 0·031	(276) 1·143 ± 0·015	(92) 1·259 ± 0·033
Fat (g/100 ml)	(48) 3·55 ± 0·18	(276) 4·19 ± 0·085	(91) 5·11 ± 0·18
Lactose (g/100 ml)	(47) 7·42 ± 0·076	(279) 7·39 ± 0·033	(94) 7·18 ± 0·081

Milk component	Fat level in diet (g per kg body weight) 1–1·5	1·5–2·0	2·0–2·5	> 2·5
Protein (g/100 ml)	(59) 1·151 ± 0·024	(168) 1·143 ± 0·021	(169) 1·167 ± 0·020	(22) 1·342 ± 0·080
Fat (g per 100 ml)	(57) 3·26 ± 0·19	(167) 3·88 ± 0·10	(171) 4·96 ± 0·10	(21) 6·06 ± 0·29
Lactose (g/100 ml)	(59) 7·40 ± 0·074	(166) 7·38 ± 0·058	(174) 7·30 ± 0·046	(22) 7·38 ± 0·076

Milk component	Carbohydrate level in diet (g per kg body weight) 5–6	6–7	7–8
Protein (g/100 ml)	(121) 1·168 ± 0·024	(215) 1·146 ± 0·017	(66) 1·177 ± 0·032
Fat (g/100 ml)	(123) 4·09 ± 0·13	(214) 4·40 ± 0·11	(67) 4·67 ± 0·19
Lactose (g/100 ml)	(121) 7·30 ± 0·052	(217) 7·38 ± 0·042	(66) 7·38 ± 0·061

Taken from Morrison (1952) by kind permission of the Director, Commonwealth Bureau of Animal Nutrition.

3. *Calcium*

Though there are considerable differences between individuals in the calcium content of milk, there is no evidence that the level can be influenced to any important extent by varying the level of calcium in the diet. Hunaeus (1909) tried giving calcium to mothers, with negative results and the data of Liu *et al.* (1940) indicate that poorly fed Chinese mothers with a history of severe calcium depletion can produce milk with normal calcium levels. On the other hand, Herz (1933) and Toverud & Toverud (1931) said that a change-over from a calcium-poor to a calcium-rich diet increased slightly the calcium content of milk.

There is certainly some reason to believe that during lactation the maternal metabolism is adjusted to conserve calcium, possibly to ensure an adequate level in the milk. According to Knapp & Stearns (1950), excretion of calcium in the urine drops abruptly after parturition, and remains below the mean normal value during the greater part of lactation. Telfer (1924) and Ritchie (1942) found no significant difference in the milk calcium of mothers with rachitic and non-rachitic infants.

4. *Phosphorus, Magnesium, Manganese, Zinc, Sodium, Potassium, Chlorine, Sulphur*

No satisfactory evidence has been produced that the level of these substances in milk depends in any way on the diet.

5. *Iodine*

The work of Turner & Weeks (1934) indicates that the iodine content of milk, which is very variable, depends largely on the iodine content of the diet. Honour *et al.* (1952) showed that the breast has the power to raise the concentration of iodine in milk above that in the blood, and many authors have commented on the danger of giving radioactive iodine to a woman who is breast feeding.

It may be mentioned, in passing, that iodine had a brief vogue as a galactagogue (Robinson, 1947); but negative results have been reported by Nicholson (1948), Dean (1950) and Miller (1951).

6. *Fluorine*

The increasing practice of adding fluorides to drinking water as a prophylaxis against dental caries gives importance to this element in relation to lactation. Levels of excretion in milk are low, and it appears that, although the fluoride content of milk can be raised by giving sodium fluoride by mouth, the rise is small in relation to the dosage (Held, 1952; Hodge & Smith, 1954).

7. *Copper*

The copper content of human milk is much higher than that of cow's milk, and has some importance in causing relatively rapid destruction of vitamin C

(Kon & Mawson, 1950). Munch-Petersen (1950) found that the intravenous administration of a copper-containing compound failed to alter the copper content of milk.

8. *Iron*

Human milk has a low iron content, a fact which has caused many a paediatrician to prescribe iron lest the baby becomes anaemic. The danger of anaemia may be real if breast milk is the sole food of the baby for more than two or three months, by which time the reserves with which it is born may be becoming exhausted. In his review, Morrison (1952) attributes the wide variation in reported iron contents of milk to contamination. He was unable to find evidence showing whether the iron level was influenced by diet or not. Since then, Neuweiler (1952) has shown that there is no correlation between levels of iron in the serum and in the milk; and that the administration of iron raises the level in serum without influencing that in the milk. This has been confirmed by Schäfer *et al.* (1955).

9. *Fat-soluble Vitamins*

More recent reports do not add materially to the literature reviewed and the original observations reported by Kon & Mawson (1950). These authors draw attention to the fact that the measurement of vitamin A and carotenoids in milk is subject to the same sampling errors as that of fat; and that, in general, the output of vitamin A and carotenoids in milk tend to increase with the fat content, but the concentration in fat decreases. They express their findings as the amount of vitamin A or of carotenoids per gram of fat.

In general, it appears that levels of the fat-soluble vitamins in milk can be affected by the intake, but to an extent which is small in comparison with the "dose." For example, Hrubetz *et al.* (1945) found that massive doses of vitamin A (50 000 to 200 000 i.u. daily) almost doubled the vitamin A content of milk, but this was insufficient to overshadow variations between individuals. Similarly, Kon & Mawson (1950) showed that a subject yielding milk with 22 to 27 i.u. vitamin A per g fat provided 37 to 48 i.u. per g fat on receiving 25 000 i.u. vitamin A daily.

The vitamin D content of milk is usually low, e.g., 0·13 to 0·41 i.u. per g fat (Kon & Mawson, 1950). According to Escudero *et al.* (1947) it can be temporarily increased 100-fold by heroic doses of vitamin D, but Polskin *et al.* (1943) concluded that as a rule increases are very small in relation to the dosage.

Breast-fed infants may develop rickets (Ritchie, 1942; Krestin, 1944) but it does not seem to be known, for example, whether the vitamin A and D contents of milk are unusually low in areas where clinical evidence of lack of these vitamins is common. The problem is, of course, complicated by the

importance of precise sampling methods and to a less extent by the difficulty of assay.

The vitamin E content of human milk appears to be much higher than that of cow's milk (Abderhalden, 1947). Neuweiler (1948) says that administration of vitamin E to the mother raises the amount in milk only if the original values are low. Burlina & Panizza (1955) claim that intramuscular injections of α-tocopheryl acetate increase the yield of milk; but their evidence is not convincing.

10. *Water-soluble Vitamins*

As mentioned above in connexion with the fat-soluble vitamins, Kon & Mawson (1950) have reviewed the literature up to about 1949. This literature is voluminous, and here we shall only summarize the findings. In general, it appears that the concentrations of the water-soluble vitamins in milk change rapidly and considerably in response to variations in the dietary intake. With a large test dose, a marked but transient increase occurs in the milk.

There are big variations in thiamine content between individual milks and during the course of lactation. Kon & Mawson (1950), however, draw attention to the fact that precise comparisons are difficult to make, owing to the unreliability of methods of estimation. Nevertheless, there is reason to believe that the thiamine content of milk is an index of the dietary intake of this vitamin. Kon & Mawson (1950) and several investigators cited by them, and—more recently—Pratt & Hamil (1951) have shown that the thiamine content of milk can be sharply increased up to a certain limit, estimated to be about 20 μg per 100 ml, when the mother's intake is increased.

Several authors have reported that the milk of women with beriberi contains less thiamine than that of healthy women (Plagnol & Dutrenit, 1956; Simpson & Chow, 1956; Concepcion & Dee, 1949). Sharma (1955) says that beriberi, leading to sudden death, is common in Burmese infants, often as early as the 3rd or 4th week of life. There are reports that such infantile "beriberi" may be due to intoxication with methylglyoxal or some similar substance excreted in the milk of thiamine-deficient mothers (Stannus, 1942; Fehily, 1944; Sato & Arakawa, 1950; Sato, 1951).

Riboflavin appears to behave somewhat similarly to thiamine, in that its level in milk is very variable, and there is a rapid transient response when a test dose is given. Sampling difficulties are considerable, owing to a marked diurnal variation in content. The level in mature milk is of the order of 30 μg per 100 ml.

Kon & Mawson (1950) do not deal with nicotinic acid. Coryell *et al.* (1945), with a microbiological method of assay and 24-hour samples of milk collected from women taking planned diets, found the mean content of mature milk to be 196 μg per 100 ml. There is a rapid transient response to a test dose, the

extent of rise depending on the size of the dose (Neuweiler, 1944; Pratt & Hamil, 1951; Nichele & Rovelli, 1952). According to Walker (1954), Bantu women taking a maize diet with an intake of 15–18 mg nicotinic acid daily had a mean concentration in milk of 70 μg per 100 ml; European women on diets containing 9·5 to 15 mg daily had a mean concentration in milk of 175 μg per 100 ml. The probable reason is that nearly all the nicotinic acid in maize is "bound," and hence unavailable (Kodicek *et al.*, 1956).

The somewhat scanty evidence available suggests that other B vitamins behave in much the same way as the above, in response to test doses.

"Because of the ease with which it can be determined the content of vitamin C in human milk has been studied more extensively than that of the other vitamins, and many facts concerning it are well established" (Kon & Mawson, 1950). Reports published since Kon & Mawson's excellent review (e.g., Squires, 1952; Bagchi, 1952; Yüceoglu, 1949) merely confirm the earlier results.

An interesting feature of ascorbic acid excretion in milk is that the level is higher than that in blood, indicating that the breast has the power to concentrate it. The concentration in milk does not vary much between feeds and different parts of the same feed, so that sampling difficulties are limited to those of preventing loss by oxidation between collection and assay. There is a marked seasonal fluctuation. Bransby *et al.* (1950a) give details of the investigation of one woman through a lactation of over 30 weeks, during which her daily vitamin C intake was maintained, successively, at about 140 mg for 8 weeks, 10 mg for 5 weeks and 50 mg for 13 weeks. The data show clearly the dependence of the level of ascorbic acid in milk on the dietary intake. There was an immediate response to a sudden change of the intake level, but the ascorbic acid level in milk did not attain stability until after about 2 weeks at a new dietary level.

The situation with the water-soluble vitamins thus contrasts sharply with that of the fat-soluble vitamins, in that the levels of the former in milk can be readily influenced by the dietary intake. Evidence that infants fed solely on the breast can develop deficiencies of water-soluble vitamins is somewhat scanty. Reference has been made above to beriberi, or a similar condition due to intoxication, among babies of lactating women themselves suffering from beriberi. It has been stated that *erythrodermia desquamativa* may arise in infants fed on breast milk with a low biotin content (Homolka & Riman, 1949; Švejcar & Homolka, 1950). There have been several suggestions, and denials, that the breast-fed infant should receive additional ascorbic acid, but we have not found any report confirming that scurvy may develop in a breast-fed baby. Rohmer & Bezssonoff (1942) suggested that the young healthy baby can synthesize ascorbic acid, but this does not seem to have been confirmed.

III. Nutritional Requirements in Lactation

It is obvious that the production of milk "costs" something. If lactation is not to represent a net drain upon the nutritional economy of the mother, the amount of energy and of any given nutrient lost in milk must be replaced. The required input is more than the output, because the energy and nutrients derived from the diet are not converted into milk with 100 % efficiency. The situation is further complicated by the fact that lactation is not necessarily maintained on a "current account" basis; in other words, the dietary intake at a given time may not be related to the immediately ensuing output of milk. In practice, the mother may use "savings" to subsidize lactation, particularly

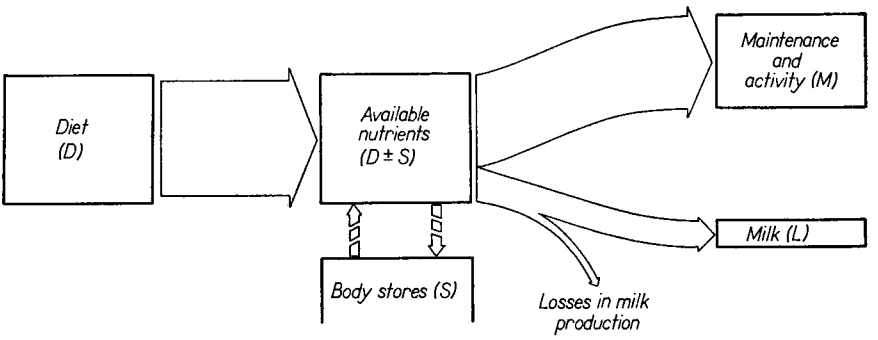

FIG. 1. Schematic representation of utilization of available energy in the diet by the lactating woman.

nutrients stored during the course of pregnancy. On the other hand, some mothers may accumulate further stores during lactation. The nutritional requirement specific to lactation is, of course, additional to that for maintenance and activity.

It is therefore possible to estimate the requirements for milk production, in terms of energy or of any given nutrient, by means of the equation

$$eR = L = e(D \pm S - M)$$

where R is the requirement, L the output of the nutrient in milk and e the factor representing efficiency of production;

D is the intake from the diet;

S is the contribution towards lactation, if any, from body stores, or the amount by which reserves are increased during lactation;

M is the cost of maintenance apart from lactation.

The relationships in this equation are shown diagrammatically in Fig. 1.

It is not unduly difficult to obtain values for L, the output in milk, by direct or indirect measurement. There is, however, considerable difficulty in

deciding what may be regarded as a representative output. The output of calories and of most nutrients varies widely during the course of a single lactation and from individual to individual. Furthermore, the output does not necessarily correspond to the needs of the baby; some mothers may produce much more milk than is needed by the baby, whereas others are unable to yield sufficient milk. In general, it seems perfectly sound to use a theoretical figure based on an assessment of the needs of a thriving baby at a given stage of development. As a rule, the stage chosen will be during the period when mature milk is being produced in amounts that can be taken to represent lactation as a whole.

The factor e, representing the efficiency of production, cannot be measured directly and must be inferred from a knowledge of all the other terms.

The intake from diet, D, may be measured directly.

The contribution from or to body stores, S, may be estimated from a knowledge of the mother's loss or gain of weight during lactation, or from an assessment of the metabolic balance.

The cost of maintenance, M, may be inferred from the requirement of a non-pregnant, non-lactating woman, similar in physique and in activity.

It must be stated at once that no experimental study of human lactation has yet been made that takes account of all these terms. The best we can do is to assemble such observations as are available and fill in the gaps by using estimates. In the account that follows, we have been able to analyse the requirement of energy fairly fully; less is known about protein, and much less about minerals and vitamins.

It should be stated here that some of the published standards referred to below were not devised primarily as physiological norms, though we shall discuss them from a physiological point of view. This matter is referred to again on page 39.

A. Energy Requirement

The most important nutritional characteristic of breast milk is its calorie value; there is little doubt that far more breast-fed babies suffer from underfeeding than from any other disability attributable to their food supply. The calorie value of the milk output is determined primarily by its volume and fat content. The volume of milk produced may be regulated by the amount removed in suckling, but there is no evidence that the baby has any influence on the composition of the milk. Some milks produced in as high a volume as the baby can readily assimilate may have such a low fat content that the energy supply is inadequate. The growth of the baby over a sufficiently long period is a more reliable clinical index of a sufficiency of calories than the result of test weighing at a single feed or over a day.

1. *The Recommendation of FAO*

The energy requirement specific to lactation has been estimated by FAO (1950, 1957a) to be 1000 kcal per day. This figure is arrived at as follows:

The average daily milk yield is assumed to be 850 ml, with a kcal value of 600. The efficiency of production is estimated to be of the order of 60 %, so that 1000 kcal per day will have to be supplied in the diet if the body stores of the mother are not to be depleted. The latest FAO report (FAO, 1957a) comments as follows: "This certainly applies to nursing women who have previously been undernourished or who are very active. In some women, however, the appetite is not always increased proportionately to the physiological need and some find it difficult to eat a diet providing 3000 Calories, which is considerably less than the [total] requirement estimated on the above basis. In practice, some women find that an increase in calorie intake of 1000 Calories daily is not easily achieved and an increase of 800 Calories may be a more reasonable estimate." It is to be presumed that the difference, 200 kcal, is derived from body stores. The same report assesses the requirement of a non-pregnant, non-lactating "reference woman" as 2300 kcal daily.

In terms of our equation, therefore, the FAO recommendation may be summarized as follows: The energy requirement specific to lactation (R) is 1000 kcal per day, on the following basis:

$$\begin{array}{cccccccc} 600 & \times & 100/60 & = & 3100 & + & 200 & - & 2300 \\ (L) & & (\tfrac{1}{e}) & = & (D) & & (S) & & (M) \end{array}$$

The relevant section of the FAO (1957a) report has been quoted verbatim, to make it clear how these figures are explained: the report does not give sources of data. In an attempt to elucidate things further, we shall now examine each figure in more detail.

The yield of "milk Calories" (L) corresponds to the assessed energy needs of an infant weighing about 5 kg and seems to be an acceptable quantity representing the period of mature milk production.

The production-efficiency figure, e, is of more dubious validity. According to the earlier report (FAO, 1950), it is an estimate derived from data published by Hoobler (1917), Deem (1931), Shukers *et al.* (1932) and Kaucher *et al.* (1946). To quote: "Since the investigations in question were not planned to estimate efficiency, they can provide only an approximate indication of energy requirements for milk production. Taken together, these studies cover 68 records of the milk yields of 19 women, all of whom were consuming a diet providing approximately 3000 Calories per day. Milk yields reported ranged from 250 milliliters during the first five days to about 1500 milliliters at full lactation. . . . Some indication of efficiency can be obtained by taking an arbitrary 'base-line' of energy expenditure which represents a constant level of maintenance and activity, and relating the excess of calorie intake over

this base-line to the energy value of milk yielded. Efficiency calculated in this way is of the order of 60 per cent. No more refined estimate can be made from the data in question. It is probable that the level of activity was not in fact constant." There is thus no doubt that the FAO estimate of production efficiency is only a very rough first approximation.

The estimated energy content of the diet (*D*), of a lactating woman, 3100 kcal per day, should correspond to observed intakes, i.e., to the data given in Table I. Not unexpectedly, the three high-yielding subjects of Shukers *et al.* (1932) had a much higher mean calorie intake. The estimate corresponds quite closely to the mean for the five unmarried mothers of Deem (1931) who "appeared to have excessive appetites", attributable to a high level of activity. The estimate is about 200 kcal greater than the average for the twelve specially-fed multiparae of Kaucher *et al.* (1946) and more than 1200 kcal greater than the average for the ill-nourished Indians of Pasricha (1958). The women of Shoreditch (Bransby *et al.*, 1950b), and the eleven primiparae studied by us in Aberdeen, all of whom took self-chosen diets, had average calorie intakes some 700 and 500 kcal lower than the estimate. As suggested above (p. 10) the "typical" lactating woman in Western society probably takes a diet providing much less than the 3100 kcal per day proposed by FAO (1957a). An average intake of this order has been recorded only for selected groups of subjects living under unusual conditions.

In our interpretation of the FAO (1957a) statement we assumed that the difference between the additional 1000 kcal recommended and the "more reasonable" estimate of 800 kcal was derived from body stores. It is at least doubtful that this is what the FAO committee intended. The (U.S.A.) National Research Council: Food and Nutrition Board (1958) adopted the findings of the FAO (1950) report, but went so far as to suggest that the additional calorie supply (i.e. from diet) should "be adjusted to actual milk production. By such means the basic diet would be supplemented by approximately 130 calories for each 100 milliliters of milk produced. Assuming an average milk yield of 850 milliliters per day, it is proposed that an additional allowance of 1000 calories daily be afforded during the lactation period." There is certainly no suggestion that part of this additional supply might be met from reserves.

The FAO (1957a) report appears to assume that its normal "reference woman," who uses 2300 kcal per day, provides a reliable base-line (*M*) on which the additional cost of lactation is superimposed. This reference woman, aged 25 years, weighing 55 kg, and living in a temperate climate with a mean annual temperature of 10°C, "may be engaged either in general household duties or in light industry. Her daily activities include walking for about 1 hour and 1 hour of active recreation, such as gardening, playing with children, or non-strenuous sport." Whether this is a fair description of the activity of a typical lactating woman is difficult to say. From our own clinical

experience we would suggest that activity in lactation is usually rather less than that of a woman who is neither pregnant nor lactating: calculations given later support this view.

It appears, then, that the FAO estimate of the additional energy required for milk production—1000 kcal per day—has a rather slender scientific foundation. The estimate of the total energy requirement of a lactating woman —3100 or 3300 kcal per day, depending on the interpretation placed on the FAO statement—fits direct observation only in special circumstances; the "typical" lactating woman apparently takes much less.

The FAO (1957a) estimate for milk production, 1000 kcal per day, may be compared with the estimate for the additional requirement in pregnancy, 40 000 kcal per pregnancy. If this is spread over, say, seven months of pregnancy, the average increment is about 200 kcal per day. Lactating women, if they require several hundred kcal per day more, should experience a very marked surge of appetite, much greater than that commonly reported by women during the first half of pregnancy. In our experience, few lactating women report an obvious further increase of appetite during lactation.

2. *A New Assessment of the Energy Requirement*

Clinical experience suggests that the typical lactating woman, in Western society at all events, does not eat very much more during lactation than she did during pregnancy. A daily intake of about 2600 kcal may perhaps be regarded as representative of lactation. This would fit the fact that most investigators have found the average diets of groups of pregnant women to provide 2000 to 2700 kcal per day (Thomson, 1958), and the proposition that the appetite is not greatly increased in lactation.

The only figure that appears to be beyond reasonable dispute is that the average lactating woman produces mature milk with a calorie value of about 600 kcal per day. Our problem may therefore be stated as follows: how does a woman taking about 2600 kcal in diet produce about 600 kcal in milk?

Unpublished data relating to the eleven Aberdeen primiparae already cited (Table I) can be used to obtain a first approximation. The following facts, useful from the present point of view, were established by direct observation. The average calorie value of their diets was 2580 kcal per day. They were losing weight at an average rate of 270 g per week. All were lactating satisfactorily, and their babies were putting on an average of 240 g per week. The mean weight of the babies in the week of the dietary survey was 4·64 kg; the average duration of lactation at the time of the dietary surveys was 7 weeks. Milk output was not measured, but since the babies were gaining weight at a satisfactory rate, the calorie value of the milk output should have corresponded to about 120 kcal per kg of baby ((U.S.A.) National Research Council: Food and Nutrition Board, 1958; FAO, 1957a).

On this basis the mean calorie value of the milk output was 555 kcal daily. This is in close accord with the mean calorie value of milks during the 5th to 13th week (see Table IX, Chapter 18). Estimates, calculated for each individual, of expenditure on basal metabolism, specific dynamic action and activity, indicated that the average cost of maintenance was a little over 2100 kcal per day. This result seems reasonable, since these women were somewhat less active than the FAO reference woman, and since it is near the measured average intake of four similar Aberdeen primiparae who were not breast feeding their babies. Deducting, say, 2150 kcal from the intake of 2580 kcal, we have 430 kcal left over for milk production. Since these women were losing weight, the contribution derived from body stores has to be added. The calorie equivalent of body weight lost or gained by a lactating woman has not been determined experimentally; in the absence of directly relevant data, 6500 kcal per kg has been taken as a first approximation, in the light of evidence derived for men by Best (1954), Keys *et al.* (1955) and Wishnofsky (1958). On this basis, the weight loss of these subjects corresponds to a "subsidy" towards lactation of about 250 kcal per day. Thus, the total energy available for the production of 555 kcal in milk was about 430 + 250 = 680 kcal. This implies an efficiency of conversion of 81·6 %, a figure which may be rounded off to 80 %.[1] There is no reason to suppose that this estimate is unduly high; Lodge (1957) found the net energy conversion efficiency in lactating sows to be approximately 85 %, a figure which is more likely to be analogous to the human efficiency than the lower efficiencies quoted for ruminants.

To summarize, our Aberdeen primiparae appear to have produced 555 kcal per day in the form of milk at a production efficiency of about 80 %, somewhat as follows:

$$\underset{(L)}{555} \times \underset{(\frac{1}{e})}{100/81{\cdot}6} = \underset{(D)}{2580} + \underset{(S)}{250} - \underset{(M)}{2150}$$

3. *Application to Previous Studies*

It seems desirable to test this estimate of production efficiency (80 %) and the allowance for energy derived from or lost to body stores (6500 kcal per kg

[1]The steps in the calculation leading to this percentage could be elaborated by making the not unreasonable assumption that energy derived from body stores can be utilized for milk production at a higher efficiency than "metabolizable energy" derived from the diet. But this additional step involves still more theory, and is unlikely to make a great difference to the final result. The efficiency given here, about 80 %, is recognized to be an approximation based on eleven sets of observations only, and no doubt includes a fairly large "experimental error." Attempts at refinement are not likely to reduce, and may increase, this error. The real need is for more measurements. As will be shown below, an assumed efficiency of about 80 % seems to fit observations reported in the literature fairly well.

body weight lost or gained) by applying them to experimental results reported in the literature. A remarkably wide range of subjects is available, from three women of average body weight about 70 kg and yielding well over 2 l. of milk daily (Shukers *et al.*, 1932) to six "poor" Indian women with an average body weight of only 37 kg and an average milk yield of only 333 ml daily. The subjects also show widely differing trends of body weight during lactation, partly because many of them were being specially fed.

Columns (1) to (4) in Table IV summarize the average experimental findings in four published investigations, in addition to our own results. One or two figures not given in the original have been estimated, as specified in the notes. Column (5) gives the calories needed for milk production on the assumption that the efficiency of conversion is 80 %. The deficit or contribution from body stores is given in column (6), on the assumption that 1 kg of weight gained or lost yields 6500 kcal. Column (7) shows what is left for maintenance and activity when the requirement for milk production is deducted from the calories provided from the diet, after adjustment is made for the change in body weight. For the sake of comparison, column (8) shows the residue available for maintenance and activity when the FAO production efficiency factor, 60 %, is used in the calculation.

The estimates for maintenance, shown in column (7), afford a gratifying degree of support for the assumptions involved in the calculations. The three wet-nurses, and the twelve specially-fed multiparae, whose diets were providing 4233 and 2928 kcal per day respectively, appear to have been expending a little over 2200 kcal per day on maintenance. The wet-nurses "were doing their own housework, tending their children and leading their customary social lives," but Shukers *et al.* (1932) considered that "their maintenance needs can be approximated only within the wide range of 2200 to 3200 calories a day." Calculation of the overall energy exchanges suggests that the lower figure is much more probable than the higher; even if they produced 1573 kcal per day in milk with 100 % efficiency and lost no weight, only 2633 kcal per day would be left for maintenance and activity. In fact, two of the subjects lost weight and one gained a considerable amount, the average being a slight gain. Very little is said about the activity of the twelve multiparae of Kaucher *et al.* (1946); according to Macy *et al.* (1945) they were chosen from a good environment, "so that the mother would not be overworked, would have ample rest, and be free from worry." The five unmarried mothers of Deem (1931) were exceptionally active and apparently expended about 2350 kcal per day on maintenance and activity; a correction for change in body weight is not possible. As stated previously, our own eleven primiparae were living rather less active lives than the FAO reference woman, and the daily expenditure on maintenance and activity, about 2150 kcal, was calculated directly for each individual and averaged for the group. The estimate

for the maintenance of the small Indian women of Narasinga Rao *et al.* (1958) and Gopalan (1958) is much lower. These women were the subjects of short-term balance experiments and were in hospital. Their activity is not specified.

The figures for maintenance given in column (8), which are based on a production efficiency of 60 %, appear to fit the known facts much less perfectly. For example the wet-nurses of Shukers *et al.* (1932) would, on this basis, have only about 1600 kcal per day left for maintenance and activity; this is not much more than would be required for continuous rest in bed. The remaining estimates are more plausible but may perhaps be regarded as unduly low.

It is of interest that Shukers *et al.* (1932) point out that a production efficiency of 50 %, proposed by Rand *et al.* (1930) gives nonsensical results when applied to their three wet-nurses; they appear to prefer 90 %, as suggested by Rose (1930), commenting that "the women of this investigation exhibited a high degree of efficiency in the transformation of food energy into milk." Kaucher *et al.* (1946) calculate the "net intake" of energy and of nutrients in the diets of their subjects by "subtracting the amounts of nutriments found in analyzing their milk from the amounts determined by analysis of their food. Thus, the 'net intake' represents, approximately, the quantities which were available for the support of the mothers' bodies." They then compare directly the "net intakes" with intake standards. This procedure implies a conversion efficiency of 100 %.

In our opinion, these findings suggest that the FAO (1950) estimate of production efficiency is too low. Our own figure, which should be regarded as provisional, suggests that the efficiency of production of energy in human milk is approximately 80 %, under a wide range of circumstances. In using this figure to calculate the total calorie requirements of lactating women, it should be remembered that the average nursing mother appears to be less active than the FAO "reference woman". Allowance may also have to be made for change of body weight. Whether it is "normal" to lose weight during lactation is a problem which requires discussion in its own right.

4. *The Role of Body Stores in Subsidizing the Energy Requirement*

It has for long been believed that most mothers store nutrients during pregnancy in amounts greater than those required for the growth of the products of conception and the enlargement of the uterus and breasts; this "extra" storage then helps to meet the requirements of lactation. Direct demonstration of storage and loss, in terms of the energy-yielding nutrients, seems to have been attempted only for protein. Experimental findings relating to protein storage and loss will be discussed later (p. 34).

If protein, fat, and possibly carbohydrate in body stores are used up during lactation, the mother must lose weight. Curiously, there seems to be very little evidence in the literature on this rather elementary point. Of the

TABLE IV

DIETARY INTAKE, MILK OUTPUT AND BODY WEIGHT CHANGE IN LACTATING WOMEN CALCULATED ON AN ASSUMED PRODUCTION EFFICIENCY OF 80 %

Subjects	(1) Mean body weight at beginning of study (kg)	(2) Mean daily weight change (g)	(3) Mean calorie value of daily milk yield (kcal)	(4) Mean daily dietary intake (kcal)	(5) Calorie requirement for milk production (kcal) $\left[(3) \times \frac{100}{80}\right]$	(6) Calorie contribution from body stores (kcal) [(2) × 6·5]	(7) Calories left for maintenance (approx.) (kcal) [(4) − (5) + (6)]	(8) Calories left for maintenance if 60 % efficiency is assumed (approx.) (kcal)
Three high-yielding multiparous wet-nurses studied throughout lactations averaging 450 days. Detroit, U.S.A. Macy *et al.* (1930) Shukers *et al.* (1932)	69·5	+ 1·8	1573	4233	1966	− 12	2250	1600
Twelve multiparae given special diets: 31 periods of study from early to late lactation. Detroit, U.S.A. Kaucher *et al.* (1946)	60·6	? Slightly positive	554	2928	693	− ?	< 2230	< 2000

Eleven primiparae. Self-chosen diets at home. One week's weighed diet survey at 5–12 wks. of lactation. Aberdeen, Scotland Unpublished data of the present authors	55·7	− 38	555[1]	2580	694	+ 247	2150	1900
Five unmarried mothers living in an institution doing "all the manual labour of the home." Auckland, New Zealand Deem (1931)	No data	No data	626	3132	783	?	2350	2100
Six "poor" Indian women fed in hospital for three 10-day periods 5th-13th week of lactation. South India Gopalan (1958) Narasinga Rao *et al.* (1958)	36·8	+ 126·3	333[2]	2976	416	− 821	1740	1600

[1]Estimated as 120 kcal per kg weight of baby.

[2]Estimated on basis of 71 kcal per 100 ml (Table IX Chapter 18).

subjects considered in Table IV, two of the wet-nurses of Shukers *et al.* (1932) lost weight, the third gained a considerable amount. Of the twelve subjects of Kaucher *et al.* (1946) weight trends after the puerperium are published for five only, and of these only one lost weight; these subjects were, of course, being specially fed, and so were the six poor Indian subjects of Narasinga Rao *et al.* (1958) and Gopalan (1958). Of our own eleven subjects, using self-chosen diets, all but one were losing weight. The weight changes, if any, of Deem's (1931) subjects are not stated.

We have additional unpublished data suggesting that a tendency to lose weight—in the absence of special feeding arrangements—is common in both lactating and the non-lactating women during the months which follow the

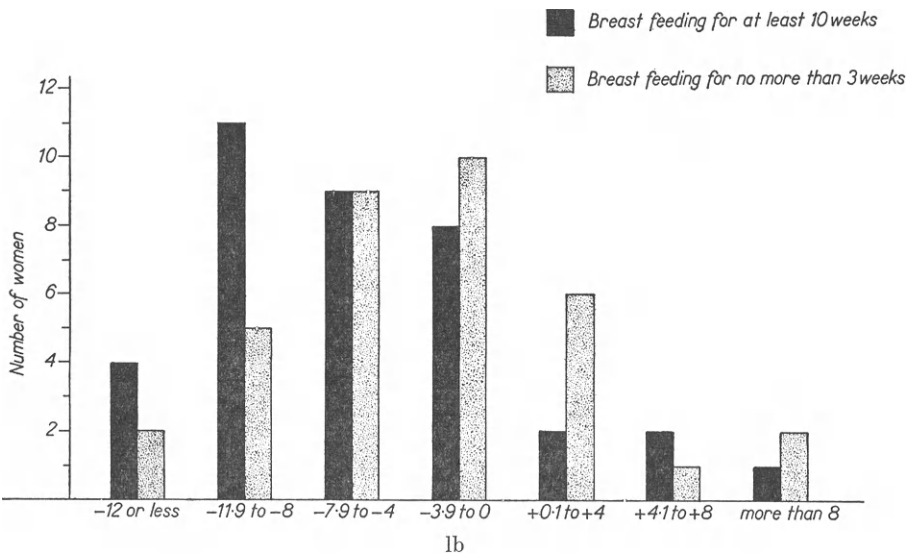

FIG. 2. Distribution of changes in body weight, between the 2nd and the 13th weeks post-partum in seventy-two Aberdeen primiparae.

puerperium. Fig. 2 shows the distribution of gains and losses of weight between the time of discharge from hospital, about a week after delivery, and the 13th week after delivery in thirty-seven primiparae who lactated for at least 10 weeks (Group B) and in thirty-five primiparae who lactated for not more than three weeks (Group A). In Group B thirty-two subjects (87 %) lost weight, and in Group A twenty-six subjects (74 %). The difference is more marked at the lower extreme: in Group B, fifteen subjects (41 %) lost 8 lb (3·6 kg) or more in the 12-week period, and in Group A, seven subjects (20 %).

The other side of the concept is that women store energy-yielding nutrients during pregnancy. Thomson & Billewicz (unpublished) have estimated the

average net weight gained during a first pregnancy—that is, the weight gained that is not lost during parturition and the week following delivery—to be about 4 kg. If this store is all available to subsidize lactation, which is by no means certain, it would suffice for about 15 weeks of lactation at the average rate of depletion of the eleven Aberdeen primiparae included in Table IV, who were all lactating satisfactorily.

Although storage of energy-yielding nutrients during pregnancy and loss during lactation may be perhaps a general rule, there is no reason to suggest that the success of lactation depends upon the existence of body stores specially provided. The indications are that when stores specially laid down are used up, the "normal" reserves in the maternal tissues will serve to ensure that the ability to breast feed is maintained, at least for some time, even when the immediately available diet is insufficient to meet the total expenditure of energy. Even though depletion of reserves does not readily influence lactation, the effects may be felt in other ways. Hytten *et al.* (1958) found that in a group of 106 breast-feeding primiparae 44 % complained of tiredness, which was often accompanied by backache, dizziness, headaches and repeated minor infections. Many of the women who felt tired were losing more than the average amount of weight during lactation and were doing an excessive amount of housework. These untoward symptoms may or may not have been exacerbated by inadequacy of the energy supply from the diet and consequent depletion of reserves; there was, at least, no economic reason for taking a restricted diet, and if there was an insufficient calorie supply it must have been associated with an insufficient appetite. The problem is greatly complicated by the social, psychological and physical strains experienced by the mother who chooses to breast feed in modern urban civilization (Hytten *et al.*, 1958).

Many women become increasingly obese as pregnancy succeeds pregnancy. This process of fattening may be due in part to failure to lose accumulated stores during lactation. If so, this would be an argument in favour of the "normality" of weight loss during lactation. It would be interesting to know if mothers who breast feed retain their figures more easily than those who do not.

If lactation is supported to some extent from stores laid down in pregnancy, it might be expected that increasing storage during pregnancy would be associated with increasing adequacy of lactation. From the results of a preliminary study, Hytten (1954e) reported that the reverse seemed to be true, milk yields rising as weight gained during pregnancy became less. This unexpected finding has not been confirmed by more extensive data now collected by us. There is no apparent correlation between the amount of weight gained during pregnancy and the yield or composition of breast milk.

Finally, the question arises as to the nature of stores laid down during

pregnancy. Storage of carbohydrate in appreciable quantity is not likely. There is now some doubt that protein stores are commonly used to subsidize lactation (see below); and, in principle, it is improbable that a reserve of energy would be stored primarily in the form of protein. It therefore seems likely that stored calories are laid down mostly as fat. This conclusion is possibly supported by the "fattening" influence of pregnancy, referred to above.

B. Protein Requirement

The estimation of additional protein required for lactation necessarily involves, with present knowledge, an even higher proportion of hypothesis to fact than we have used in estimating energy requirements. The question of quality as well as quantity of protein arises. The problem of a "requirement" cannot be resolved, as with energy, by a final appeal to thermodynamic equilibrium. It is difficult even to appeal to metabolic equilibrium, since most human beings take diets containing much more nitrogen than is required for balance.

In the following account, we shall quote the statements of various authoritative bodies and make a few comments on them in the light of metabolic and dietary data.

1. *The Recommendations of FAO and other Authoritative Bodies*

The report of FAO (1957b) states that "Protein needs increase as soon as lactation is established and continue to mount as the quantity of milk secreted increases, reaching a maximum sometime after the fifth postpartum month. Human milk contains on an average 1·2 grams of protein per 100 cc. and the average quantity of milk secreted by a woman daily is approximately 850 cc. Although concrete evidence is lacking, the assumption might be made that about 2·0 grams of food protein of high nutritive value is needed to produce 1·0 gram of milk protein. On such a basis, a simple calculation suggests that about 20 grams of additional protein daily will be needed to sustain lactation in the average woman." The report then points out that some women, such as wet-nurses, secrete more than the average amount of milk, and suggests the provision of 30 g of protein daily in order to meet the requirements of almost all nursing women. The protein supplied should be of high nutritive value, corresponding to that of the protein of milk, eggs and meat, or to the amino acid composition of a theoretical "reference protein." This supply is additional to the requirement of a normal non-lactating woman, who (assuming age 20 years or over and weight 55 kg) is stated to need 19·3 g of "reference protein" daily. The total dietary requirement during lactation is therefore about 50 g of "reference protein" daily, an amount which should

cover even the requirements of women with an exceptionally high milk yield. Since some, or even most, of the protein actually taken by women is of a lower biological value than the reference protein, correction may be made by using a system of "protein scores", details of which are given in the FAO report. For example, in a country with an average diet providing 2030 kcal and 57 g of protein, of which 14 % comes from pulses, and 51 % from cereals, chiefly maize, the theoretical protein requirement should be increased by a factor of 1·6, so that the total protein requirement during lactation will be $50 \times 1{\cdot}6 = 80$ g daily. In a country where about 12 % of the calories are derived from protein (95 g protein and 3200 kcal) and 60 % of the protein is of animal origin, the appropriate correction factor is 1·25, giving a total requirement for lactation of $50 \times 1{\cdot}25 = 62{\cdot}5$ g protein daily.

The (U.S.A.) National Research Council: Food and Nutrition Board (1958) proposed that a supplement of 40 g protein daily be provided in the diet of the lactating woman. This amount may be presumed to include a high proportion of animal protein.

The British Medical Association: Committee on Nutrition (1950) proposed that lactating women should receive diets in which 14 % of the calories are derived from protein. On their assumption that the diet provides 3000 kcal daily and that each gram of protein provides 4 kcal, the total daily requirement of the protein is 105 g.[1] Since the normal non-lactating woman doing moderate work is said to require 2250 kcal of which 11 % should be derived from protein, it can be calculated that the additional protein requirement specific to lactation is about 43 g, in good agreement with the N.R.C. estimate.

It should be noted that the additional daily requirement recommended by FAO, 30 g of "reference protein," includes a "safety factor" of 50 %; the requirement of an "average woman" is 20 g. The N.R.C. recommended allowance also includes a safety factor, not specified but presumably of a similar degree. On the other hand, the B.M.A. says that its recommendations, in general, "are believed to be sufficient to establish and maintain a good nutritional state in *representative* individuals of the groups concerned" (our italics); in other words, a factor of safety has not been consciously included.

2. *Comment*

The FAO estimate of the average amount of protein secreted in milk is 10 g daily (850 ml milk containing 1·2 g protein per 100 ml). This total excretion corresponds fairly well to the daily intakes of infants quoted by Macy & Kelly in Table IX of Chapter 18. On the other hand, Table I of the

[1]The actual figure given in the B.M.A. report is 111 g. This appears to assume that 1 g protein is equivalent to 3·78 kcal.

same chapter gives the mean protein content of mature milk as 1·1 g per 100 ml. Provisionally, the FAO estimate of output may be regarded as possibly erring on the side of generosity. It may be noted here—not as a comment on the FAO estimate—that many figures for milk "protein" given in the literature are derived from measurements of the total nitrogen in milk, multiplied by a constant—usually 6·38—representing the percentage of nitrogen in milk protein; since total nitrogen contains a fairly high proportion of non-protein nitrogen, figures for protein so derived are of course too high.

The origin of the FAO factor for efficiency of conversion of dietary protein to milk protein, 50 %, is obscure. This figure was recently suggested by a speaker at the Princeton Conference (1957); however, he misquoted the efficiency factor for calories used by FAO (giving 50 % instead of 60 %) and appears to have thought that the same figure might be applied to protein. In the same discussion, another speaker remarked that the cow has a conversion efficiency for protein of about 75 %; and another suggested that the sow has a conversion efficiency of almost 100 %. As we noted previously, an assumed conversion efficiency of 80 % for energy seems to give "sensible" results when applied to human data, and in the absence of more relevant information the same figure may perhaps be used for protein. The FAO efficiency factor may therefore be too low. If 80 % efficiency is assumed, an intake of about 13 g protein of good quality will suffice for the production of about 10 g of protein in milk. If a factor of 1·25 is applied, as in the second FAO example given above, 16 to 17 g protein in the average Western diet should suffice to support the additional needs of lactation.

It has long been believed, and is stated in many reviews and standard texts, that nitrogen is stored during pregnancy in excess of the amount required for the growth of the foetus, placenta, uterus and breasts, and that this excess is available to subsidize lactation. This idea appears to have received considerable impetus from the unique study by Hunscher *et al.* (1935) of a single highly selected multipara with an enormous capacity for lactation. Writing from the same centre (Detroit) in the previous year, however, Macy & Hunscher (1934) noted that: "Only fragmentary information exists upon the nutritive needs of the average nursing woman as lactation progresses, because there are too few figures on the nitrogen retention during mature milk flow. The methods used, the duration of the metabolic balance studies, and the variability in types of subjects observed are too diverse to warrant an interpretation in terms of daily protein requirements of the average lactating woman." Garry & Wood (1945–46) probably overlooked this cautious statement when preparing their review; they say that ". . . breast feeding may easily lead to a negative nitrogen balance. Since, however, as a result of pregnancy, a woman normally stores a not inconsiderable amount of protein, the lactational negative balance may be provided for (Hunscher *et al.*, 1935)."

However, they then proceed to question the normality of a negative nitrogen balance: "It might be worth investigating whether this negative nitrogen balance is conditioned by an energy intake too low for all the demands made on a lactating woman."

Though little additional evidence has been published, it now seems permissible to doubt that a negative nitrogen balance is characteristic of lactation in its mature phase. Oberst & Plass (1940) found negative nitrogen balances in two women studied during the 1st and 2nd weeks after parturition, and positive balances in the 3rd week. There is, of course, an outpouring of nitrogen during parturition and the puerperium, and the majority of nitrogen balance studies during lactation appear to have been made during this early phase.

More fundamentally, however, it has become necessary to regard metabolic balance data with reserve, since many investigations of animals have shown the apparent storage of nitrogen to be considerably greater than the amounts detected by carcass analysis (Nehring *et al.*, 1957).

It has been shown (Cuthbertson, 1940-41; Dole, 1957; Thomson, 1959) that in most ordinary diets some 10 to 14 % of the calories are provided in the form of protein. If we take the higher end of this range as "adequate," a diet providing 2600 kcal per day will contain about 85 g protein; of this amount, about 17 g will be required to support lactation, leaving 68 g for maintenance. If only 10 % of the dietary calories are derived from protein, 48 g protein will be available for maintenance.

It appears that all the average diets shown in Table I should provide more than enough protein to support lactation. In Western society at least, it would seem to be true that a diet that is adequate in calories will also be adequate in protein. This conclusion may not be tenable in societies where the dietary protein is of low biological value.

C. Calcium Requirement

The (U.S.A.) National Research Council: Food and Nutrition Board (1958) and the British Medical Association: Committee on Nutrition (1950) agree in recommending that the total intake of calcium by a lactating woman should be 2 g daily. The allowance for a non-pregnant, non-lactating woman is given as 0·8 g daily, so the requirement specific to lactation may be taken as 1·2 g daily. The N.R.C. links these figures to a milk yield of 850 ml daily, a quantity which is likely to contain about 0·3 g calcium. On the face of it, therefore, a loss of 75 % in the transfer from diet to milk is assumed.

The available information seems to be quite inadequate to support firm conclusions on the physiological requirement of calcium for lactation. Studies with radioactive calcium have shown beyond doubt that the non-pregnant non-lactating adult loses "endogenous" calcium by excretion into the gut with

incomplete reabsorption, as well as by loss through the kidney (Blau *et al.*, 1957). It is also probable that the absorption and retention of calcium depend, not only on the dietary intake, but also on the state of the reserves in the body, so that the results of balance experiments will vary according to the previous dietary history. Long-continued shortage of calcium probably leads to economy of calcium utilization, so that the apparent requirement is reduced. The problem is further confused by the fact that "availability" of calcium is greater from some foods than from others, and may be influenced by the intake of vitamin D and of other nutrients.

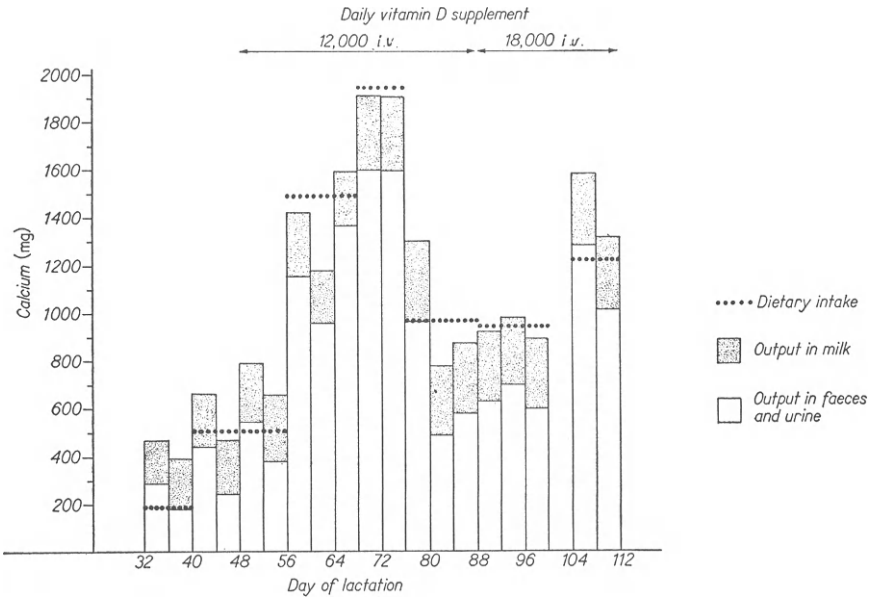

FIG. 3. Calcium balances in a Chinese wet-nurse. (Redrawn from the data of Liu *et al.*, 1940.)

Again, balance studies with calcium have now come under suspicion because experiments with ruminants show cumulative estimates of retention far in excess of increments found by carcass analysis (Duncan, 1958).

It has for long been believed that normal pregnancy is accompanied by storage of calcium and normal lactation by loss. Duckworth & Warnock (1942–43) have summarized the data. Their tables, based on balance studies, show considerable average retentions of calcium during the later stages of pregnancy, accompanied by rising percentages absorbed and increased losses in the urine. In lactation, the retentions are nearly all negative, and percentages absorbed are variable and generally lower than in pregnancy, with urinary excretions that are mostly well below the pregnancy levels but higher

than the normal average excretions of adults. Inspection of Duckworth & Warnock's table relating to lactation shows that the original data must have been a curious mixture; thus the daily calcium excretion in milk in each month of lactation appears to vary erratically from 0·2 to 1 g, without any relationship to the stage of lactation. The net absorption percentages in pregnancy have a range of + 12 to + 47; those for lactation vary from — 12 to + 38. Duckworth & Warnock conclude by accepting that a total requirement of 1·5 to 2 g calcium during lactation may be reasonable. Since their estimated requirement for the non-pregnant, non-lactating woman is 0·6 g, the requirement specific to lactation is within the range 0·9 to 1·4 g. They believe a negative calcium balance to be "normal" in lactation, and doubt whether it would be wise to attempt to overcome it by giving calcium salts during pregnancy.

The interesting investigations of Liu *et al.* (1940) do not appear to have been included in Duckworth & Warnock's review. These investigations, made in Peiping, were concerned for the most part with cases of osteomalacia, and their interpretation in relation to normal lactation is not easy, as treatment with calcium and vitamin D was in progress. Fig. 3 summarizes the calcium balance in a "presumably normal lactating woman" who wet-nursed a baby born to a mother with osteomalacia. During the previous year, she had delivered her own child, and taken a diet consisting of "rice, wheat flour, millet, corn-meal and salted or fresh vegetables, but practically no meat or eggs"; there is therefore no reason to think that she had well-stocked reserves of calcium. The figure shows that the losses of calcium in the milk and faeces followed very closely the changes of calcium intake, and it is perhaps doubtful whether the small positive and negative balances found in each 4-day metabolic period had any biological significance. Totalling the balances for the 80-day period of observation indicates an overall loss of about 4·3 g calcium, although the intake of calcium during the last 56 days exceeded 0·9 g daily. There is some suggestion that this subject ceased to lose calcium when her intake was between 1·5 and 2 g daily. Another Chinese wet-nurse reported on by Liu *et al.* (1937) eventually achieved calcium equilibrium with an intake of 0·4 g daily, apparently by extreme economy of utilization. The loss of calcium in milk was not unusually low.

It is of interest that Liu *et al.* (1937) summarized their views as follows: "Current conception about calcium requirement implies that it is a fixed quantity below which there is danger of running into negative balance; whereas it must be a very variable affair. Aside from the increased demand in growth and reproduction, many other modifying factors may be operative. The requirement may seem to be greater when dietary habits accustom the subject to larger intake, and when there is a larger prior store in the body. On the other hand vitamin D when present in adequate amounts will conserve

calcium metabolism to such an extent as to make the usually quoted requirement superfluous. Therefore in defining calcium requirement for a given period of life, such factors as previous dietary custom, the state of the skeletal store and the amount of vitamin D must be taken into consideration."

It appears, then, that the requirement of calcium during lactation depends upon the context. At worst, lactation will accentuate the removal of calcium from a skeleton that is already depleted. The effect may be brought into focus by considering a hypothetical extreme instance. Let us assume that half the average output of calcium in milk—say 0·15 g daily—has to be met by depletion of reserves in the maternal skeleton. The total loss from the body during a lactation of 180 days will be about 27 g. This loss will be from a skeleton likely to contain over 1000 g of calcium. The loss is therefore relatively small and seems unlikely, by itself, to assume importance from the point of view of the mother's health. But if such a loss is not made good between lactations and is repeated several times, and especially if there is further depletion during and between previous pregnancies, the cumulative losses may become deleterious. Such chronic depletion is probably not uncommon in Western civilization. In a study of pregnant women in Aberdeen, Scotland, Thomson (1958) found that the mean calcium intake among wives of semi-skilled and unskilled workers was 0·88 g daily, with one-quarter taking less than 0·68 g daily. Of our eleven lactating primiparae referred to previously (Table I), six were taking less than 1 g and two less than 0·7 g daily. Diets between pregnancies and lactations are unlikely to have had as high a calcium content, since cheap milk would not be available. Similarly, Toverud *et al.* (1950) noted that "in America also the calcium intake of pregnant women was often below 1 gm daily and . . . negative calcium balances were common (during pregnancy) with this intake level."

Since satisfactory evidence is lacking, we are unable to suggest a physiological requirement for calcium during lactation, that may be used as a yardstick to assess the adequacy of the diet of a "typical" individual or small group of such individuals. The "recommended allowance" of the N.R.C. should be used with great caution as a physiological norm, though it may be valid as a target towards which society should aim, in order to be sure that the health of lactating women is fully safeguarded.

D. Iron Requirement

The average iron output in mature milk is between 1 and 1·5 mg daily; since losses of iron are minimal, in the absence of bleeding, it may perhaps be assumed that the physiological requirement of iron specific to lactation is of a similar order. As stated previously, the iron content of milk is not readily influenced by the iron content of the diet, or by the serum iron level.

E. Requirement for Vitamins

It has been shown previously that the level of the water-soluble vitamins in milk can be raised or lowered by change of the maternal diet. Whether the maintenance of high levels in breast milk is beneficial to the infants is entirely speculative. At present, therefore, it seems reasonable to relate requirements for lactation to the vitamin content of milks that nourish apparently healthy babies. Representative figures are given in Table IX, Chapter 18, and it can probably be assumed that average requirements specific to lactation are of a similar order.

F. Some Reflections on "Recommended Allowances" and Requirement Standards

The (U.S.A.) National Research Council: Food and Nutrition Board (1958) says specifically that it uses the term "recommended allowances" to avoid misinterpretation of the allowances as representing either minimal or optimal requirements. On the other hand, "They are meant to afford a margin of sufficiency above minimal requirements [which] varies widely among the single nutrients listed." Neither the minimum requirements nor the margins of sufficiency are specified.

Since there is no equally authoritative statement of requirements more suitable for use with experimental measurements, it is not surprising that many investigators have used the recommended allowances of the N.R.C. and similar authorities as if they were indeed physiological yardsticks.

In respect of standards referring to the energy intake of healthy adults, the distinction between a "recommended allowance" and a "requirement" does not arise. Both a deficit and a surplus of calories are likely to be harmful, and therefore there is no question of adding a margin of safety to the estimate of the physiological need.

For individual nutrients, the problem is more complicated, and to superimpose a factor of safety on an average physiological requirement may be wise, in the calculation of the supplies which are desirable for populations or large groups. FAO (1957b) recommends that the requirement of protein for the "average woman" should be increased by 50 % in order to cover the higher needs of women with an exceptionally high milk yield. If some "average" women receive more protein than they need, this in itself will be harmless. But the factor of safety should not be added if the FAO requirement is being used as a yardstick with which to compare the protein intakes of average experimental subjects.

In this Chapter, our interest has been restricted to the physiological aspect of the problem of requirements. We have discussed what milk production

"costs" in terms of energy and of certain nutrients, so far as possible with reference to a hypothetical average healthy mother who is using the whole of her milk production to feed an average healthy baby. We have been able to make rough estimates of the cost—the physiological requirement—in terms of calories and of protein. The position in respect of calcium is too uncertain to enable us to come to any conclusion. Our suggestions for iron and for the vitamins make the possibly unwarranted assumptions that the efficiency of transfer to milk is about 100 %, and that the levels in milk currently accepted as normal are indeed adequate.

Throughout, we have been aware that experimental data on this subject are extremely meagre. When more information is available, there will probably be no universal flat-rate requirement standard for any nutrient. It is already common practice to adjust the calorie requirements of women for age, body size, activity and climate, and our own suggestions include a method of adjusting for the level of milk production and for the use of body reserves. The requirements for protein and other nutrients may also depend on the context, but we are not yet in a position to define the adjustments which should be made under particular circumstances.

But planning for a healthy society cannot await the acquisition of a complete and detailed knowledge of metabolism. We need guides to practice now: hence "recommended allowances," which are not "requirements," and should not be used as such unless their physiological basis is explicit. Much of the confusion that has arisen in the literature may be due to attempts to justify such allowances by reference to physiological experiments when, in fact, there is insufficient evidence for any physiological conclusion to be drawn. There is no need to appeal to physiology when devising allowances intended as social targets, that is, the quantities of nutrients which, if taken by most of the people in a large population, will safeguard health. Such allowances may be specified by reference to the diets of persons known to be healthy. Allowances derived empirically in this way will be both practical and safe, because they are derived from real diets taken by people who are observed to be healthy. Perhaps "recommended allowances" proposed in future should differentiate clearly between standards derived empirically, and those derived from a knowledge of physiology. The "factor of safety," if any, added to the latter should be specified.

IV. Lactation and Health

In previous Sections of this Chapter, we have touched on possible deficiency states that may arise in infants fed solely from the breast; and we have stated that underfeeding (insufficiency of calories) is by far the most important "deficiency disease" of breast-fed infants.

In this Section we will consider whether the mother's nutritional status and general health influence lactation and, conversely, what effect lactation has upon the mother's health. Very little reliable evidence is available, either way.

In Section II B, it was noted that, so far as is known, famine conditions are by no means necessarily accompanied by an obvious deleterious effect upon lactation; and we found no studies that definitely contradict the common belief that easy breast feeding is almost universal in primitive communities where malnutrition is common. In Australia, Wardlaw & Dart (1934) found that short women had a significantly lower output of milk than tall women; but the "state of nutrition," as judged by the fatness of the subject, was not related to the yield. The fat content of the milk did not seem to be related either to stature or fatness. Hytten (unpublished) found that tall women and women assessed as being in a very good state of general health had a slight tendency to produce more milk of higher fat content than small women and those in a less satisfactory state of general health. Provisionally, it seems that maternal physique, health and nutritional status exert an inconspicuous and unimportant effect upon lactation, in comparison with such factors as age, parity and the functional capacity of the breast. It should be noted that we are dealing with lactation, not breast feeding. The undoubted improvement in nutritional conditions in Britain during the last three decades has been accompanied by a steady decline of breast feeding (Hytten, 1958); but we are far from suggesting that this is a matter of cause and effect.

When we turn to the question, whether lactation causes maternal ill-health, the analyses in Section III of this Chapter indicate that under generally adverse nutritional conditions lactation depletes the maternal reserves of calcium. The literature, however, is scanty. Anderson & Brown (1941) have reported from Scotland that a woman suffering from tetany responded to rest, a good diet and cessation of lactation; this woman, aged 42, had had seven children, all breast fed, and the youngest was still at the breast at the age of 21 months, when tetany was diagnosed in the mother. The mother herself was "a poorly nourished sallow rachitic female of short stature"; not atypical of the inhabitants of Glasgow slums a generation ago. Osteomalacia has long been known to be precipitated by pregnancy and possibly by lactation; it was once not uncommon in Glasgow, but most reports concern severely malnourished women in eastern countries (Maxwell, 1935, Wolfe, 1935). Vitamin D deficiency is almost certainly a factor (Liu *et al.*, 1941).

These are relatively rare states which arise under nutritional conditions that are doubtless exceptional. That lactation predisposes to more common complaints such as "rheumatic" pain, and dental caries is a not uncommon popular belief, but according to Garry & Wood's (1945–46) review, the published evidence is meagre and conflicting.

We have noted elsewhere (Hytten *et al.*, 1958) that in modern urban civilization breast feeding is by no means usually associated with maternal well-being. Whether dietary inadequacy, especially of calories, contributes to tiredness and other minor symptoms of ill-health during lactation is a question we cannot answer (see p. 31).

Whatever the facts are, satisfactory lactation represents the greatest nutritional stress imposed by a physiological process on the human body. It seems reasonable to suppose that the mother who embarks upon pregnancy and lactation in a poor nutritional state, the result of prolonged dietary inadequacy in the past, possibly exacerbated by previous pregnancies and lactation, should sometimes "break down." It is therefore just as important to safeguard the nutrition of women before and between lactations, as during each lactation.

V. Summing Up

Our main impression, on completing this review, is of a large, confused, and unbalanced literature which yields relatively little factual information on many important questions. This is not altogether surprising. The scientific investigation of human lactation necessarily involves very close team work between scientists and clinicians who, together, must gain the confidence and co-operation of mothers who are already pre-occupied by the care of their babies. Sustained and comprehensive research on lactation represents a major feat of perseverance and planning; it is by no means sufficient to bring together some laboratory facilities and a few fairly docile subjects. Really comprehensive research on human lactation has not yet been attempted. Most clinicians are too pre-occupied by day-to-day problems of supervision and treatment to worry unduly about academic problems of lactational physiology and metabolism; conversely, physiologists and biochemists can readily find much easier subjects to study under controlled conditions than nursing mothers and their babies. It is in this light that tribute should be paid to the massive contribution of the Detroit workers, led by Icie Macy Hoobler. Even though this research was biased towards the laboratory and used as subjects mothers who were mostly either exceptional physiological specimens or were observed under special conditions, it is likely to remain unique for a long time. What seems to be needed now is an equally purposeful study of "typical" lactating women living under ordinary dietetic and social conditions, with as much emphasis on the epidemiological and clinical setting as on laboratory measurements.

We ourselves began to study lactation in Scotland with this idea in mind, but have now been forced to face the fact that breast feeding is rapidly losing importance in Western civilization. The decline of lactation may

accelerate if most clinicians come to accept—as many mothers do already —that artificially fed babies thrive very well nowadays, and that the well-worn slogans emphasizing the importance and safety of breast feeding have seemingly lost nearly all their force (Hytten, 1958). Under these circumstances, there are more pressing problems to study than those of lactation and breast feeding.

The decline of breast feeding may not yet be significant in civilizations less well-endowed, and we can but hope that intensive studies will be made there before it becomes too late. For this reason, research such as that undertaken by Gopalan and his associates in India will be followed with the liveliest interest. We hope that laboratory investigations will be accompanied by parallel clinical and epidemiological studies, and that as much attention will be paid to well-fed as to ill-fed subjects.

The fact is that much can be done with relatively simple "research tools." As we have pointed out already, no certain answer can be given to such apparently elementary questions as the incidence of full and partial breast feeding in an ill-nourished community, and the adequacy of lactation under such circumstances; nor is it known whether a loss of body weight during lactation usually occurs and, if so, whether it should be regarded as "normal." Perhaps clear answers to such questions should be obtained before intensive laboratory studies are undertaken, especially since they will help to indicate what kinds of subjects can be most profitably investigated by laboratory methods.

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CHAPTER 14

Nutrition of Lactating Farm Animals

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I. Introduction

As previous chapters in this book record, the nature of lactation is complex. The metabolism of lactation includes a great many processes among which are the digestion and absorption of nutrients and the transport, mobilization and biosynthesis of the metabolites used by the mammary gland to form milk. In a consideration of the nutritional requirements of lactating farm animals there are, superimposed upon these complex functions, others associated with the peculiarities of the animal species. These peculiarities are primarily inter-species differences: in the architecture of the digestive tract and, therefore, in the character of the natural diet; in the degree of symbiosis between the host animal and the microbial population inhabiting its digestive tract; and in the composition and quantity of the milk secreted.

It is the purpose of this chapter to deal with the nutrition of the lactating ruminant, mare and sow, giving special emphasis to the nutritional requirements.

II. Digestion, Absorption and Gastro-intestinal Synthesis

A. Some Inter-species Peculiarities

1. *Digestive Capacity and Agents and End-products of Digestion*

In different species, the length, capacity, location and histological characteristics of the parts of the alimentary tract vary markedly. As a consequence,

there are differences in the natural diets consumed, the digestive processes employed and the nutrients required by the ruminants, the horse and the pig.

Since it is impossible within the confines of this chapter to discuss in detail the architecture, capacity and digestive agents of the gastro-intestinal apparatus, attention will be given only to a few major peculiarities that effect differences in the nutritive requirements among species. Also, more attention will be given to newer, less well-known work than to earlier findings.

Relative to body size the capacity of the gastro-intestinal canal of farm animals is greatest in cattle, somewhat less in sheep and goats, intermediate in horses and least in pigs. Despite the fact that the digestive tract of the horse is 80 % as large per unit of body weight as that of the cow and that its fermentation compartments are large, the horse has a distinctly lower digestive capacity, particularly for crude fibre and highly lignified feeds (Crasemann, 1945). On the other hand, the ruminant, horse and pig digest low-fibre feeds to about the same extent.

Although enzymic digestion of protein and lipids appears to be similar and equally important for all farm animals, and enzymes effect the digestion of simple carbohydrates in the simple-stomached animals, the microflora is the agent responsible for the digestion of carbohydrates in the large intestines of all animals and in the reticulo-rumen of the ruminants. The highly developed symbiotic relationship existing between the micro-organisms of the gastro-intestinal tract and the host is the salient feature of the nutrition of the ruminant. Though symbiosis exists in other farm animals, its degree of development is much less marked than in the ruminant. As a result chiefly of bacterial action (the protozoa present in the rumen appear to be largely commensal), sugars, starch, hemicelluloses and cellulose undergo fermentative digestion to form fatty acids and gases. Approximately 85 to 95 % of the total volatile fatty acids produced in the rumen and large intestine of ruminants are composed of the following acids in the listed proportions: acetic, 60 to 70 %; propionic, 15 to 20 %; and butyric, 10 to 15 %. It is noteworthy that the same proportions of volatile fatty acids are produced in the main sites of fermentation in all farm animals. The proportions of the volatile fatty acids and of lactic acid produced are to some extent influenced by the nature of the diet though, as Shaw (1957) has shown, the tendency is great for the proportions to remain constant. The volatile fatty acids are absorbed into the blood directly from the rumen and large intestine (Barcroft *et al.*, 1944) and, in the ruminant, they represent a quantitatively significant source of energy (Carroll & Hungate, 1954). In contrast, the chief end-products of carbohydrate digestion absorbed by the simple-stomached animals are monosaccharides.

a. *Contributions of volatile fatty acids to lactation and energy economy.* Several uses of the volatile fatty acids in lactation and the other body functions have been demonstrated. Propionate is a precursor of carbohydrate and probably serves, in the absence of glucose, as a source of oxaloacetate which, upon condensation with acetyl coenzyme A, allows the entrance of acetic acid into the tricarboxylic-acid cycle (Armstrong & Blaxter, 1957). Acetate is used in the biosynthesis of milk fat (Folley, 1956; Kleiber *et al.*, 1952). In addition it is a precursor of lactose and glycerol in milk (Popják *et al.*, 1952) and provides carbon chains for the biosynthesis of the non-essential amino acids of casein (Black *et al.*, 1957). Butyrate is used in the synthesis of milk fat and lactose (Kleiber *et al.*, 1954) and provides carbon chains for the synthesis of the non-essential amino acids of casein (Black *et al.*, 1952). Further evidence was reported recently that the energy of acetic, propionic and butyric acids is metabolized by ruminants to support maintenance (Armstrong & Blaxter, 1957) and fattening (Armstrong *et al.*, 1957).

2. *Microbial Synthesis of Protein and Vitamins*

In addition to fermenting carbohydrates, the micro-organisms of the rumen effect the synthesis of protein (Reid, 1953) and vitamins (Kon & Porter, 1954). The action of bacteria upon protein or non-protein nitrogenous compounds provided in the diet results in the production of ammonia in the rumen. Concurrently, a net synthesis of protein occurs (Pearson & Smith, 1943; Loosli *et al.*, 1949) which is associated with an increase in the number of certain bacteria and a reduction in the quantity of ammonia (McNaught & Smith, 1947–48). As a result of microbial synthesis, ruminants are able to utilize non-protein nitrogen which is valueless to simple-stomached animals consuming natural foodstuffs. (Non-protein nitrogen is a potential source of amino groups for non-essential amino acids; however, amino groups for the synthesis of non-essential amino acids are provided abundantly by the non-essential amino acids and surplus essential amino acids of natural foodstuffs.) Since the synthesized protein is stored in the bacterial cells, peptic digestion in the abomasum and the further enzymic degradation of such products as the proteoses and peptones in the small intestine are required to avail the ruminant of peptides and amino acids. Effecting this digestion would appear to be the chief function of the abomasum in the ruminant.

The ruminal microflora also effects the synthesis of thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, biotin and vitamins B_{12} and K in the rumen. As a consequence, these vitamins are not dietary essentials for the mature ruminant, though a dietary need for certain of them has been demonstrated in the very young. It would appear that the amounts of the vitamins synthesized by, and made available to, the ruminant, horse and pig are different by degree. To some extent the differences in the vitamin (and

protein) requirements of these animals are certainly the result not only of the magnitude of the microbial processes, but also of the location in the tract of the major sites of fermentation and synthesis.

Although many phases of digestion and absorption in the ruminant are similar to those of the pig and horse, gastric and intestinal digestion (with the exception of that involving protein) are much less essential in the ruminant, as simple carbohydrates reach the small intestine only as the result of entrapment in plant or bacterial cells or of hydrolysis of bacterial polysaccharide, and usually the amount of fat in the ruminant's diet is small. Differences among animal species in the activity of gastro-intestinal microflora result in the absorption of different proportions of end-products and, thereby, in different economies of energy use. Differences in the degree of synthesis and absorption of protein and vitamins result in differences in the dietary requirements of the various farm animals.

III. Nutritional Requirements and Related Metabolism

A. General Considerations

The lactating animal requires nutrients of a kind and amount determined by her maintenance needs, the amount and character of the milk produced and the extent of wastage in the use of nutrients for the support of these two functions. An additional allowance of nutrients is needed, particularly near the termination of gestation, when pregnancy and lactation proceed simultaneously. Under the usual conditions of management of the common farm animals, pregnancy and lactation may coincide only in the cow, goat and mare. Needless to state, separate quantitative requirements for lactation have been derived by direct experimentation only for those animals used in commercial milk production and for which the milk yield is known. This is to say that the existing information on the nutrient needs specifically for the secretion of milk was obtained in experiments mainly with the cow and to a much lesser extent with the goat. However, several feeding standards such as those proposed by Morrison (1956), the (U.S.A.) National Research Council (1949, 1953, 1957) in America and the Ministry of Agriculture and Fisheries in Britain (Woodman, 1948) set forth nutrient allowances for the combined needs of maintenance and lactation in one or more of the ewe, sow and mare. From the standpoint of feeding practice these allowances are probably adequate, but at best they are only very rough guides. On the other hand, it is difficult to improve the existing feeding standards for farm animals that normally suckle their young without more information than that available at the present time on: (1) variations in the yield and composition of milk, (2) milk yield-time trends, (3) the influence of level of feed intake upon absorptive and

metabolic efficiency, (4) the energetic efficiency of maintenance and lactation, and (5) the biological value of protein, mineral elements and vitamins for both maintenance and lactation. Naturally, the strain or breed within a species might influence at least the first two items and, therefore, need to be studied.

Since lactation cannot proceed except for very short periods (the duration depending upon the extent of body reserves) unless the maintenance requirements are first satisfied by the diet, the requirements of each of the lactating farm animals for maintenance and the secretion of milk will be outlined here separately. It is my intention to employ data obtained experimentally where they exist and, mainly for the non-commercial milk-producing animals that normally suckle their young and for which such data do not exist, to estimate the requirements from established nutritional principles. Although some degree of variation attaches to the bases which will be used, the estimates derived should be at least of comparative value. Also, this Section will deal briefly with the normal metabolism of energy and nutrients as related to the nutritional requirements.

B. Energy

Of all dietary deficiencies affecting the lactational performance of animals, an inadequate intake of energy-providing nutrients is the most serious and most commonly encountered. Although deficiencies of some mineral elements and vitamins occur in animals under practical farm conditions and others can be produced under rigorous experimental conditions, these are of relatively minor importance as compared to an energy deficiency.

The gross energy values of common feeds, though not greatly different, depend mainly upon the proportions of carbon, hydrogen and oxygen in the feed. As an indication of the nutritive value of feedstuffs, the gross energy value is essentially worthless because, in the metabolism of the diet by the animal, variable proportions of energy are dissipated in the form of combustible gases, faeces, urine and heat.

1. *Digestive Losses*

As a result of fermentative digestion, methane (which has no nutritive value) is formed in quantities equivalent to 5 to 12 % of the dietary gross energy consumed by ruminants and horses and generally to less than 1 % of that consumed by pigs (Forbes *et al.*, 1928; Armsby, 1917). The heat produced by fermentation in the rumen is equivalent to about 6 % of the gross energy of the fermented substrate (Armstrong *et al.*, 1957).

The largest and most variable loss of dietary energy is represented by the faeces. In general the gross energy value of the faeces is 10 to 35 % of that

consumed as concentrates by all farm animals. Of the gross energy consumed in the form of forages, the following ranges of losses occur in the faeces: ruminants, 20 to 60 %; horse, 20 to 80 %; pig, 40 to 90 %.

The magnitude of the energy loss in the faeces of certain farm animals is influenced by the following conditions: (1) the amount of feed consumed per unit of time, (2) the physical nature of the diet, (3) the associative effects of feeds and nutrients, (4) the growth stage of forages and (5) the fibre content of the diet. Whether or not the level of feed intake affects the proportion of dietary energy lost in the faeces is contingent upon the species of animal being fed and upon the nature of the diet. As the amount of mixed (concentrate and forage) rations consumed by ruminants (Forbes *et al.*, 1928; Mitchell *et al.*, 1932) and horses (Edin & Eriksson, 1929) increases, the percentage of dietary energy lost in the faeces increases. However, the faecal loss from all-forage diets is not affected by the level of intake (Reid, 1956) unless the forage is finely ground, under which condition the faecal loss is markedly increased (Blaxter & Graham, 1956). Level of intake does not affect the size of the faecal losses by the pig (Mitchell & Hamilton, 1929). Certain combinations of feeds are more digestible than the digestibility of the individual feeds would indicate. This effect, known as "associative digestibility" is seldom greater than 7 to 8 % of the matter or energy digested. Although other dietary conditions influence the degree of associative digestibility, the most marked changes in digestibility by ruminants are effected by the relative amounts of sugar and nitrogenous substances consumed. In general, high levels of sugar or low levels of nitrogen or both depress digestibility and vice versa.

2. *Urinary Loss*

In the processing of absorbed energy a small amount of energy is lost in urine. This loss as a proportion of the dietary energy generally does not exceed 5 % in the ruminant and horse and 3 % in the pig. The absorbed energy remaining is available for transformation in the processes of maintenance, milk secretion, body gain and other body functions. This fraction of energy is called metabolizable energy (ME).

3. *Utilization of Metabolizable Energy*

The utilization of ME results in the loss of variable quantities of energy in the form of heat. The energy remaining after the loss of heat is known as the net energy (NE); it is this fraction of dietary energy that is put into the animal product. Some of the most important determinants of the magnitude of the heat loss are: (1) the level of feed intake, (2) the balance of nutrients in the diet (including dietary deficiencies and supplementary effects of nutrients), (3) the nature of the absorbed end-products of digestion, (4) the body function

for which ME is being used, (5) the extent of physical activity, and (6) the environmental temperature. The first four items will be considered here because these are of especial significance to the dietary requirements for lactation.

a. *Effect of plane of nutrition.* The total production of heat and the percentage of ME lost in the form of heat increases (or, conversely, the net availability of ME decreases) as the dietary intake is increased for cattle (Forbes *et al.*, 1928, 1930; Mitchell *et al.*, 1932); sheep (Henneberg & Pfeiffer, 1890; Marston, 1948; Blaxter & Graham, 1954); and pigs (data of Fingerling *et al.*, 1914, computed by Armsby, 1917). The heat increment per unit of feed consumed is lower for pigs than for ruminants. The consumption of increasing quantities of feed results in a curvilinear relationship between the ME intake and the production of heat and the retention of energy. Thus, the utilization of ME over the range of intakes, from fasting to about three and a half times the requirement for energy equilibrium, is not constant. However, much of the curvilinearity is at intakes below that needed for energy equilibrium; at intakes above this level the relationship is essentially rectilinear. The base heat-production value (i.e., fasting or energy equilibrium) used has a decided effect upon the value obtained for the net availability of ME. For example, when the fasting heat production is employed as the base value, the net availability of the ME of the same ration for body gain by cattle in the experiments of Forbes *et al.* (1928, 1930) ranged from 85 to 63 % as the level of intake was increased from that of maintenance to that of about three times maintenance. When the energy expenditure of maintenance is applied as the base value, the net availability of ME above the maintenance level for the same range of intakes becomes 62 to 55 %.

b. *Effect of nutrient balance.* There is a great body of evidence (reviewed by Reid, 1956) to support the view that the availability of metabolizable energy is higher (i.e., the heat loss is lower) for nutritionally adequate diets than for those deficient in certain nutrients. In ruminants, deficiencies of protein (Möllgaard, 1929) and phosphorus (Kleiber *et al.*, 1936) cause a reduction in the net availability of metabolizable energy, whereas a deficiency of vitamin A has no effect (Ritzman *et al.*, 1945). Deficiences of vitamin D (Colovos *et al.*, 1951) and magnesium (Blaxter & Rook, 1955), though having no effect upon the heat increment, increase the basal heat production. Kleiber (1945–46) cited evidence for the reduced efficiency with which dietary energy is utilized by laboratory animals in a number of other dietary deficiency states.

It was on such bases that Forbes (1929) suggested that feedstuffs express their characteristic net energy values only as they are components of "nutritively complete" rations, and Mitchell (1934) concluded that the utilization of the metabolizable energy of all "balanced" rations is maximal

and equal. Naturally, as Blaxter (1956) recently pointed out, the tenability of these hypotheses is contingent upon the definition of the terms, "balanced" and "nutritively complete." Blaxter (1956) has attacked these concepts on several apparently valid bases: (1) a deficiency of three nutrients (vitamins A and D, and magnesium) essential for a "balanced ration" does not in fact affect the utilization of metabolizable energy; (2) the net availability of metabolizable energy tends to decline as the fibre content of the ration increases, though, in his own experiment (Blaxter & Graham, 1955) purporting to demonstrate this, the depression in net availability may have been to some extent the result of a deficient protein intake and to differences in the heat of fermentation and in muscular activity (which seem more logically charged as digestive losses); and (3) the net availability of the metabolizable energy provided to sheep by a hay in long form was lower than that provided by the same hay which had been finely ground and fed as small pellets (Blaxter & Graham, 1956). Although this was offered as evidence that the physical form of the ration influences the efficiency with which ME is used, it seems possible that, since the heat of digestion is not distinguishable from the heat arising from other sources, the apparently greater heat increment for the long hay than for the pelleted hay might have been the result of an increased heat of fermentation and muscular activity. Since these losses are incidental to digestion, they should not be charged to the utilization of ME. Thus, it is possible that the proportion of ME loss as heat might not be affected by the physical nature of the ration.

c. *Effect of end-products of digestion.* As pointed out earlier in this chapter, the nature of the ration influences the molar ratios of the volatile fatty acids produced in the digestion of carbohydrates by herbivorous animals. The recent experiments of Armstrong & Blaxter (1957) have demonstrated that the administration of acetic acid into the rumen of fasting sheep results in a heat increment (41 %) which is considerably higher than that for propionic acid (14 %), butyric acid (16 %) or glucose (6 %). However, the heat increments (13 to 17 %) caused by dripping into the rumen various mixtures of the three volatile acids, were lower than those that would have been predicted from the separate effects of the fatty acids. It would appear that this synergism is the result of propionate serving, in the absence of glucose, as a source of oxaloacetate which upon condensation with acetyl coenzyme A provides a means for the entrance of acetic acid into the tricarboxylic acid cycle (Armstrong & Blaxter, 1957). Although these findings indicate that the proportions of fatty acids in the mixture that would be produced by usual rations would have no effect upon the efficiency with which sheep use ME for maintenance, Armstrong *et al.* (1957) reported that the composition of the fatty-acid mixture has a marked effect upon the utilization of ME for fattening. For mixtures of acetic, propionic and butyric acids containing 25

and 75 % of acetic acid, the heat increments were 42 and 68 %, respectively. Thus, it is apparent that differences in the proportions of the fatty acids produced and absorbed when different rations are consumed by ruminants represent a form of "associative" effects of feeds which has a marked effect upon the efficiency with which ME is used.

Although the ruminant, by virtue of its vast population of bacteria, has a unique capacity for dissimilating complex carbohydrates and, thus, of utilizing feeds having less value for simple-stomached animals, the resulting fatty acids are used by the ruminant and horse less efficiently than the monosaccharides are used by the omnivorous and carnivorous simple-stomached animals. Attention has been drawn to the higher heat increments and the consequent reduced energetic efficiency of ruminants as compared to non-ruminants (Ritzman & Benedict, 1938; Marston, 1948; McClymont, 1952; Armstrong *et al.*, 1957). In general the heat increment produced both above and below the maintenance intake is about twice as great for ruminants as for non-ruminants, e.g., the heat increment associated with the metabolism of starch for fattening is approximately 20 % in the pig (Fingerling *et al.*, 1914) and rat (Kriss *et al.*, 1934) and 40 % in the steer (Kellner & Kohler, 1900) and sheep (Jucker, 1948, cited by Armstrong *et al.*, 1957). That the absorbed end-product of carbohydrate digestion influences the heat increment has been demonstrated by the experiments of Armstrong *et al.* (1957). Indeed, they showed that a sheep whose rumen had been flushed out and treated with penicillin to minimize fermentation, utilized for maintenance the energy provided by glucose considerably more efficiently than that provided by the volatile fatty acids. Also, the energy of glucose was used for maintenance as efficiently by a sheep so treated as by simple-stomached animals. In addition to causing this loss of energy associated with the kind of end-product formed from carbohydrate digestion, bacterial breakdown of higher carbohydrates also results in the formation of methane and in the production of fermentation heat which represent further losses of energy to the host. The micro-organisms attack sugars and starch, even in preference to the higher carbohydrates and, therefore, prevent their absorption as monosaccharides from the lower gut. Under these circumstances, in which volatile fatty acids are formed, the energy value of sugar and starch to the animal is lower than it is when these nutrients reach the tissues as sugar.

d. *Effect of physiological function.* Since the relationship between the retention of energy and the level of intake is curvilinear, it is axiomatic that the use of dietary energy for the prevention of energy loss by the body (i.e., for the maintenance of energy balance) is a more efficient process than that for fattening. The experiments of Armstrong *et al.* (1957) demonstrated that, whereas the ME provided to sheep by mixtures of volatile acids is utilized to the extent of 85 % for maintenance, the efficiency of fattening ranged

from 32 to 58 %, depending upon the composition of the mixture. In the experiments reported by Fries *et al.* (1924) and Forbes *et al.* (1926), it was found that the ME provided to cows in excess of energy equilibrium is used for milk secretion and body increase 98 and 76 %, respectively, as efficiently as ME is used for maintenance. The author's summary (Reid, 1957) of the data reported for twenty-five experiments conducted with cattle by Kriss (1943) and Forbes & Kriss (1931) gives a value for the net availability of ME provided above the energy-equilibrium level, of 58·4 $\pm$ 3·5 % for fattening. Despite the fact that seven different hays and seven different concentrates were fed in rations containing from 33 to 50 % hay, and at levels from one to three times that required for energy equilibrium, and that the protein and fibre concentrations ranged from 10 to 15 % and 12 to 20 %, respectively, the coefficient of variation between animals was only 6 %. The rations fed presumably conformed with the definition of a "balanced ration" as proposed by Forbes (1929) and Mitchell (1934). On the assumption that this value (58·4 %) is accurate, the net utilization of ME for maintenance in cattle would be about 70 %.

The availability of ME (above energy equilibrium) for the secretion of milk by cows appears to be approximately 69 % (Fries *et al.*, 1924; Forbes *et al.*, 1926; Möllgaard, 1929; Armsby, 1917). A similar value was obtained (Reid, 1956) in a study of Brody & Procter's (1935) data when it was assumed (as most data indicate) that 1 lb of total digestible nutrients (TDN) is equivalent to about 1625 kcal of ME rather than 1814 kcal of digestible energy.

Such data as those cited above are the basis of Mitchell's (1934) view that the utilization of the ME of "balanced" rations is maximal for the same species of animal and for the same body function. Notwithstanding Blaxter's (1956) very credible criticisms of this concept, the apparent constancy with which cattle utilize ME for maintenance, body gain and milk secretion is remarkable, at least with the usual rations fed. In an attempt to derive the energy requirements for lactation of those ruminants for which the requirements have not been determined and of which the milk yield usually is not measured, these values for the availability of ME offer a reasonable physiological basis.

No studies with the sow and mare similar to those with the cow appear to have been made. From the data of Popoff (1928), Fingerling (1932) and Breirem (1935), who found that the net energy value of a feed for fattening is 89 % of that for maintenance in pigs, Mitchell & Kelley (1938) estimated that the availability of ME for maintenance in pigs is 80 %. The data reported by Breirem (1935), Popoff (1928) and Mitchell & Hamilton (1929) indicate that the net utilization of ME is approximately 70 % for growth and fattening and probably about 65 % when relatively less protein growth and more fattening occurs.

Although the net utilization for the secretion of milk by sows does not appear to have been studied directly, Mitchell & Kelley (1938) proposed the value, 80 %. A recomputation of the data recorded by Brody (1945) for the "gross efficiency" of lactation in rats and women indicates that the net utilization of ME for lactation by these species is approximately 77 % (Reid, 1957). Thus, it would appear that for maintenance, fattening and lactation, simple-stomached omnivorous animals are somewhat more efficient in their use of ME than are the ruminants. In view of the end-products of digestion absorbed, it seems probable that in this respect the mare is intermediate between the sow and ruminants, but possibly more like the ruminant than the pig.

The more efficient use of ME for milk secretion than for fattening might be partly explained on the bases to follow. In ruminants milk fat contains a considerable quantity of fatty acids with a chain length less than C_{16}, whereas body fat consists of fatty acids with chain lengths mainly of 16 and 18 carbon atoms. It has been demonstrated that most of the fatty acids up to and including palmitic acid in the milk glycerides are synthesized in the mammary gland from acetate (Folley, 1956). Presumably some body fat also can be synthesized by similar condensation processes. The synthesis of a C_{16} fatty acid (such as predominates in body fat) from acetate would involve seven C to C condensations each of which is an energy-absorbing process requiring a free energy supply. As a consequence of the expense of lengthening the carbon chain, the synthesis of body fat results in a greater wastage of energy than does that of milk fat. The expense is less, however, when butyrate and propionate are predominant than when acetic acid is the chief precursor for body-fat synthesis. (It is of interest in this connexion that the mammary tissue of non-ruminants can utilize glucose, but not acetate by itself, for the synthesis of milk fat (Folley, 1956).)

The concentration of protein in milk is high, and fat can be deposited in the fattening animal, even concurrently with a loss of protein from the body. When amino acids are in excess of the needs of the product being formed (as they may be when body fat is being produced), they are deaminated, resulting in an appreciable heat increment. Since the quantity of amino acids in excess of those used in milk secretion is less than the excess of amino acids used in fattening, the heat increment is lower and the net efficiency of milk secretion is higher. In addition, the dissimilated amino acids contribute to the wastage of energy in the urine.

4. *Requirement for Maintenance*

The quantity of dietary net energy required for maintenance is equivalent to the energy expended in the maintenance of the non-productive but essential life processes. In its usual application, the term "maintenance"

includes the expenditure of energy for basal metabolism (fasting heat production) and physical activity. The energy increment for physical activity would be expected to vary with the kind of animal (Armsby, 1917; Mitchell & Kelley, 1938), the degree to which various animals are phlegmatic and the method of animal management. For example, cows expend approximately 40 % more energy for "maintenance" when grazing than when they are stall-confined (Smith & Reid, 1956). Other conditions such as temperature, light and humidity (Benedict & Ritzman, 1935) undoubtedly affect the expense of maintenance, though an allowance for their effects is difficult to prescribe. As a consequence, it is impossible to set forth a standard for the maintenance of even a particular species that will be precisely accurate under all conditions.

Since the basal metabolism constitutes an important part of maintenance, it can serve as a starting point for estimating the maintenance requirements. During the 120-year period elapsing after Sarrus & Rameaux (1837–38) suggested that the production of heat by animals is related to body surface area, this principle was verified, extended and made adaptable for various uses. Notable among these developments are the findings that: (1) the production of heat by dogs is directly proportional to body surface area (Rubner, 1883), (2) heat production per unit of surface area is relatively constant for all animals (Voit, 1901), and (3) body surface area and body weight are related exponentially and, therefore, the basal metabolism varies with body weight raised to the power of 0·7 (Kleiber, 1932; Brody & Procter, 1932). Thus, the following equation was proposed by Brody (1945) for estimating the basal metabolism of all mature animals:

$$\log \text{basal metabolism (kcal)} = \log 70{\cdot}5 + 0{\cdot}734 \log \text{body weight (kg)}.$$

The data tabulated in Table I represent the daily energy requirements in terms of total digestible nutrients (TDN), starch equivalent (SE), and Scandinavian feed units (FU) for the maintenance of mature, non-pregnant farm animals of approximately average size. These requirements were computed by the use of Brody's equation and on the assumption that the basal metabolic expenditure is 77 % of the energy requirement for maintenance. Thus, the basal metabolism in calories times the factor, 1·3, represents the net calories required for maintenance. It will be noted that these requirements in general are slightly less than the minimum allowance recommended in feeding standards. In order for these values to be used satisfactorily in feeding practice, additional provisions for growth, pregnancy, and physical activity are required, depending upon the circumstances.

Included in Table I are the values used for the net utilization of ME (discussed previously) and the ME value of TDN (kcal/lb) as determined in the experiments of many workers. The value (1795 kcal/lb TDN) that was applied to rations consumed by horses is based upon the limited data reported

by Zuntz & Hagemann (1898). In computing the maintenance requirements for SE and FU of the various animals, the following conversion factors were used: for pigs, 1 lb SE = 1464 net kcal and 1 FU = 2260 net kcal; for horses, 1 lb SE = 1372 net kcal and 1 FU = 2119 net kcal; and for ruminants, 1 lb SE = 1281 net kcal and 1 FU = 1978 net kcal.

TABLE I

ENERGY AND PROTEIN REQUIREMENTS FOR MAINTENANCE OF FARM ANIMALS

Animal	Body weight	Metabolizable energy value of TDN[1]	Net utilization of ME[2]	Daily requirement for maintenance: Net energy[3]	TDN[1]	SE[4]	FU[5]	Digestible protein
	(lb)	(kcal/lb)	(%)	(kcal)	(lb)	(lb)	(units)	(lb)
Sow	400	1860	80	4170	2·8	2·8	1·8	0·21
Mare	1500	1795	75	11000	8·2	8·0	5·2	0·71
Ewe	150	1625	70	2029	1·8	1·6	1·0	0·17
Goat	125	1625	70	1775	1·6	1·4	0·9	0·13
Cow (*Bos taurus*)	1000	1625	70	8168	7·2	6·4	4·1	0·55
Cow (*Bos babulis*)	1000	1625	70	8168	7·2	6·4	4·1	0·55

[1]TDN = total digestible nutrients.
[2]ME = metabolizable energy.
[3]NE for maintenance = 1·3 (70·5 × body weight $^{0.734}$ in kg).
[4]SE = starch equivalent.
[5]FU = feed units (Scandinavian).

5. *Requirement for Milk Production*

The chemical composition and caloric value of milk represent the net requirements for milk secretion. Although an examination of the proximate chemical composition of the milks produced by farm animals suggests that the nutritional requirements of these animals might be qualitatively similar, the concentrations of the various constituents in milk and, therefore, the quantitative nutritional needs, vary with species of animal. Superimposed upon these considerations are inter-species differences in the quantities of

nutrients synthesized microbiologically and in the net utilization of nutrients.

In previous Sections of this Chapter, some of the peculiarities of the metabolism of energy by farm animals were outlined with a view to obtaining information of a quantitative nature that might be used to derive the energy requirements for lactation, particularly for those animals that are normally allowed to nurse their young. As a consequence of applying the principles that appear to govern the utilization of dietary energy to data representative of the composition of the milks of the various animals (such as those shown in Table II) the energy requirement could be derived in terms of TDN, SE and FU per pound of milk produced. The quantities of these feed units so determined for lactation in the common farm animals and the water buffalo, camel and reindeer are tabulated in Table III. Since the composition of milk varies even within a given species, it is emphasized that the values listed here represent the minimum dietary energy requirement for the production of milks having the caloric values specified. The energy requirement increases as the fat content of milk increases. In practice, this increased need is satisfied by increasing the level of the total ration or by increasing the proportion of concentrates in the ration. Of the animals listed, the cow is the only one for which the energy requirements for milk production have been studied extensively. The values in Table III derived on a theoretical basis for the cow are almost identical with those determined by direct experimentation and recorded in feeding standards in terms of the various feed units, e.g., 0·31 lb TDN, Morrison (1956); 0·26 lb SE, Woodman (1948); and 0·17 FU, Hansson (1926).

The conversion factors and basic data employed to derive the energy requirements for lactation are: (1) 1 lb of TDN = the ME values listed in Table I, (2) 1 lb of SE = 1830 kcal ME, (3) one FU (Scandinavian) = 2825 kcal ME, (4) the caloric values per pound of milk shown in Table III, and (5) the factors for the net utilization of ME tabulated in Table III.

In discussing these energy relationships both in the text and in Tables I and III several conversion factors have been used together with generalizations made above about the utilization of ME. When the conversion factors do not agree with those of Fingerling or Kellner, it is chiefly because all available data on the efficiency of energy utilization have been used. The factors applied are believed to be the best overall values available at the present time.

6. *Feed Energy Input—Milk Output*

a. *Effects upon lactation of concurrent energy intake.* Feeding-standard energy allowances are casually considered to represent the levels required by animals to produce milk at their inherited maximum capacity. However,

numerous input–output experiments (reviewed by Reid, 1956) have demonstrated that increasing the feed intake above the feeding standard allowance for current production results in an increased yield of milk by cows until the stomach capacity or the maximum production capacity of the cow is reached.

TABLE II

REPRESENTATIVE COMPOSITION OF THE MILKS OF VARIOUS DOMESTIC ANIMALS

Animal	Total solids	Protein	Fat	Lactose	Mineral matter
	(%)	(%)	(%)	(%)	(%)
Sow	20·1	5·8	8·5	4·8	1·0
Mare	10·1	2·2	1·6	6·0	0·3
Ewe	18·4	5·6	7·5	4·4	0·9
Goat	13·2	3·3	4·5	4·4	1·0
Cow (*Bos taurus*)	12·8	3·4	4·0	4·7	0·7
Cow (*Bos babulis*)	16·8	3·8	7·5	4·9	0·6
Camel	12·9	3·7	4·2	4·1	0·9
Reindeer	36·7	10·3	22·5	2·5	1·4

TABLE III

ENERGY AND PROTEIN REQUIREMENTS FOR MILK PRODUCTION OF VARIOUS DOMESTIC ANIMALS

Animal	Gross energy value of milk	Net utilization of ME	Requirement per lb of milk			
			TDN	SE	FU	Digestible protein
	(kcal/lb)	(%)	(lb)	(lb)	(units)	(lb)
Sow	590	77	0·41	0·42	0·27	0·083
Mare	230	72	0·18	0·17	0·11	0·035
Ewe	536	69	0·48	0·42	0·27	0·093
Goat	351	69	0·31	0·28	0·18	0·055
Cow (*Bos taurus*)	340	69	0·30	0·27	0·17	0·056
Cow (*Bos babulis*)	499	69	0·45	0·40	0·26	0·063
Camel	344	69	0·31	0·27	0·18	0·062
Reindeer	1260	69	1·12	0·99	0·64	0·172

Increasing the feed intake by 25 % above the feeding standard level effects an increase in milk yield of about 15 to 20 %. However, the production response to increased amounts of feed is less at high levels of intake than at low levels. Therefore, the principle of diminishing increments operates in the conversion of feed to milk. The basic causes of diminishing returns are nutritional in nature and concern the efficiency with which feed energy is utilized. When dry matter is the input unit, the diminishing output of milk per unit of feed, as the input is increased, is the result of: (1) the decreasing nutritive value (or net energy value) of the ration (much of this depression is the result of increased digestive losses, while a part of it results from slightly increasing heat losses per unit of metabolizable energy being used), (2) the increasing diversion of energy into body fat, and (3) the relative inefficiency with which energy is utilized for body gain as compared with lactation.

The extent to which the milk production increments diminish varies with the feed unit in which the input is rationed. For example, the rate of diminishing production is considerable when TDN is the input unit, whereas it is nil when true net energy is the input unit. However, in feeding practice the various units of feed value are assumed to be constant and invariant with level of intake. The rate of diminishing returns is least when the input is rationed in terms of the feed unit that most nearly allows for the effect of increasing intake upon the energy losses in digestion and metabolism. As a result of further studies of energy metabolism, it should become possible to correct the existing units of nutritive value (i.e., TDN, DE, ME, SE and FU), which are based primarily upon digestibility, to obtain a closer nexus between level of feed intake and response.

Of the farm animals, only the cow has been used in "input–output" experiments. Nevertheless, some of the basic principles governing the utilization of energy indicate that certain qualitative features of the lactation response of other animals to increasing intakes in feed might be predicted. Presumably the causes and extent of diminishing increments in the lactation response of other ruminants are similar to those of the cow. Since the plane of intake is without effect upon the digestibility of the ration by the sow, the diminishing output of milk per unit of feed consumed is mainly the result of increased fattening. Also, since the heat loss per unit of ME used is markedly less in the sow than in the ruminants, the principle of diminishing returns operates much less pronouncedly in the lactation process of the sow than in that of the ruminant. It seems probable that the lactation response to increased feed intake by the mare would be more similar to that by the ruminant than to that by the sow. Even within species it appears probable that heredity determines the tendency to fatten and, therefore, influences the extent to which the lactational increments diminish as the level of dietary energy consumed increases.

Prolonged underfeeding of energy causes, in addition to a loss of body condition and a reduction in milk volume, a reduction in the concentration of solids-not-fat in the milk (Rowland, 1944). In practice, the energy intake generally is intensified by increasing the proportion of the ration comprised of concentrates. When the ration of the cow consists mainly of concentrates and as little as 5 lb of forage, the fat content of the milk is depressed and the proportion of milk fat consisting of short-chain fatty acids is reduced (Balch *et al.*, 1953). These effects upon milk composition are accompanied by a reduction in the relative molar proportion, and total amount, of acetic acid in the rumen contents. Similar depression of the fat content of milk is produced by feeding finely ground hay in rations consisting of even the usual proportions of concentrates and hay.

b. *Effect upon lactation of energy intake during rearing.* During the last 50 years the effects upon the performance of farm animals of imposing different planes of nutrition during various stages of life have been studied. In the experiments involving lactation, generally the effects of providing levels of feed ranging from 60 to 140 % of the feeding-standard allowances during the growing period before parturition and of providing standard levels beginning with lactation have been investigated. During the first lactation period the trend in the milk yield of dairy cattle as affected by level of feeding during early life has been different for the various experiments. However, in general the milk yield of first-lactation cows reared on a high level of feed intake has been either less than (Hansson, 1956; Swanson & Spann, 1954; Eskedal & Klausen, 1958) or the same as (Hansen & Steensberg, 1950; Reid *et al.*, 1957) that of cows fed on the standard, or less than the standard, amount of feed. The concentration of fat in the milk produced during the first-lactation period by cows reared on a high plane is greater than that of the milk yielded by cows given less feed before calving. In subsequent lactation periods the yield of cows reared on low planes becomes increasingly superior to that of cows reared on high levels of feeding. Whether or not a high degree of fattening causes poor lactation performance by impairing the development of mammary secretory tissue as suggested by Swanson & Spann (1954) requires detailed study.

In addition to the milk yield per lactation period, the length of the productive-life span is important to the economics of dairying. The results of Danish (Hansen & Steensberg, 1950; Eskedal & Klausen, 1958) and Swedish (Hansson *et al.*, 1953) experiments indicating that the production of milk is higher and the incidence of reproductive disturbances and of other diseases is lower and, therefore, the span of productive life is greater in cows reared at a low level of intensity than they are in cows grown at a rapid rate, have suggested that restricted feeding of growing dairy cattle might be an economical practice. Thus, it would appear that retarded early growth is associated

with a prolongation of the life span in cattle as it is in rats, mice, silkworms, crustacea, protozoa and fruit flies.

The milk yield (as determined by weaning weight of the pigs) of gilts fed on 75 to 80 % of full-feed from the time they weighed 120 lb to the time of farrowing was slightly lower than that of their full-fed littermates (Hansen, 1955). Restricting the feed intake to an amount equivalent to 88 % of full-feed or diluting the full-fed ration with 35 % of corn cobs had no effect upon the production of milk. Although somewhat less feed than self-fed gilts would voluntarily consume does not impair lactation performance, the effects of the plane of nutrition during the growing period upon the milk yield after the first lactation period has not been determined. Based upon the weaning weight of lambs, the milk yield by ewes fed on a high and a low level of feed from birth to 12 months or from 4 to 18 months was not different in any of the first four lactation periods during which the level of feeding was the same (Coop & Clark, 1955).

The plane of nutrition imposed upon farm animals during the rearing period seems to have much less effect upon the milk yield than the level of feeding imposed after lactation has commenced.

c. *Effect upon lactation of energy intake during the dry period.* It has been demonstrated many times that dairy cows in some state of fatness at the time of parturition produce more milk of a higher fat content than if they calve in a thin condition. Also, fat sows produce a milk higher in fat than do thin sows. The difference between the percentages of fat in the milk of fat cows and thin cows usually disappears after about the first 3 months of lactation. A considerable store of body fat is needed to support lactation during the peak production period of high-producing cows because their stomach capacity relative to their feed needs is limited. Nevertheless, the level of feed intake during the dry period that promotes the most desirable condition and the degree of fatness which is desirable have not been determined. In practice the feeding level during the dry period ranges from approximately that of maintenance to that advocated by Boutflour (1943) in the "steaming up" procedure. In the latter procedure the level of concentrates is gradually increased during the dry period until, at the time of parturition, it reaches about 14 lb of concentrates per day in addition to forages fed *ad libitum*.

The minimum plane of nutrition required during the dry period to produce the maximum lactation response in the subsequent period depends upon the body condition of cows at the end of the prior lactation period and their inherited capacity for milk yield. When cows begin the dry period in a moderate to good state of flesh, high levels (12 to 15 lb per day) of concentrates resulting in gains of 75 to 150 lb do not produce during the ensuing lactation milk yields different from those of cows given no concentrates and

gaining only 40 lb of body weight (Gardner & Greenhalgh, 1955; Schmidt, 1958). The milk yield of cows beginning the dry period in a thin state responds to a high plane of nutrition to a greater extent than that of fatter cows (Campbell & Flux, 1948). Cows of a high-producing capacity, having a relatively limited stomach capacity, would be expected to respond to high-plane, dry-period feeding more markedly than low-producing cows. From the standpoint of energy economy the amount of body fat needed to support the ensuing lactation should be gained during the dry period because the wastage of energy per unit of feed is less then than it is during lactation when the level of intake is greater.

The milk output by ewes is positively correlated with the energy intake during the period of 6 weeks pre-partum (Wallace, 1948).

C. Protein

1. *Qualitative Considerations*

Proteins constitute an important part of the tissues and secretions of the body. As a consequence, amino acids, which are the main constituents of proteins, are metabolic essentials for all animals. Whether or not specific amino acids are dietary essentials depends upon the species of animal, the body function being performed, and the extent of symbiosis between the host and the microbial population of the gastro-intestinal tract.

a. *Ruminants.* Present evidence suggests that ammonia is the only source of nitrogen required by the micro-organisms of the rumen for the synthesis of protein, preformed amino acids are not required, and that all dietary sources of nitrogen have about the same value per unit of nitrogen for the ruminant. This nutritional feature makes the ruminant distinctly different from the pig which requires preformed "essential" amino acids in its diet and for which the value of protein feeds is variable. Although, because of its rumen flora, the ruminant is able to utilize simple forms of nitrogen and upgrade protein of low biological value, the action of bacteria at the same time can be detrimental to the protein economy of the host as it degrades for ruminants the biological value of protein having high quality in the form in which it was consumed. As evidence for this, the retention of nitrogen by sheep is greater when casein (a high-quality protein) is administered into the duodenum (thus circumventing rumen microbial action) than when it is put directly into the rumen (Cuthbertson & Chalmers, 1950).

The nitrogen provided to rations at levels of 10 to 13 % of protein equivalent (% N $\times$ 6·25) by each of many different high-protein feeds and by urea has a biological value of approximately 60 for growth (and probably for maintenance and lactation) for ruminants (Miller & Morrison, 1942; Johnson *et al.*, 1942). The relative constancy of this value for ruminants as contrasted with a range

of 0 to 90 for the biological value of nitrogen fed in the same sources to rats led to the view that microbial protein is the common intermediary between dietary sources of ammonia nitrogen and the amino acids absorbed by the ruminant.

b. *Simple-stomached animals.* Since 1930 when Rose (1938) began his studies which eventually delineated 10 amino acids which the body of the rat is unable to synthesize in sufficient quantity to support normal growth, it has been suspected that the pig requires the same amino acids. As the result of studies conducted since 1949, it became apparent that the provision of adequate protein nutrition for pigs is resolved in the provision of certain amounts of specific amino acids and an additional source of amino groups. These studies culminated in the findings that pigs can grow normally on diets containing the amino acids essential for rat growth and ammonium salts as the sole sources of nitrogen (Shelton *et al.*, 1950) and in the measurement of the quantitative requirements of the amino acids for growth.

The quality or biological value of dietary proteins depends upon the degree to which the relative quantities of their constituent amino acids presented to the body tissues are similar to those of the products being formed, e.g., proteins needed for maintenance and growth of body tissue and the secretion of milk. The closer the similarity, the higher the "quality" or "biological value" of the dietary protein and the better the "balance" or "assortment" of the essential amino acids in the dietary protein.

Since more than half of the protein of the body tissues of the sow and of her milk is constituted by the non-essential amino acids, these are metabolic essentials too. Therefore, in addition to the "essential" amino acids, sources of amino groups for the synthesis of "non-essential" amino acids are needed. The required amino groups can be provided by amino acids or other non-protein nitrogenous sources.

The quantities in the diet of certain non-essential amino acids affect the requirement for specific essential amino acids and vice versa. Also, certain vitamins may spare the amino acids in the nutrition of all animals. The rate and time at which the individual amino acids reach the sites of synthesis affect the efficiency of protein synthesis because: (1) the required amounts of the amino acids needed currently must reach the tissues simultaneously and (2) the amino acids *per se* are not stored in the body, but, if they are present in an incomplete mixture, the quantity of those in excess (which is determined by the amount of the one amino acid that is most limiting) of the proportions needed is catabolized almost immediately. Even though the diet contains the proper assortment and amounts of amino acids, the extent of digestion and absorption of individual amino acids can influence the completeness of the mixture supplied to the tissues in the pig.

2. *Dietary Deficiency*

Regardless of the form of dietary nitrogen which is metabolized to satisfy the protein requirement of animals, the ultimate structural units are the amino acids. Since the protein content of the milk and the amino acid composition of milk and body tissues tend to remain constant regardless of the level of protein intake, it is axiomatic that a deficiency of nitrogen limits the yield of milk (or the amount of body tissue laid down) to the quantity of component protein which can be synthesized from the quantities and assortment of amino acids made available to the tissues. As the protein intake of lactating animals is reduced, milk production declines and eventually ceases (though, to some extent, dissimilated body protein contributes to the secretion of milk protein) and body protein continues to be catabolized to support the vital functions of the body until these are terminated by death.

3. *Requirement for Maintenance*

In order for lactation to proceed, sufficient dietary protein must be provided, first, for maintenance. The size of this requirement depends upon the quantity of protein lost as urinary endogenous and faecal metabolic nitrogen and that used for adult growth.

a. *Essential cellular activity.* In the maintenance of the life processes, the body requires an essential catabolism of nitrogen which is represented by the minimum excretion of nitrogen (known as endogenous nitrogen) in the urine. Since this loss of nitrogen represents a considerable proportion of the total nitrogen needed by the body to support non-productive life, its magnitude can serve as the starting point for estimating the dietary requirement of nitrogen for maintenance. As a consequence, several relationships involving endogenous nitrogen will be recorded here: (1) the minimum catabolism of nitrogen essential to cellular activity bears a constant relationship to the basal metabolism of energy, irrespective of animal species, size or age (Terroine & Sorg-Matter, 1927), (2) the basal catabolism of both energy and nitrogen increases at a rate in accordance with body weight raised to the power, 0·7 (Brody *et al.*, 1934), and (3) approximately 2 mg of endogenous nitrogen is excreted per kcal of energy output in basal metabolism (Smuts, 1935).

b. *Metabolic faecal nitrogen loss.* Also included in the protein requirements for maintenance is an amount of nitrogen equivalent to the fraction of faecal nitrogen (called metabolic nitrogen) contributed by the body, i.e., residues of bacteria, bile, and digestive juices and fragments of intestinal mucosa. The absolute output of metabolic nitrogen is governed by the level of feed intake, the digestibility of the ration and, to a lesser degree, the size of animal within a species. Also inter-species differences, partly contingent upon

the natural diet consumed, exist in the output. For example, the amount of metabolic nitrogen excreted per gram of dry matter consumed is about 0·001 g for pigs and from 0·003 to 0·007 g (depending upon the fibrousness of the ration) for ruminants. Though the metabolic faecal loss of nitrogen is only about 50 % as large as the urinary endogenous loss for pigs, the metabolic fraction is generally larger than the endogenous loss for ruminants. Since a part of the nitrogen in the bacterial residue is derived directly from the diet, the entire fraction of bacterial nitrogen is not strictly a metabolic loss. However, the size of this fraction is difficult to assess.

The absolute size of both the endogenous and metabolic nitrogen fractions is independent of the amount and quality of protein consumed. At the same level of feed intake, the proportion of total nitrogen comprised of metabolic nitrogen is inversely related to the digestibility of the dietary protein. Because of the nature of the usual diet consumed, metabolic nitrogen constitutes a much larger proportion of the total faecal nitrogen output by pigs than by herbivorous animals. For all farm animals the apparent digestibility of protein increases as the concentration of protein in the diet increases. (This is partly the result of an inverse relationship between the protein and fibre content of the ration.) Since this is the reflection of a decreasing proportion of metabolic nitrogen in the total faecal nitrogen as the percentage of nitrogen in the diet increases, the true digestibility of protein is not affected by the concentration of protein provided in a given diet.

c. *Adult growth.* The nitrogen requirement for the growth and replacement of hair, wool, horns, hoofs and integument represents a further portion of the maintenance requirement. Since this process continues throughout life, holding priority even over the use of nitrogen for the maintenance of the remainder of the body, it has been called "adult growth." At the expense of the catabolism of other tissue protein, adult growth in farm animals requires the following amounts of protein per 100 lb of body weight per day: horse, 1·1 g; cow, 0·1 g; sheep, 6·1 g (computed from data recorded by Armsby, 1917). Presumably the requirement of the pig approaches that of the cow. With the exception of that of sheep, the requirements for "adult growth" are trivial.

d. *Digestible protein: ruminants, mare, sow.* The basic data discussed above were used to establish the minimum requirements, for maintenance, for digestible protein which are recorded in Table I. In this scheme of estimating requirements, the endogenous nitrogen output of all animals was computed by use of the equation of Brody *et al.* (1934) : log EN (mg per day) = log 146 + 0·72 log body weight (kg); where, EN = urinary endogenous nitrogen. For the sow it was assumed that the quantity of protein represented by the faecal metabolic nitrogen and "adult growth" is 75 % as large as the endogenous protein loss and that the biological value of digestible protein for maintenance

is 70. The amount of metabolic nitrogen excreted by the mare and ruminants was assumed to be equal to the endogenous loss. Daily allowances of protein in grams per 100 lb of body weight were made for "adult growth" as follows: cattle and goats, 0·11; horse, 1·05; and sheep, 6·12. The biological value of digestible protein was assumed to be 60 for the ruminants and 62 for the mare. The use of these data produced values (Table I) that are intended to be the minimum requirements of maintenance for digestible protein. For application to feeding practice, these quantities should be increased by about 10 to 15 % to allow a margin of safety. Indeed the minimum requirements listed in Table I are lower than present-day feeding standard allowances (where they exist) by about that much.

4. *Requirement for Milk Production*

a. *Digestible protein: ruminants, mare, sow.* The minimum amount of dietary protein required for milk production is equivalent to the amount secreted in the milk plus that wasted in digestion and metabolism. Since the amount of protein lost in digestion is variable and depends upon the composition of the ration, the requirement usually is expressed in terms of digestible protein. As discussed earlier, the proportion of absorbed protein lost in metabolism by ruminants appears to be relatively constant regardless of dietary source and is of the order of 40 %. This conclusion is supported further by the results of Haecker's (1914) practical experiments. He found that while cows, receiving an allowance of digestible protein somewhat less than 140 % of that in the milk, secreted milk coincidentally with a loss in body weight, the provision of 40 % more than the amount in the milk adequately supported both milk production and the maintenance of the body tissues. Although the wastage of absorbed protein by the sow depends upon the completeness of the amino-acid assortment reaching the site of synthesis, it is expected that the digestible protein of the usual ration containing 5 to 10 % of animal-protein feeds would be utilized by sows to the extent of about 70 %.

The minimum requirements of digestible protein for milk secretion by the common farm animals and the water buffalo, camel and reindeer as recorded in Table III were computed on the basis that: (1) the biological value of protein is 70 for the sow, 62 for the mare and 60 for the ruminants, and (2) the various milks contain the amounts of protein listed in Table II.

b. *Amino acids: sow.* Of the farm animals, the sow is the only one for which the quality of protein consumed affects lactation performance. Although in general the dietary proteins that have high quality for growth also have high quality for milk production, neither the qualitative nor the quantitative requirements of the lactating sow for amino acids have been determined by direct experimentation.

In a study of the amino acid composition of the total protein of the body of the pig as reported by Williams *et al.* (1954) and of that in the milk of sows as reported by Beacom & Bowland (1951) and Morrison (1956), the author observed that the amino acids essential for pig growth exist in about the same proportions in sow's milk as in body tissues. Also, the ten essential amino acids comprise approximately 45 % of the total protein in both milk and body tissue. As shown in Table IV, the major apparent

TABLE IV

AMINO ACID REQUIREMENTS OF SOWS FOR MILK PRODUCTION

Amino acid	Relative amounts of amino acids in protein		Concentration in milk	Dietary requirement	Concentration in dietary protein
	Body[1]	Milk[2]			
	(As % of amount of lysine)		(g/lb)	(g/lb of milk)	(%)
Arginine	83	68	1·55	2·77	5·9
Histidine	31	32	0·73	1·31	2·8
Isoleucine	45	50	1·14	2·04	4·3
Leucine	84	96	2·18	3·89	8·3
Lysine	100	100	2·27	4·05	8·6
Methionine	21	22	0·51	0·91	1·9
Phenylalanine	44	42	0·95	1·70	3·6
Threonine	44	42	0·95	1·70	3·6
Valine	70	70	1·59	2·84	6·0
Tryptophan	9	12	0·27	0·49	1·0

[1]Based upon data of Williams *et al.* (1954).
[2]Based upon data of Beacom & Bowland (1951) and Morrison (1956).

difference is in the relative amount of arginine. These relationships, however, do not necessarily mean that all amino acids essential for growth, with or without the exception of arginine, are essential for milk production. Nevertheless, on the assumption that the same amino acids are essential for milk production, an attempt has been made to estimate the requirements for this function. These values, recorded in Table IV, were derived from the following bases: (1) that sow's milk containing 5·8 % protein has the concentrations of amino acids listed in Table IV, and (2) that the biological value and digestibility of each amino acid is 70 and 80 %, respectively.

Since, in practice, the rations commonly fed to sows contain as much as 10 to 15 % of forage, the value of 80 % for the digestibility of protein was

adopted even though a higher one is indicated when all-concentrate rations are fed. Likewise the biological value (70) used here is intended to allow for differences in the rate at which individual amino acids may become available and for imbalances resulting from other causes when natural diets are fed. When diets containing pure amino acids are fed, it is expected that their absorption would be nearly complete. Also, assuming that they exist in the proper proportions, their biological value should be much higher than the value used here and, as a consequence, the requirements should be somewhat less than those recorded in Table IV. In computing these requirements, no consideration was given to sparing relationships that may exist among the amino acids (values for the cystine and tyrosine contents of sow's milk are not available) or between the amino acids and certain B-complex vitamins. Since approximately 55 % of the protein in sow's milk consists of non-essential amino acids, these amino acids or the materials (particularly, the amino groups) from which they can be synthesized must be provided ultimately by the diet. During the past 15 years the results of delineating the separate nutritive effects of some of the vitamins and mineral elements and the amino acid requirements for growth have suggested that protein supplements have been fed in the past at levels sufficiently high to cater for certain non-nitrogenous needs as well as for the essential amino acids. Thus, as Mertz *et al.* (1952) have demonstrated with a purified diet containing 7·4 % of protein equivalent as essential amino acids and 3·9 % of protein equivalent as diammonium citrate, that a low level of nitrogen (which is lower than the usual allowance) promotes satisfactory growth (1·2 lb body weight gain per day) in weaning pigs, provided that the essential amino acids are in proper balance and a source of amino groups for the synthesis of non-essential amino acids is included. (Though demonstrating the adequacy of a low-nitrogen diet, the experiment of Mertz *et al.* (1952) did not demonstrate that non-protein nitrogen is utilized by pigs. It would appear that the quantities of essential amino acids fed were sufficient to supply both the essential amino acids required and the amino groups needed to synthesize non-essential amino acids.) Although a similar reduction in the present allowances of protein for lactating sows seems feasible on the same basis, an allowance ranging from 13 to 16 % of total protein is needed by sows yielding from 7 to 15 lb of milk per day when the amino acids are provided by usual natural diets.

D. Fat

1. *Dietary Requirements*

Present knowledge indicates that lipids are required in the diet chiefly to provide the essential fatty acids and choline. The results of early experiments demonstrating that farm animals are able to (1) use dietary protein

and carbohydrates to produce body fat (Lawes & Gilbert, 1852) and milk fat (Jordan & Jenter, 1897) and (2) synthesize phospholipids and cholesterol within their body, together with the difficulty of preparing fat-free diets, led to the view that fat *per se* is not needed in the diet. Further, the viewpoint developed that fat serves merely as a source of energy exchangeable on an isodynamic basis with other organic nutrients. However, it was found that rats consuming a diet devoid of fat fail to grow, reproduce and lactate successfully, develop dermatitis and eventually die. Subsequently it was established that linoeleic, linolenic and arachidonic acids are essential nutrients and that certain of these acids more or less effectively than the others prevent and cure the various deficiency signs.

a. *Pigs*. Very limited study has been made of fat deficiency in farm animals and, then, only very young animals have been studied. When diets containing 0·12 % or less of ether extractives were fed to growing pigs, the following conditions were noted (Witz & Beeson, 1951): (1) scaly dermatitis, (2) loss of hair, (3) necrotic areas in the skin, (4) very small gall bladders containing little or no bile, (5) enlarged thyroid glands, (6) some haemorrhage and necrosis in kidneys, and (7) testes and ovaries of approximately half normal size. When the diet contained 0·12 % of fat, the growth rate was 97 % of that obtained with the control ration containing 5 % of fat. On the other hand, reducing the level of ether extractives to 0·06 % of fat reduced the growth rate to 76 %.

b. *Ruminants*. None of these signs, save the retarded growth rate and alopecia, is observed in weanling sheep and goats (Cunningham & Loosli, 1954a) and in very young calves (Cunningham & Loosli, 1954b; Lambert *et al.*, 1953). In addition the young ruminant consuming fat-free diets develops leg weaknesses, muscular twitches and diarrhoea and dies after 3 to 7 weeks. Although the animals receiving the fat-free diets supplemented with various sources of fat, e.g., corn (maize) oil, lard and hydrogenated coconut oil, did not exhibit these signs, it still remains to be proven that the fatty acids essential to the rat, and possibly the pig, are also required by the young ruminant. No studies of the needs of mature farm animals have been made. From the practical standpoint, the provision of the essential fatty acids to farm animals does not require special attention because natural feedstuffs appear to contain adequate amounts.

Much attention has been given to the quantity of fat provided to lactating cows. Noteworthy among the findings resulting from the study of this problem are: (1) the replacement of an isocaloric amount of fat by starch results in a small reduction in milk yield (Loosli *et al.*, 1944); (2) slightly more milk is produced by cows consuming concentrate mixtures containing 5 to 7 % of ether extractives than by those consuming concentrates containing from 0·7 to 4 % of ether extractives (Loosli *et al.*, 1944; Dijkstra *et al.*, 1953); (3)

although the fat concentration in the milk is not generally affected by the level of dietary fat in usual rations, high intakes of such high-fat feeds as soya beans, coconut meal and palm kernel meal have caused at least temporary increases in the percentage of milk fat; and (4) the response to high-fat rations is not the result of an increased intake of the fat-soluble vitamins (Lucas *et al.*, 1943) or of an improvement in the digestibility of the ration (Lucas & Loosli, 1944).

2. *Role in Energy Economy*

Although the problem has not been studied very much in ruminants, the absorbed end-products of fat digestion are probably more efficiently utilized than are those of carbohydrate digestion. Forbes *et al.* (1946) found that the ME provided by high-fat diets is more efficiently utilized for lipogenesis by rats than that provided by isocaloric, high-carbohydrate diets. The heat increment (9 %) of dietary fat (arachis oil) used for fattening by pigs is lower than that (17 %) of starch (Fingerling, 1938). Although this appears to reflect the thermodynamic expense of lengthening the carbon chain when carbohydrate is metabolized for the synthesis of body fat, no similar data exist for the synthesis of milk fat by the sow. With sheep fed on isocaloric diets containing from 2 to 8 % of fat, Swift *et al.* (1948) found no difference in the heat increment. However, when the same basic diet contained 10·5 % of fat, the heat increment was only 80 % of that for the other diets even though the diet was fed at a higher level of intake. No similar studies have been made with the lactating ruminant.

E. Mineral Elements

Although a large number of mineral elements are found in the tissues and milk of animals, not all of these are dietary essentials. Some occur in the feed and fail to be excreted and some may have an unknown essential function, but at least fourteen elements have one or more known essential functions in one or more species of animal. The mineral elements recognized at present to be dietary essentials are: calcium, phosphorus, magnesium, sodium, potassium, chlorine, sulphur, iodine, iron, copper, cobalt, manganese, zinc and molybdenum. That other elements such as fluorine and selenium are essential nutrients has not yet been established. Only those elements that generally require attention in the feeding of lactating farm animals will be dealt with here. It will be assumed that the lactating animal is mature and non-pregnant; therefore, no consideration will be given to the function of minerals in growth and reproduction except as the requirement for these functions might provide information on the requirements for maintenance and lactation.

a. *Calcium and phosphorus.* As a calcium-deficiency state develops, the calcium phosphate stores of the bones are mobilized by the action of the parathyroid glands to maintain the calcium level of the blood and tissues. As a consequence, the penalty of a calcium deficiency in the diet is long deferred. However, no mobilizing mechanism for phosphorus exists except indirectly when the deficiency of phosphorus occurs concurrently with that of calcium. Therefore, even when the bone stores of phosphorus are large, the animal lives on a meal-to-meal basis for this element, and an inadequate dietary supply very quickly leads to biochemical and structural disturbances. The maintenance and growth of the skeleton requires, in addition to calcium and phosphorus, vitamin D which functions in the absorption of calcium and phosphorus from the gut and in the deposition of these minerals at the sites of bone formation.

A deficiency of phosphorus causes abnormal conditions earlier and is more commonly encountered than that of calcium, though the end results of each are similar. As the deficiency of calcium or phosphorus or both becomes more severe and the bone stores are either not extensively used (i.e., as in phosphorus deficiency) or become depleted (i.e., as in calcium deficiency), milk production declines and eventually ceases for the mineral concentration in the milk remains constant and the endogenous losses continue. The resulting condition known as "osteomalacia" in mature animals is characterized by rarified and broken bones in cattle (Eckles *et al.*, 1926; Becker *et al.*, 1933), horses (Svanberg & Johansson, 1936), sheep (Fraser *et al.*, 1933) and pigs (Evans, 1929; Davidson, 1930).

It is well established that negative calcium and phosphorus balances occur during the first part of the lactation period when the milk flow is greatest and that a replenishing of the stores occurs during the latter part of the lactation period and during the dry period (Huffman *et al.*, 1930; Ellenberger *et al.*, 1931). Normally this cycle is completed with a net positive balance for the lactation period as a whole. Although supplementation of the diet with calcium and phosphorus generally improves the balance, negative balances are not always circumvented during peak lactation. Thus, the early withdrawal and later replacement of the mineral stores must be considered a normal cycle of events for the cow and, presumably, other farm animals.

Relatively large quantities of calcium and phosphorus are required by lactating animals because: (1) the faecal endogenous loss is large, e.g., cows excrete about 8 g of calcium (Visek *et al.*, 1953) and 12 g of phosphorus (Kleiber *et al.*, 1951) per day; (2) the rate of absorption is low and, as a consequence, the proportion of dietary calcium and phosphorus assimilated is generally less than 35 % (though, under certain conditions such as a low level of intake, the rate of utilization can be as high as 50 %) for all mature farm animals (pigs, Mitchell *et al.*, 1931; horses, Scheunert *et al.*, 1923; cattle,

Huffman *et al.*, 1930; sheep, Gallup & Briggs, 1950); and (3) the quantities of these elements in milk are relatively large. Some representative percentages of calcium and phosphorus, respectively, in the milk of farm animals are: cow and goat, 0·13 and 0·10; ewe, 0·20 and 0·17; sow, 0·25 and 0·21; and mare, 0·09 and 0·05. Regardless of the intake of calcium and phosphorus the concentration of these elements in the milk remains quite constant.

The quantitative requirements of maintenance and lactation for calcium and phosphorus are listed in Table V. With the exception of those for the cow, very few data on the quantitative needs of these two functions have been obtained by direct experimentation.

b. *Sodium, potassium and chlorine.* Because the diets of farm animals are mainly of plant origin, the provision of potassium does not require special care, whereas special additions of sodium chloride to the diet are required for all farm animals, with the possible exception of pigs, receiving feeds of animal origin.

The dietary need of lactating cows for sodium chloride (Babcock, 1905; Smith & Aines, 1952) appears to be approximately of the same order as that for calcium or phosphorus. A drastic restriction in the intake of sodium chloride generally for 8 or more months results in the following conditions in cows: (1) a poor appetite and a reduction in the milk yield and body weight, (2) shivering, (3) reduction in body temperature, (4) cardiac arrhythmia, (5) hypertrophy of the adrenal glands, and (6), in a prolonged deficiency state, complete physical collapse terminating in death. The signs of a sodium chloride deficiency in lactating cows are caused primarily by a lack of sodium (Aines & Smith, 1957). The concentrations of sodium, potassium and chloride in the blood and milk remain practically constant regardless of the level at which these elements are consumed. In deriving the sodium chloride requirements for the maintenance and lactation of farm animals as summarized in Table V, the data obtained with milking cows were used as the basis for computing the requirements for other animals.

c. *Iodine.* From the practical viewpoint the provision of adequate dietary iodine to farm animals is a problem prevalent only in certain geographic areas and concerns mainly the reproductive function. When a deficient diet is consumed during pregnancy, stillbirths may occur or, if the young live, they may be weak and unthrifty as a result of the reduced production of thyroxine. Although the mothers of goitrous pigs appear in a visual examination to be healthy, their thyroid glands generally are several times larger than those of sows consuming iodine-adequate diets (Hart & Steenbock, 1918). Thus, it is possible that lactation performance might be reduced by a deficiency of iodine sufficient to impair thyroid function, though this effect has not been examined critically. The amount of iodine in milk is usually small but it is related to the level of the element in the diet.

TABLE V

MINERAL AND VITAMIN REQUIREMENTS FOR MAINTENANCE AND LACTATION OF VARIOUS DOMESTIC ANIMALS

Animal	Body wt.	Maintenance[1] (quantity/day) Ca	P	NaCl	Carotene[12]	Vit. A[12]	Milk production (quantity/lb) Ca	P	NaCl[17]
	(lb)	(g)	(g)	(g)	(mg)		(g)	(g)	(g)
Sow	400	3·2[2]	4·0[2]	1·8[8]	5·3	0·94	1·8[13]	1·1[13]	0·6
Mare	1500	12·0[3]	15·0[3]	30·0[9]	19·8	3·54	0·6[14]	0·4[14]	0·2
Ewe	150	1·2[4]	1·5[4]	1·4[10]	2·0	0·35	1·8[14]	0·2[14, 15]	0·6
Goat	125	1·0[5]	1·2[5]	1·1[10]	1·7	0·30	0·9[14]	0·7[14]	0·6
Cow (*Bos taurus*)	1000	8·0[6]	10·0[6]	9·0[11]	13·2	2·36	0·9[16]	0·7[16]	0·8[11]
Cow (*Bos babulis*)	1000	8·0[7]	10·0[7]	9·0[10]	13·2	2·36			

[1]Values do not include allowances for growth and reproduction.

[2]Based on requirement of 0·2 % of Ca in diet (Mitchell & McClure, 1937) and assumption that the P requirement is 125 % of that of Ca; the value for Ca is slightly higher than, and the value for P is the same as, that estimated by Mitchell & McClure (1937). Combining the maintenance and lactation requirement recorded here for a 400 lb sow producing 5 and 10 lb of milk per day, respectively, would be equivalent to 0·46 and 0·57% of Ca and 0·35 and 0·4% of P in the usual ration.

[3]Ca requirement based on data obtained with the cow; assumed same rate of utilization. P requirement assumed to be 125 % of Ca requirement. Values slightly lower than those suggested by Breirem (1938), considerably lower than those recommended by Jesperson (1949), but much higher than those estimated by Mitchell & McClure (1937).

[4]Ca requirement assumed to be same per unit of body weight as that of the cow; P requirement assumed to be 125 % of the Ca requirement. The P requirement recorded here is identical with that determined by Gallup & Briggs (1950) using the balance-trial method and the requirement for maintenance + lactation is about the same as that determined experimentally (Beeson *et al.*, 1945). As a percentage of the ration for a ewe producing 3 lb of milk per day, the Ca and P requirements (0·26 and 0·20 %, respectively) are slightly higher than those (0·23 and 0·19 %, respectively) estimated by Mitchell & McClure (1937) but about the same as those (0·28 and 0·20 %, respectively) recommended by Pope *et al.* (1957).

[5]Ca requirement assumed to be the same per unit of body weight as that of the cow; P requirement assumed to be 125 % of the Ca requirement.

[6]Ca requirement based on average endogenous excretion value (8 g per day) determined by Visek *et al.* (1953) and on a reappraisal of metabolism data by Loosli *et al.* (1956). P requirement based on average endogenous excretion of 10 g per day (Kleiber *et al.*,

1951) which also is 125 % of the Ca requirement; Mitchell & McClure (1937) estimated the P requirement to be 8·9 g.

[7]Ca and P requirements assumed to be the same as those of the domestic cow (*Bos taurus*).

[8]Based on Na requirement of 40 mg per kg body weight per day determined by Meyer *et al.* (1950); this is equivalent to 0·46 g NaCl per 100 lb body weight per day.

[9]No real basis; approximately twice the allowance for the cow per unit body weight to provide for sweating; Ehrenberg (1932) recommended 27 g per day. Kellner & Fingerling (1924) suggested a supplement of 15 to 25 g (i.e., total intake of about 25 to 35 g per day); Jesperson (1949) recommended 10 to 15 g of supplemental NaCl (i.e. about 20 to 30 g total intake). Requirement is probably much higher for heavy work resulting in profuse sweating than that recorded here.

[10]NaCl requirement of ewe, goat and water buffalo cow assumed to be 0·9 g per 100 lb body weight and the same as that of the domestic cow. Depending on the extent of sweating by the water buffalo cow the requirement may be higher than that recorded here.

[11]Based on an appraisal of the data reported by Babcock (1905) and Smith & Aines (1952); Babcock's data indicate a requirement of 0·8 g per lb of milk; data of Smith & Aines (1952) suggest that 30 g of supplemental NaCl (or a total intake of about 40 g per day) is adequate for the maintenance of 1250 lb cows producing 11 000 lb of milk per 10-month lactation period. Therefore, the cow appears to require 0·9 g NaCl per 100 lb of body weight per day for maintenance.

[12]Based on data of Guilbert *et al.* (1940) showing that the daily minimum requirement for the prevention of nyctalopia in mammals is 2·4 μg of vitamin A or 13·2 μg of carotene per lb of body weight. Requirements for reproduction are probably two to five times the values recorded here (Guilbert *et al.*, 1940; Pope *et al.*, 1957) depending upon body stores.

[13]Ca and P values based upon the concentrations in the milk and the requirements for lactation by the cow; assumed that sow utilizes dietary Ca and P to same extent as does the cow. Total requirements listed here for maintenance and lactation are similar to those estimated by Mitchell & McClure (1937) by other means (see footnote 2).

[14]Based on the Ca and P concentrations in the milk and the requirements for lactation by the cow, assuming similar rates of utilization of dietary Ca and P.

[15]See footnote 4.

[16]Requirements for Ca and P based upon results of many metabolism experiments; however, values are probably about 0·1 to 0·2 g higher than the minimum requirements (Mitchell & McClure, 1937). Also, Hart *et al.* (1932) maintained bone health in cows producing over 10 000 lb of milk per annum during each of 4 lactation periods by feeding 25 to 28 g of Ca and 28 g of P per day. A similar performance by cows over 3 lactation periods was reported by Converse (1954). Although an allowance of about 0·5 to 0·6 g each of Ca and P per lb of milk (Hart *et al.*, 1932) and about 0·36 g of Ca per lb of milk (Converse, 1954) appeared to be satisfactory for the duration of these experiments, the values listed in this table probably offer some insurance for satisfactory reproduction and a long and healthy life.

[17]NaCl requirements based upon the Na concentrations in the milks and the apparent NaCl requirement for milk production by the cow (Babcock, 1905). Since Na appears to be more limiting than Cl (Aines & Smith, 1957; Meyer *et al.*, 1950) and the satisfaction of the Na needs by NaCl ensures a surplus of Cl, the amount of NaCl equivalent to the amount of Na in the milk was used as the starting point for computing the NaCl requirement. (See footnote 11.)

Although it is known that certain quantities (e.g., 0·2 mg per 100 lb of body weight per day for the sow (Beeson *et al.*, 1953)) of iodine prevent the reproductive disturbances ascribed to its deficiency, the minimum amount required is not known. As a result of the studies of Mitchell & McClure (1937), it would appear that the minimum requirement for iodine is equivalent to 20 to 40 μg per 100 kcal of heat produced. On this basis the various animals of the sizes listed in Table V would require for maintenance the following amounts (μg per day): sow, 160; mare, 440; ewe, 80; goat, 70; and cow (both domestic and water buffalo), 330.

d. *Iron and copper.* Although both iron and copper are required for haemoglobin formation and the function of certain enzymes, information on the specific roles of these elements in lactation is lacking. Milk has a low concentration of iron and copper and supplementation of the diet of lactating animals with these elements does not increase the amount in the milk above the normal level.

Under usual feeding conditions a deficiency of iron is not encountered in lactating farm animals; however, a wasting syndrome in ruminants attributed to a dietary deficiency of copper (or to an excess of molybdenum or both) has been associated with certain geographic areas. The requirements of lactation for iron and copper are not known, but the requirements of growing animals may indicate the approximate size of the needs of mature, lactating animals. To at least one year of age the requirements of growing calves for iron and copper are 30 and 6 mg per day, respectively (Matrone *et al.*, 1957). Per unit of body weight the growing pig requires more of these elements than does the calf. According to Venn *et al.* (1947) the pig needs to retain 7 mg of iron per day during the first 8 weeks of life; about 0·5 mg of copper per 100 lb of body weight is an adequate daily allowance for pigs (Teague & Carpenter, 1951).

e. *Cobalt.* The occurrence in various parts of the world of a wasting syndrome in ruminants characterized by inappetence, anaemia and severe losses of body weight and milk production is prevented and cured by supplementation of the diet with cobalt. Since horses remain healthy while grazing pastures on which cattle, sheep and goats die, and simple-stomached animals remain healthy on diets containing less than 10 % as much cobalt per unit of diet as is required by sheep, a function of cobalt in the symbiosis between the ruminant host and its microflora was indicated. After the discovery of vitamin B_{12} (a growth or anti-anaemic factor for non-ruminant animals) and the finding that this vitamin contains cobalt as a molecular component, it was demonstrated that the parenteral administration of vitamin B_{12} is therapeutic for cobalt-deficiency disease (Smith *et al.*, 1951; Marston & Lee, 1952). Thus, vitamin B_{12} is an intermediary in the metabolism of cobalt by ruminants and it is a metabolic essential for all animals; however, vitamin

B_{12} is not a dietary essential for ruminants because of bacterial synthesis. On the other hand, cobalt is not a dietary essential for simple-stomached animals provided vitamin B_{12} is supplied.

The cobalt needs of lactation have not been determined, but the requirements (0·07 to 0·08 mg per day) for the maintenance of general health in sheep (Marston, 1952) appear to be adequate for both maintenance and lactation. Presumably about the same quantity is needed by goats and, at the same rate per unit of body weight, the requirement of lactating cows would be 0·5 to 1·0 mg per day.

f. *Magnesium.* Ordinary rations appear to provide an abundance of magnesium for all farm animals. Nevertheless, hypomagnesaemia has been observed in ruminants consuming magnesium-adequate diets, apparently as the result of impaired absorption of the element (Head & Rook, 1955). The requirement (g per day per 100 lb body weight) for growing calves is 0·54 to 0·68 according to Huffman *et al.* (1941) or 0·65 to 0·75 based on the data of Blaxter *et al.* (1954). The balance data reported by Balch *et al.* (1956), suggest a daily requirement of 0·3 to 0·5 g of magnesium per 100 lb for milking cows. Growing pigs require approximately 1·0 g per 100 lb of body weight (Mayo *et al.*, 1957).

g. *Manganese.* Although manganese is required in the diet of certain simple-stomached animals for normal reproduction, bone development and enzyme function, the quantitative requirements of the various body functions of farm animals have not received much attention because ordinary diets provide adequate amounts. Sows consuming diets containing less than 12 p.p.m. of manganese have less well developed mammary glands and produce less milk than sows fed on diets containing 40 to 100 p.p.m. of manganese (Grummer *et al.*, 1950; Outler *et al.*, 1955; Plumlee *et al.*, 1956). It would appear that a diet containing 20 p.p.m. of manganese would adequately support maintenance and lactation in the sow. This is equivalent to an intake of 30 to 35 mg per day by a 400 lb sow.

Since growth, reproduction and lactation were not different in cattle consuming diets ranging in manganese content from 10 to 60 p.p.m. (Bentley & Phillips, 1951) and since the ordinary diet generally contains more than 30 p.p.m., it is unlikely that a deficiency will occur naturally in lactating ruminants.

F. Vitamins

The significance of the vitamins to lactation is two-fold: they may be essential to the secretion process and their presence in the milk may enhance its nutritive quality. The present knowledge concerning the requirements of lactation for the vitamins is scanty. In most instances when the given intake

of a vitamin is known to maintain general health, prevent a particular structural change, allow satisfactory growth or support satisfactory reproduction, it has been assumed that the same level with or without some additional allowances for safety will suffice also for lactation. For those animals that normally suckle their young, the allowance may be made sufficiently high to ensure a considerable secretion of the vitamin in the milk as insurance for the young. Since the maintenance of general health obviously can influence lactation performance, the requirements of farm animals for the vitamins will be considered here.

a. *Vitamin A.* A source of vitamin A or of its provitamins is required in the diet of all farm animals. Of several specific functions in the animal body ascribed to this vitamin, its role in the maintenance of epithelial health is the only one that might have a direct effect upon lactation. Although a dietary deficiency results in a decreased concentration of vitamin A in the milk and the amount in the milk of cows can be greatly increased by giving large amounts of vitamin A, there is no evidence that the milk yield is increased by the giving of quantities of vitamin A (or carotene) greater than those needed to maintain general health.

According to Guilbert *et al.* (1940) the daily minimum requirement for the prevention of nyctalopia in all mammals is 2·4 μg of vitamin A or 13·2 μg of carotene per lb of body weight. It seems reasonable to assume that these amounts would be adequate for non-pregnant, lactating farm animals. On this basis the requirements recorded in Table V were computed for the various farm animals of representative sizes.

b. *Vitamin D.* The growth and maintenance of bone in all farm animals requires, in addition to calcium and phosphorus, a dietary supply of vitamin D. Since a large proportion of the vitamin D needed is normally provided by solar irradiation, the dietary requirement depends to a great extent upon the degree of exposure of animals to sunlight.

The vitamin D need of farm animals is less critical for reproduction and lactation than it is for growth. Although the extensive metabolism of calcium and phosphorus in lactation implies a great need for vitamin D, neither the milk yield nor the mineral balance of cows or goats under usual feeding and management conditions is improved by supplementation of the diet with vitamin D (Hart *et al.*, 1927, 1930) or by exposure to ultraviolet light radiations (Hart *et al.*, 1924, 1926). However, as a result of feeding cows confined in darkness on a ration devoid of roughages, Wallis (1938) demonstrated that vitamin D is essential for lactation. Under these conditions the onset of the deficiency signs required at least 4 months to develop, but very brief exposure to sunlight or the feeding of only 2 lb per day of sun-cured hay effected a rapid recovery.

The vitamin D requirement of lactating farm animals, with the exception of that of the cow has received almost no research attention. Inadequate information exists even on the growth requirements of some animals, and, as a consequence, the recommended allowances for certain farm animals are based upon data obtained with other species. The recommended allowances of the American National Research Council range from 250 to 400 i.u. per 100 lb of body weight per day, which probably is two to four times the minimum requirement. Since Wallis (1944) found that levels of vitamin D between 1000 and 2000 i.u. per day were curative for rachitic milking cows, the minimum requirement of animals not in a deficient state probably would not exceed 100 i.u. per 100 lb of body weight. On this basis, farm animals of the sizes listed in Table V would have the following approximate requirements (i.u. per day) for vitamin D: sow, 400; mare, 1500; ewe, 150; goat, 125; and cow, 1000.

c. *Vitamin E.* Whether or not vitamin E has a specific role in the lactation of farm animals has not been demonstrated. Gullickson & Calverley (1946) found that thirteen of twenty-eight cows and bulls consuming vitamin E-free diets died of cardiac failure between 2 and 5 years of age, but there was no apparent effect upon the milk yield (Gullickson *et al.*, 1949). Thus, it would appear that for mature farm animals consuming ordinary diets the intake of vitamin E is sufficient to maintain muscle health (which probably represents their major need for this vitamin). The concentration of vitamin E in the milk is positively correlated with the level of tocopherols in the diet and milks high in tocopherols resist the development of oxidized flavours more than those low in this vitamin (Krukovsky *et al.*, 1949).

d. *B-Complex vitamins.* Because of their function in enzyme systems, the members of the B-complex group are assumed to be metabolic essentials for all farm animals. Whether they are dietary essentials and the extent to which they are dietary essentials depend upon the degree of synthesis in, and absorption from, the gastro-intestinal tract. It has been demonstrated (McElroy & Goss, 1939, 1940; Wegner *et al.*, 1940; Lardinois *et al.*, 1944; Porter, 1953) that the following vitamins are synthesized in the rumen: thiamine, riboflavin, nicotinic acid, pyridoxine, biotin, pantothenic acid, folic acid, and vitamin B_{12}. During the first few weeks of life at least certain of the B-complex vitamins are dietary essentials for the young ruminant; after the ruminal flora becomes established, none of these is a dietary essential.

The same vitamins are synthesized in the intestinal tract of the horse (Carroll *et al.*, 1949). Since most of the synthesis occurs in the caecum, it is questionable whether the vitamins are absorbed to a very great extent (Pearson *et al.*, 1949). Deficiencies of the B-complex vitamins in the horse can be avoided by the careful selection of dietary components. Nevertheless the following approximate requirements (mg per 100 lb of body weight per

day) for certain vitamins have been proposed: thiamine, 2·5 (Carroll *et al.*, 1949); riboflavin, 2·0 (Pearson *et al.*, 1944a,b); pantothenic acid, 1·7 (Pearson *et al.*, 1944b; Pearson & Schmidt, 1948).

Although all of the B-complex vitamins are either known, or suspected, to be dietary essentials for the pig and considerable attention has been given to the requirements of the growing-fattening pig, there is no adequate basis for deriving from existing data the requirements of the lactating sow. On the assumption that the needs per unit of body weight of the lactating sow are the same as those of the growing-fattening pig, the daily requirements for a 400 lb sow would be: thiamine, 8 mg (Hughes, 1940b; Van Etten *et al.*, 1940; Ellis & Madsen, 1944; Jacobsen *et al.*, 1950); riboflavin, 12 mg (Hughes, 1940a; Miller & Ellis, 1951; Mitchell *et al.*, 1950; Moustgaard, 1952; Krider *et al.*, 1949); nicotinic acid, 28 mg (Hughes, 1943; Luecke *et al.*, 1948; Braude *et al.*, 1946; Moustgaard, 1952); pantothenic acid, 56 mg (Hughes & Ittner, 1942; Luecke *et al.*, 1952; Moustgaard, 1952); vitamin B_6, 16 mg (Hughes & Squibb, 1942; Wintrobe *et al.*, 1943; McMillen *et al.*, 1949; Moustgaard *et al.*, 1952); and vitamin B_{12}, 80 μg (Catron & Culbertson, 1949; Vohs *et al.*, 1951). Deficiencies of the other B-complex vitamins are unlikely to occur under practical conditions.

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CHAPTER 15

Metabolic Disturbances Associated with Lactation

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I. Introduction

The three major metabolic diseases of cattle, and particularly lactating cows, are parturient paresis (milk fever), hypomagnesaemia (grass tetany) and ketosis. Although a great deal of information has been obtained during the past three decades on the nature of these disorders, their aetiologies remain obscure. These diseases occur most frequently in high-producing and older cows in the earlier part of lactation.

In this chapter no attempt will be made to cite all of the literature, since a number of comprehensive reviews of all three disorders have appeared recently (Allcroft, 1954; Blaxter & McGill, 1956; Boda & Cole, 1956; Hibbs, 1950; Shaw, 1955, 1956; Thaddea & Kuhn, 1937). For the most part, reference will be made only to articles in which conclusions or postulations or both are based upon valid experimental data.

II. Parturient Paresis

Parturient paresis or milk fever is a metabolic disease occurring in cattle at the time of parturition and beginning lactation. The characteristic signs are a

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generalized tetany followed by paralysis and a semicomatose state, and if untreated the disease usually terminates in death. This condition was first mentioned in Germany in 1793 and during the next half century reports of its occurrence were appearing in the literature from all over the world (Hibbs, 1950). It is generally agreed that the condition occurs most frequently in older, high-producing cows, and that Jerseys are more susceptible than the other breeds of dairy cattle (Henderson, 1938; Hibbs *et al.*, 1946b; Metzger, 1936). In contrast to grass tetany and ketosis, which occur most frequently from a few days to a few weeks post-partum, milk fever generally occurs within the 24-hour period after parturition. There is probably no seasonal influence on the incidence of milk fever (Hutyra & Marek, 1926; Smith, 1957) and apparently no correlation with climatic conditions (Hallgren, 1955; Hibbs, 1948; Hutyra *et al.*, 1938). It is generally recognized that cows that have had one attack are more susceptible to attacks at subsequent parturitions (Hutyra & Marek, 1926).

A. Hypocalcaemia

Under the mistaken impression that milk fever was caused by a virus infection of the udder, Schmidt (1897) injected with notable success a potassium iodide solution into the udders of cows with milk fever. This led to the finding that water alone was equally adequate, and finally to the discovery that air inflation of the udder was a highly effective treatment (see Hutyra & Marek, 1926), a treatment which is still used to some extent in refractory cases (Reid, 1951).

For a few years (1923–26) it was believed by many that milk fever was due to hypoglycaemia associated with the demands of the udder for glucose (Maguire, 1926; Widmark, 1926; Widmark & Carlens, 1925a,b,c,d). It appears quite likely that some of the recoveries elicited by intravenous injections of glucose actually represented a cure of ketosis, a syndrome which was little understood at that time and which is still frequently confused with milk fever. A series of studies from 1924 to 1927 (Fish, 1927; Greig, 1926; Hayden, 1927; Hayden & Sholl, 1923–24; Little & Wright, 1925; Moussu & Moussu, 1926, 1927) demonstrated that hypocalcaemia is accompanied by hyperglycaemia rather than hypoglycaemia.

Dryerre & Greig (1925) proposed, without experimental evidence, that milk fever was due to a parathyroid deficiency resulting in an accumulation of toxic substances such as guanidine, and a fall in blood calcium, the fall in calcium being further accentuated by lactation. They further proposed that the curative effect of mammary inflation was due either to oxidation of toxins due to adrenal stimulation or to prevention of passage of blood calcium to the milk or both; these latter proposals have since been shown not to be valid

(Auger, 1926; Hayden, 1929). A short time later, Little & Wright (1925) reported hypocalcaemia in milk fever and a relationship between the degree of hypocalcaemia and the severity of the signs. Numerous reports confirmed the existence of hypocalcaemia in milk fever (Dryerre & Greig, 1928; Fish, 1929; Greig, 1930b; Sjollema, 1928).

Little & Wright (1926) attached a great deal of importance to the drain on blood calcium by the secretion of colostrum; more recently it has been shown (Hibbs, 1948) that cows with milk fever produce no more colostrum than normal cows during the post-parturient period and that the ash and calcium content of the colostrum of cows with milk fever is no higher than that of normal post-parturient cows.

Hypophosphoraemia (Fish, 1929; Hibbs *et al.*, 1946a; Sjollema, 1928; Wilson & Hart, 1932) and also hypermagnesaemia (Godden & Duckworth, 1935; Hibbs *et al.*, 1946a; Seekles *et al.*, 1932d; Sjollema, 1928; Sjollema & Seekles, 1930, 1932) in addition to hypocalcaemia occur in cows with milk fever. The diffusible blood calcium has been reported to decrease to a greater extent than total blood serum calcium (Kronfield, 1956; Sjollema & Seekles, 1930, 1932). Since the blood Ca : Mg ratio in milk-fever cows approaches that observed in magnesium narcosis, it has been suggested that this may be the primary cause of the signs typical of milk fever (Hibbs, 1948; Hibbs *et al.*, 1946a; Pribyl, 1933; Sjollema, 1928). A decrease of serum citric acid (Blosser & Smith, 1950; Ward *et al.*, 1953b) and higher serum pyruvic acid (Ward *et al.*, 1953b; Van Soest & Blosser, 1954) and lactic acid levels (Ward *et al.*, 1953b) have also been reported in milk-fever cows. The interpretation of these findings is not clear although it has been suggested that a failure of the glycolytic cycle may occur (Ward *et al.*, 1953b). Lymphopenia and eosinopenia were reported in milk-fever cows by Carlstrom (1950), Garm (1950), Merrill & Smith (1954) and Shaw *et al.* (1952), probably due to the stress of parturition.

Excellent cures were obtained by intravenous injections of calcium chloride (Mattick & Little, 1933; Sjollema, 1928, 1929) and both intravenous (Greig, 1930a; Stinson, 1929) and subcutaneous (Dryerre & Greig, 1935) injections of calcium gluconate. Seekles *et al.* (1931a,b, 1932b,c) demonstrated an antagonism between calcium and magnesium in the bovine animal. They observed an increased rate and disturbed rhythm of the heart of cows with grass tetany and milk fever after the injection of either calcium chloride or magnesium chloride alone, but no such effects when the two were injected together. These workers (Seekles *et al.*, 1931c) also injected sodium oxalate into cattle and obtained hypoglycaemia and signs somewhat similar to those of milk fever. Since the signs did not coincide with the lowest point of the calcium level it was concluded that they cannot be explained on the basis of decreased blood calcium alone.

Robertson (1949) was unable to confirm the classification of milk fever cases suggested by Barker (1939) who proposed that the variations observed in the signs were related to the level of blood magnesium.

Air inflation of the udder for milk fever treatment has been shown to increase blood calcium (Dryerre & Greig, 1928; Fish, 1930; Greig, 1930c; Niedermier & Smith, 1950; Sjollema, 1928) and serum inorganic phosphorus (Fish, 1930; Niedermier & Smith, 1950) but it does not alter blood magnesium materially (Niedermier & Smith, 1950; Reid, 1951). Mastectomy (Niedermier *et al.*, 1949) prevented the decline in blood calcium partially and the increase in blood magnesium which occurs immediately post-partum, but did not prevent the decrease in blood inorganic phosphorus. Neither pre-partum milking (Smith & Blosser, 1947) nor complete milking immediately post-partum (Owen, 1954; Smith *et al.*, 1948) was found to influence the incidence of milk fever. It appears strange that greater attention has not been given to the possible aetiological role of phosphorus in milk fever.

B. Milk Fever in Relation to Vitamin D and the Parathyroids

Particular attention in recent years has been given to the possible involvement of the parathyroids in milk fever and to the use of vitamin D as a preventive. It is generally recognized that, with the exception of dietary calcium *per se*, vitamin D has the greatest influence on the absorption of calcium and has a direct effect on the mineralization of bone (Greenberg, 1945; Nicolaysen & Eeg-Larsen, 1953). Hypercalcaemia has been induced in dogs and rats (Hess *et al.*, 1931; Jones & Rapoport, 1931; Shelling, 1932) on low-calcium diets by the administration of vitamin D and in cattle by massive doses of vitamin D (Duncan & Huffman, 1934; Hess *et al.*, 1932; Hibbs & Pounden, 1955; Lewis & Burrow, 1953).

Vitamin D was early suggested as a possible preventive in milk fever (Greig, 1930b; Little & Mattick, 1933; Sjollema, 1930). Whereas 60 000 i.u. of vitamin D daily did not increase the blood calcium of cows (Hess *et al.*, 1932), increases were obtained in lactating goats receiving 32 million i.u. of vitamin D for four days (Campbell & Turner, 1943). High doses of a vitamin D preparation were shown to be toxic to rats and to depress parathyroid activity (Bastenie & Zylberzac, 1939; Campbell & Turner, 1943).

Hibbs & Pounden (1955) in a review of their work stated that the administration of 30 million i.u. of vitamin D daily for at least 4 or 5 days, but no longer than 7 days, pre-partum was highly successful in preventing the development of milk fever. The customary decrease in blood calcium and phosphorus at parturition was prevented. In earlier studies Hibbs *et al.* (1946a, 1947) were unsuccessful in preventing milk fever by feeding 1 to 5 million i.u. of vitamin D daily for from 2 to 4 weeks pre-partum. Dell &

Poulton (1958) have confirmed the reports of the value of vitamin D as a milk fever preventive. In studies with over 400 cows, the addition of 30 million i.u. of vitamin D_2 daily to the grain for approximately 72 hours pre-partum and 48 hours post-partum decreased the incidence of milk fever by approximately 70 % below that of the controls. Thus it has been demonstrated that massive doses of vitamin D properly administered will prevent milk fever. A serious disadvantage of this method is the necessity of accurately estimating the calving date.

Ward *et al.* (1952, 1953a) conducted calcium balances pre-partum on normal cows and on cows that subsequently developed milk fever, and observed a severe negative calcium balance in the latter. They suggested that it may have been due to a period of lowered calcium absorption or to excessive calcium excretion through the intestines, and that it may explain the value of vitamin D in preventing milk fever.

Since the early postulation of Dryerre & Greig (1925) that milk fever is due to a parathyroid involvement, considerable attention has been directed to these glands in connexion with this disorder. The parathyroid glands have long been known to be one of the most important regulators of blood calcium (Collip, 1925; Greenwald & Gross, 1925b; Salrensen, 1923). Salrensen (1923) noted that parathyroidectomized dogs survived when fed on high-calcium diets and that the intravenous injection of soluble calcium salts prevented and also cured the tetany resulting from parathyroidectomy. The parathyroid hormone also has been shown to be needed for maintenance of normal urinary phosphate excretion (Greenwald & Gross, 1925a; Talmage & Kraintz, 1954a,b); the evidence is rather convincing that there is a close interrelationship between calcium and phosphorus metabolism with respect to the parathyroid hormone. The mode of action of the parathyroids involves both a regulatory action in controlling blood calcium and phosphorus by renal excretory mechanisms and a direct effect on bone resorption (Barnicot, 1948; Bodansky & Duff, 1941; Hastings & Huggins, 1933).

Since the young growing animal exhibits a greater response to injections of parathyroid hormone (Fish, 1930) than do older animals, it was suggested by Hibbs (1950) that this may be one of the reasons why older cows are more susceptible to the development of milk fever than cows in their first lactation.

The report of Seekles *et al.* (1932a) that milk fever could be cured in cows with parathyroid extracts was not confirmed by the more recent work of Hibbs *et al.* (1947) although some increase in serum calcium and phosphorus occurred in normal cows after the injection of a parathyroid extract. In calves, marked increases in blood serum calcium occurred after the injection of high levels of a parathyroid extract. In contrast to the work of Talmage *et al.* (1953) with rats, Lotz *et al.* (1954) reported that parathyroid extract administered to sheep caused removal of previously deposited ^{32}P, but not ^{45}Ca, from the bones.

From these results, and also from observations that while the blood phosphorus increased the blood calcium remained normal, it was concluded that in sheep the parathyroid hormone acts directly on bone to remove phosphorus.

Parathyroidectomy has been shown to decrease the blood calcium level of goats (Campbell & Turner, 1943; Smith *et al.*, 1957). In calves, thyroparathyroidectomy resulted in a marked lowering of serum calcium, less marked decrease of serum inorganic phosphorus and death (Stott & Smith, 1957a). The elevation of serum inorganic phosphorus, which is usually noted in other animals, did occur but only immediately before death. Stott & Smith (1957b) reported that thyroparathyroidectomized cows exhibited a marked decrease in serum calcium and a lesser decrease in serum inorganic phosphorus for a few days and then a return to normal, but did not exhibit signs of milk fever. On a normal diet (high Ca : P ratio) the absence of the parathyroids did not have any adverse effect on pregnancy or parturition and did not effect a cessation of lactation. It was suggested that the results could be explained on the basis of a proposed mechanism whereby there is a chemical equilibrium between blood serum and bone which operates independently of the parathyroids (McLean & Urist, 1955).

An interesting approach to the prevention of milk fever is that of Boda (1956) and Boda & Cole (1954) who reported that the use of low-calcium, high-phosphorus pre-partum diets was effective in decreasing the incidence of milk fever in high-producing Jersey cows. Sixty-nine aged Jersey cows were divided into four groups and fed on diets containing various Ca : P ratios for varying periods of time pre-partum. Thirty % of the cows receiving diets with a calcium to phosphorus ratio of 6 : 1, 15 % of those receiving diets with a calcium to phosphorus ratio of 1 : 1 and none of those receiving diets with a calcium to phosphorus ratio of 1 : 3·3 exhibited clinical signs of milk fever. The rationale of this regime is that low-calcium, high phosphorus diets stimulate the parathyroids to greater secretion before the initiation of lactation and the resulting increased demand for calcium mobilization.

That this approach is valid is indicated by the numerous reports that low-calcium diets increase the size and activity of the parathyroids and that high-calcium diets exert the opposite effect (Campbell & Turner, 1943; Carnes *et al.*, 1942; Luce, 1923; Saxton & Ellis, 1941; Sinclair, 1941; Stoerk & Carnes, 1945). The ingestion of high levels of phosphate salts for long periods of time has been reported to produce bone changes characteristic of hyperparathyroidism (Saxton & Ellis, 1941).

Ender *et al.* (1956) reported that of nineteen cows on experimental high-calcium diets, seven developed typical pictures of milk fever with all of the classic signs, and eight developed moderate signs. Hypocalcaemia was noted in all of the experimental cows. The diets were also low in phosphorus,

but the authors state that since milk fever was also induced on high-calcium diets containing ample phosphorus, the effect appears to be primarily one of high dietary calcium. It was also concluded that an increase of the dietary phosphorus immediately post-partum may contribute to the initiation of milk fever.

III. Hypomagnesaemia

This discussion will be confined to the condition known as "grass tetany" which occurs primarily in older cattle and especially in lactating cows. Clinical signs of this disease were described in great detail by Sjollema (1930, 1932). The signs vary greatly but may include initial signs of nervousness, restlessness, lack of appetite, unsteady gait, abnormal eye-muscle contraction, abundant salivation, mild convulsions and tetanic contraction of certain muscles. Excitement is often followed by a paretic or comatose state resembling milk fever.

The clinical manifestations were known for about half a century before the first of a series of extensive reports by Sjollema & Seekles (1929, 1930) and Sjollema *et al.* (1939a,b,c) which were reviewed by Sjollema (1930, 1932). Dryerre (1932) presented the first report on hypomagnesaemic tetany or "lactation tetany" in England.

The incidence of this disease is much lower than that of milk fever (Blaxter & McGill, 1956) and of ketosis (Shaw, 1956). However, owing to the high death loss, the disease does assume considerable economic importance. The condition is seasonal, the incidence being especially high when cattle are turned out to grass in the spring (Dryerre, 1932; Sjollema & Seekles, 1929), but also occurs in the winter months in cattle receiving winter rations (Allcroft, 1947a; Allcroft & Green, 1938; Inglis *et al.*, 1954). It is apparently not confined to any age, breed or stage of lactation (Allcroft, 1953). Both "grass tetany" (Muth & Haag, 1945) and "winter tetany" (Metzger, 1936; Nolan & Hull, 1941; Udall, 1947) have been described in the United States.

Sjollema (1930, 1932), and later Blaxter & McGill (1956) in a recalculation of Allcroft's (1947a) data, noted that old cows are more susceptible to hypomagnesaemia than younger cows; the latter authors suggested that the present apparent increase of tetany might be the result of an increased number of older cows due to improvements in the control of cattle diseases.

A. Nutritional Aspects of Hypomagnesaemia

Low blood-magnesium values were first associated with "grass tetany" by Sjollema & Seekles (1929, 1930) in the Netherlands; these same investigators also reported hypocalcaemia as well as hypomagnesaemia in cattle affected

with this condition, as did Allcroft & Green (1934). Allcroft (1947a) noted that of a total of 406 cases of hypomagnesaemia, 76 % also exhibited hypocalcaemia.

In one of his earlier reports Sjollema (1930) suggested that the disease must have some connexion with alterations in the methods of feeding and manuring practised by the farmers, and stated that most veterinarians believed that the condition was most likely to occur when cows were pastured on quick-growing grass resulting from heavy manuring. Later Sjollema and Seekles became interested in the possibility of a relationship between hypomagnesaemia and high potassium intake, but were unable to correlate the occurrence of the disease with a high potassium, high protein or high nitrate content of the grass (Seekles & Sjollema, 1932; Sjollema & Seekles, 1933). Kunkel *et al.* (1953) reported that excessive potassium elicited a slight decrease in serum magnesium but this was not observed by others (Eaton & Avampato, 1952; Odell *et al.*, 1952; Pearson, 1948; Pearson *et al.*, 1949). A potassium deficiency has been reported to have no influence on magnesium metabolism (Blaxter & McGill, 1956). The abnormal acid-base content of the ash of some spring grasses has been suggested as a possible contributing factor (Brouwer, 1952; Schultz, 1958) but has not been shown experimentally to induce hypomagnesaemia.

Many believe (Allcroft, 1954; Blaxter & McGill, 1956) that the disorder is not merely that of a nutritional deficiency of magnesium, since the condition occurs on varying levels of magnesium intake. However, it appears that the nutrition of the animal can be a very important factor in the development of the hypomagnesaemia occurring post-partum. For example, in long-term experiments, Breirem *et al.* (1949, 1954) and Ender *et al.* (1949) induced hypomagnesaemia and tetany in dairy cows on high-protein rations which were deficient in energy and low in magnesium. Hypomagnesaemia has been produced on as much as 11 g of magnesium daily, but it appeared more readily in cows on lower levels of magnesium. According to Blaxter & McGill (1956), 11 g is sufficient for a cow milking 30 lb per day. Larger amounts of magnesium prevented the nervous signs and tetany. It was concluded that lack of magnesium together with an energy deficiency was instrumental in producing tetany. It was reported by Blakemore & Stewart (1934–35) that the feeding of magnesium oxide (31 g/day) had a preventive effect on the occurrence of seasonal hypomagnesaemia, a finding which was confirmed by others (Allcroft, 1947b, 1953).

The comprehensive studies by Swan & Jamieson (1956a,b) confirm the relation of undernutrition to hypomagnesaemia. Clinical cases of "grass staggers" were produced by limited grazing of cows early post-partum; both hypomagnesaemia and hypocalcaemia occurred. However, hypomagnesaemia was produced in many animals that did not exhibit clinical signs. They

concluded that although hypomagnesaemia is a necessary condition for the tetany-paresis syndrome, it is not by itself a sufficient condition.

In studies on the influences of certain fertilizer treatments of pasture plots (Bartlett *et al.*, 1954) a high incidence of hypomagnesaemic tetany was observed in milking cows grazing on plots that had been heavily fertilized with nitrogen, but not on plots treated with both nitrogen and magnesium and on untreated plots. The heavy fertilization with nitrogen (sulphate of ammonia) was quite evidently a major factor in the development of tetany on these plots. Studies with several kinds of grasses demonstrated that the occurrence of hypomagnesaemia was not confined to a particular species of grass.

In the United States, in the Appalachian areas of the States of Kentucky, Maryland, Virginia and West Virginia, a high incidence of hypomagnesaemic tetany has been evident for many years during the early post-partum period in beef cows wintered primarily on hay and silage (Metzger, 1936; Nolan & Hull, 1941; Udall, 1947). In studies by Leffel *et al.* (1958), hay from one of the farms affected was transported to a non-tetany area 200 miles away and fed to older cows, three beef and three dairy, as the sole ration the following winter. Of the six cows receiving the hay for 5 to 6 months pre-partum and a month post-partum, three beef cows developed hypomagnesaemia and typical signs of grass tetany 2 to 3 weeks post-partum. Bone magnesium was decreased by 50 %. Two recovered after an intravenous injection of calcium and magnesium salts. The remaining three (dairy cows) exhibited hypomagnesaemia post-partum but no signs of tetany were evident. The hay contained 0·08 % magnesium, which is lower than the concentration (0·1 %) proposed by Blaxter & McGill (1956) as being sufficient to prevent a dietary deficiency of the element in lactating cows. High protein was not a factor since the hay contained only 8 % protein. The hay was not consumed in sufficient quantity to prevent a loss in body weight, the position thus being reminiscent of the observations of Ender *et al.* (1949) and Swan *et al.* (1956b).

In the Maryland studies it must be concluded that the high incidence of winter tetany occurring in beef cows within a few weeks post-partum and covering such a wide area of the Appalachian region, does represent a hypomagnesaemic tetany of nutritional origin since, in the experimentally induced tetany, the cattle, year and environment were completely different from those of the area where the hay had been obtained. The hay fed was the only factor common to both the farm where the incidence of tetany was high and the experimental feed lot at the University of Maryland where the tetany was reproduced.

The conditions under which tetany has been reported to occur are varied. This is perhaps not too surprising considering the number of factors that can

influence the magnesium content of feed substances and the availability of the magnesium. Repeated observations that hypomagnesaemic tetany may occur at relatively high levels of magnesium intake suggest an abnormality of absorption, retention or mobilization. It has been suggested that the magnesium of different feeds is not utilized equally well by cattle (Huffman *et al.*, 1941; Carbery *et al.*, 1937; Thomas & Okamoto, 1958) and therefore the amount of magnesium in the ration is not necessarily a guide to its adequacy. The report of Kunkel & Pearson (1948) with rats is particularly interesting in this connexion since they found that the availability of magnesium in various forms differed greatly, the order of availability being magnesium sulphate, wheat plant magnesium, magnesium oxide.

Blaxter & Rook (1954) on the basis of trials with calves on an artificial magnesium-free diet, with added increments of magnesium oxide, concluded that the availability of magnesium was only 30 to 50 %, owing to a large faecal loss. As noted above, appreciable variation can be expected depending on the source of the magnesium. With older animals the availability should be less. Balance trials with calves by Smith (1957) showed that the availability of magnesium varies with age, being about 75 % at 5 weeks of age and 0 to 10 % at 24 weeks of age. An intake of 6·7 g was found by Ray (1942) to be necessary for a positive magnesium balance in 400 to 500 lb animals fed on natural feedstuffs. Thomas & Okamoto (1958) found that an intake by calves on milk of 1 g of magnesium per 100 lb body weight was insufficient to meet requirements. Thus it is possible that the minimal requirements suggested by Blaxter & McGill (1956) may be low. Balch *et al.* (1956) concluded that hypomagnesaemia in dairy cows during the spring grazing period is due to a low availability to the cow of the magnesium of the grass combined with a low intake of magnesium as compared with winter feeding.

The magnesium content of plants varies greatly depending upon fertilization, rainfall and other environmental factors (Bartlett *et al.*, 1954; Blaxter & McGill, 1956; Krackenberger & Peterson, 1954). For example, high content of potassium and calcium in the soil, high rainfall and heavy nitrogen fertilization have all been associated with a decrease in the magnesium content of plants. Magnesium tends to be low in the leaves of plants and higher in the seed. Legumes are usually higher in magnesium than the grasses.

The earlier observations of Sjollema and others of a possible relation between heavy nitrogen fertilization and the occurrence of grass tetany and the definite implication of such a relationship in the well-controlled experiments by Bartlett *et al.* (1954) have a possible explanation in recent studies which suggest a causative relationship between excess ammonia production in the rumen and grass tetany. It was reported that in hypomagnesaemia of cows on spring pasture, urinary magnesium excretion decreased which was associated with a low blood serum magnesium (Head & Rook, 1955); this had earlier

been observed by Cunningham (1936b). Hypomagnesaemia was also noted to be associated with high ammonia production from grass (Rook, 1954). Ammonium salts (Head & Rook, 1955; Rook, 1954) were shown to decrease urinary magnesium excretion but produce little or no decrease in blood serum magnesium. It was suggested that the decrease in urinary magnesium excretion was due to impaired magnesium absorption.

Blaxter & McGill (1956) have called attention to the fact that only a small amount of magnesium is excreted in the urine. They suggest that the decrease in urinary magnesium might be due to an inability of the animal to excrete magnesium in urine made alkaline by an excess of ammonia. Head & Rook (1956) noted a three-fold increase in the concentration of ammonia in rumen fluid of cows grazing on spring grass as compared to that on a ration of hay and concentrates. In differently fertilized pastures a close relationship was noted between the level of crude protein in the grass and both the serum non-protein nitrogen and the blood ammonia; serum magnesium exhibited an inverse relationship with the level of protein in the grass, decreasing from 1·9 to 1·6 to 1·0 mg % as the protein content of the grass increased from 15·9 to 18·8 to 23·8 % respectively. The addition of starch to a high-protein grass ration decreased urinary nitrogen excretion, which confirms earlier reports (Head, 1953; McDonald, 1952). Though it is not clear how the ammonia may affect magnesium metabolism, the proposal that hypomagnesaemic tetany may be due to excessive ammonia formation in the rumen would explain many observations which tend to associate nitrogen metabolism with the disease. The decrease in rumen ammonia induced by the feeding of starch could explain why the incidence of hypomagnesaemic tetany is lower in animals receiving concentrates. The high incidence of hypomagnesaemia in cows on the high-protein, low-starch diets of Ender *et al.* (1949) could possibly be explained on this basis. On the other hand, Line *et al.* (1958) have noted that in studies at Reading the feeding of high-starch concentrates to grazing cows failed to reduce the incidence and severity of hypomagnesaemia, and Meyer & Rustige (1958) were unable to induce more than a slight fall in blood calcium and magnesium by the oral administration of urea even when toxic doses were given. They concluded that high concentrations of ammonia in the rumen are not the cause of hypomagnesaemia in grass tetany.

B. Metabolic Relationships

The question of whether the hypomagnesaemic tetany which occurs in cows during the early post-partum period is nutritional or metabolic or a combination of both remains undetermined. That this condition can be induced by nutritional means appears to be fairly well established.

However, there are a number of well-known occurrences which suggest that the condition could also be of metabolic origin. Certainly some of these occurrences are difficult to explain on a purely nutritional basis. For example, blood magnesium can fall very rapidly within a few days in older animals after a change in the ration (Allcroft, 1954). Blaxter & McGill (1956) argue that this cannot be a nutritional deficiency *per se* since the serum magnesium level returns to normal even when an animal is maintained on the same ration. However, it is conceivable that this merely represents a physiological adaptation in which a temporary imbalance in magnesium metabolism, which has been induced by dietary means, is overcome by homeostatic mechanisms normal to the animal. Also in animals predisposed to hypomagnesaemia, excitation may result in the sudden occurrence of the signs of tetany (Sjollema, 1930). However, this could be the result of inadequate nutrition; in the Maryland studies referred to earlier this phenomenon was observed and the hypomagnesaemic tetany which occurred was almost certainly of dietary origin.

Regardless of whether most cases of hypomagnesaemic tetany which occur in the milking cow are primarily of nutritional or metabolic origin, certain physiological mechanisms can have a profound influence on the development of this disorder. Blaxter (1956) has reported that calves mobilize magnesium from the bone without a concomitant loss of calcium, which he explains on the basis of a simple exchange between the extracellular fluid bathing the bone-crystal snrface and the surface ions of the bone crystal. The bone-crystal surface acts as a reservoir for a rapidly available source of magnesium when the demands for magnesium for various body functions are suddenly increased or the availability from the alimentary tract is decreased. Recrystallization from bone is known to take place but is a relatively slow process. Blaxter & McGill (1956) have made the interesting observation that when the serum calcium concentration of calves is maintained at high levels for long periods, the serum magnesium level decreases regardless of the adequacy of the content of the diet. Thus appreciable decreases in serum magnesium can take place without affecting serum calcium (or phosphorus). Loss of magnesium from the bone does not necessarily affect the bone calcium or phosphorus, but loss in bone calcium or phosphorus does involve a decrease in the magnesium content of the bone. The low serum calcium which is usually evident in hypomagnesaemic tetany may thus be taken as evidence that hypomagnesaemic tetany is not due to a simple magnesium deficiency in the usual sense.

It has been demonstrated by means of radioactive tracers that in adults there is much less bone surface available for exchange reactions than in young animals (Copp *et al.*, 1951; Hansard *et al.*, 1951). Thus, in the adult animal only a small part of the skeleton provides a readily available source of

magnesium in contrast to the young animal. It has been suggested (Blaxter & McGill, 1956) that this is the explanation for the higher incidence of tetany in older animals as well as for the observations that the bones of adult animals with hypomagnesaemic tetany do not exhibit depletion of magnesium (Cunningham, 1936a,b) whereas in magnesium deficiency in young calves there is a large loss of magnesium from the bones (Blaxter & Sharman, 1955). However, as was noted earlier, the Maryland study shows that the magnesium content of the bones of mature beef cows with winter tetany may be quite low.

It would appear that hypomagnesaemic tetany may occur under two different conditions, (1) a dietary deficiency of magnesium possibly complicated by undernutrition and perhaps other factors, and (2) a temporary metabolic disturbance in which the animal is unable to mobilize magnesium from the bones at a sufficient rate to maintain blood serum magnesium. Both types of hypomagnesaemia would be complicated by high milk production which increases greatly the need for magnesium and for energy.

In a sense most cases of hypomagnesaemia could be construed to be of nutritional origin, with their occurrences being dependent in part upon certain physiological aspects inherent in the animal such as age, stage of lactation, and individuality. It is conceivable that the actual onset of tetany in the rapidly occurring type of hypomagnesaemia may be "triggered" by certain rapidly occurring stresses, after a conditioning period of a few days or weeks in which a relative insufficiency of magnesium has developed owing to interferences with magnesium metabolism by dietetic factors such as that of excessive ammonia production as proposed by Head & Rook (1955).

IV. Bovine Ketosis

What is now recognized as ketosis or acetonaemia was described about the middle of the nineteenth century and was referred to as "mania puerperalis." It has also been variously diagnosed as retention of second cleansing, gastrointestinal catarrh, after-calving indigestion, chronic milk fever, and toxaemia of pregnancy. Chloral hydrate was the principal early therapeutic substance used in treatment (see Fleming, 1879; Sampson, 1947; Udall, 1943). The presence of excess ketone bodies in the blood and urine of animals suffering from this disease was first reported by Sjollema & Van der Zande (1923). Five years later, Hupka (1928) made the important observation that this condition was associated with hypoglycaemia and that sugar administration was beneficial. Stinson (1929) reported the successful use of glucose, injected subcutaneously, for this condition which he described as "post-parturient dyspepsia." Most of the signs and conditions associated with ketosis today were described in detail by Sjollema & Van der Zande (1923) in their original article.

A. Incidence of Bovine Ketosis

Ketosis is one of the major metabolic diseases of dairy cattle and has been observed in almost all areas where dairy cattle are found. The signs as described by Sjollema & Van der Zande (1923) leave little doubt that they were dealing with classic ketosis as we now know it. They observed that fat, high-producing cows were the most susceptible. The cows exhibited a rapid loss in body weight, inappetence, dry faeces, decreased milk production and, in many cases, nervous disturbances. It was noted that the liver of a cow that died with ketosis was yellow and enlarged and had undergone fatty degeneration. A marked improvement occurred when cows were turned out to pasture. It was also noted that the condition usually occurred from 7 to 10 days post-partum. Hutyra & Marek (1926) stated that ketosis was most prevalent in cows after the third to sixth parturitions and especially during the winter period when the animals were stabled; they credited Joehnk with the observation that many cows develop ketosis in subsequent lactations after an initial attack. All of the above observations have been confirmed repeatedly (Allcroft, 1947a; Carlstrom *et al.*, 1939; Durrell, 1943; Fincher, 1936; Hayes, 1931; Shaw *et al.*, 1942, 1953). Sjollema (1932) reported that cows that suffer from milk fever are often liable to acetonaemia, both disturbances occurring after the same parturition. This has also been noted by others (Hutyra & Marek, 1926; Shaw, 1956) and undoubtedly has a bearing on the aetiology of bovine ketosis.

Ketosis is usually most prevalent during the winter or stall-feeding period (Boddie, 1935; Duncan *et al.*, 1939; Fincher, 1936; Hutyra & Marek, 1926; Shaw *et al.*, 1942; Sjollema & Van der Zande, 1923), but in certain areas such as on the island of Jersey (Allcroft, 1947a) and in the Los Angeles area in the United States (Shaw *et al.*, 1955c) there is a rather uniform occurrence of ketosis throughout the year; in the latter area the incidence is equally high in animals kept in dry lots and receiving either alfalfa hay or freshly cut alfalfa and in animals on alfalfa pasture. In this study of ninety-seven cases of ketosis, it was observed that there was an incidence of approximately 6·5 % in the cows which had been in the herds one or more years, as contrasted to approximately 14 % in the cows which had been shipped into the area immediately pre-partum from areas 300 to 600 miles distant. This relationship has also been noted in the State of Florida where the importation of dairy cows late in gestation is also a common practice.

Although it has been reported that ketosis may be an inherited characteristic (Harrison, 1951), this does not appear to be an important consideration in most cases (Shaw, 1943; 1956). Among dairy animals it may be concluded that the incidence of ketosis is not influenced greatly by breed (Fincher, 1936; MacKay, 1943). Ketosis also occurs frequently in the heavier milking beef breeds (Kingman *et al.*, 1945).

The economic importance of bovine ketosis is emphasized by the fact that the incidence in individual herds may be 40 % or greater (Harrison, 1951; Shaw, 1943). The incidence in the United States appears to be approximately 5 % of the total dairy cow population (Shaw, 1956). The economic loss is high because of loss of milk, loss of body weight, cost of medication, death due to complications resulting from lowered resistance of the animals, and disposal of animals that have had repeated attacks of the disease (Duncan, *et al.*, 1939; Sampson, 1947; Sjollema & Van der Zande, 1923).

B. Diagnosis

Bovine ketosis is often difficult to diagnose, yet an accurate diagnosis is an extremely important factor in the effective treatment of this condition (Klussendorf, 1952; Shaw *et al.*, 1953). Unfortunately, ketosis is frequently accompanied by various complications such as metritis, retained placenta, nephritis, and other abnormalities. In such cases it is difficult to determine whether the ketosis is primary or secondary, that is, whether the ketosis is the true syndrome of deranged carbohydrate metabolism in which the complication may be either coincidental or a major stress factor, or whether the ketosis may be merely that of fasting ketosis, or ketonaemia and ketonuria due to certain pathological conditions other than the true ketotic syndrome.

It is conceivable that, from time to time, certain abnormalities, such as metritis, may constitute the final stress factor responsible for the development of primary ketosis. On the other hand, many cows with metritis and other infections have poor appetites and exhibit a fasting ketosis, rather than primary ketosis, from time to time, so that it is often difficult to differentiate between primary and secondary ketosis. Thus, a positive urine qualitative test does not necessarily mean that a cow has a primary ketosis. A negative urine qualitative test, however, rules out ketosis.

It becomes essential, therefore, to pay particular attention to the signs, as well as possible complications. A poor appetite, loss in milk production, and either lethargy or high excitability are usually in evidence. These signs may be accompanied by paresis or incoordination. A rapid emaciation will be observed in cows that are not treated promptly or that do not recover quickly after treatment.

A low level of blood glucose and a high level of blood or urine ketone bodies are not sufficient evidence alone for a correct diagnosis of ketosis. It has been pointed out that underfeeding will produce a hypoglycaemia and ketonaemia, but will not produce the signs typical of primary ketosis. In fact, the blood glucose of cows maintained on a low energy intake post-partum was decreased in many cases to values much lower than that usually observed in primary

ketosis (Shaw & Leffel, 1949). In these studies, the blood and urine ketone bodies did not increase to the levels often observed in primary ketosis but were sufficiently high to give a positive urine qualitative test and to make it impossible to differentiate the condition from primary ketosis on the basis of the qualitative test alone. A failure to get some definite response to a glucose injection is good evidence that ketosis is not the primary ailment. A failure to get a good response to an adequate dose of one of the more effective glucocorticoids or ACTH is even better evidence that one is not dealing with primary ketosis. For example, in the Los Angeles area study referred to earlier, of a total of 97 ketotic cows treated with varying dosages of glucocorticoids and ACTH, less than 5 % failed to show a definite response to as little as 0·5 g of cortisol (hydrocortisone).

In screening the effectiveness of various substances for the treatment of ketosis, it is advisable to use only animals which exhibit definite hypoglycaemia and ketonaemia or ketonuria and typical signs of ketosis, uncomplicated by various abnormalities, even though it is recognized that a normal level of blood glucose may exist from time to time and that primary ketosis is frequently associated with various abnormalities. It is important also to recognize that spontaneous recovery does occur without treatment (Shaw, 1946b).

Approximately 30 % of some 300 cases diagnosed as ketosis in the field were observed to be secondary rather than primary (Shaw *et al.*, 1953). This emphasizes the importance of diagnosis in the treatment of ketosis, since treatments generally used for primary ketosis would not be expected to be particularly effective in such cases.

C. Intermediary Metabolism in Normal and Ketotic Animals

1. *Blood and Liver Substances in Normal, Fasted and Ketotic Cows*

All reports without exception are in agreement that bovine ketosis is accompanied by both hypoglycaemia and ketonaemia. The blood glucose and acetone body changes before and during the development of ketosis are shown for two cows in Figs. 1 and 2 (Shaw, 1943). Both cows had developed ketosis of a severe nature for several consecutive years; repeated glucose injections over a period of several weeks were required before recovery was effected. Fig. 1 shows the blood picture of a cow in which there had been a gradual development of ketosis for a period of 9 days before the first signs were observed. Fig. 2 shows, in graphical form, the data for a cow in which the ketotic syndrome developed more rapidly, incoordination occurring on the day of calving associated with a precipitous drop in blood glucose and before the blood acetone bodies were appreciably above normal; inappetence

and lethargy were noted on the 5th day post-partum. Of major importance is the fact that these cows were fed liberally pre-partum and post-partum on a ration of excellent quality alfalfa hay and an equally good quality 16 % protein concentrate, in addition to a complex mineral mixture which was

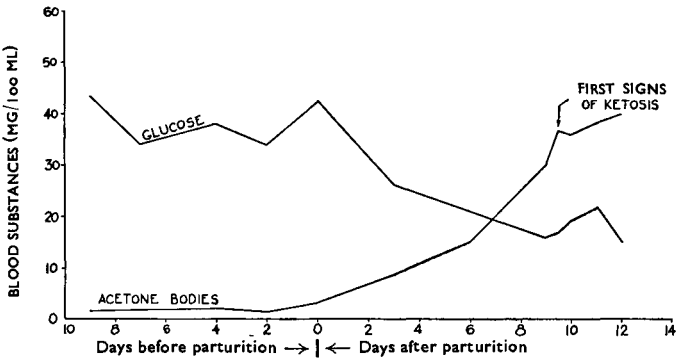

FIG. 1. The Development of Ketosis (Cow 830)

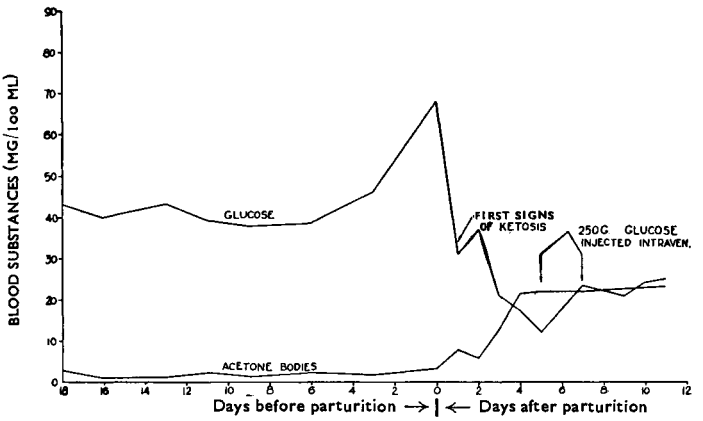

FIG. 2. The Development of Ketosis (Cow 760)

given *ad libitum*. Feed consumption data showed that the calorie intake was adequate up to 9 days post-partum in one cow (Fig. 1) and for 5 days post-partum in the other (Fig. 2); ketosis was not due to fasting.

Hypoglycaemia and ketonaemia can be induced in cows by underfeeding during the early post-partum period (Forbes, 1943; Leffel, 1953; Leffel & Shaw, 1957; Robertson & Thin, 1953; Shaw, 1946c, 1955; Shaw & Leffel, 1949) but the signs of clinical or primary ketosis are not reproduced. In the studies at Maryland (Leffel & Shaw, 1957; Shaw, 1955) cows were fed on

high-, medium- and low-protein rations pre-partum, and half of them received only 35 % of their estimated calorie requirements for 10 days post-partum. A marked hypoglycaemia, moderate ketonaemia and low liver glycogen (lower than in many cases of ketosis (Shaw & Leffel, 1949)) were noted but none of the signs characteristic of primary ketosis were observed. The level of protein before fasting had no significant effect on the fasting blood acetone body levels of these cows in contrast to reports of studies on rats (MacKay *et al.*, 1941; Tidwell & Treadwell, 1946); likewise it did not influence liver glycogen or blood glucose levels.

Numerous studies have demonstrated conclusively that the principal source of ketone bodies in the non-ruminant is fat (Hurtley, 1916), although it is recognized that metabolic pathways exist for the transformation of almost any substance to ketone bodies (Bloch, 1947). The fasting of a normal person for several days results in an increase in blood ketone bodies and the excretion in the urine of as much as 10 g per day. In diabetes, several hundred grams of ketone bodies may be excreted per day. The normal bovine animal, on the other hand, has appreciable amounts of ketone bodies in the blood, usually varying between 2 and 4 mg per 100 ml of blood with a maximum, for cows adequately fed, not greater than 6 to 7 (Boddie, 1935; Knodt *et al.*, 1942; Sampson & Hayden, 1935). Fasting the cow during the early post-partum period may result in increases of the blood ketones to as much as 25 or 30 mg per 100 ml (Forbes, 1943; Shaw, 1946c; Shaw & Leffel, 1949). Rather severe fasting is required in order to increase the blood ketone bodies above these levels. Considerable importance may be attached to the fact that cows with primary ketosis, however, usually exhibit much higher levels of blood ketones than fasted cows, although the degree of hypoglycaemia is often not as marked as that observed under fasting conditions (Shaw & Leffel, 1949). Thin & Robertson (1953) have also noted that the intense degree of ketonaemia in ketotic cows is often out of proportion to the reduction in food consumption. Since ketosis is observed, from time to time, in cows which have a normal level of blood glucose, the question may be raised as to whether bovine ketosis is due to a lack of carbohydrate or to an impairment in the utilization of carbohydrate. The fact that glucose-tolerance curves of ketotic cows are normal suggests that there is no impairment of the utilization of carbohydrate.

Wick & Drury (1941) and others have demonstrated that the utilization of ketones is not impaired in conditions which are associated with ketosis. This appears to be true, also, in bovine ketosis. For example, it was shown that the lactating mammary gland of the normal cow utilizes approximately 2 mg of β-hydroxybutyric acid from each 100 ml of blood traversing the gland, whereas the active mammary gland of the ketotic cow utilizes approximately twice this amount, presumably because of the relatively high concentration in the blood (Shaw, 1942; Shaw & Knodt, 1941). It has been shown by the use

of radioactive β-hydroxybutyrate that this substance is one of the principal precursors of the short-chain fatty acids of milk fat (Shaw *et al.*, 1955c), and a competition has been shown to exist between acetate and β-hydroxybutyrate for this, and perhaps for other purposes, with the mammary gland showing a preference for acetate. It is apparent, therefore, that β-hydroxybutyric acid is an important metabolite in both normal and ketotic cows (see also Folley & McNaught, Chapter 12).

Glucose-tolerance tests (Shaw, 1943) were made on the two ketotic cows referred to in Figs. 1 and 2. The tolerance curve did not differ appreciably from normal, indicating that the cause of bovine ketosis is not an inability of the animal to utilize glucose. After the intravenous injection of glucose into the ketotic cows the blood glucose decreased within 2 hours to a level somewhat above the previous low level, but considerably below the normal level. These results were confirmed by Holmes (1951). In the above-mentioned study, Shaw (1943) also obtained evidence of low levels of liver glycogen and noted normal uptake of glucose by the udders of ketotic cows.

Very little β-hydroxybutyrate is present in the blood of the calf during the first 2 months of life (Knodt *et al.*, 1942). After that there is an increase in both the blood and urine acetone bodies, due primarily to an increase in β-hydroxybutyric acid. Since the gradual increase in the acetone bodies in the calf parallels the development of the rumen, it was concluded that the rumen is responsible for the relatively large concentration of acetone bodies in the blood and urine of the bovine animal. Brouwer & Dykstra (1938) had demonstrated acetonaemia after the feeding of grass silage high in butyric acid. It appeared, therefore, that the blood β-hydroxybutyrate was being derived from rumen butyric acid. However, as will be indicated later, some modification of our concepts of the origin of the blood acetone bodies normally found in the blood of ruminants may be necessary.

Thin & Robertson (1953) reported that the blood ketone bodies of cows with ketosis consisted primarily of acetone and β-hydroxybutyric acid, some acetoacetic acid, and an appreciable amount of isopropanol. It was suggested that acetone and β-hydroxybutric acid are formed from acetoacetic acid. Since the isopropanol concentration was greater in the rumen than in the blood, they suggested that it was produced in the rumen from acetone, which diffuses in from the blood stream. Observing that the isopropanol concentration in the blood and rumen of cows with primary ketosis was greater than that of cows with starvation ketosis, they suggested that the formation of isopropanol may play an important part in the development of ketosis. The possible significance of isopropanol is not apparent at the present time.

Saarinen & Shaw (1950a,b,c) observed that the blood and liver lipids of ketotic cows differed from those of normal cows. Changes of a similar nature

were observed in cows on a low calorie intake post-partum, indicating that the differences were due to an insufficient calorie intake. The increased fat content of the adrenals of ketotic cows was reproduced by maintaining normal cows on a low calorie intake immediately post-partum. The cholesterol content of the adrenals of ketotic cows was about the same as that of normal cows. The ascorbic acid content of the adrenals of ketotic cows was somewhat low, but a similar lowering was obtained by withholding feed from normal cows. The flabbiness and hypertrophy observed in adrenals of ketotic cows was not reproduced by withholding feed from normal cows in the early post-partum period. The enlargement of the adrenals of ketotic cows could not be explained on the basis of the water or fat content of these glands. It was found that the fat content of the liver often approached normal post-partum values in the early stages of ketosis. The extremely fatty liver was observed in the later stages of ketosis. The effect was reproduced by fasting post-partum; it was concluded that the fatty liver associated with primary ketosis is due to inanition. The total cholesterol and especially the ester cholesterol fraction followed the same pattern. It was also concluded that a fatty liver is not a predisposing factor in the development of most cases of spontaneous or primary ketosis. Cows that received a low-protein ration, both before and after parturition, exhibited livers with a higher fat content than cows receiving a high-protein ration. As noted earlier, however, (Leffel & Shaw, 1957; Shaw & Leffel, 1949) the protein level did not exert any significant effect upon the degree of fasting ketonaemia.

2. *Rumen Metabolism*

It is the rumen which renders the cow, goat, sheep and other ruminants so different, nutritionally and physiologically, from monogastric animals. This difference together with the relatively enormous net synthesis of organic substances by the udder makes it necessary to proceed with great caution when attempting to explain occurrences in the lactating cow on the basis of biological phenomena observed in small laboratory animals.

It is generally recognized that the organic acids produced in the rumen by microbial action are of major importance as sources of energy and tissue syntheses within the animal body. It has been demonstrated both *in vivo* (Balch *et al.*, 1955; Phillipson, 1942; Phillipson & McAnally, 1942; Shaw *et al.*, 1957) and *in vitro* (Leffel *et al.*, 1956) that the ruminal production of the volatile fatty acids (VFA) and lactic acid may be varied within wide limits on different kinds of substrates (or rations). Cellulose, starch, sugars and even some of the amino acids (Doetsch *et al.*, 1953; Lewis, 1955; Sirotnak *et al.*, 1953) are precursors of rumen VFA.

Although a number of workers have attempted to ascertain the rates of absorption of the VFA from the rumen there has been no general agreement

in the results obtained (see Annison *et al.*, 1957). The methods used have ranged from the measurement of the disappearance of VFA added to the normal rumen as well as the washed-out rumen, the examination of the blood draining the rumen or other individual organs and the analysis of portal blood. Although each of the studies has served to emphasize the importance of absorption from the rumen, none has been sufficiently precise to give the desired information on either the rate of absorption or the possible factors which may control absorption. The recycling of some of the acids when added to the rumen contents may result in a considerable error in calculations of the rate of absorption based on the disappearance of the added acids. For example, when [1-^{14}C]butyrate was added to the rumen contents during a rumen perfusion, approximately 25 % of the label was found in the acetate in the rumen (McCarthy *et al.*, 1959b; Shaw *et al.*, 1958). The addition of acids to the washed-out rumen is non-physiological since no appreciable amount of recycling would be expected to take place and, in addition, other influences which the rumen contents may exert on absorptive mechanisms are removed. Analysis of blood draining the rumen is of considerable value in showing that certain substances are absorbed from the rumen, but the values fluctuate widely (Annison *et al.*, 1957); this would be expected in view of the relatively rapid rate of blood flow. In addition, lack of information on quantitative aspects of absorption due to lack of information on blood-flow rate renders any precise quantitative measurement impossible.

Recognizing these difficulties, Pennington (1956) and Pennington & Sutherland (1956a,b) have studied the metabolism of sections of sheep-rumen ephithelial tissue. Pyruvate was metabolized with the production of lactate and acetone bodies; lactate and butyric acid were also metabolized with the formation of ketone bodies, the latter being converted primarily into acetoacetic acid. Propionic acid was metabolized by the ephithelial tissue of the rumen and it was concluded that a substantial part of this acid produced in the rumen is metabolized by this tissue. Propionate was observed to supress ketone body formation from pyruvate. Studies on the metabolism of carboxyl-labeled propionate indicated the formation of succinate from propionate by a direct path involving carbon dioxide fixation. Though of obvious importance to our understanding of some of the metabolic reactions that may take place within the rumen ephithelial tissue, these studies do not provide information on the net absorption of the VFA from the rumen. Annison *et al.* (1957) have measured the ketone bodies in the portal and carotid blood during the addition of successive doses of VFA to the sheep rumen. Although they concluded that considerable quantities of butyrate were metabolized by rumen ephithelium with the production of ketone bodies, the data presented on portal and carotid blood show one comparison with no difference in concentration, one in which the concentration was higher in the carotid blood,

and two in which the concentration was higher in the portal blood. Since the differences were small it is difficult to interpret their possible physiological significance.

A new approach to the problem of measuring ruminal production and absorption has been the use of a perfused rumen preparation (McCarthy, 1958; McCarthy *et al.*, 1957, 1958, 1959b; Shaw *et al.*, 1958). In one-hour perfusions of goat rumens in which the rumen content and the blood perfusate were analysed at the beginning and end of the perfusion, not only was a large production of rumen VFA observed, but their rates of absorption were found to be rather directly related to their production within the rumen. Carboxyl-labelled propionate was recycled only slightly within the rumen; 97 % of the label in the blood was in the propionate showing that it is absorbed directly as such. The addition of carboxyl-labelled butyrate to the rumen contents demonstrated a conversion of about 26 % of the butyrate to acetate and 11 % to propionate. Of greatest importance was the fact that of the label that appeared in the blood, 72 % was in the butyrate and only traces were observed in the blood acetone bodies. In four perfusions of goat rumens there was an increase in acetone bodies in the blood perfusate in two and no increase in the other two. Since butyrate appears to be absorbed as such, the sources of these acetone bodies are somewhat obscure. Both formate and glucose were used by the perfused rumen. That absorption of VFA from the perfused rumen reflects their production within the rumen has been confirmed by Davis *et al.* (1958). This is a technique that offers a real possibility for elucidating the mechanisms controlling the production and absorption of VFA and other organic metabolites by the rumen.

A slightly lower proportion of propionic acid was observed in the rumen fluid of ketotic cows than in normal cows by Schultz (1954) who proposed that this difference indicated a possible aetiological relationship of ketosis. Hoflund & Hedstrom (1948) and Johnson (1951b) have also suggested that disturbances in the normal rumen fermentation were aetiological factors in ketosis. Brown & Shaw (1957) also observed somewhat lower proportions of propionate but were able to reproduce this finding by fasting the animals; normal levels and proportions of rumen VFA were found in cows with chronic ketosis but with normal appetites. Thus, although apparent digestive disturbances and abnormal faeces have been observed in ketotic cows, there is no evidence that such abnormalities represent primary aetiological factors.

3. *Liver Metabolism*

Alterations of a pathological nature have been observed in the livers of cows with ketosis (McIntosh, 1944; Seekles, 1950; Shaw *et al.*, 1948, 1950); and in the lipid and glycogen content as was noted earlier (Leffel & Shaw,

1957; Saarinen & Shaw, 1950c). Additional evidence of liver damage in cows with ketosis, based on studies with the bromsulphalein fractional clearance method, was obtained by Robertson *et al.* (1957b) who concluded that liver function in ketotic cows is depressed. This did not appear to be due to fasting (Mixner *et al.*, 1957).

As was noted earlier, the bulk of the VFA produced by the rumen is absorbed as such and reaches the liver directly via the portal system. The liver is then responsible for the alterations both in the proportions and the amounts of these substrates available for the rest of the body. It may be assumed that the ruminant liver is very similar in its behaviour to the liver of the monogastric animal. The major metabolic dissimilarity between these animals must then arise from the differences in the substrates available to the liver through the portal blood (glucose for monogastric animals and VFA for ruminants) and in enzymic and endocrine adaptation to these differences. In studies by McCarthy (1958), and McCarthy *et al.* (1959b), designed to ascertain the nature of the metabolites made available to the various body tissues of the ruminant, goat livers were perfused with blood containing added carboxyl-labelled organic acids. Unlabelled acetate, propionate and butyrate were added to the blood in the proportions in which they were absorbed from the perfused rumen. The label from formate, propionate and butyrate recovered in liver glycogen and blood lactic acid accounted for 64, 74 and 81 % respectively of the total ^{14}C recovered. Little of the ^{14}C from acetate was recovered in liver or blood carbohydrate, the acetate passing through the liver and being made available to the various body tissues as such. The blood acetone bodies were not labelled. In the perfusion with n-[1-^{14}C]butyrate there was no labelling in the blood acetone bodies although an increase in total blood acetone bodies of 10 mg/100 ml occurred in this perfusion. It appears that the acetone bodies were derived from the lipids of the liver since the latter were only slightly labelled. It was suggested that butyrate is not ketogenic in the ruminant unless the normal levels of butyrate reaching the portal system are exceeded, as may occur temporarily after feeding or when butyrate is administered *per se*. There was a high incorporation of label in the nucleotide fraction containing coenzyme A, the percentage incorporation from formate, propionate, acetate and butyrate being 15, 10, 6 and 4 % respectively. It is apparent, therefore, that propionate and butyrate are metabolized by the body tissues of the ruminant primarily as carbohydrate, whereas acetate is metabolized as such. Some fruitful approaches to an elucidation of the possible metabolic mechanisms involved in bovine ketosis would be turnover studies on acetate and glucose as well as investigation of possible alterations in the conversion of butyrate, propionate and formate into carbohydrate. Since Kronfield (1956) observed an increase in the blood glucose of fasted but not of fed sheep after the administration of butyrate, it

is possible that the nutritional status of the animal may exert an influence on relative glucogenicity or ketogenicity of butyrate.

Pennington (1956), working with liver slices and ^{14}C-labelled substrates, observed that the oxidation of acetate and pyruvate but not butyrate was decreased markedly by the addition of propionate. The conversion of acetate into acetone bodies was also lowered by the addition of propionate. In liver-slice studies, Pritchard & Tove (1958) also noted that propionate reduced the incorporation of ^{14}C from carboxyl-labelled acetate into carbon dioxide and, in addition, they noted a reduction in the incorporation into lipids and protein. Acetate, glucose or butyrate increased the incorporation of ^{14}C from carboxyl-labelled propionate into carbon dioxide, glucose, succinate, malate, and fumarate, whereas citrate caused no differences. Hueter (1958) and Chung *et al.* (1959) studied the oxidative metabolic pattern of normal and ketotic cow liver slices with 1-^{14}C-labelled C_1 through C_5 aliphatic acids, using a chromatographic–autoradiographic technique. As the severity of the ketotic syndrome increased, a corresponding increase in the utilization of propionate and a decrease in utilization of acetate and butyrate occurred. Since the major oxidative pathway of acetate and, at least in part, of butyrate is via acetyl CoA entering the Krebs cycle, it appears that some mechanism associated with this pathway is altered during ketosis. Since propionate appears to enter the Krebs cycle primarily as succinate, the above-mentioned alterations should not affect its utilization; therefore, in the ketotic cow, it would be logical to expect a decreased utilization of acetate and butyrate followed by an increased utilization of propionate. This may explain the effectiveness of propionate in the treatment of bovine ketosis (Schultz, 1952). In addition, the studies by Chung *et al.* demonstrated a marked decrease in the oxidation and in the incorporation of formate carbon into glucose by the liver slices of cows with ketosis. It is believed that the altered formate metabolism must play an important part in any metabolic explanation of bovine ketosis. Also, with liver slices of ketotic cows there was a marked increase in incorporation of formate, acetate, and butyrate into aspartate and an increased incorporation of the label from butyrate into glutamate was noted. This increased production of aspartate and glutamate may be related to faulty gluconeogenesis in ketotic cow liver slices.

Peeters *et al.* (1958) observed that the sulphonamide-acetylating capacity of liver slices from ketotic cows was 15 to 20 % lower than of those from normal cows. This disturbance was reported to be improved by means of glucose and made completely normal by glucose plus insulin. They suggested that in the liver of ketonaemic cows the citric acid cycle is reduced to a low value resulting in a decreased acetate-activating mechanism.

Sauer *et al.* (1958) incubated various substrates with liver homogenates prepared from ketotic cow livers and noted that the oxygen uptake was

greatly decreased. Bach & Hibbitt (1959) observed increased levels of pyruvate and α-oxoglutarate in the blood of cows with ketosis and suggested that bovine ketosis is associated with a deficiency of coenzyme A. Bouckaert *et al.* (1958) reported that injected acetate disappeared more slowly from the blood of normal than from that of ketotic cows and that the injection of glucose increased its rate of removal. This is of considerable interest with reference to the observation of Shaw (1959) of a level of 60 mg acetic acid per 100 ml blood in a cow with refractory ketosis. More recently McCarthy & Shaw (1960) have noted that the perfused liver of ketotic cows utilized much less acetate and butyrate than normal, produced much less formate, exhibited no conversion of labelled butyrate to acetone bodies (similarly to the normal liver) and, in surprising contrast to the perfused liver of the normal ruminant, produced little or no acetone bodies. It was suggested that the metabolism of the extrahepatic tissues could provide the answer.

It is evident from the above that certain biochemical processes of the liver of the ketotic cow, including the citric acid cycle, are altered and reduced. A continuation of these researches appears to offer the greatest potential for the further unravelling of the many puzzling aspects of this syndrome. For example, it will be interesting to ascertain whether the pituitary-adrenocortical involvement (histopathological changes, remarkable therapeutic effect of glucocorticoids, etc.) are primary or secondary.

4. *Lactation*

The liver perfusion studies help to explain a number of phenomena which have been observed in the lactating cow. For example, Kleiber *et al.* (1954) found that after the intravenous injection of labelled butyrate into lactating cows there was less labelling in milk fat than in casein or lactose. Shaw and collaborators (see Shaw & Lakshmanan, 1957) on the other hand, found that the perfusion of a lactating udder with blood containing labelled β-hydroxybutyrate resulted in a high level of incorporation into fat and casein but almost none into lactose. This has appeared puzzling because of the general belief that butyrate is metabolized to an appreciable extent via β-hydroxybutyrate in the cow. The liver perfusion studies, however, explain the above results in terms of the conversion of butyrate into carbohydrate rather than into β-hydroxybutyrate. The utilization of formate and propionate (Kleiber *et al.*, 1953, 1954) for lactose synthesis can also be explained on the basis of the liver perfusion work showing the conversion of these materials into liver glycogen. Similarly the insignificant incorporation of acetate into milk lactose can now be readily understood, since it is not converted into carbohydrate by the liver to any appreciable extent.

The earlier arterio-venous studies on the udder (Shaw *et al.*, 1938), the perfusion of the lactating udder with labelled glucose (Dimant *et al.*, 1953) and

the demonstration with the lactating cow by means of intravenous injections of labelled glucose that 85 % of milk lactose may originate from the plasma glucose (Baxter *et al.*, 1956), all point to the fact that milk lactose is produced primarily from blood glucose and that this constitutes the major direct use of glucose by the lactating dairy cow.

Granting that most of the milk lactose is derived from blood glucose, it can be calculated that a cow producing 25 kg of milk per day will require somewhat more than a kilogram of glucose for this purpose. Thus an appreciable increase in gluconeogenesis is imposed upon the animal with the initiation of lactation. An obvious approach to further studies of the aetiological factors in bovine ketosis is that of the metabolism by the liver of the carbohydrate precursors, propionate, butyrate, formate, and other volatile acids, as well as the possible alterations in gluconeogenesis from amino acids. The numerous alterations observed by Chung *et al.* (1959) in the metabolism of the various volatile fatty acids by the ketonaemic liver provide substantial evidence of alterations in various metabolic pathways within the liver of the ketotic cow. Cornelius *et al.* (1957) have demonstrated, by the intravenous injection of labelled butyrate into lactating dairy cows, that butyrate may have a high ketogenicity. However, in view of the high incorporation of injected labelled butyrate into lactose (Kleiber *et al.*, 1954), the high incorporation of the label from butyrate into carbohydrate by the perfused liver (Shaw *et al.*, 1958; McCarthy *et al.*, 1959b), and the failure of the perfused udder to incorporate β-hydroxybutyrate into lactose, it appears valid to conclude that butyrate is primarily a glycogen former under normal conditions. The lack of conversion of labelled butyrate to acetone bodies by the perfused ruminant liver suggests that extrahepatic tissues may be responsible for the ketogenicity of injected butyrate. An alterative is that the liver converts butyrate to acetone bodies only when there is an excess of the former. However, no such conversion was noted when the liver of a ketotic cow was perfused with blood containing a high concentration of added butyrate containing labelled butyrate (McCarthy & Shaw, 1960).

5. *Therapeutic Efficacy of Various Glucogenic Metabolites*

Hypoglycaemia was demonstrated by Hupka (1928) to be associated with ketosis and since that time carbohydrate therapy in bovine ketosis has received wide acceptance (Sampson & Boley, 1941; Shaw *et al.*, 1942; Stinson, 1929). Glucose, to be effective, must be injected intravenously. However, the percentage of "glucose resistant" cases is relatively large (Gingras, 1947; Harrison, 1951; MacKay, 1943; Shaw, 1956). The feeding of soluble carbohydrate has not been very helpful for either the treatment or the prevention of bovine ketosis (Fincher, 1950; Shaw, 1943), undoubtedly because it is metabolized rapidly by microbial action in the rumen and thus little, if any,

soluble carbohydrate is absorbed from the alimentary tract (Schambye & Phillipson, 1949). Indeed the rumen has been shown to utilize carbohydrate in appreciable amounts (McCarthy *et al.*, 1957, 1959a).

In view of the fact that the intravenous injection of glucose into ketotic cows maintains the blood glucose at the normal level or above for only 80 to 100 min (Shaw, 1943), it is not surprising that many cases are not improved by a single injection of glucose. It appears quite likely that glucose acts by promoting a homeostasis in which the organism recovers its ability to maintain normal body metabolism. Peeters *et al.* (1958) have suggested that the efficacy of carbohydrate administration is due to its acting as a sparking phenomenon since they believe that, in the ketotic cow, the citric acid cycle is reduced to a very low value. Whatever the mechanism may be, it is evident that a homeostasis is achieved in the majority of cases after the intravenous injection of glucose.

An obvious approach to oral therapy becomes that of the administration of glucogenic substances which are either absorbed without being acted upon appreciably by the rumen micro-organisms or are dissimilated within the rumen to end-products which are glucogenic.

Several substances, which may be considered as glucose precursors (or glucose-sparing substances) have been used with varying degrees of success. The first report of the use of a glucose precursor, other than various forms of sugar, was that of Seekles (1951), who used ammonium lactate. Approximately 4 oz was administered per os twice a day for 5 or more days.

Propionate was shown to have a hyperglycaemic effect in sheep and goats (Ray, 1942; Schultz & Smith, 1951) and Schultz (1952) showed that it was effective for the treatment of bovine ketosis. He also demonstrated some advantage from the feeding of propionate to cows early post-partum, in terms of lower blood ketones, higher blood sugar, and higher milk production in herds in which the incidence of ketosis was high (Schultz, 1958). Shaw *et al.* (1953) also obtained favourable results with the use of propionate for the treatment of bovine ketosis. Some limitations of propionate are its frequent failure to produce a prompt recovery and the necessity of daily drenching for either prevention or treatment, since propionate is relatively unpalatable. It was reported that the feeding of propionate to normal cows did not increase milk production (Schmidt & Schultz, 1958). In studies at Maryland it was found that sodium lactate was absorbed much more rapidly when given per os to cows than calcium lactate (Hueter *et al.*, 1956); some was converted to propionate in the rumen. Calcium lactate was found to be effective for the treatment of ketosis and somewhat more palatable than propionate (Shaw *et al.*, 1955b); highly soluble lactates are more effective. Other glucogenic substances which have been used with some success have been tripropionin, glycerol and propylene glycol (Johnson, 1951a, 1954). None of them, however,

are palatable. In recent studies by Shaw *et al.* (unpublished) combinations of sodium lactate, aluminium lactate and calcium lactate were found to be useful and to be more palatable than propionate or calcium lactate. Lactamide was found to be ineffective and lactide, though glucogenic and palatable, was observed to be too toxic. A commercial wood by-product ("Marathon") was observed to be glucogenic for ketotic cows when administered in large amounts. Though of some effectiveness and fairly palatable, it produced diarrhoea when fed at high levels. It was found to contain approximately 9 % propionic acid, which may account for its glucogenicity.

Acetate, which has been shown to have a glucose-sparing effect on extra-hepatic tissues (Drury & Wick, 1953), was used by Miller & Allen (1952) for the treatment of bovine ketosis with some degree of success. Shaw *et al.* (1953) observed a definite increase in blood glucose and an initial decrease in blood acetone bodies in most cases after the use of acetate but the response was so slow that other treatments were needed. In view of the work of Chung *et al.* (1959) showing an increase in incorporation of acetate into glucose by liver tissue of the ketotic cow, the moderate hyperglycaemic effect of acetate in ketotic cows but not in normal ruminants (Schultz & Smith, 1951) appears to have a valid explanation. More recently, however, McCarthy & Shaw (1960) failed to observe any appreciable incorporation of [1-^{14}C]acetate into glucose by the perfused liver of a ketotic cow.

D. Concept of Ketosis as a Pituitary-adrenocortical Syndrome

1. *Histopathology and Certain Physiological Aspects*

It was reported by Shaw (1947) that an extract of the adrenal cortex was effective in the treatment of four cases of bovine ketosis. Since these extracts were known to have a hyperglycaemic effect on normal animals it was concluded that the evidence was too meagre to postulate an adrenal insufficiency. This initial study was prompted by observations of enlarged and fatty adrenals in two cows with primary ketosis and, of course, hypoglycaemia. The report of Greenewald *et al.* (1941) that severe fasting induced fatty degeneration of the adrenal cortex of ewes also suggested that an investigation of the possible role of the adrenals might prove fruitful. A histopathological study was initiated in 1946 (Hatziolos & Shaw, 1950; Shaw *et al.*, 1948, 1949, 1950) which was directed at the adrenal and pituitary glands but included various other tissues of normal and ketotic cows. Recognizing that various types of stress tend to produce cellular changes and that the ketotic cow is usually on a low calorie intake, studies were also conducted on lactating cows on a low level of energy intake post-partum and on cows with other abnormalities, but which were diagnosed erroneously as ketosis. The histopathological study of the

pituitaries and adrenals of twenty-four cows has now been published in detail (Hatziolos & Shaw, 1958). All of the animals that were killed for this study were in the early post-partum period and were mature cows. Six normal cows were studied, three of which were subjected to varying degrees of inanition; six were cows in which ketosis had been diagnosed but actually represented pathological abnormalities other than ketosis; six cows were in the early stages of primary ketosis and six were classed as chronic ketosis; the last were animals that had responded and relapsed repeatedly after treatment. Of the six cows classed as having primary ketosis, two represented the usual form of ketosis, two were of the nervous form and two were cows that had exhibited ketosis for several consecutive years (recurrent form). There were marked and consistent differences in the histopathology of the pituitaries and adrenals of the ketotic cows as compared to those of normal cows, normal fasted cows, and cows with abnormalities other than ketosis.

It was found that primary ketosis is associated with characteristic changes in the pituitary and adrenal glands, which are believed to interfere with the normal functioning of these glands and to results in hormonal disturbances. These lesions of the pituitary were located in the anterior lobe and were extensive and often reversible. Usually the β cells, allegedly the producers of adrenocorticotrophin (ACTH), were involved in the degenerative process. In the early stages of ketosis the β cells, although increased in number, showed marked vacuolation due to fatty degeneration. Cyclical changes such as degeneration, vesiculation and hyalinization often occurred and appeared to account for the shrinkage and pyknosis of the cells that were observed. Tiny haemorrhages and emboli in the blood vessels and increased stroma and reticulization of the β cells also occurred.

The zona fasciculata of the adrenals showed lipid depletion, extensive degeneration of the cortical cells, reticulization (fibrinoid degeneration), extensive polymorphonuclear infiltration and cytolysis or disintegration of the cords, resulting in lumen formation.

The nervous form of ketosis was accompanied by extensive haemorrhages of the anterior-pituitary lobe. Whether these lesions are associated with others located in some part of the brain is a question that needs further clarification.

In recurrent ketosis, the marked degenerative changes noted in the early stages of ketosis were evident but were associated with permanent lesions such as cystic degeneration and folliculization of the anterior lobe of the pituitary. Likewise, the adrenals showed extensive degeneration of the inner cortical zones, lipid depletion in patches, and polymorphonuclear cell infiltration in areas of ischaemic necrosis; this was due to injuries to the walls of the blood vessels.

In ketosis of a tenacious form (chronic), degenerative changes similar to those described in the other forms were noted in the β cells as well as in the α

cells in association with an increase of colloid accumulation or folliculization of the cords in the basiphil area. There were many residual or permanent lesions such as the development of cysts, increased stroma, and an unusually enlarged cleft causing pressure atrophy of the anterior lobe.

The circulatory disturbances noted in the pituitaries and adrenals of cows with ketosis are similar to those usually occurring during the stages of alarm reaction and exhaustion of the general adaptation syndrome, which actually represent a relative adrenal insufficiency.

Unlike the above-noted changes, fasting, infections, and other conditions simulating ketosis caused an increase in both the number and the size of the β cells, the majority of which became sparsely granulated. In these cows there was proliferation of the chromophobes and early differentiation to chromophils. The β cells often exhibited cyclical changes but seldom exhibited degeneration. These changes were interpreted as signs of increased pituitary activity related to ACTH discharge.

Since the histopathological findings on the cows killed at various stages of ketosis were similar to the tissue changes which Selye (1946, 1950) described as being characteristic of the alarm reaction phase of the adaptation syndrome, and because of the early success in the treatment of ketotic cows with extracts of the adrenal cortex, cortisone acetate was used in the early part of 1950 for the treatment of ketosis with remarkable success. As a result, it was proposed by Hatziolos & Shaw (1950) and Shaw *et al.* (1950) that bovine ketosis is due to an adrenal insufficiency, possibly associated with the pituitary-adrenocortical system. Later, more extensive studies were reported on the effective treatment of bovine ketosis with cortisone acetate, and an initial report was made on the effectiveness of ACTH in the treatment of bovine ketosis (Shaw *et al.*, 1951).

Most cows are able to adapt themselves to the normal stresses of parturition and early lactation. On the other hand, cows that develop ketosis year after year regardless of environment may do so because they are unable to adapt themselves to these normal physiological stresses. If it is true that primary ketosis is due to a temporary adrenal insufficiency, absolute or relative, caused by a temporary exhaustion of the pituitary as the result of stress, it would appear that most ketotic cases are caused by unknown stresses superimposed upon the normal stresses incident to parturition and early lactation.

Carlstrom (1950) confirmed the early report of Shaw (1947) that adrenal-cortical extracts were beneficial in the treatment of bovine ketosis. He further stated that adrenal-cortical extracts and deoxycorticosterone corrected the hypokalaemia, which he reported to exist in cows with ketosis, and as a result proposed that bovine ketosis is due to a reduced production of ACTH as the result of a high production of prolactin. It has since been shown

that these substances produce a hypokalaemia in the cow (Chung & Shaw, 1957) in conformity with occurrences in other animals.

It is interesting that Hutyra & Marek (1926) speculated on the possibility of adrenal exhaustion being involved, based on the reports of the presence of acetone compounds in the body; hypoglycaemia was not known to exist at that time.

If a temporary depression of the secretion of ACTH exists in cows with ketosis, such animals should not exhibit a decrease in blood eosinophils after the injection of adrenaline. It was shown to be so by Shaw (1952) and Shaw *et al.* (1952). Thirteen normal cows exhibited marked eosinophil decreases of 21 to 46 % 4 hours after the injection of 5 ml of adrenaline (1 : 1000), whereas of seven cows with ketosis, six exhibited no significant decrease in the eosinophil count and in one the count decreased by 9 %. Later studies on six normal cows maintained on a low energy intake demonstrated that the normal eosinophil response was diminished. Thus, it is apparent that the pituitary of the ketotic cow does not function normally, but it is not certain to what extent this may be due to lowered food intake. It should also be recognized that the adrenaline test has not proven to be too dependable in screening adrenal-cortical insufficiency.

Puntriano (1952) presented some interesting evidence in support of the concept that bovine ketosis is a pituitary-adrenocortical disturbance. Urine glucocorticoid assays showed a decrease in adrenocortical activity in two ketotic cows as contrasted with two normal cows. However, no work was done on fasted cows. Since cows with ketosis are usually in a fasting state, the use of fasted cows as controls would appear to be essential.

Vigue (1955) reported a failure of cows to develop ketosis after administration of 2 g of cortisone or 600 Avu (Armour veterinary units) of ACTH for as long as 2 weeks. In the Maryland laboratories, A. C. Chung has injected larger doses of both glucocorticoids and ACTH over a longer period of time without observing a hypoglycaemia or ketonaemia after cessation of treatment. These injections were made immediately pre-partum and discontinued at parturition since cows are most susceptible to ketosis in the early post-partum period. It is apparent that it will be necessary to resort to more drastic measures to establish the role of the adrenals in the bovine animal. Shaw & Lehman (unpublished) adrenalectomized a non-lactating cow. The animal exhibited marked hyperglycaemia after injections of 0·5 g cortisone for the first several weeks after operation. Both deoxycortisterone and cortisone were administered daily for several weeks, after which the animal adjusted to the adrenalectomy either owing to regeneration of adrenal tissue or to other organs assuming adrenal-like functions.

In unpublished work by the author, ketotic cows were observed to be extremely hypersensitive to insulin, two cows with primary ketosis exhibiting

severe tremors in contrast to normal cows which exhibited no effects after similar dosage.

A somewhat puzzling observation by Shaw *et al.* (1951, 1953) was the gradual decrease of blood eosinophils to very low levels during the treatment of several cases of ketosis with propionate, followed by a rapid increase to normal levels as recovery took place. It appeared that propionate may have been acting by permitting or stimulating certain enzymic reactions temporarily inhibited in ketosis, or that it acts as a stimulant to glucocorticoid secretion. This may explain, in part, why relatively small amounts of propionate are beneficial in the treatment of bovine ketosis, since marked reductions in blood eosinophils were usually observed within 12 to 24 h after the treatment of ketotic cows with cortisone or ACTH.

Robertson *et al.* (1957a) reported that ketotic cows have elevated levels of plasma-free 17-hydroxycorticosteroids and low levels of plasma protein-bound iodine, as compared to control cows, and suggested that bovine ketosis is due to a relative adrenal-cortical insufficiency, as a result of hypothyroidism. However, because the metabolism and conjugation of corticoids have been shown to be defective in patients suffering from liver diseases (Brown *et al.*, 1953; Klein *et al.*, 1955) it was considered that liver disorders could be the cause of the elevated corticoids in ketotic cows. As was mentioned earlier in this review, they did indeed observe evidence of a decreased liver function (Robertson *et al.*, 1957b). It appears quite likely, therefore, that the high plasma levels of corticoids observed in ketotic cows are due to the marked decrease in liver function which has been observed by several workers.

In view of the relatively severe degenerative changes noted by Hatziolos & Shaw (1959) in the pituitaries and adrenals of cows that had exhibited ketosis for some period of time (chronic), the biochemical changes indicated by the work of Chung *et al.* (1959) which were especially marked in persistent ketosis, the indications of malfunctioning of the liver obtained by indirect means by Peeters *et al.* (1958) and the evidence of Robertson *et al.* (1957b) suggesting poor utilization of corticoids, it is not surprising that ketotic cows frequently do not recover rapidly after treatment.

2. *Relationship of Pituitary and Adrenals to Ketosis*

It is now quite generally recognized that adrenalectomy is followed by a decrease of the concentration of carbohydrate in the blood, liver and muscle and that hypoglycaemia during fasting is a common manifestation of adrenal-cortical insufficiency in some species, including man. Also, in the adrenalectomized animal, the conversion of protein into carbohydrate is impaired, the rate of oxidation of administered glucose may be accelerated and sensitivity to insulin may be strikingly increased. In the diabetic animal, adrenalectomy effects a marked amelioration of the diabetic state.

At the time of the initial proposals of Hatziolos & Shaw (1950) and Shaw *et al.* (1950) that bovine ketosis represents a relative adrenal insufficiency associated with the pituitary-adrenocortical system, considerable confusion existed regarding the relationship of the pituitary and the adrenals to ketosis. Researches since that time, however, have clarified the picture greatly and emphasize the extremely important role of the pituitary and adrenals in ketosis. The early report of MacKay & Barnes (1937) that adrenalectomy reduces the ketonuria induced by ketogenic pituitary extracts was explained by Mirsky (1938) who demonstrated an increase in renal threshold for ketone bodies in adrenalectomized animals treated with extracts of ox anterior-pituitary glands. Welt & Wilhelmi (1950) concluded that adrenalectomy is followed by an increased rate of liponeogenesis from carbohydrates and that the adrenalectomized animal tends to utilize a greater proportion of carbohydrates over the pathway of fat synthesis and oxidation; activation of the adrenal cortex partially inhibited the process of fat synthesis from carbohydrate. Kinsell *et al.* (1951) demonstrated that the administration of ACTH and cortisone in adequate amounts results in partial or complete suppression of fasting ketosis in man. Scott & Engel (1953) confirmed these results with rats and concluded that the general effect of adrenal hormone action is to inhibit ketosis.

Engel & Engel (1954b) summarized the current concept at that time as follows: "The production of ketone bodies by the liver may be looked upon as an alternative pathway in the metabolism of acetate, occurring whenever acetate (acetyl CoA) exists in excess and either cannot be oxidized via the citric acid cycle because of a paucity of oxalacetate from carbohydrate or protein catabolism or cannot be converted to fat. In the fasted normal animal, ketosis is presumably a reflection of an acetate excess due to the increased production of this substance from fat catabolism, coupled with a decreased ability to synthesize fat from acetate and to provide sufficient oxalacetate from preformed carbohydrate or from enhanced tissue protein catabolism to allow full oxidation of the acetate in the liver via the citric acid cycle. One might speculate that following adrenalectomy, continued lipogenesis from carbohydrate and acetate might persist in contrast to the normal animal in which this process comes to a standstill. In terms of acetate utilization, however, presumably this process is overbalanced by enhanced fat catabolism. The latter, added to the combination of decreased carbohydrate stores and diminished protein catabolism to serve as sources of oxalacetate, then sets the stage for the development of ketosis. Conversely, while cortisone treatment of the normal animal favours ketosis by inhibiting fat synthesis, this effect may be more than overbalanced by a greater decrease in fat catabolism, by an increase in carbohydrate production by the liver and an increase in regeneration of citric acid cycle precursors from protein catabolism, thus inhibiting

ketosis during fasting. Thus, observed effects of cortisone and adrenalectomy on fasting ketosis can be fitted readily into current concepts of ketosis and of the mode of action of the adrenal cortex in intermediary metabolism." Engel & Engel (1954b) observed that adrenalectomized rats maintained with deoxycorticosterone acetate, exhibited ketosis during a four-day fast and that cortisone treatment restored the ketonaemia and the glycaemia to normal. It was suggested that enhanced ketonaemia might be a feature of the state of adrenal insufficiency. They cited an earlier report by Thaddea & Kuhn (1937) indicating that adrenal insufficiency in the cat may be associated with elevated blood ketone levels during fasting, and that these may be restored to normal by treatment with adrenal-cortex extract.

Astwood *et al.* (1953) and Engel & Engel (1954a) demonstrated that oxycel-purified ACTH induced ketonaemia in mice and rats. The latter noted that the ketonaemia was associated with a concurrent fall in blood sugar and persisted in adrenalectomized rats maintained with deoxycorticosterone acetate or cortisone. Although exceedingly good correlation was observed between the fall in blood sugar and the rise in blood ketones in many studies, nevertheless they observed that neither a fall in blood sugar nor a hypoglycaemia *per se* was a necessary prerequisite to ketosis. This report is of added interest because of the observations noted earlier in this review that a certain percentage of clinical or primary ketosis has been noted to occur in dairy cows not exhibiting hypoglycaemia. In later studies it was demonstrated (Engel & Engel, 1958b) that the ketogenic activity of purified ACTH is due to the ACTH molecule itself and is an extra-adrenal metabolic response. Engel & Engel (1958a) noted that pretreatment of normal rats with cortisone or hydrocortisone prevented the development of ketosis during fasting, cold exposure, or poisoning with sodium fluoroacetate which blocked the Krebs cycle. Cortisone was without effect on the ketosis secondary to the action of oxycel ACTH.

Glucocorticoid and ACTH Therapy. The efficacy of intramuscular injection of cortisone acetate was first reported by Hatziolos & Shaw (1950) and Shaw *et al.* (1950). In subsequent years an initial report (Shaw *et al.*, 1951) and extensive studies (Shaw *et al.*, 1953) were published showing the effectiveness of ACTH. Extensive studies demonstrated the remarkable efficacy of a number of glucocorticoids (Gessert *et al.*, 1955; Shaw *et al.*, 1952, 1954, 1955b,d) and ACTH. These studies demonstrated that when adequate dosages are used, very few cases of primary ketosis fail to recover promptly and completely. A dosage of at least 1·5 g of cortisone acetate was found to be necessary to obtain a high percentage of recoveries, and in one area (Shaw *et al.*, 1955c) it was found necessary to administer approximately 2 to 2·5 g of cortisone acetate during a period of 2 to 3 days to obtain rapid recovery. The usual pattern after treatment with a glucocorticoid is for the

blood glucose to return to normal within 8 to 10 h and to rise above normal within 24 h, remaining above normal for 2 or more days before levelling off. The blood ketones or acetone bodies usually return to normal by the 3rd to 5th day. Milk production usually shows the greatest improvement after 2 to 3 days and the appetite ordinarily improves within 18 to 24 h. Inadequate dosage was often observed to result in refractive cases, probably due to the development of extensive tissue damage.

Intravenous injection of smaller amounts of cortisone (alcohol form) resulted in temporary improvement of the animals, but was followed by relapses (Shaw *et al.*, 1952). The demonstration that ACTH is also effective (Shaw *et al.*, 1951, 1952, 1953) was important in showing that the adrenal glands of the ketotic cow are still functional. Based on the above-mentioned studies the following dosages are recommended for treatment, 1500 to 2500 mg cortisone acetate, 1500 to 2000 mg cortisol, 600 to 900 Armour veterinary units of ACTH, 200 to 400 mg of either prednisone or prednisolone, 50 to 100 mg 9-α-fluorocortisol, 25 to 50 mg Δ′1-dehydro-9-α-fluorocortisol, and 100 to 200 mg 9-α-chlorocortisol. However, because of the danger of the halogenated glucocorticoids due to their sodium-retentive properties, especially when administered to ruminants with added sodium chloride or other sources of sodium (Chung & Shaw, 1957), these substances must be used with caution. On the basis of the extensive degeneration observed in the adrenals of cows with ketosis, the altered liver function and the presence in commercial pituitary preparations of a hypoglycaemic-ketonaemic entity, glucocorticoids might be expected to be more effective than ACTH particularly in the more refractory cases.

ACTH injections have been shown to decrease milk production (Cotes *et al.*, 1949; Shaw *et al.*, 1955a; see also Cowie, Chapter 4 and Meites, Chapter 8). The question was raised as to why a relatively small dose of ACTH (100 i.u.) produces such a marked hyperglycaemia in normal cows whereas 1·5 to 3 g of cortisone acetate results in only a slight increase. Studies by Shaw *et al.* (1954) demonstrated that intramuscular injections of normal cows with 5 g of cortisol produced a marked hyperglycaemia and a sharp reduction in milk production, thus suggesting that the effect of ACTH on normal cows is merely that of eliciting a substantial increase in the production of highly active glucocorticoids by the adrenals.

E. Other Nutritional Aspects

As was noted earlier, numerous attempts have been made to produce primary ketosis in dairy cows by nutritional means but none has succeeded. Indeed, primary ketosis in cattle has never been reproduced experimentally. Fasting ketosis in dairy cattle has been induced by partial or complete

inanition post-partum (Forbes, 1943; Leffel, 1953; Leffel & Shaw, 1957; Robertson & Thin, 1953; Shaw, 1946c, 1950; Shaw & Cairns, 1947; Shaw & Leffel, 1949). Pre-partum diets, high or low in protein, high or low in fat, and high or low in soluble carbohydrates, had no influence on the degree of fasting hypoglycaemia and ketonaemia post-partum.

These data provide a possible explanation as to why it is so much easier to produce hypoglycaemia and ketonaemia in the early post-partum period than during the later part of the lactation period. It was observed that a low calorie intake during the early post-partum period did not result in the marked decrease in milk production invariably observed when cows were maintained on a low calorie intake during the later part of the lactation period (Leffel, 1953). It is obvious that the stimulation for milk production during the very early part of the lactation period is such that various body reserves are called upon for the secretion of milk, whereas during the later part of the lactation period this marked stimulation does not exist. Thus, milk production of cows on a low energy intake continues at a rather high level during the early part of the lactation period and, among other things, the glucose requirements of the mammary gland remain high at a time when the exogenous sources of glucose are reduced. Cows fasted during the later part of the lactation period, however, decrease sharply their milk production and thus the glucose requirements of the mammary gland are lessened materially. In the vicinity of Los Angeles, California, where the cow population is extremely high and the incidence of ketosis approaches 10 %, the dietary regime is uniformly excellent; the cattle receive a high-quality alfalfa hay, green-cut alfalfa or pasture, and excellent mixed concentrates (Shaw *et al.*, 1955c). In addition, all these animals are liberally fed during the early post-partum period. Further evidence that bovine ketosis is not due to a nutritional deficiency, in the classical sense, is the demonstration of the occurrence of ketosis in cows on a high level of energy intake (Shaw, 1943).

A study by Duncan *et al.* (1939) demonstrated normal levels of plasma calcium, magnesium and chloride in ketotic cattle. The inorganic phosphorus values were somewhat low, but it was concluded that this was due to the poor nutritional condition of the cows. Saarinen & Shaw (1950a,b) did not observe any significant alterations in plasma potassium, inorganic phosphorus or blood chloride in bovine ketosis. Carlstrom (1950), on the other hand, concluded that potassium levels were somewhat below normal and stated that potassium chloride administered intravenously was beneficial for the treatment of bovine ketosis. Talsma (1952) reported that both potassium chlorate and sodium chlorate were beneficial for the treatment of ketosis and more effective than potassium chloride and concluded that the beneficial effect was due to the chlorate ion. Shaw (1956) also observed what were believed to be low levels of potassium in ketotic cows, the average plasma potassium

of forty-three such cows being 16·2 mg per 100 ml. However, by the same analytical method, similar values have since been observed in normal cows.

Several have suggested that cobalt is effective for the treatment of ketosis (Gingras, 1947; Henderson, 1947; White, 1955). In rather extensive studies in Norway, Breirem *et al.* (1949, 1954) were unable to prevent ketosis with supplements of phosphorus, copper or cobalt; they suggested that the ketosis associated with cobalt deficiency is a fasting ketosis. Since some degree of inanition usually exists in animals exhibiting evidences of cobalt deficiency, it appears quite likely that the ketosis observed is indeed that of the secondary or fasting type. Hatziolos & Shaw (1958) noted injuries of the vascular walls of the adrenals of cows with ketosis reminiscent of the *periarteritis nodosa* experimentally produced in rats by high salt intake, and suggested that the relatively high salt intake of dairy cows might play a role in the aetiology of bovine ketosis. In a preliminary trial (unpublished) inappetence and a moderate degree of hypoglycaemia and ketonaemia occurred post-partum in two cows fed 2 lb of salt daily for 2 months pre-partum. Further studies of a longer-term nature would appear to be indicated.

MacKay (1943), Owen (1954) and Patton (1945) reported that ketosis was due to a vitamin A deficiency and could be cured by vitamin A therapy. However, Shaw *et al.* (1945) demonstrated that the blood plasma carotene and vitamin A values were normal in eight cases of uncomplicated bovine ketosis and that the administration of from 1 million to 4 million i.u. of vitamin A, for as long as 3 weeks, had no value in the treatment of ketosis. Hayden *et al.* (1946) confirmed the latter observation. Later, Shaw (1950) made a detailed study of thirteen cows which were maintained for 4 months pre-partum and 3 weeks post-partum on an inadequate carotene intake. Vitamin A deficiency signs were observed in the cows and in their calves at birth but primary ketosis did not develop, even in the cows that were fasted post-partum.

It was noted earlier that the levels and proportions of volatile fatty acids in the rumen of ketotic cows are approximately normal for cows on a low plane of nutrition (Brown & Shaw, 1957).

Thiamine was reported to be helpful for the treatment of ketosis by Carlstrom *et al.* (1939) and Compton (1944). However, Shaw (1946a) obtained negative results by the administration, orally and intravenously, of massive doses of thiamine, alone or in combination with riboflavin, pyridoxine, nicotinic acid, pantothenate, inositol, *p*-aminobenzoic acid, and biotin.

Lewis & Jacobson (1956) did not observe any statistical differences in post-partum blood glucose values or in rumen volatile acids in thirteen cows receiving an all-hay ration pre-partum compared to a similar group receiving

hay and grain; in later analyses no differences in blood acetone bodies were observed between the two groups.

That the rumen volatile acids may be controlled within rather wide limits is indicated by the work of Balch *et al.* (1955) with rations low in roughage and high in flaked maize and of Shaw *et al.* (1957) working with a variety of cooked grains and varying levels of roughage, or high levels of ground and pelleted hay plus relatively low levels of cooked concentrates. Indeed, rations have been developed with which not only acetate and propionate can be varied within wide limits but also butyrate and the higher acids (Shaw, 1958). In view of the demonstrated glucogenicity of propionate and butyrate, as was noted in Section IIIC 3, it should now be possible to assist materially in the prevention of ketosis by feeding rations that result in the ruminal production of large proportions of the organic acids which are glucogenic in the ruminant. Examples have been given of diets for cows which should be antiketogenic as well as of some which should be ketogenic (Shaw, 1958).

V. Conclusions

The bulk of the evidence indicates that of the three major metabolic diseases of cattle parturient paresis and ketosis are primarily of metabolic origin, whereas certain nutritional aspects appear to be of importance in grass tetany, although that disorder should also probably be classed as a physiological dysfunction. All three disorders in the dairy cow are associated with early lactation, ketosis and parturient paresis to a greater extent than grass tetany. Some of the confusion regarding their aetiology may be the result of our attempts to assign a single major causative factor to each.

In all three metabolic disorders there are indications of an impairment of homeostasis. Parturient paresis appears to result from a failure of the blood calcium regulatory mechanism (primarily the parathyroid glands) to induce the mobilization of calcium from the skeleton rapidly enough to maintain blood calcium at a normal level, at a time when there has been a sudden and large demand for calcium for milk secretion. The prevention of parturient paresis by feeding low-calcium rations pre-partum and its production by feeding high-calcium diets pre-partum adds support to the belief that this condition is associated with a hypofunctioning of the parathyroid glands.

In grass tetany in dairy cows on fresh pasture, the rapidity of its occurrence, lack of depletion of bone magnesium, spontaneous recovery, and rapid response to a single injection of magnesium are usually quite different from the characteristics of hypomagnesaemic tetany which develops in cattle under winter feeding conditions. It appears that hypomagnesaemic tetany may occur in animals on an adequate as well as on an inadequate intake of magnesium.

In the former this would not represent a magnesium deficiency in the usual sense. The researches indicating that a high ammonia production in the rumen may inhibit the absorption of magnesium of grass owing to the formation of ammonium complexes in the digestive tract, could provide an explanation for the early observations of a possible relationship between heavy nitrogen fertilization and hypomagnesaemic tetany and the more recent demonstrations of such a relationship under experimental conditions.

The experimental production of hypomagnesaemic tetany by maintaining cows on low levels of energy and moderately low levels of magnesium on dry rations and on pasture, and its experimental production on a ration of hay only but deficient in energy, in which the hay was obtained from a farm with a high incidence of tetany and fed in a different area, all demonstrate that typical post-partum hypomagnesaemic tetany can be induced by nutritional means.

There is little or no experimental evidence relating primary ketosis in lactating cows to nutritional deficiencies in the usual sense. On the other hand, there is a growing volume of experimental evidence that bovine ketosis is a disease of metabolic origin. That the pituitary and adrenal activity is impaired is indicated by the marked degenerative changes observed in these organs, the hypersensitivity to insulin, the lowered response of the adrenals to ACTH and the remarkably rapid recovery which can be effected by adequate doses of suitable glucocorticoids or ACTH. Secondary indications that the disease may be a pituitary-adrenocortical syndrome are the changes that have been noted, such as involution of the thymolymphatic system, acute involution of the pancreas, gastro-intestinal inflammation and ulcers, nephrosis, and fatty changes in the liver.

Additional evidence which favours the view that bovine ketosis is of metabolic origin is the depression of general liver function and, of even greater significance, what appear to be marked alterations from normal in certain metabolic pathways in the liver. The substantial number of cows which have been observed to develop ketosis for several years consecutively, regardless of changes in nutritional and environmental conditions, is irrefutable evidence that such cases are of metabolic rather than nutritional origin. Further evidence is that the intense degree of ketonaemia in cows with ketosis is usually out of proportion to the reduction in food consumption as well as the decrease in blood glucose. The ever-increasing evidence of the enormous importance of the pituitary and adrenals to the development of ketosis in man, the mouse and the rat adds support to the concept that bovine ketosis is a syndrome involving the pituitary-adrenocortical system.

Though it is possible that a "digestive" or nutritional form of ketosis may eventually be found to be related to certain feeding or management practices, no concrete evidence of such is available at the present time. In fact, the

incidence of ketosis is relatively high in many herds which, on the basis of our present knowledge, are being fed on rations that are nutritionally adequate in every respect. Field observations of a possible relationship of nutrition to the aetiology of primary ketosis must be treated with reserve since ketosis occurs in cattle on widely different diets.

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CHAPTER 16

Dietary Requirements for Lactation in the Rat and Other Laboratory Animals

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I. Introduction

For over a quarter of a century we have known that many nutritive factors essential for normal growth in mammals are needed in far greater amounts during lactation. However, with rare exceptions, our knowledge of dietary requirements for this physiological process has not kept pace with our knowledge of the requirements for growth and pregnancy.

In the 1940s the availability of crystalline B vitamins stimulated lactation studies in many laboratories to determine whether this physiological stress would reveal the presence of additional, unidentified factors which could be considered dietary essentials. Also, the possibility of "specific" dietary lactational factors defined by Folley *et al.* (1938) as "dietary constituents which are essential for lactation alone of the physiological processes" has been explored periodically. So far, all proposed "specific" lactational factors have been found also necessary for growth, but since they are needed in considerably smaller amounts during growth, they may be supplied by biosynthesis or by trace contamination of the diet. In recent years the emphasis has been on the interrelations and balance of nutritive essentials, and the goal of optimal rather than adequate lactation has been increasingly stressed.

This review and evaluation of experimental data on the nutritive needs for lactation has been based primarily on the rat, as insufficient information is available for other small laboratory animals.

A. Experimental Mammals

The rat has occupied for many years a unique position as an experimental mammal for nutrition studies. This has largely been due to its omnivorous habits and its co-operative behaviour in eating dietary mixtures of pure or semi-purified ingredients. The ease of handling large numbers of these animals and their resistance to infections (except those of the respiratory system) have no doubt aided in their popularity. This concentration of effort on a single species has produced considerable information on nutritional requirements of the rat whereas similar knowledge for other species is meagre or non-existent. For example, many laboratories have reported adequate lactation for two or more generations of rats given highly purified diets, and Schultze (1957) succeeded in maintaining rats on a completely synthetic diet for four succes-

sive generations, though growth was subnormal. A few long-term studies with purified diets have been carried out with mice (Cerecedo & Vinson, 1944; Fenton & Cowgill, 1947; Mirone, 1948) and with guinea-pigs (O'Dell *et al.*, 1957; Everson & Hurley, 1958). A single study with dogs maintained on semi-purified diets has recently been reported (Ontko & Phillips, 1958), but no similar long-term studies with either semi-purified or purified diets are available for hamsters, cats or monkeys.

The dietary requirements for both pregnancy and lactation in rats and other laboratory animals were reviewed in detail by Russell (1948) and those for rats by McCoy (1949). Recent reviews on general nutritive requirements are those of Cuthbertson (1957) on rats and mice, Mannering (1949) and Reid (1958) on guinea-pigs, and the (U.S.A.) National Research Council (1953, 1954) publications on nutrient requirements for dogs and rabbits. In addition, other recent reviews are those of Sebrell & Harris (1954) on vitamins, Underwood (1956) and Gilbert (1957) on trace elements and minerals, Block & Weiss (1956) on amino acids, Deuel (1955) and Deuel & Reiser (1955) on fat and fatty acids.

B. Experimental Procedures

Both long-term studies for one or more generations, and short-term studies of a single lactation period have been used for lactation studies in rats and other laboratory mammals. Long-term studies extending over one or several successive generations are necessary for the gradual depletion of certain trace elements or vitamins stored in body tissues and provide the most thorough test of the adequacy of a diet. However, short-term studies of only 3 weeks in the rat have been used successfully for the study of many essential nutrients. It is remarkable that at the time of parturition the rat will endure an abrupt transfer from a diet of natural foodstuffs to a "purified" diet, i.e., one composed of separate purified components. This circumstance has greatly facilitated the study of the role of dietary factors in lactation.

In either long-term or short-term procedures, litters may be exchanged, i.e., one-half of the litter from the mother on the experimental diet given to a normal lactating mother of equivalent age and body weight and one-half of the normal litter given to the experimental rat. This exchange of litters decreases the variability resulting from genetic factors and aids in distinguishing dietary effects on the foetus during embryonic development from those changes due to dietary effects during lactation. The latter can also be accomplished with dietary essentials which do not require long depletion periods, by a comparison of the lactational performance when the experimental diet is given only during lactation, with that observed when the experimental diets are given during both pregnancy and lactation.

C. Criteria for Lactational Performance

Milk production is seldom measured directly in small animals. Therefore, indirect methods of evaluating lactational performance have been commonly used. The criteria most frequently used are: (1) percentage of young weaned; (2) weaning weights of the young, usually expressed as the average weight per young but occasionally as total litter weight or average weight gains during the nursing period; and (3) maternal weight change during the lactation period. The percentage of young weaned may be calculated from the number of young living at birth or from the number of young living on the 4th day of lactation. The latter calculation was proposed by Mirone *et al.* (1948) for long-term studies in which the diet may be inadequate during pregnancy. For comparison of average weaning weights, the number of young per litter should be uniform and is frequently limited to six for the rat. Raising a large litter is undoubtedly a more severe strain on the mother and may test the adequacy of the diet to a greater extent, but weaning weights of the young and mother cannot then be compared with those in which the litters are limited to a smaller number. The lactating mother should gain as much weight as non-lactating controls during the same period,[1] since a loss in body weight indicates that maternal body tissues rather than dietary supplies are being used for milk production. Many investigators have emphasized the importance of using optimal rather than adequate performance as a standard, e.g. Dunn *et al.* (1947) and Fenton & Cowgill (1948), as standards lower than the optimum will result in underestimating the dietary requirements being studied. However, optimal weaning weights have not been determined for most strains of rats.

Additional criteria used for specific deficiencies during lactation have included carcass analyses, X-ray studies, tracer studies, tissue levels of vitamins or of the corresponding enzymes, peripheral blood counts, morphological studies of the liver, etc. Such biochemical or morphological measurements are valuable as they are more sensitive indicators of normality or abnormality and should be used more extensively in addition to body weights.

II. Protein Requirement

The total protein requirements for growth, reproduction and lactation in the rat, mouse, guinea-pig and rabbit appear to be similar when expressed as percentage of protein in the diet. Commercially prepared rations for these species usually contaiu 20 to 25 % of proteins furnished by several sources. The amino acid requirements of the rat (Rose, 1938; Rose *et al.*, 1948) and mouse (Bauer & Berg, 1943) likewise appear to be similar. However, the

[1]Usually 10 to 20 g for the Long-Evans rat.

guinea-pig (Woolley & Sprince, 1945) and probably the rabbit (Hove & Herndon, 1957b) have a higher arginine requirement for growth than the rat and mouse. For this reason and because of the apparent limited availability of arginine in casein, higher levels of dietary casein may be needed by the guinea-pig than by the rat (Heinicke *et al.*, 1955, 1956).

A. Total Protein[1]

The predominant role of protein in regulating milk secretion has long been known. Meigs's (1922) review of the literature, although limited to dairy cows for the most part, showed that there was general agreement that the quantity of milk yielded depended on the quantity of protein supplied. Changes in the quantity of protein fed affected primarily the volume of milk with only minor effects on its composition.

Mueller & Cox (1946) demonstrated that the volume of milk produced by the lactating rat was likewise dependent upon the dietary protein level. These investigators placed normal litters at parturition on semi-purified diets varying in casein level. Samples of milk were obtained by means of an ingenious milking machine.[2] Both the volume of milk and the weaning weights of the young increased directly with the casein level up to 30 % dietary casein. The protein concentration of the milk showed no significant differences within the range of dietary casein from 5 to 50 %. The optimal casein level was 30 % as judged by the volume of milk produced, and between 20 and 30 % as judged by weaning weights of the young.

When rats have been maintained for one or more generations on diets of natural foodstuffs or on semi-purified diets in which casein[3] was the principal source of protein, 20 to 25 % levels of protein have usually been considered best for lactation. For example, Macomber (1933) used semi-purified diets containing 5, 10, 17 or 21 % protein. He concluded that the 21 % protein level gave the best lactational performance. Weaning weights of the young as

[1]The value of considering a requirement for total protein may well be questioned inasmuch as the amount and availability of specific amino acids are the determining factors for each protein. The studies reviewed in this section have been limited to those in which casein was the principal source of protein.

[2]The use of mechanical milking methods for small laboratory animals has been questioned by many investigators, e.g., by Brody & Nisbet (1938), Luckey *et al.* (1954).

[3]When other protein sources have been used, the multiplicity of dietary variables and deficiencies has made interpretation difficult, e.g. in the studies of Slonaker (1939) in which diets furnishing from 10 to 26 % protein were prepared by addition of meat scraps to each preceeding diet. Although Slonaker concluded that the 14 % protein level seemed to be the most generally satisfactory over six generations, his data showed that the weaning weights of the young and the maternal weight increased with increasing protein level.

well as the maternal weight during the lactation period improved as the protein level was increased. Weaning weights averaging 38 g resulted with the 21 % protein diet but the mothers still showed a slight loss of weight during the lactation period. Kao *et al.* (1941) carried out a similar study with three generations of rats fed on diets composed of natural foodstuffs which contained 14·4, 18·8 or 25 % protein. When litters were weaned at 4 weeks of age, the 14·4 % protein diet resulted in young averaging 40 g, the 18·8 % protein diet in young averaging 47·5 g, and the 25 % protein level in young averaging 49 g. The authors concluded that the optimal degree of enrichment with protein was reached at about the 18 % level in such diets.

In a carefully conducted study McCoy (1947) maintained Wistar rats for five generations on diets of natural foodstuffs which furnished 15, 25 or 40 % protein. Eighty female rats were used for each protein level. On the 15 % protein diet approximately 60 % of the young were weaned with average weights of 34 g. With 25 % dietary protein, 78 % of the young were weaned with average weights of 42 g. The 40 % protein ration was superior for reproduction and resulted in the largest number of litters and young per litter, but did not improve lactation; 77 % of the young were weaned with average weights of approximately 38 g. McCoy concluded that the 25 % protein level was to be preferred for lactation.

In all of these studies the young were usually inferior in body weight to animals reared on stock diets and rarely attained weights over 40 g at 3 weeks of age.

When purified diets in which casein was the sole source of protein have been used for lactation studies, dietary levels of 25 to 30 % casein have been preferred. Nelson & Evans (1948c) used the Long-Evans rat in a 3-week test of lactational performance on purified diets varying in casein level from 6 to 48 %; twenty to thirty-six rats were used for each casein level. Normal female rats, together with their litters, were placed on the diets at parturition; litters were limited to six young and weaned at 21 days .Table I[1] demonstrates the immediate and striking effects of dietary casein level on lactational performance. On the 6 % casein diet only 69 % of the young were weaned with low average weaning weights of 17 g. For all casein levels above 6 % the percentage of young weaned was in the normal range, 90 to 100 %. Weaning weights of the young increased progressively with increasing casein levels and averaged 53 g when the diet contained 30 % casein. Further increases in

[1]The analysis of variance showed extremely high F values (over 100) for weaning weights of the young, maternal weight change, and average daily food intake. The high proportion of variance due only to casein levels was noteworthy, i.e. 82 to 89 %. A more detailed analysis of variance in which weights of individual young were used instead of average weaning weights per litter gave the following results: protein level variance 84·5 %, inter-litter variance 12 %, and intra-litter variance, 3·5 %.

TABLE I

EFFECT OF DIETARY CASEIN LEVEL ON LACTATION[1]
(Three-week test with Long-Evans rats)

Dietary casein level (%)	No. of litters	No. of young	Young weaned, day 21 (%)	Av. wt.[2] of young, day 21 (g)	Wt. change of mother during lactation (g)	Av. daily food intake (g)
Purified diets with casein as source of protein						
6	24	144	69	17 ± 1[3]	-100 ± 3[3]	13 ± 1[3]
12	21	126	98	25 ± 1	-61 ± 4	20 ± 1
18	23	138	97	39 ± 1	-15 ± 4	25 ± 1
24	36	216	98	48 ± 1	$+18 \pm 2$	34 ± 1
30	24	144	93	53 ± 1	$+15 \pm 2$	33 ± 1
36	23	138	98	53 ± 1	$+15 \pm 2$	34 ± 1
48	24	144	97	51 ± 1	$+12 \pm 2$	32 ± 1
Stock diet I plus lettuce twice weekly[4]						
28	102	612	96	49 ± 1	$+14 \pm 2$	35 ± 1

[1]Nelson & Evans (1948c). The basal purified diet was composed of 24 % alcohol-extracted casein, 64 % sucrose, 8 % hydrogenated vegetable oil (Crisco or Primex), and 4 % modified salts no. 4 (Hegsted *et al.*, 1941; see Table V); casein and sucrose were interchanged isocalorically in the diet. Crystalline vitamins per kilogram of diet were: 5 mg 2-methyl-1, 4-naphthoquinone, 5 mg thiamine HCl, 5 mg pyridoxine HCl, 10 mg riboflavin, 10 mg *p*-aminobenzoic acid, 20 mg nicotinic acid or nicotinamide, 50 mg D-calcium pantothenate, 400 mg inositol, and 1 g choline chloride; in addition 0·5 % of liver eluate powder furnished biotin, folic acid, and vitamin B_{12} (T. H. Jukes, personal communication) together with a small amount of amino-acid nitrogen. One ml of a fat-soluble vitamin mixture containing 6 mg DL-α-tocopherol, 115 chick units vitamin D, 800 U.S.P. units vitamin A, and 650 mg corn (maize) oil (Mazola) was given weekly to each litter. This basal purified diet with minor changes noted in each table has been used for the experiments reported in Tables I, II, III, VI, VII, VIII, X and XI.

[2]This column gives the average weaning weight of the young as calculated from average litter weights, each litter being corrected to the 3 : 3 sex ratio by means of the mid-sex correction factor (CF).

[3]Standard error of the mean $= \sqrt{\frac{(\sum X^2)}{n(n-1)}}$.

[4]This stock diet of natural foodstuffs (Nelson & Evans, 1947a) contained approximately 24·3 % protein (N × 6·25) furnished by wheat, milk powder and casein, and was equivalent to the protein in a 28 % casein purified diet. All rats were maintained on this diet during pregnancy.

casein did not result in significant improvement. On the 6 % casein diet lactating rats lost 100 g in body weight (over one-third of their initial weight) in 3 weeks. The weight change of the lactating mother improved with increasing casein levels, with an average gain of 18 g when the diet contained 24 % casein. Higher casein levels did not significantly change the maternal weight gain. The average daily food intake increased with the proportion of casein in the diet and paralleled the improvement in maternal body weight.

The marked differences in lactational performance between the rats on the 18 % and 24 % casein levels may be noted in Table I. All casein levels below 24 % resulted in a maternal weight loss and significant decreases in food intake and weaning weights of the young. The 18 % casein level resulted in young averaging 39 g at weaning and an average loss of 15 g for the lactating mother. This lactational performance would be considered "adequate" or "average" for some stock diets, but was markedly inferior when compared with that obtained with either the stock diet used in this study or the purified diets with optimal casein levels. The purified diets with optimal casein levels (24 to 30 %) resulted in equivalent maternal weight gains and slightly heavier young at weaning than the stock diet. Weaning weights on these purified diets closely approached the optimal weaning weights of the Long-Evans rat, 54 g for females and 56 g for males as reported by Dunn *et al.* (1947). These data serve to emphasize the importance of having optimal or near optimal performance as a standard.

Table II presents information on maternal blood counts at weaning. Total erythrocytes (RBC) and leucocytes (WBC) increased directly with dietary casein levels from 6 % to 24 % when normal levels of these cells, almost equivalent to those in animals maintained on the stock diet, were attained. The leucopenia observed at the lower casein levels was primarily a decrease in mononuclear cells with little change in polymorphonuclear leucocytes (PMN) in contrast to the severe granulocytopenia observed in pteroylglutamic acid deficiency during lactation (compare Tables II and XI).

On the basis of the available data it seems probable that 20 to 25 % protein from different sources or 24 to 30 % casein should be used during lactation in the rat.

B. Amino Acids

The classic studies of Rose (1938) on the amino acid requirements for post-weaning growth and nitrogen balance in rats have not been extended to the study of amino acid requirements during lactation. Numerous reports have been made, however, on the beneficial effects of cystine or methionine supplementation for lactation in rats maintained on diets containing protein sources known to be low or lacking in the sulphur amino acids, e.g., casein (Daggs &

Lidfeldt, 1938; Sure, 1941a), alfalfa proteins (Wright & Haag, 1939; Smuts & Du Toit, 1941), peanut meal (Haag & Wright, 1940), and yeast protein (Klose & Fevold, 1947). In fact, cystine and methionine have been proposed as specific dietary lactagogues by Daggs & Lidfeldt (1938).

TABLE II

EFFECT OF DIETARY CASEIN LEVEL ON MATERNAL BLOOD COUNTS AT WEANING[1]
(Three-week test with Long-Evans rats)

Dietary casein level %	No. of rats	RBC (millions per mm^3)	Total WBC (thousands per mm^3)	Mono-nuclears (thousands per mm^3)	Polymorpho-nuclears (thousands per mm^3)	Mono-nuclears %
Purified diets with casein as source of protein						
6	15	6·82 ± 0·10[3]	5·97 ± 0·82[3]	3·38 ± 0·62[3]	2·41 ± 0·39[3]	57·3 ± 4·5[3]
12	21	7·58 ± 0·31	6·17 ± 0·75	3·52 ± 0·49	2·65 ± 0·30	56·0 ± 2·1
18	17	7·69 ± 0·10	8·20 ± 0·59	4·81 ± 0·34	3·39 ± 0·33	58·9 ± 2·4
24	34	8·65 ± 0·15	9·81 ± 0·56	6·90 ± 0·43	2·91 ± 0·21	70·1 ± 1·4
30	23	8·33 ± 0·20	9·03 ± 0·61	6·24 ± 0·37	2·80 ± 0·31	70·0 ± 1·6
36	11	8·54 ± 0·18	9·31 ± 0·50	6·44 ± 0·48	2·87 ± 0·35	69·1 ± 3·0
48	18	8·36 ± 0·13	8·86 ± 0·77	5·88 ± 0·46	2·98 ± 0·34	67·6 ± 2·0
Stock diet I plus lettuce twice weekly[4]						
28	94	8·20 ± 0·15	10·58 ± 0·27	7·30 ± 0·21	3·28 ± 0·13	68·9 ± 0·9

[1]Footnotes 1, 3 and 4 same as for Table I.

Marked improvement in lactational performance during a short 3-week test resulted from cystine supplementation (0·5 % level) of diets varying in casein content from 12 to 30 % (Table III, Nelson & Evans, 1958). In the presence of sufficient cystine, the optimal casein level was between 18 and 24 %, but probably closer to the 18 % level. The data indicate that slightly more than 1 % of total sulphur amino acids (SAA) was necessary for optimal lactation in the Long-Evans rat and that cystine may furnish at least half of this requirement. This SAA requirement for lactation is considerably higher

than that needed for the growth of weanling rats,[1] i.e., 0·5 to 0·6 % SAA furnished by 0·3 to 0·4 % methionine and 0·2 % cystine (Wretlind & Rose, 1950). There are several possible explanations for this difference, e.g. (1) higher SAA requirements for lactation than for growth after weaning, (2) strain differences in SAA requirement, and (3) loss of methionine or cystine or non-availability of these amino acids resulting from the alcohol-extraction of casein.

TABLE III

EFFECT OF SUPPLEMENTARY CYSTINE FOR LACTATION ON CASEIN-CONTAINING DIETS[2]
(Three-week test with Long-Evans rats)

Dietary casein level (%)	Cystine suppl. (%)	No. of litters	No. of young	Av. wt. of young, day 21 (g)	Maternal wt. change during lactation (g)	Av. daily food intake (g)	Total SAA[3]
12	0	11	66	31 ± 2	− 43 ± 8	23 ± 1	0·37
	0·5	10	60	41 ± 2	− 14 ± 3	29 ± 1	0·87
18	0	13	78	41 ± 1	− 24 ± 2	27 ± 1	0·56
	0·5	12	72	54 ± 1	0 ± 3	34 ± 1	1·06
24	0	34	204	49 ± 1	− 1 ± 2	31 ±1	0·74
	0·2[4]	23	138	54 ± 1	+ 2 ± 3	33 ± 1	0·94
	0·5	36	216	54 ± 1	+ 17 ± 2	35 ± 1	1·24
30	0	12	72	50 ± 1	− 2 ± 3	32 ± 1	0·93
	0·5	12	72	54 ± 1	+ 9 ± 4	34 ± 1	1·43
36	0	12	72	52 ± 1	+ 19 ± 4	33 ± 1	1·11
48	0	12	72	51 ± 1	+ 18 ± 5	34 ± 1	1·48

[1]In adult rats the SAA requirement for maintenance of weight and nitrogen balance is only 0·16 % with cystine furnishing approximately three-quarters of this amount (Womack *et al.*, 1953).

[2]Nelson & Evans (1958). The basal purified diet (see Table I) contained ten crystalline B vitamins with 300 μg D-biotin and 5·5 mg pteroylglutamic acid per kilogram of diet being substituted for 0·5 % liver eluate powder.

[3]Percentage of total sulphur-amino acids (SAA) calculated from standard tables (Block *et al.*, 1956) and expressed in relation to 16 % nitrogen.

[4]0·2 % L-cystine was used for twelve litters and 0·2 % DL-methionine for eleven litters. The data have been combined as there was no significant difference between the two groups.

Lactation has been achieved in two strains of rats given purified diets containing only amino acids as the source of protein (Schultze, 1955, 1956; Greenstein *et al.*, 1956, 1957). In fact, Schultze (1957) was able to maintain rats of the "L" strain for four generations on a completely synthetic diet. Greenstein *et al.* (1957) were able to maintain two generations of Sprague-Dawley rats on a similar diet which was synthetic except for corn (maize) oil. However, optimal lactation performance as judged by weaning weights of the young was not observed in either study.

In the extensive studies of Schultze (1955, 1956) with amino acid rations, the use of 15·9 % of a mixture containing the ten essential amino acids and diammonium citrate resulted in the weaning of approximately 90 % of the young. However, growth of the young was retarded and maternal weight loss was great. Larger litters of heavier young were produced when glutamic acid was used instead of diammonium citrate in a mixture of equivalent nitrogen content. The use of only 12·2 % of a mixture containing sixteen amino acids permitted equivalent lactation performance,[1] i.e., 96 % of the young weaned with weaning weights of 30 g at 28 days. Weights of young and mother at weaning were improved when the mixture of sixteen amino acids was increased to 18·3 % of the diet. A further increase to 24·4 % did not result in additional improvement so that lactation performance equivalent to that of stock rats was not obtained. In addition, all amino acid diets resulted in production of fatty livers in the lactating mothers (suggesting a maternal amino acid deficiency or imbalance) whereas lactating animals maintained on stock diets did not develop fatty livers (Hallanger & Schultze, 1956). The high content of liver fat declined 2 or 3 weeks after lactation ended, although the rats were maintained on the same amino acid diets.

In contrast to the amino acid mixtures used by Schultze (1955, 1956) which contained several racemic acids, Greenstein *et al.* (1956, 1957) used only the L-isomers and these were given at a higher level, approximately 20 % of the diet. The use of a mixture containing fourteen amino acids resulted in 93 % survival of the young during the lactation period of 29 to 31 days, but in poor growth of the young (average weaning weights of 39 g) and in loss of maternal weight. Young given to foster mothers maintained on a stock diet of commercial laboratory chow averaged 65 to 70 g at the same age. When the mixture contained eighteen amino acids with the non-essential amino acid proportions similar to that in casein, the lactating mothers gained weight and pre-weaning and post-weaning weights of the young were improved, though still subnormal. Poorer growth was obtained when amounts of

[1]These findings are in agreement with those reported by Rose *et al.* (1948, 1949) that amino acid mixtures which include the non-essential amino acids are of higher nutritive quality and result in better growth than mixtures containing only ten essential amino acids.

TABLE IV

COMPARISON OF AMINO ACID MIXTURES USED FOR GROWTH, REPRODUCTION AND LACTATION WITH AMINO ACID CONTENT OF PURIFIED DIETS RESULTING IN OPTIMAL LACTATION IN THE LONG-EVANS RAT
(Expressed as percentage of diet)

Amino acids	Rose (1938) Rose *et al.* (1948) Requirements for growth	Rose *et al.* (1948) Mixture XXIII for maximal growth	Schultze (1956) Mixtures for diets AA 16 and 19 for growth, reproduction and lactation	Greenstein *et al.* (1957) Mixture for diet 26 for growth, reproduction and lactation	Amino acid content[1] of optimal-lactation diets containing 0·5 % L-cystine with 18 %–24 % casein
Essential					
L-Arginine	0·2	0·4	0·63 – 0·84	0·62	0·63 – 0·84
L-Histidine	0·4	0·7	0·42 – 0·56	0·40	0·45 – 0·60
L-Isoleucine	0·5	0·8	1·00 – 1·33	0·50	0·99 – 1·32
L-Leucine	0·8	1·2	1·90 – 2·53	0·80	1·52 – 2·02
L-Lysine	1·0	1·2	0·84 – 1·12	0·99	1·23 – 1·64
DL-Methionine	0·6	0·8	0·54 – 0·72	0·60	0·50 – 0·66
+ L-cystine		0·2	0·50 – 0·64	0·09	0·56 – 0·58
DL-Phenylalanine	0·9	1·2	0·80 – 0·97	0·90	0·87 – 1·16
+ L-tyrosine		0·6	0·99 – 1·32	1·46	0·94 – 1·26
L-Threonine	0·5	0·7	0·60 – 0·90	0·50	0·68 – 0·90
DL-Tryptophan	0·2	0·4	0·28 – 0·37	0·20	0·22 – 0·30
L-Valine	0·7	1·0	1·08 – 1·44	0·70	1·11 – 1·48

Non-Essential				
L-Alanine	0·2	0·43 – 0·57	0·75	0·47 – 0·62
L-Aspartic acid	0·2	0·49 – 0·65	1·63	0·97 – 1·30
L-Glutamic acid	2·0	3·67 – 4·90	5·24	3·54 – 4·72
L-Glycine	0·1	0·08 – 0·11	0·48	0·32 – 0·42
L-Hydroxyproline	0·1	—	—	—
L-Proline	0·2	—	2·99	1·84 – 2·46
L-Serine	0·1	—	1·55	0·94 – 1·26
Total physiologically active amino acids	12·1	14·25 – 18·95	20·4	17·8 – 23·5
Amino acid mixture as used in diet	17·0	18·3 – 24·4	22·5	18·5 – 24·5

[1] Calculated from standard tables (Block & Weiss, 1956) and expressed in relation to 16 % nitrogen. See Table III for data on lactation performance of Long-Evans rats in 3-week test on these diets.

the non-essential amino acids added were similar in proportion to those of other animal proteins or when DL-amino acids were used. The use of the eighteen amino acid mixture (modelled after casein) also decreased the incidence of fatty livers in lactating mothers.

The composition of the amino acid mixtures used for growing of weanling rats by Rose *et al.* (1948) and those used by Schultze (1956) and Greenstein *et al.* (1957) for reproduction and lactation may be compared with the amino acid content furnished by the purified diets which resulted in optimal lactation in Long-Evans rats (Table IV). It may be noted that the level of physiologically active amino acids used by Schultze was lower than the level used by Greenstein *et al.* or that furnished by the 24 % casein diet. In addition, two of the non-essential amino acids present in casein were not supplied by Schultze. On the other hand, the levels for nine of the essential amino acids used by Greenstein *et al.* were the same as those proposed by Rose as requirements for growth but were lower than those given for maximal growth. The sulphur amino acid level used by Greenstein *et al.* was 0·7 %, which is lower than the requirement (more than 1 %) for optimal lactation in Long-Evans rats (Nelson & Evans, 1958, Table III).

In summary, both essential amino acids and the so-called non-essential amino acids appear to be necessary for optimal lactation in the rat.

III. Mineral (Elements) Requirements

The mineral requirements of the rat and the mouse, as far as is known, appear to be similar and are satisfied by dietary levels of 4 to 5 % of the commonly used salt mixtures (Table V). On the other hand, the guinea-pig and the rabbit, being herbivorous, are accustomed to a high mineral intake and commercially prepared diets usually contain 7 to 9 % minerals (Reid, 1958). The guinea-pig has a higher requirement for potassium and magnesium (Roine *et al.*, 1949) and the rabbit for potassium (Wooley, 1954). Otherwise, little is known about quantitative needs for individual elements in these two species.

A. Calcium and Phosphorus

The majority of studies on the interrelated requirements for calcium and phosphorus have been concerned primarily with optimal calcium levels and with the Ca : P ratio. However, there is a single study of severe calcium deficiency during pregnancy and lactation in the rat (Boelter & Greenberg, 1943). The semi-purified diet used contained only 0·01 % calcium and resulted in the production of stillborn young or in high mortality of the young during lactation. In the few young that survived to weaning susceptibility to

paralysis (induced by galvanic stimulus) and haemorrhage were noted as well as greatly retarded growth. This study should be repeated with purified diets which are complete according to present-day nutritional knowledge.

Cox & Imboden (1936) studied ten consecutive reproductive cycles in rats maintained on semi-purified diets varying in calcium and phosphorus levels from 0·245 to 2·45 % for each element. These workers concluded that successful reproduction and lactation were dependent upon both the actual levels of these elements and on the Ca : P ratio. Excellent lactation performance resulted with a Ca : P ratio of 1 : 1 and dietary levels of 0·49 % of both calcium and phosphorus, as judged by weaning weights of the young, ash content of the young, and maternal weight gain. Good lactation was observed with somewhat higher dietary levels of calcium and Ca : P ratios of 1·5 : 1 and 1·7 : 1. Poor performance was observed regardless of the ratio when mineral levels as high as 2·45 % were used; however, phosphorus was better tolerated in excess than calcium.[1]

Although Cox & Imboden (1936) did not examine maternal bones in their studies, it is well known that depletion of bone salts has occurred in lactating rats on diets which were adequate for normal calcification and bone formation during growth and pregnancy. Warnock & Duckworth (1944) have shown that bone resorption in well-nourished lactating rats was restricted to the spongiosa of the "bone ends" and that the spongiosa was replenished after the lactation period. Extensive osteoporosis is, therefore, not a necessary consequence of lactation in the rat. These authors suggested that lactation requirements might be defined as "those intakes which, for a given output of minerals in milk, would restrict Ca and P withdrawals to the spongiosa during this period." Fournier (1954) has emphasized the protective effects of milk and specifically of lactose in decreasing the loss of skeletal calcium during lactation in the rat; this author believes that calcium loss during lactation can be prevented by optimal dietary regimens.

Calcium levels furnished by some of the commonly used salt mixtures vary from 0·44 to 0·87 % and phosphorus levels from 0·20 to 0·48 % (Table V). It should be remembered that these salt mixtures are usually used with high-phosphorus dietary components such as casein or yeast. Salts No. 12 of Jones & Foster (1942) is the only mixture formulated for use with dietary components either high or low in phosphorus.

The rat, mouse and rabbit are similar in that all have a low level of calcification at birth and show a large increase in calcium and phosphorus content during the suckling period (Spray & Widdowson, 1950; Spray, 1950). In contrast, the new-born guinea-pig has high levels of both elements at birth and these do not increase during the first 2 weeks of life (Spray, 1950).

[1]In contrast, the guinea-pig can tolerate excess levels of calcium but not of phosphorus (see Reid, 1958).

B. Potassium

Potassium has been known to be a dietary essential for the rat since 1923 (Miller, 1923). Yet our knowledge of the potassium requirement for pregnancy and lactation is based upon a single study, that of Heppel & Schmidt (1938), with diets not optimally supplemented according to present nutritional

TABLE V

Composition of Salt Mixtures Used in Purified Diets for Rats
(Expressed as mg per 100 g of diet
when the salt mixture is used as 4 % of the diet[1])

Element	Wesson (1932)	Hubbell *et al.* (1937)	Hegsted *et al.* (1941) Salts No. 4[2]	Jones & Foster (1942) Salts No. 12	Richardson & Hogan (1946)	U.S.P. XIV and Hawk–Oser[3]
Calcium	567·3	868·8	480·0	576·0	651·9	441·5
Phosphorus	350·4	204·8	283·4	334·0	478·3	266·7
Potassium	607·5	478·1	579·5	421·0	325·0	654·4
Sodium	166·3	110·6	263·1	206·0	161·5	122·2
Chlorine	483·4	380·8	406·9	319·0	278·7	424·5
Magnesium	72·7	41·8	40·4	43·0	32·5	68·4
Iron	14·8	20·6	18·4	20·0	16·1	10·3
Copper	0·40	1·43	0·31	0·46	1·30	—
Manganese	0·29	0·51	4·90	4·94	19·68	0·24
Zinc	—	—	0·48	0·47	0·36	—
Iodine	0·15	0·24	2·44	2·28	1·46	0·12
Fluorine	1·03	1·80	—	—	1·45	9·20
Cobalt	—	0·71	—	0·02	0·05	—

[1]Salt mixtures are ordinarily used at 4 to 5 % dietary levels except for the Hubbell *et al.* (1937) mixture which has been frequently employed at 2 to 3 % levels.

[2]The modified salt mixture No. 4 used by Nelson & Evans is made principally from anhydrous salts and furnishes in mg/4 g salt mixture: calcium 577·5, phosphorus 309·6, potassium 617·2, sodium 280·3, chlorine 433·4, magnesium 42·8, iron 19·6, copper 0·33, manganese 5·27, zinc 0·53, iodine 2·60.

[3]Hawk *et al.*, 1947.

knowledge. These investigators showed that pregnancy was possible in rats reared on diets containing 0·010 to 0·015 % potassium. However, the mothers were in negative potassium balance, lost weight, and usually ate their young at birth. When dietary potassium was raised to 0·14 %, the rats were usually in positive balance during pregnancy but in negative balance during lactation.

Many mothers maintained on this diet failed to lactate. Increasing the dietary potassium to 0·58 % for both pregnancy and lactation (on a diet of natural foodstuffs) usually resulted in positive potassium balance during lactation. Heppel & Schmidt also demonstrated the immediate effects of potassium deficiency on lactation. When they placed stock female rats at

TABLE VI

POTASSIUM REQUIREMENT FOR LACTATION IN THE RAT[1]
(Three-week test with Long-Evans rats)

Dietary potassium (%)	No. of litters	No. of young	Young weaned, day 21 (%)	Av. wt. of young, day 21 (g)	Wt. change of mother during lactation (g)	Av. daily food intake (g)
0·0	24	143	39	16 ± 2	− 82 ± 5	11 ± 1
0·1	13	78	90	27 ± 2	− 72 ± 6	18 ± 1
0·2	12	72	100	40 ± 2	− 35 ± 4	23 ± 1
0·3	12	72	97	53 ± 2	− 4 ± 5	31 ± 2
0·4	12	72	100	56 ± 2	+ 2 ± 5	34 ± 1
0·5	12	72	99	55 ± 1	+ 12 ± 5	34 ± 1
0·6	11	66	97	57 ± 2	+ 24 ± 3	34 ± 1
			Non-lactating rats[2]			
0·0	12	—	—	—	− 46 ± 4	10 ± 1
0·6	12	—	—	—	+ 14 ± 3	14 ± 1

[1]The basal purified diet contained 24 % casein (Nutritional Biochemicals Corporation, Cleveland, Ohio), 0·5 % L-cystine, high levels of the 11 B vitamins, including 50 μg vitamin B_{12} per kg of diet, and 3·15 % of the potassium-free salt mixture of Grunert & Phillips (1949). By analysis this diet contained only 0·008 % potassium. In the supplemented diets potassium was added in the form of KCl.

[2]The non-lactating rats in this and other tables are rats equivalent in age, body weight and dietary history to lactating animals but whose litters had been discarded at birth.

parturition on a diet containing 0·018 % potassium the mothers lost as much as 30 % of their body weight and the young averaged only half the normal weight when weaned.

Preliminary studies (summarized in Table VI) with Long-Evans rats maintained on purified diets supplemented with all dietary essentials known at the present time, have confirmed these findings of Heppel & Schmidt,

and have emphasized the importance of potassium for optimal lactation. In the 3-week test (with a basal diet containing only 0·008 % potassium) the requirement for optimal lactation as judged by weights of young and mother at weaning appeared to be 0·4 to 0·6 % potassium.[1] Potassium levels furnished by the commonly used salt mixtures vary from 0·33 to 0·65 % (Table V).

C. Sodium and Chlorine

Present knowledge of the sodium and chlorine requirements for reproduction and lactation in the rat is based on two studies carried out in 1925–26. Olson & St. John (1925) reported that a wheat ration containing 0·23 % sodium was insufficient for growth and reproduction, one containing 0·53 % was most satisfactory, and others with 0·785 % sodium or higher were somewhat detrimental. Miller (1926) found that satisfactory growth, reproduction and lactation resulted when 0·46 % sodium was used in a yellow-corn ration. These values may be compared with the sodium requirement of 0·05 % (Grunert *et al.*, 1950) for growth, and with sodium levels of 0·11 to 0·28 % furnished by the commonly used salt mixtures (Table V). The severe effects of sodium deficiency during lactation on weights of the young and mother at weaning in comparison with slight effects of this deficiency in non-lactating rats may be seen in Table VII. The salt mixture used in the control diet furnished 0·28 % sodium and resulted in optimal lactation in the 3-week test.

Miller (1926) also observed in this study with a yellow-corn ration that about 5 mg of chlorine per day were apparently sufficient to supply the requirements for growth, reproduction and lactation. This daily intake, equivalent to 0·02 to 0·025 % chlorine, may be too low since losses of tissue chlorine, sodium, potassium and nitrogen as well as retarded growth have been reported in rats maintained on a diet containing 0·02 % chlorine (Voris & Thacker, 1942; Thacker, 1943). The commonly used salt mixtures furnish 0·28 to 0·48 % chlorine (Table V).

D. Magnesium

Magnesium was first shown to be a dietary essential for the rat in 1932 (Kruse *et al.*, 1932). However, only a single study has been reported on the requirement of this element for reproduction and lactation. Tufts & Greenberg (1938) found that 5 mg magnesium per 100 g diet permitted growth and pregnancy but was entirely inadequate for lactation. Many rats failed to lactate and ate their young. The few young raised to weaning showed signs of this deficiency (e.g. hyperexcitability and convulsions) at 2 to 3 weeks.

[1]This may be compared with the requirement of 0·18 % potassium for post-weaning growth of rats (Grunert *et al.*, 1950).

Although these young had a normal carcass magnesium content at birth, they contained less magnesium in their tissues at weaning than rats raised on stock diets. Even the control diet containing 50 mg magnesium per 100 g diet did not result in young with a normal magnesium content. These findings should be confirmed with diets known to be complete according to our present knowledge.

The commonly used salt mixtures furnish 32 to 73 mg magnesium per 100 g diet (Table V).

TABLE VII

EFFECTS OF SODIUM DEFICIENCY ON LACTATION[1]
(Three-week test with Long-Evans rats)

Experimental group	No. of litters	No. of young	Young weaned, day 21 (%)	Av. wt. of young, day 21 (g)	Wt. change of mother during lactation (g)	Av. daily food intake (g)
Sodium-deficient	11	66	100	26 ± 1	− 86 ± 6	18 ± 1
Sodium-supplemented (controls)	11	66	97	57 ± 1	+ 24 ± 3	34 ± 1
			Non-lactating rats			
Sodium-deficient	12	—	—	—	− 7 ± 3	13 ± 1
Sodium-supplemented (controls)	12	—	—	—	+ 14 ± 3	14 ± 1

[1]The basal purified diet is the same as that quoted in Table VI, with 3·45 % of the sodium-free salt mixture (Grunert & Phillips, 1949) being used. Sodium was added as NaCl for the supplemented group.

E. IRON AND COPPER

Copper is essential for the utilization of iron in haemoglobin formation in the rat (Hart *et al.*, 1928) as well as in many other mammals (Underwood, 1956). In studies of the iron and copper requirements of the rat, milk diets

have been used to a great extent, since mammalian milk, in general, is a poor source of both iron and copper (Underwood, 1956). The iron and copper content of milk of several species cannot be raised above normal levels by dietary excess of salts of these elements. Rat's milk is exceptional compared to the milk of other mammals and contains approximately ten times the amount of both iron and copper present in cow's milk (Cox & Mueller, 1937).

When rats were given a whole-milk diet during pregnancy and lactation the first litter had normal haemoglobin values at birth and did not show anaemia during the suckling period (Alt, 1938). Young of the second litter had only about one-half the normal haemoglobin content at birth and rarely survived for more than 10 to 12 days. The second pregnancy also resulted in moderate anaemia of the mother. Daily supplements of 5 mg of iron protected both mother and young from the anaemia.

Daniels & Everson (1935) observed excellent reproduction and lactation for five generations of rats maintained on boiled milk with a mineral supplement which included 5 mg of iron and 0·8 mg of copper per 100 ml (equivalent to the daily intake). The 28-day weaning weights of the young in the fifth generation were equivalent to those obtained for young from stock-diet mothers, i.e., 69 and 70 g. Difficulties with reproduction or lactation have been reported when similar diets containing lower levels of iron and copper were used (Evans & Phillips, 1939; Richardson & Hogan, 1940), although factors other than low concentration of these elements may have been involved.

Salt mixtures commonly used in purified diets for rats furnish 10 to 21 mg of iron and 0·3 to 1·4 mg of copper per 100 g diet (Table V).

F. Manganese

The importance of manganese during the lactation period in the rat was first recognized by Orent & McCollum (1931). Lactation failed completely in rats reared on a semi-purified diet free of manganese. Supplementation with 5 mg manganese per 100 g diet permitted some lactation and 50 mg improved performance considerably.

Since this original demonstration, diets better supplemented with other essential nutrients but somewhat higher in manganese (0·1 to 0·3 mg per 100 g diet) have been devised. The majority of diets have contained milk as a major component since milk is low in this element (Underwood, 1956). For example, Daniels & Everson (1935) used a mineralized milk diet that maintained reproduction and lactation in rats for five generations when supplemented with 1·2 mg manganese per 100 g diet. With manganese omitted, high mortality of the suckling young resulted. Only a few of the manganese-deficient

young could be raised by stock-diet mothers, whereas manganese-deficient mothers weaned 80 % of suckling young from normal mothers. These investigators concluded that the high mortality during the lactation period was due primarily to a "congenital debility" of the young. Furthermore, recent studies by Hurley *et al.* (1958) have shown that manganese supplementation of deficient rats beginning on or before the 14th day of pregnancy prevented high mortality and deficiency signs in the young during the lactation period whereas withholding manganese until the 18th day of pregnancy was ineffective. However, Skinner *et al.* (1931) had previously shown that mammary transfer of manganese was limited, so that this factor may also be involved. Suckling rats from manganese-deficient mothers showed retardation of growth, skeletal abnormalities, ataxia and lack of equilibrium, or high mortality during the lactation period, depending on the severity of the deficiency (see, e.g. Shils & McCollum, 1943; Hill *et al.*, 1950; Frost, 1951).

Estimates[1] of the manganese requirement for lactation in the rat have ranged from 0·1 to 12·5 mg daily or from 0·3 to 50 mg per 100 g of diet. In the majority of studies a requirement of approximately 1 mg daily has been reported, e.g. 0·8 mg daily (stock diet of Daniels & Everson, 1935), 1·2 mg daily (mineralized milk diet of Daniels & Everson, 1935), 1·5 mg daily (purified casein-containing diet of Frost, 1951). Not all of the commonly used salt mixtures furnish sufficient manganese for a daily intake of 1 mg during lactation, i.e. 3 to 5 mg per 100 g of diet (Table V).

The mouse, like the rat, develops the syndrome of manganese deficiency in second-generation young from manganese-deficient mothers (Shils & McCollum, 1943). In the rabbit, retardation of growth and skeletal defects were produced in first generation manganese-deficient animals (Smith *et al.*, 1944).

G. Zinc

Zinc was shown to be an essential dietary factor for the rat in 1934 (Todd *et al.*, 1934) and is presumably required by all mammals inasmuch as the enzyme, carbonic anhydrase, contains this element in the prosthetic group (Keilin & Mann, 1939). Special treatment of the protein components (hydrolysis followed by dithizone extraction) of the diet is usually necessary to produce a diet low in zinc.

The zinc concentration of mammalian milk is high compared to that of other trace elements, and colostrum is four to five times higher in zinc than later milk (Underwood, 1956). Nishimura (1953) produced a zinc deficiency in

[1]These estimates have been made on the basis of 25 to 35 g of dry diet or 100 ml of milk daily for lactating rats.

mice during the lactation period by the simple but ingenious procedure of depriving the young of colostrum and giving them foster mothers from a later stage of lactation (usually 13 to 18 days). The suckling mice showed retardation in growth and hair development, and skin changes, with death resulting in severe cases. Surviving mice showed a pronounced decrease in total zinc content in comparison with control young. Single doses of zinc chloride or zinc acetate (providing about 7 μg zinc) given orally on the day of birth effectively protected the suckling mice. Providing foster lactating mothers with 0·1 % zinc acetate solution in the drinking water did not prevent the skin changes in suckling young, indicating limited mammary transfer of inorganic zinc. This study emphasizes the importance of zinc during early post-natal growth in the mouse despite appreciable body stores of this element in new-born young (Widdowson, 1950). There have been no similar studies employing the rat.

Not all of the commonly used salt mixtures for rats contain a zinc compound but, when present, the amount furnished to the diet is approximately 0·35 to 0·5 mg of zinc per 100 g of diet (Table V).

H. Iodine

It is well known that "iodine is unique among the trace elements in that it functions solely as an indispensable constituent of a hormone—that of the thyroid gland" (Underwood, 1956). Therefore, the minimum iodine requirement is usually defined in terms of thyroid function. Using as a criterion "the smallest amount of iodine necessary to prevent significant thyroid enlargement," Levine *et al.* (1933) found that the minimum iodine requirement in young rats was 1 to 2 μg daily (i.e., per 10 g of diet). These investigators used a goitrogenic diet which was deficient in factors other than iodine and resulted in subnormal growth (Remington, 1937). With a presumably better balanced diet Remington & Remington (1938) reported that 2·65 μg iodine per 10 g of diet protected growing rats from this deficiency as judged by weight and iodine content of the thyroid.

It is usually considered that the iodine requirement is increased during pregnancy and lactation, at least in man and farm animals, since cretinism and other indications of thyroid deficiency have been observed in goitrous areas. However, for the rat, Parker *et al.* (1951) concluded that the minimum iodine requirement, when expressed as proportion of iodine in the diet rather than daily intake, was no greater for pregnancy and lactation than it was for growth. These investigators maintained rats for three generations on commercially prepared diets containing 0·25 to 6·25 μg iodine per 10 g of diet. The minimum dietary level necessary for excellent reproduction and lactational performance was found to lie between 1 and 2·25 μg iodine per 10 g of

diet. Lower iodine levels, e.g., 0·25 μg per 10 g diet, did not interfere with reproduction and lactation but thyroids of new-born young from mothers given this level were embryonic morphologically.

I. Other Trace Elements

1. *Aluminium, Arsenic, Boron and Cobalt*

It has not yet been established that these trace elements are essential for growth, reproduction and lactation in the rat. The reports of several investigators indicated that possible requirements were satisfied by the following amounts: 1 μg daily of aluminium (Hove *et al.*, 1938a); 2 μg daily of arsenic (Hove *et al.*, 1938b); < 1 μg daily of boron (Hove *et al.*, 1939; Teresi *et al.*, 1944); $< 0{\cdot}03$ μg daily of cobalt (Houk *et al.*, 1946).

2. *Bromine*

The only attempt to produce a bromine deficiency in rats is that of Winnek & Smith (1937). Supplementation of a semi-synthetic diet (containing less than 0·5 p.p.m. bromine) with 20 p.p.m. bromine (as KBr) did not improve growth, reproduction or lactation. However, the young born to mothers on the bromine-low diet had a greatly decreased bromine content of their tissues as compared with young born to stock-diet females. Probably a better supplemented diet could now be prepared in accordance with modern nutritional knowledge and the effects of a bromine deficiency studied in second and third generation animals. Another experimental approach is indicated by the recent report (Huff *et al.*, 1956) that mice require bromine for growth when fed on a purified diet containing iodinated casein. Increased growth occurred in 12 days when only 3·75 p.p.m. of bromine were added to the diet.

3. *Fluorine*

Evans & Philips (1939) maintained five generations of rats on a mineralized milk diet furnishing only 2 to 10 μg of fluorine daily. There appeared to be no changes in bones or teeth nor was there a depletion of fluorine stores or increased demand for this element throughout the five generations. However, no data were given on lactation performance. Increasing the fluorine level 200-fold did not result in any discernible improvement.

4. *Molybdenum*

Teresi *et al.* (1942) concluded that if molybdenum was necessary for growth in the rat, a daily intake of 0·5 μg on a mineralized milk diet should satisfy this requirement. However, recent studies showing that molybdenum is an integral part of the prosthetic group of xanthine oxidase and necessary for the maintenance of normal tissue levels of this enzyme in the rat (Richert &

Westerfeld, 1953; De Renzo *et al.*, 1953) should stimulate further interest in this trace element. Dietary supplements furnishing 0·2 to 0·3 μg molybdenum per day were sufficient to produce "saturation" levels of this enzyme (De Renzo *et al.*, 1953).

5. *Selenium*

Although the toxicity of this element for many animal species has long been known, only recently has any evidence regarding its essential nature in mammalian nutrition been reported. Schwarz & Foltz (1957) have recently demonstrated that dietary liver necrosis in rats maintained on Torula yeast rations was prevented by selenium compounds. As little as 0·25 μg of selenium daily, given as sodium selenite, was completely protective. The basal diet used was low in cystine and deficient in vitamin E and "factor 3" (now identified as organic selenium compounds); the resulting necrotic liver degeneration may be prevented by any one of these factors (Schwarz, 1954).

IV. Water-soluble Vitamin Requirements

The B vitamin requirements of the rat, the mouse, and the guinea-pig, appear to be similar. However, the guinea-pig apparently needs a dietary supply of nicotinic acid even though it is able to synthesize this vitamin from tryptophan (Reid, 1958). It has been shown that the rabbit needs dietary nicotinic acid (Wooley & Sebrell, 1945), choline (Hove *et al.*, 1954), and pyridoxine (Hove & Herndon, 1957b) but the practice of habitual coprophagy or "pseudo-rumination" undoubtedly decreases the need for the other B vitamins (see (U.S.A.) National Research Council, 1954).

A. Vitamin "B"—Thiamine (Vitamin B_1)

As early as 1924 both Sure and Hartwell noted that for successful lactation in rats the vitamin "B" content of the diet had to be increased considerably above that needed for growth. When yeast or wheat-germ extract was used as a source of the B vitamins, three to five times as much was necessary for lactation as for growth and pregnancy (Hartwell, 1925; Sure, 1927; Evans & Burr, 1928a). The inefficiency of the lactating rat in transferring dietary vitamin "B" to her milk, 60 % being "lost" in this process according to Sure (1928), was believed to be one reason for the increased requirement. Lactating rats on B-vitamin deficient diets showed a 50 % reduction in food intake. However, pair-feeding of control rats demonstrated that vitamin B deficiency had deleterious effects on lactation greater than those resulting

from decreased food intake, as the pair-fed rats exhibited more successful lactation and increased survival and growth of suckling young (Sure & Walker, 1931).

Although the principal dietary deficiency in many early studies was probably that of thiamine, deficiencies of the other B vitamins undoubtedly existed at the same time. For example, the only study on the relation of B vitamins to the volume of milk secreted by the rat is one in which a 10 % casein, semi-purified diet was used with yeast extracts as the source of the B vitamins (Mueller & Cox, 1946). The volume of milk secreted and weaning weights of mother and young increased with increasing amounts of yeast extract, whereas there was no significant change in protein, fat, and ash content of the milk. Houston & Kon (1939) have reported that the thiamine content of rat's milk decreased to trace amounts when lactating animals were deprived of this vitamin. Administration of large amounts of vitamin B_1 increased the vitamin content of the milk but did not raise it above the normal level, suggesting some limitation in mammary transfer of this vitamin. However, increased amounts of riboflavin raised the riboflavin content of rat's milk.

The immediate effects of thiamine deficiency on lactation were seen when Long-Evans rats, together with their litters, were placed on the vitamin-deficient diet at parturition (Table VIII). Only 77 % of the young were weaned with average weights of 25 g, approximately half the weight of control young. The weight loss of the lactating rats and the percentage decrease in food intake during the 3-week period were greater than that in any other B vitamin deficiency studied in Long-Evans rats. Non-lactating rats of similar age and body weight maintained their weight for 3 weeks when placed on the same deficient diet. Inasmuch as all lactating rats have access to faeces, the severity of this vitamin deficiency during lactation was surprising.

No studies have been made of the minimum requirement of thiamine for lactation in the rat, but thiamine levels varying from 0·5 to 2 mg per 100 g of diet have been successfully used in both short-term and long-term lactation studies (Table IX).

B. Riboflavin (Vitamin B_2)

Many of the early studies indicated the necessity not only of vitamin B_1 but also of the vitamin B_2-complex for lactation (e.g., Hussemann & Hetler, 1931). Daniel *et al.* (1942) reported stunting of growth and loss of fur in suckling young from stock rats placed on a vitamin B_2-deficient diet shortly before parturition. When Long-Evans rats, together with their litters, were given a riboflavin-deficient diet at parturition, the young had subnormal weaning weights of 35 g (Table VIII) and soft downy fur, while the lactating mothers showed an average loss of approximately 40 g during this 3-week period. The

deficiency was not accentuated by addition of 1 % succinylsulphathiazole (SST) to the diet during lactation.

Levels of riboflavin varying from 1 to 2 mg per 100 g of diet have been used in both short-term and long-term studies of lactation in the rat (Table IX).

TABLE VIII

EFFECT OF B-VITAMIN DEFICIENCIES ON LACTATION[1]
(Three-week test with Long-Evans rats)

Vitamin deficiency	No. of litters	No. of young	Young weaned, day 21 (%)	Av. wt. of young, day 21 (g)	Wt. change of mother during lactation (g)	Av. daily food intake (g)	Reference
Thiamine	24	144	77	25 ± 1	− 83 ± 4	19 ± 1	This paper
Riboflavin	23	138	99	35 ± 1	− 38 ± 3	25 ± 1	This paper
Pyridoxine	22	132	13	22 ± 1	+ 3 ± 2	22 ± 1	Nelson & Evans (1951)
Pantothenic acid	50	300	96	30 ± 1	−55 ± 3	22 ± 1	Nelson & Evans (1945) and this paper
Pteroyl-glutamic acid	55	330	100	44 ± 1	− 30 ± 3	26 ± 1	Nelson & Evans (1948a)
Biotin	47	282	95	45 ± 1	+ 7 ± 2	30 ± 1	Nelson & Evans (1948b)
Inositol or *p*-aminoben-zoic acid	40	240	96	49 ± 1	− 6 ± 3	31 ± 1	This paper
Choline	24	143	99	45 ± 1	− 4 ± 3	29 ± 1	This paper
Controls ± vitamin B_{12}	34	204	97	49 ± 1	− 1 ± 2	31 ± 1	Nelson & Evans (1948a,b) and this paper

[1]The basal purified diet is the same as in Table III. Since L-cystine was not added to the basal diet, optimal lactation was not obtained in control animals. In this table a difference of 3–4 g in average weaning weights of the young is statistically significant at the 1 % level ($P < 0{\cdot}01$).

C. Vitamin B_6

Many of the early studies mention convulsions, screaming, and running fits in suckling rats when the mothers were given vitamin "B"-deficient diets. At that time these signs were considered indicative of polyneuritis resulting from vitamin B_1-deficiency. However, to the authors' knowledge polyneuritis has never been demonstrated in vitamin B_1-deficient suckling rats. The studies

TABLE IX

B-Vitamin Levels in Diets Used for Lactation Studies[1] in the Rat
(Expressed as mg per 100 g of diet)

Investigators	Vinson & Cerecedo (1944) Sica *et al.* (1948)	Schultze (1950)	Richardson & Brock (1956)	Nelson & Evans (1948b)
	Length of test			
	4 generations	5 generations	1 generation	3 weeks
Thiamine HCl	2·0	0·5	1·0	0·5
Riboflavin	2·0	1·0	1·0	1·0
Pyridoxine HCl	2·0	0·5	1·0	0·5
Calcium pantothenate	4·0	5·0	4·0	5·0
Choline chloride HCl	50·0	100·0	100·0	100·0
Nicotinic acid or nicotinamide	—	2·0	5·0	2·0
Inositol	—	40·0	10·0	40·0
p-Aminobenzoic acid	—	1·0	0·1	1·0
Pteroylglutamic acid	0·5–2	0·2	0·1	0·55
Biotin	—	0·02	0·02	0·03
Vitamin B_{12}	—	—	0·0025	—
Casein (%)	30·0	24·0	25·0	24·0[2]

[1]The lactational performance observed in studies with these purified diets was equivalent to that obtained with stock diets for the same period of time.
[2]Supplemented with 0·5 % L-cystine.

of Hartwell (1921, 1925) on the toxic effects of high-protein diets during lactation with prevention by high levels of vitamin "B" (supplied by marmite) probably furnished the first description of vitamin B_6 deficiency in suckling rats.

Modern recognition of the specific syndrome came in 1942 when Daniel *et al.* (1942) observed convulsions in suckling young of mothers maintained on a semi-purified diet considered satisfactory for growth and reproduction. Between the 11th and the 18th days the animals suddenly showed emaciation and tremors, uttered sharp cries and "exhibited a series of running fits accompanied or followed by convulsions, and in most cases by death on the same or following day." Daniel *et al.* also observed this syndrome in the young when stock rats were placed on purified vitamin B_6-deficient diets during pregnancy or shortly before parturition; they were able to cure or prevent the convulsions with crystalline pyridoxine. These findings have been confirmed by many investigators, e.g. Patton *et al.* (1944). Convulsions and epileptiform fits also result occasionally from vitamin B_6 deficiency in older rats as found by, e.g., Chick *et al.* (1940).

As can be seen in Table VIII, deficiency of vitamin B_6 during lactation is unique among the B vitamin deficiencies for its markedly deleterious effects on the suckling young. When Long-Evans rats, together with their litters were given the vitamin B_6-deficient diet beginning with the day of parturition, survival of the young to weaning rarely occurred, whereas the lactating mothers showed no external signs of the deficiency (Table VIII, Nelson & Evans, 1951). Addition of either deoxypyridoxine (a vitamin B_6 antimetabolite) or SST did not accentuate the deficiency during lactation, whereas instituting the deficiency at the beginning of pregnancy markedly decreased survival of the young during the lactation period (Nelson & Evans, 1951). The beneficial effects of direct feeding of vitamin B_6 to the young (Daniel *et al.*, 1942; Patton *et al.*, 1944) indicated that vitamin B_6 deficiency, in contrast with protein deficiency, affected the quality of the milk rather than quantity produced (see Section II).

Daily doses of 10 to 50 μg pyridoxine to lactating mothers have been reported to prevent convulsions in the young (Daniel *et al.*, 1942; Patton *et al.*, 1944). In short- and long-term lactation studies levels of 0·5 to 2 mg pyridoxine per 100 g of diet have been used (Table IX).

D. Pantothenic Acid

A deficiency of this vitamin during lactation in the rat was first demonstrated by Morgan & Simms (1939, 1940) with rats subjected to a chronic, partial deficiency in "filtrate factor" (primarily pantothenic acid). The offspring of such deficient rats had normal body weights at birth but did not survive to weaning. Daniel *et al.* (1942) found that suckling young from normal females placed on a pantothenic acid-deficient diet shortly before parturition were characterized by sudden respiratory failure, with death occurring between the 10th and 20th days of life. When Long-Evans rats, together with their

litters, were given a pantothenic acid-deficient diet during lactation, the young survived to weaning but their growth was markedly retarded (Table VIII). Characteristic signs of the deficiency such as greying of the fur, porphyrin deposition and dermatitis, were never seen in the young before weaning, although lipid depletion in the adrenals was observed at autopsy. The lactating mothers lost considerable weight during this period.

The levels of calcium D-pantothenate usually used in purified diets for lactation studies have been 4 to 5 mg per 100 g of diet (Table IX). The minimum level necessary for optimal lactation of Long-Evans rats, as judged

TABLE X

PANTOTHENIC ACID REQUIREMENT FOR LACTATION[1]
(Three-week test with Long-Evans rats)

Pantothenic acid (μg/100 g diet)	No. of litters	No. of young	Young weaned, day 21 (%)	Av. wt. of young, day 21 (g)	Wt. change of mother during lactation (g)	Av. daily food intake (g)
0	12	72	97	30 ± 2	-51 ± 3	26 ± 1
250	11	66	97	37 ± 2	-28 ± 4	30 ± 1
500	12	72	97	45 ± 2	-10 ± 5	31 ± 1
750	11	66	97	47 ± 2	$+6 \pm 5$	34 ± 1
1000	11	66	98	53 ± 2	$+15 \pm 3$	38 ± 1
			Non-lactating rats			
0	12	—	—	—	-15 ± 4	14 ± 1
250	12	—	—	—	$+1 \pm 4$	16 ± 1

[1]The basal purified diet contained 18 % casein, 0·5 % L-cystine and 10 crystalline B vitamins (see Table IX).

by weaning weights of young and maternal weight gain, was found to be 1 mg pantothenic acid per 100 g of diet[2] (Table X). This requirement, equivalent to 380 μg daily, for lactation may be compared with the requirement for non-lactating rats of similar ages and body weights, i.e., < 25 μg daily (Table X) (Unna & Richards, 1942). Accentuation of the maternal deficiency by lactation was shown by the smaller weight loss of non-lactating rats given the deficient diet during the same period (Table X).

[2]The basal diet used in this study contained 18 % alcohol-extracted casein with 0·5 % L-cystine in order to reduce the sparing action of protein on the pantothenic acid requirement of the rat (Nelson & Evans, 1945).

Barboriak *et al.* (1957) have reported recently that rats maintained on purified diets throughout life were rarely successful in weaning litters even when 2 mg of calcium pantothenate per 100 g of diet were employed. However, 83 % of the young were weaned when their mothers had been given 10 mg of the vitamin per 100 g diet. This high pantothenic acid requirement may be due to the longer experimental period, to the use of different strains of rats, or to suboptimal levels of vitamin B_{12}, cystine, and pteroylglutamic acid.

E. Pteroylglutamic Acid (PGA or Folic Acid)

In the early 1940s several laboratories found that purified diets supplemented with the five to eight crystalline B vitamins[1] then available resulted in excellent growth and reproduction in the rat but lactation was still below normal (Vinson & Cerecedo, 1944; Richardson & Hogan, 1945; Nelson & Evans, 1947a,b). Nelson & Evans (1947b) also reported that lactational performance was almost as severely affected in a short-term test as in long-term studies of these diets. Synthetic pteroylglutamic acid given during lactation (Nelson & Evans, 1947c) or throughout life (Sica *et al.*, 1948) markedly improved weaning weights of young and maternal body weight.

Table VIII shows that the effects of this vitamin deficiency during the 3-week lactation period were more severe on the lactating mother than on suckling young, as judged by changes in body weight. The lactating mother on this diet also developed a marked leucopenia and granulocytopenia (Nelson *et al.*, 1946) which was prevented by PGA (Table XI). Addition of SST to depress intestinal vitamin synthesis accentuated the vitamin deficiency. In fact, the deficiency was so acute that almost 20 % of the mothers receiving the SST-containing PGA-deficient diet at parturition died before the end of the lactation period (Nelson & Evans, 1948a). High levels of PGA were as effective in the presence as in the absence of SST. Williamson (1949) has reported similar findings on accentuation of PGA deficiency during lactation by SST.

Levels of PGA varying from 100 to 550 μg per 100 g of diet have been used in lactation studies for the rat (Table IX). When Long-Evans rats were placed on purified diets at parturition, the minimal amount of PGA necessary for excellent lactation was found to be 275 μg per 100 g of diet (Nelson & Evans, 1947c).

Similar studies on reproduction and lactation in mice have emphasized the importance of PGA for optimal performance in this species. When mice were reared for four generations on a purified diet supplemented with eight crystalline B vitamins,[1] growth and reproduction were normal whereas

[1]Thiamine, riboflavin, pyridoxine, pantothenic acid, choline, nicotinic acid, inositol and *p*-aminobenzoic acid.

TABLE XI

EFFECT OF SUCCINYLSULPHATHIAZOLE (SST) ON PTEROYLGLUTAMIC ACID (PGA) DEFICIENCY DURING LACTATION[1]
(Three-week test with Long-Evans rats)

Supplement per 100 g of diet	No. of litters	Av. weaning wt. of young (g)	Maternal wt. change (g)	Av. daily food intake (g)	Maternal blood counts at weaning: RBC (millions per mm³)	WBC (thousands per mm³)	Mononuclears (thousands per mm³)	PMN (thousands per mm³)
				Basal diet				
0	55	44 ± 1	− 30 ± 3	26 ± 1	7·37 ± 0·19	4·85 ± 0·27	3·89 ± 0·24	0·96 ± 0·11
275–550 μg PGA	56	49 ± 1	+ 5 ± 2	31 ± 1	8·06 ± 0·13	8·51 ± 0·44	5·84 ± 0·33	2·67 ± 0·16
				Basal diet + 1 % SST				
0	45	36 ± 1	− 61 ± 3	23 ± 1	5·99 ± 0·31	2·66 ± 0·21	2·48 ± 0·20	0·19 ± 0·05
550 μg PGA	41	49 ± 1	+ 10 ± 2	33 ± 1	8·39 ± 0·25	9·19 ± 0·52	5·83 ± 0·41	2·31 ± 0·20

[1]Nelson & Evans (1948a); the basal purified diet is the same as in Table III.

lactation was barely adequate and considerably below that obtained with a stock diet (Foster *et al.*, 1943). Addition of 50 to 1000 μg PGA per 100 g of diet improved lactation in three strains of mice (Cerecedo & Mirone, 1947; Fenton & Cowgill, 1947).

F. Biotin

The necessity of including biotin in a casein-containing purified diet for the rat has been doubted by many investigators. However, Nelson & Evans (1948b) demonstrated in a 3-week lactation test (Table VIII) that addition of 30 to 60 μg biotin per 100 g of diet significantly improved weaning weights of the young. The data showed that a mild biotin deficiency was produced during lactation in the absence of avidin or sulphonamide from the diet. Addition of SST to the diet did not accentuate biotin deficiency during lactation as in PGA deficiency, but the young from such mothers showed signs of biotin deficiency soon after weaning, e.g., alopecia, dermatosis, pigmentation and spasticity.

Previous negative or inconclusive findings regarding the value of biotin for lactation can be understood by noting the weaning weights (45 g) obtained when biotin was omitted from the vitamin supplements (Table VIII). These weaning weights are higher than those reported for many strains of rat on adequate stock diets, yet are significantly below the control weaning weights (49 g) obtained when biotin was included in the vitamin supplement. In addition, the beneficial effects of biotin on lactation were more marked in the presence of dietary PGA than in its absence (Nelson & Evans, 1948b).

All other studies on biotin deficiency during lactation have included a simultaneous deficiency of PGA. Nevertheless, the findings of Kennedy & Palmer (1945) on pregnancy and lactation in rats maintained on a 30 % egg-albumin diet, which was deficient in both vitamins, should be mentioned. Both the lactating mothers and the young showed signs of biotin deficiency. Lactational performance was poor with 2 to 6 μg biotin daily even when 15 % casein was substituted for 15 % egg albumin in the diet.

Levels of biotin varying from 20 to 30 μg per 100 g of diet have been used for lactation studies in the rat (Table IX). Beneficial effects of biotin supplementation (15 to 20 μg per 100 g of diet) for lactation in mice maintained on casein-containing purified diets have been reported by Mirone & Cerecedo (1947) and by Fenton & Cowgill (1947).

G. Inositol, *p*-Aminobenzoic Acid, and Nicotinic Acid

At the present time there is no convincing evidence to indicate that dietary supplies of inositol, *p*-aminobenzoic acid (PABA), or nicotinic acid are

essential for growth, reproduction and lactation in the rat. The studies of Sure (1941b, 1943) and of Climenko & McChesney (1942) on the beneficial effects of inositol or PABA or both on lactation were carried out before biotin and PGA were available. Purified diets ordinarily used contain 20 to 30 % casein and so furnish adequate tryptophan from which the rat may synthesize the required nicotinic acid.

Table VIII shows that omission of either inositol or PABA during lactation in the Long-Evans rat had no deleterious effects on weaning weights of young or maternal body weight. The presence of 1 % SST in a diet deficient in either factor did not change lactation performance. In addition, the omission of inositol during both pregnancy and lactation, i.e., a 6-week test, had no deleterious effects.

Regardless of the lack of evidence, almost all investigators have included these three B vitamin factors in the diets used for lactation studies. The vitamin levels customarily used are 10 to 40 mg inositol, 0·1 to 1 mg PABA, and 2 to 5 mg nicotinic acid per 100 g of diet (Table IX).

H. Choline

In view of the lack of experimental data, the general use of dietary supplies of choline for lactation in the rat is somewhat surprising. The choline requirement for growth, as is well known, varies with the dietary protein level, or more specifically with the dietary methionine level.

Reports on the production of paralytic signs (Sure, 1940) or brain haemorrhages (Jervis, 1942) in suckling rats from mothers reared on diets lacking choline have not been confirmed. However, Brown *et al.* (1947) reported the occurrence of brain haemorrhages in offspring from rats given similar diets and prevention of these changes by vitamin K or lard.

Omission of choline from the diet of Long-Evans rats during lactation (Table VIII) resulted in decreased weaning weights of the young. The levels of choline usually used in purified diets for lactation studies in the rat vary from 50 to 100 mg per 100 g of diet (Table IX).

I. Vitamin B_{12}

The importance of vitamin B_{12} for lactation in the rat was revealed by the use of two different types of diets, one in which the proteins were vegetable in origin and the other in which casein exhaustively extracted with alcohol was the source of protein. When vegetable-protein diets (composed of yellow-corn, soya-bean meal, alfalfa or cottonseed meal) were supplemented with minerals and the known vitamins, lactation in the rat was seriously impaired

(see, e.g., VanLandingham & Lyon, 1947; Zucker & Zucker, 1948). Simultaneous studies by Cary & Hartman (1947) demonstrated impaired lactation resulting from a vitamin-supplemented diet containing casein exhaustively extracted with alcohol. The decreased growth of suckling young and high pre-weaning mortality were prevented by animal proteins (unless highly purified) and by anti-pernicious anaemia liver extracts. Less than 0·5 μg crystalline vitamin B_{12} daily improved weaning weights and prevented early mortality in suckling young from mothers reared either on the alcohol-extracted casein diet (Dryden *et al.*, 1952a) or on vegetable protein diets (Schultze, 1950; Borson *et al.*, 1950).

Vitamin B_{12}-deficient suckling young exhibited decreased growth, kidney hypertrophy or failed to survive to weaning (Dryden *et al.*, 1952b). With a severe deficiency[1] acute uraemia, leucopenia and granulocytopenia occurred with high mortality during the first 3 days of life (Zucker & Zucker, 1948; Borson *et al.*, 1950). The uraemia could be prevented by subcutaneous injections of 0·05 μg vitamin B_{12} within 6 hours after birth (Schultze, 1949; Halvorson & Schultze, 1950). Such vitamin B_{12}-deficient young still showed a high mortality when given to normal lactating rats whereas vitamin B_{12}-deficient mothers were able to raise normal young (Lepkovsky *et al.*, 1951). The high mortality of vitamin B_{12}-deficient young when given to normal lactating rats may be due to congenital defects in the new-born young or possibly to insufficient vitamin B_{12} in the normal rat's milk. Daniel *et al.* (1953) have shown that the vitamin B_{12} content of rat's milk varies directly with the vitamin B_{12} content of the maternal diet, but the efficiency of transfer from diet to milk has not been determined.

Jaffé (1956) maintained rats of the Sprague-Dawley strain for eighteen generations on a vegetable-protein diet supplemented with 5 μg vitamin B_{12} per kg of diet. Increasing the vitamin B_{12} level six-fold did not improve lactation performance which was equivalent to that obtained with a stock diet, i.e. average weaning weights of 42 g. With a diet containing the usual type of "vitamin-free" casein (which has trace amounts of vitamin B_{12}) beneficial effects of vitamin B_{12} supplementation on lactation were not observed in the 3-week test with Long-Evans rats (Table VIII). However, when a 6-week test period was used, beneficial results have been obtained (Nelson & Evans, unpublished). As a precaution, 25 to 50 μg vitamin B_{12} per kg of diet are usually added to purified diets for long-term generation studies.

In mice maintained on a vegetable-protein diet (20 to 25 % protein), 3 μg vitamin B_{12} per kilogram of diet prevented all signs of vitamin B_{12} deficiency, and were just as effective for reproduction and lactation as 30 μg vitamin B_{12} per kg of diet (Jaffé, 1954). When mice were maintained on similar diets

[1]Vitamin B_{12} deficiency may be accentuated by high protein levels, dietary lactose, or by diets containing thyroid-active materials (see Zucker & Zucker, 1950).

containing 40 % protein, a single injection of 3 μg vitamin B_{12} given to the mother before parturition almost completely prevented the high mortality of suckling young (Jaffé, 1951).

J. Additional Factors

Additional factors necessary for survival of suckling young rats have been proposed but not confirmed. In the majority of such studies, diets composed of crude or partially purified foodstuffs have been used, and it has not been shown that all known dietary essentials were present in optimal amounts and proportions.

Nakahara & Inukai (1933) postulated additional factors needed for lactation by rats maintained on a rice-powder diet. The need for and beneficial effects of these factors, later identified as anthranilic acid (vitamin L_1, Nakahara *et al.*, 1945) and adenylthiomethyl pentose (vitamin L_2, Nakahara *et al.*, 1943) have not been confirmed in other laboratories, e.g. by Folley *et al.* (1942).

Piccioni *et al.* (1951) postulated an "animal protein factor" distinct from vitamin B_{12} which was needed to prevent lactation failure in rats maintained on a diet composed principally of mixed cereal grains. Gander & Schultze (1955) were only partially successful in confirming the need for this factor, later identified as orotic acid[1] by Moruzzi *et al.* (1956). Schultze (1953) observed a curative effect of orotic acid in one lactation study using a purified diet containing soya-bean protein but was unable to demonstrate a prophylactic effect in several other experiments. Moreover, Schultze (1955, 1956) was able to maintain rats for four generations on amino acid diets, and even on completely synthetic diets (Schultze, 1957). These studies by Schultze did not provide evidence for existence of unknown factors for survival of the young, but raise questions regarding amounts and proportions of the known dietary essentials for optimal growth during the lactation period.

K. Ascorbic Acid (Vitamin C)

Only the guinea-pig and the primates are known to require a dietary supply of vitamin C throughout their life cycle. Although a deficiency of ascorbic acid has been studied more fully in the guinea-pig than that of any other dietary factor for this species, there are few studies on vitamin C deficiency

[1]Some relationship of orotic acid to vitamin B_{12} appears likely as addition of 10 mg orotic acid per kg of vitamin B_{12}-deficient diet was only slightly less effective than that of 20 μg vitamin B_{12} for growth and increased liver transmethylase activity (Marchetti *et al.*, 1956).

during lactation. Mouriquand *et al.* (1936) reported that macroscopic signs of scurvy resulted in young guinea-pigs suckled by mothers given a scorbutogenic diet during lactation. Day (1947) found that when guinea-pigs with two young were placed on a vitamin C-deficient diet at parturition, weaning weights of the young were subnormal and the lactating mothers lost approximately 31 % of their body weight during the 20-day period. Both mother and young showed scorbutic changes in the bones and dental tissues. Daily supplementation with vitamin C (given as orange juice) improved weaning weights of young and maternal body weight in proportion to the dosages given. Protection against microscopic changes in dental tissues in mother and young and the rate of incisor growth in the lactating mothers were likewise proportional to the vitamin dosages. The highest dosage given (8 ml daily) was not as effective as supplementation with cabbage and lettuce suggesting either sub-optimal vitamin C levels or the presence of other dietary deficiencies in the basal diet.

Crampton & Bell (1947) reported that 5 mg of ascorbic acid daily satisfied the requirement for pregnancy (and lactation) in guinea-pigs, though optimal performance was not obtained with the diets used. Purified diets used for generation studies with guinea-pigs furnish 15 to 25 mg ascorbic acid daily, e.g., those of O'Dell *et al.* (1957) and Everson & Hurley (1958).

V. Fat-soluble Vitamin and Lipid Requirements

The rat and the mouse both require dietary sources of vitamins A and E and of the essential fatty acids. When differences in body weight are considered, the requirements appear to be similar except for the lower vitamin A requirement of the mouse (McCarthy & Cerecedo, 1953). In comparison with the rat, the guinea-pig has a limited ability to convert carotene and also to store vitamin A, so is considerably more sensitive to the lack of this vitamin (Bentley & Morgan, 1945). In addition, the guinea-pig is more sensitive than the rat to deficiencies of vitamin E and essential fatty acids and may require dietary vitamin K for pregnancy and lactation (Reid, 1958). The rabbit is known to require vitamins A and E (see (U.S.A.) National Research Council, 1954).

A. Vitamin A

The need for vitamin A during lactation in the rat was first shown by Mason (1935) who reported that rats subjected to a moderate vitamin A deficiency frequently delivered stillborn young. The living young usually survived less than 5 days, but the few that were weaned exhibited xeroph-

thalmia and subnormal body weights. Other investigators have reported similar findings, and in addition have noted retardation in retinal development (Tansley, 1936), abnormalities in dental tissues (Mellanby, 1941) and hydrocephalus (Rokkones, 1955). The incidence of hydrocephalus in suckling rats from mothers on a diet low in vitamin A (i.e., 15 i.u. daily) increased to 100 % after several successive reproductive cycles but was prevented by 105 i.u. of the vitamin daily during lactation. Hydrocephalus has also been observed in young rabbits from mothers given a vitamin A-deficient diet for 3 to 4 months before mating (Millen *et al.*, 1953).

In all of these studies vitamin A deficiency was present to some degree during pregnancy as well as during lactation. Thus, it is possible that some of the pathological changes observed were initiated during embryonic development and accentuated during the lactation period. Placental transfer of vitamin A is exceedingly limited in rats and rabbits (Dann, 1932) and in mice (McCarthy & Cerecedo, 1952). For example, the livers of new-born rats contained only 5 to 10 i.u. of vitamin A, even when the vitamin A content of the diet was high and the maternal vitamin A liver reserves varied from 30 to 20 000 i.u. (Henry *et al.*, 1949). Mammary transfer of dietary vitamin A, unlike placental transfer, is not limited, as the vitamin A content of milk and of the liver in suckling rats varied with the maternal dietary vitamin content during lactation (Henry *et al.*, 1949).

Sherman *et al.* (1945) and Sherman & Trupp (1949) reported that the Sherman Diet A,[1] which furnished 30 i.u. of vitamin A per 10 g of diet, maintained adequate reproduction and lactation for fifty-eight generations of rats. When the vitamin A level was increased to 60 i.u. by addition of cod-liver oil, the reproductive period and life span of female rats were prolonged. Increasing the level to 120 i.u. per 10 g of diet resulted in further improvement but 240 i.u. had no greater effect. These findings are in agreement with those reported by Fraps (1947) who tested varying levels of carotene furnished by alfalfa meal and found that amounts equivalent to 67 and 133 i.u. vitamin A per 10 g of diet, were slightly superior to lower levels for lactational performance.

Since optimal lactational performance was not observed in either of these studies (as far as could be ascertained from the data) and since the basal diets used in both cases could be improved in regard to dietary factors other than vitamin A, the question of the vitamin A requirement for reproduction and lactation in the rat needs further investigation. Moreover, it has been demonstrated that many factors influence the vitamin A requirements in mammals, e.g., dietary level of tocopherols, types and levels of dietary fat and proteins, antibiotics, vitamin B_{12}, and stress factors (Rubin & De Ritter, 1954).

[1]Composed of $\frac{5}{6}$ ground whole wheat and $\frac{1}{6}$ dried whole milk plus NaCl.

Inasmuch as dietary carotene is frequently the major source of vitamin A, factors affecting its utilization must likewise be taken into consideration.

B. Vitamin D

The specific antirachitic activity of vitamin D can be demonstrated in the rat only by the use of special diets containing abnormal proportions or restricted amounts of calcium and phosphorus.[1] The usual rachitogenic diets contain cereals (with phytate phosphorus) and are free of vitamin D and low in phosphorus, with a calcium to phosphorus ratio of 4 : 1 or 5 : 1.

When diets containing the usual amounts and proportions of calcium and phosphorus were used, vitamin D appeared to be unessential or needed in very small amounts. For example, Bethke *et al.* (1932) concluded that the most favourable Ca : P ratio for growth and bone formation in the rat was between 1 : 1 and 2 : 1 and that the vitamin D requirements were minimal at these ratios. Cox & Imboden (1936) reported good to excellent growth, reproduction and lactation with Ca : P ratios of 1 : 1 to 1·7 : 1 in semi-purified diets containing only trace amounts, if any, of vitamin D. These findings are typical of those reported by many investigators, so there seems to be general agreement that vitamin D supplementation is not needed by the rat when the diet contains favourable amounts and ratios of calcium and phosphorus. However, in view of the invariable occurrence of negative calcium balances in lactating rats, the vitamin D requirement during this period needs further investigation.

C. Vitamin E

The importance of vitamin E for the rat during lactation was first demonstrated by Evans & Burr (1928b). Second generation suckling young, from deficient mothers which had received a single dose of vitamin E at the beginning of pregnancy, suddenly developed paralysis a few days before weaning. Many young died but a few showed spontaneous recovery. This acute paralytic syndrome, termed muscular dystrophy and characterized by hyaline necrosis of the skeletal muscle (Olcott, 1938), has also been observed in suckling mice from vitamin E-low mothers (Goettsch, 1942), though in lower incidence. Herbivorous animals such as the guinea-pig and the rabbit developed muscular dystrophy from this deficiency during the first generation (Mason, 1944).

Placental transfer of vitamin E appears to be limited, a condition similar to that observed for vitamin A and other fat-soluble vitamins. Mason & Bryan (1940) found that the concentration of vitamin E in the uterus and

[1] In addition, it may be necessary to prevent exposure of the animals to sunlight, or at least to ultraviolet light.

placenta of pregnant rats was five times greater than in the full-term foetus or new-born rat. After 24 hours of suckling, the vitamin E content of new-born rats increased more than three-fold, indicating mammary transfer of this vitamin and also the high vitamin E content of colostrum. The fact that a single dose of α-tocopherol administered to the mother at littering protected the young from developing muscular dystrophy 3 weeks later also demonstrated mammary transfer of this vitamin (Evans & Emerson, 1940). Similar findings in regard to placental and mammary transfer and the high vitamin E content of colostrum have been reported in other mammals, e.g., by Parrish *et al.* (1947, 1950).

Evans & Emerson (1940) reported complete protection of the young from muscular dystrophy when a single dose of 10 mg α-tocopherol was administered to the mother at parturition or when the young were given 1 mg daily beginning with the 10th day or 3 mg daily beginning with the 15th day of lactation. Goettsch & Ritzmann (1939) had earlier observed protection of the young when dosage levels as low as 0·33 mg were given to the young from the 10th to the 25th day of lactation. When 0·75 mg daily of α-tocopherol was given prophylactically to female rats from weaning through three pregnancy and lactation periods, there was still a low incidence of dystrophy in the third litter (Evans & Emerson, 1943). However, a daily intake of only 0·10 mg was sufficient for the birth of living young. In all of these studies the usual high-fat, vitamin E-low diet containing cod-liver oil was used. When the diet was low in fat and cod-liver oil, the vitamin E requirement was considerably lower for growth and reproduction and presumably also for lactation (Gottlieb *et al.*, 1943; Emerson & Evans, 1944).

D. Vitamin K

There is no convincing evidence that dietary sources of vitamin K are required by the rat unless a sulphonamide (Daft & Sebrell, 1945) is used. In the few studies which have reported the occasional occurrence of hæmorrhage associated with low blood prothrombin (e.g., Dam & Glavind, 1939; Greaves, 1939), the semi-purified diets used have not been optimally supplemented with all known dietary essentials. It is not known whether the brain haemorrhages observed by Brown *et al.* (1947) in new-born or suckling young from mothers given diets deficient in vitamin K (and prevented by vitamin K or lard) were accompanied by decreased blood coagulation. As a precaution, however, purified diets used for rats usually contain 25 to 50 μg 2-methyl-1, 4-naphthoquinone (menadione, menaphthone) per 10 g of diet.

Dietary vitamin K may be necessary for pregnancy and lactation in guinea-pigs (Hogan & Hamilton, 1942; Reid & Briggs, 1953) and in rabbits (Hogan & Hamilton, 1942).

E. Essential Fatty Acids (EFA)

The importance of the essential fatty acids for lactation in the rat has been known since the original extensive studies by Evans *et al.* (1934a). When rats of the Long-Evans strain were reared to maturity on a basal "fat-free" diet[1] and then bred, 80 % of the young were born dead and the remainder died soon after birth. Supplementation with 150 mg daily of the unsaturated fatty acid preparation (80 % purity and equivalent to 120 mg linoleate) made lactation possible, but only one-half of the young were weaned and the weaning weights were considerably below normal (28 g). Twenty-five per cent butterfat or lard in the diet resulted in lactation closely approaching that produced by the stock diets used at that time, i.e. 88 % of the young were weaned with average weights of 39 g. High dietary levels of saturated fatty acids furnished by 60 % hydrogenated coconut oil produced the same failure in lactation as the basal, fat-free diet (Evans *et al.*, 1934b), and supplementation with essential fatty acids made lactation possible but not normal. These findings have been confirmed by many investigators who reared rats to maturity on similar or better-supplemented semi-purified or purified diets (e.g., Mackenzie *et al.*, 1939; Quackenbush *et al.*, 1942; Kummerow *et al.*, 1952; Deuel *et al.*, 1954, 1955).

A less severe deficiency can be produced by giving the fat-free diet during pregnancy according to Guggenheim & Jurgens (1944). Suckling young from such mothers developed scaly necrosis of the tail during the first 10 days, and then grew slowly with a high mortality during the 3rd week. Daily dosage with 50 to 100 mg linoleate was necessary for curative effects. Using the same procedure Van Dam (1948) observed the scaly tail syndrome in the young of the second litter from mothers maintained on a fat-free diet.

The rapid production of this deficiency during pregnancy and lactation is probably made possible by the limited placental transfer of essential fatty acids from maternal to foetal tissues. New-born young from mothers maintained on a fat-free diet contained no linoleic acid in their tissues at birth. However, some linoleic acid was present in maternal tissues but in smaller amounts than found in the controls (Bernard & Bodur, 1946). Decreased tissue levels of phospholipids, especially those containing arachidonic acid, have been reported by other investigators in similar young (Kummerow *et al.*, 1952; Beauvallet & May, 1953). Transfer of essential fatty acids from the maternal diet to the milk is apparently efficient in rats since Hallanger & Schultze (1957) have shown that the EFA concentration of rat's milk may be

[1]No diet except a completely synthetic one can actually be "fat-free." The semi-purified or purified diets used in the studies discussed in this Section contained 0·2 to 0·6 % lipids (Mackenzie *et al.*, 1939) but were low in or free of the essential unsaturated fatty acids (linoleic, linolenic and arachidonic acids).

markedly elevated by increased dietary consumption, i.e., "a 5·2-fold increase in dienoic acid content of the diet brought about a 3·6-fold increase in dienoic acid of milk fat."

Approximately 100 mg daily of linoleic acid (given as the methyl or ethyl ester) have usually been recommended for lactation in the rat (by e.g. Quackenbush *et al.*, 1942). Deuel *et al.* (1954, 1955) carried out two separate studies in which daily levels of linoleate varying from 10 to 100 mg were used

TABLE XII

REQUIREMENT OF METHYL LINOLEATE FOR PREGNANCY AND LACTATION IN RATS OF THE U.S.C. STRAIN[1]
(Litters limited to seven young 3 days after birth)

Methyl linoleate (mg/rat/day)	No. of litters	No. of young per litter	Survival, day 3 (%)	Young weaned, day 21[2] (%)	Av. weaning wt. of young (g)
0	14	4·9	0		
	14	7·1	0		
10	16	6·9	92	0	
	14	9·6	25	26	
20	16	7·5	57	68	23
	15	9·7	59	86	26
40	15	7·2	73	84	26
	15	8·7	80	88	29
80–100	16	7·2	86	87	29
	15	9·8	84	86	30

[1]Modified from Deuel *et al.* (1954, 1955).
[2]Calculated from the number of young living on day 3.

(Table XII). They concluded that the requirement for pregnancy and lactation in rats of the U.S.C. strain was 80 to 100 mg daily. Inasmuch as optimal weaning weights were probably not attained, the requirement may have been underestimated. Schultze (1957) used a lower level, 55 to 60 mg daily, in a synthetic diet which maintained rats for four generations, but weaning weights were subnormal. Arachidonic acid was found to be as effective as linoleic acid whereas linolenic acid was relatively ineffective for lactation (Quackenbush *et al.*, 1942). However, combinations of linolenic with linoleic acid were as beneficial for lactation as equivalent amounts of linoleic acid (Deuel *et al.*, 1955).

F. Fat *per se* (Other Lipid Factors)

Whether fat *per se* or components of natural fats and oils, other than the essential fatty acids, are required for optimal lactation and other functions in the rat has long been controversial. Deuel (1955) concluded that the beneficial effects of high-fat diets on pregnancy and lactation could be "largely, if not entirely, ascribed to their essential fatty acid content." In some studies, lactational performance as judged by weaning weights of young has improved progressively with increasing dietary levels of fat, e.g., in those of Maynard & Rasmussen (1942), Loosli *et al.* (1944), Scheer *et al.* (1947). In other studies higher levels of fat did not improve lactation or may even have been deleterious (Deuel *et al.*, 1947; Nelson & Evans, 1947 a,b; French *et al.*, 1952). However, the use of 3 to 5 % of corn or cottonseed oil has usually resulted in better lactational performance than supplementation with minimal levels of 50 to 100 mg linoleic acid daily (see, e.g., Schultze, 1957). In all of these studies the levels of dietary essential fatty acids have varied for each experimental group, and conclusions cannot be drawn until equal amounts of essential fatty acids with and without supplementary fatty acids or triglycerides have been tested.

Recent reports on the beneficial effects of cholesterol (Raulin, 1954) and squalene, an intermediate in cholesterol biosynthesis (El Ridi *et al.*, 1955) for lactation in rats maintained on diets presumably adequate in the essential unsaturated fatty acids have not yet been confirmed. The basal diets for these studies contained 5 % lard with 10 % margarine, or 9 % lard, respectively.

No significant differences in lactational performance have been observed when rats were maintained on diets with 10 to 40 % fat from different sources: e.g., cottonseed oil as compared with margarine fat for one generation (Deuel *et al.*, 1947); butter or margarine for five generations (von Euler *et al.*, 1947); butter fat or margarine fat for forty-six generations (Deuel *et al.*, 1950; Alfin-Slater *et al.*, 1957); and butter or hydrogenated vegatable oil for four generations (Dryden *et al.*, 1957). Purified diets used for lactation studies in the rat usually contain 5 to 15 % fat furnished either by natural vegetable oils or by their hydrogenated products.

VI. Water and Calorie Requirements

A. Water

Although water is of extreme importance in any dietary regimen, there are few studies on either water intake or the effects of water restriction in laboratory animals. Crampton & Lloyd (1954) reported that a 50 % restriction of water intake in young growing rats resulted in an immediate reduction in

food intake to 73 % of the *ad libitum* intake; this was followed by decrease in efficiency of food utilization by more than 30 %, and depression of the growth rate. In a similar study Sarett & Snipper (1956) found that restriction of water intake to 80 % and 60 % of the voluntary consumption decreased the food intake of young growing rats to 85 % and 78 % of control values when the animals were given a powdered milk diet. When the diet contained added carbohydrate, water consumption was less and the effects of water restriction on food intake were much less marked. The increased water requirement of high-protein and mineral-containing diets and the water-sparing effect of carbohydrate have long been known (Adolph, 1933). Similar studies employing lactating rats have not been reported but the effects would probably be similar, if not more severe.

The daily water intake of adult female rats, approximately 250 g in body weight, averaged 20 g and increased as much as four-fold during the peak periods of lactation (Bruce, 1950). In self-selection studies, Richter (1955) observed that the voluntary fluid intake of adult female rats, which ranged from 32 to 38 ml daily, increased to 51 to 67 ml daily during lactation. However, this fluid intake included separate solutions of calcium lactate, potassium chloride, dibasic sodium phosphate, and sodium chloride as well as drinking water.

B. Food and Calorie Intake

Food consumption and calorie intake during the lactation period usually average two to three times those of non-lactating rats. The food intake increases progressively during this period (see Russell, 1948). Reported values for average daily food intakes of lactating rats have varied from 27 to 42 g; all values included the food eaten by the young after 16 to 18 days of the lactation period. The average daily food consumption varies with the number of young suckled (Slonaker, 1925; Murray, 1941), the length of the lactation period (21 to 30 days), the level of lactational performance (Hitchcock, 1927, and this paper), the composition and calorie value of the diet, the strain of rat, etc. Nelson & Evans (1948c) (see Table I) observed an average daily intake of 35 g when Long-Evans rats with litters of six young were maintained on a stock diet of natural foodstuffs; weaning weights of the young at 21 days averaged 49 g. When a purified diet containing 30 % casein and furnishing more than 4 kcal per g of diet was used, the daily food intake averaged 33 g and weaning weights of 53 g resulted (Table I). These daily intakes were 250 to 260 % of those observed in non-lactating rats maintained on the same diets. Mirone (1948) reported that the food intake of mice during a 21-day lactation period on purified diets averaged 7·5 g daily or 214 % of the average intake for growth.

The calories available for milk production, after allowance for maintenance, have been estimated by Slonaker (1927) to be 279 kcal per suckling rat for 22 days of lactation, and by Hitchcock[1] (1927) to be 234 kcal. Optimal lactational performance as judged by weaning weights of the young was not obtained in either study. Brody & Nisbet (1938) have shown that the gross energetic efficiency of milk production (i.e., the ratio of milk energy to food energy consumed) is the same for the rat and the dairy cow, within the limits of the experimental methods used. The volume of milk produced by the rat, as judged by its calorie value, may exceed 50 ml daily during the 3rd week of lactation (Brody & Nisbet, 1938).

The immediate effects of food restriction or fasting on milk production in the rat have not been studied by many investigators. Brody *et al.* (1938) have shown that milk production declined from 3 g to zero within 12 hours after food was removed from lactating rats; with refeeding, milk production returned to normal levels. Milk production was measured by the weight increase in the litter during 45-minute nursing periods 3 hours apart. These investigators concluded that normal lactation or milk production did not occur in the absence of food, i.e., in starvation, fasting, or the post-absorptive state. Preliminary studies by Nelson & Evans (unpublished) have indicated that lactational performance, as judged by weaning weights of young and mother, decreased proportionately with progressive restriction of an optimal lactation diet. The mechanism of this effect is unknown but the effects of food restriction on milk production are similar to those observed for protein restriction. The observations of Meites & Reed (1949) on the decreased lactogenic hormone content in the pituitaries of rats subjected to 50 % or more food restriction may be pertinent to this problem.

C. Carbohydrates

There is no direct evidence that dietary carbohydrate for the rat has any essential function other than supplying energy needs for growth, reproduction and lactation. Follis & Straight (1943) reported good growth in twelve rats maintained on a purified, carbohydrate-free diet for 77 days. Two female rats maintained on this diet were bred and had litters. One litter was weaned. The substitution of 10 % sucrose for 5 % of the protein and 5 % of the fat in this diet had no effect on growth, reproduction, or lactational performance. In diets for rats, mice, guinea-pigs and rabbits, carbohydrate has been supplied either as a mixture of different components or as a single source with little, if any, effect on lactational performance. The relation of dietary carbohydrate levels to optimal lactational performance has not been studied in these species,

[1]Estimated by Russell (1948) from the data given by Hitchcock (1927).

presumably because dietary levels of protein and fat have been considered more important.

VII. Summary

This review of the literature and new experimental data has emphasized the fact that there are increased dietary needs during lactation and that severe deficiencies can be produced during this period of physiological stress. This has been clearly established in the rat. The requirements of lactating rats for the majority, if not all, of the essential nutrients are at least two to ten times the amounts needed by non-lactating rats, whether these requirements are expressed in proportion to body weight, as daily intakes, or as dietary levels. This increase is probably due to the two-fold demand for dietary essentials during this period: (1) the maternal needs for the production of milk for the needs of the rapidly growing and developing young, and (2) maternal needs for body maintenance.

Two dietary deficiencies, those of protein and calories, appear to have an immediate effect on milk production, with the volume of milk being proportional to the protein and calorie intake. Other dietary deficiencies such as a lack of vitamin B_6 or vitamin E apparently affect the quality or composition of the milk rather than the quantity produced. Still other deficiencies such as of thiamine and potassium, which result in markedly decreased protein and calorie intakes, probably affect both the volume and composition of milk. However, accurate data on the changes in the volume and composition of milk are lacking for the majority of the dietary deficiencies.

The severity of a deficiency during lactation will vary with the magnitude of the requirement in relation to body stores in both mother and young and the intake furnished by the diet and by faeces (inasmuch as coprophagy cannot be prevented during this period). Although all dietary essentials are stored to some extent, severe effects can be produced in normal rats and their new-born young in the 3-week lactation test by deficiencies of protein, calories, sodium, potassium, and several of the B vitamins. However, deficiencies of the fat-soluble factors and of many of the trace elements such as iron, copper, and manganese require lengthy depletion periods and may not be observed until the second generation.

The effects of dietary deficiencies during lactation are usually more severe in lactating mothers than in the suckling young, as progressive depletion of maternal body stores protects the young. The loss of maternal body weight in deficiencies of protein (and calories), sodium, potassium, and thiamine may exceed 30 % in the 3-week lactation period. Deficiency signs such as bone demineralization with inadequate calcium and phosphorus intakes, anaemia in iron, copper, protein and pteroylglutamic acid deficiencies, and fatty livers

TABLE XIII

PROVISIONAL ASSESSMENT OF DIETARY REQUIREMENTS FOR LACTATION IN THE RAT

Dietary factor	Investigators	Requirements per 100 g of diet: Reported requirements	Suggested amounts	
Protein	Kao *et al.*, 1941; McCoy, 1947	20–25 g mixed proteins	25 g mixed proteins	
	Mueller & Cox (1946)	24–30 g casein	30 g casein or more unless supplemented with cystine or methionine	
	Nelson & Evans (1948c)			
Amino acids	Nelson & Evans (1958)	1 g or more SAA	Both essential and non-essential amino acids, L-forms (see Table IV)	
Calcium	Cox & Imboden (1936)	0·5–0·7 g	0·6–0·7 g	
Phosphorus	Cox & Imboden (1936)	0·4–0·5 g	0·4–0·5 g	
Ca/P	Cox & Imboden (1936)	1 : 1 to 1·5 : 1	1·5 : 1 (McCoy, 1949)	
Potassium	Heppel & Schmidt (1938)	0·5–0·6 g	0·5–0·6 g	
	Nelson & Evans, this paper			
Sodium	Olson & St. John (1925)	0·5 g	0·3 g	(See Table V)
	Nelson & Evans, this paper	0·3 g or less		
Chlorine		(1)	0·3–0·5 g	,,
Magnesium	Tufts & Greenberg (1938)	50 mg or more	50 mg	,,
Iron	Daniels & Everson (1935)	15–20 mg or less	20 mg	,,
Copper	Daniels & Everson (1935)	2–3 mg or less	2–3 mg	,,
Manganese	Daniels & Everson (1935)	3–5 mg	5–10 mg	,,
	Frost (1951)			
Zinc (mice)		(1)	0·4–0·5 mg	,,
Iodine	Parker *et al.* (1951)	10–25 μg	50–100 μg	,,
Bromine, fluorine, molybdenum and selenium		(1)	1–10 μg	

Thiamine		(1)	0·5–2 mg	(See Table IX)
Riboflavin		(1)	1–2 mg	,,
Pyridoxine		(1)	0·5–2 mg	,,
Pantothenic acid	Nelson & Evans, this paper	1 mg	5–10 mg	,,
	Barboriak *et al.* (1957)	2–10 mg		
Pteroylglutamic acid	Nelson & Evans (1947c)	275 μg	0·5–2 mg	,,
Biotin	Nelson & Evans (1948b)	30 μg or less	10–30 μg	,,
Vitamin B_{12}	Jaffé (1956)	0·5 μg (2)	2–5 μg	,,
	Richardson & Brock (1956)	1·0 μg or less		
p-Aminobenzoic acid		Not needed in the rat	0·1–1 mg	,,
Nicotinic acid		,,	1–5 mg	,,
Inositol		,,	10–100 mg	,,
Choline		(1)	100 mg	,,
Vitamin C		Not needed in the rat	None	
Vitamin A	Sherman *et al.* (1945, 1949)	1200 i.u.	2500 i.u.	
Vitamin D	Bethke *et al.* (1932)	Unnecessary with Ca/P between 1·0 to 2·0 although 300 i.u. slightly beneficial	100 i.u. (Cuthbertson, 1957)	
Vitamin E	Evans & Emerson (1940)	2–3 mg	5 mg	
Vitamin K		Not needed by the rat unless intestinal synthesis inhibited	0·5–2 mg	
Essential fatty acids	Deuel *et al.* (1954, 1955)	300–400 mg	0·5–1·0 g	
Fat		Need has not been proven	5–15 g	

[1]No satisfactory data available.

[2]Vegetable-protein diets.

TABLE XIII.—*continued*

PROVISIONAL ASSESSMENT OF DIETARY REQUIREMENTS FOR LACTATION IN THE RAT

Dietary factor	Investigators	Requirements per 100 g of diet	
		Reported requirements	Suggested amounts
Water		2–3 times that needed for non-lactating rats	*Ad libitum*
Calories		2–3 times that needed for non-lactating rats	*Ad libitum*
Carbohydrate		Not needed by the rat	50–65 g
Bulk		Not needed by the rat	None

with certain amino-acid deficiencies also indicate maternal depletion. However, in the complete absence of protein or calories or when the intake falls below the critical levels, milk production ceases in spite of considerable maternal protein stores, and the young are unable to survive.

The suckling young are more severely affected than the mothers in deficiencies of vitamin B_6, vitamin B_{12}, manganese, and the fat-soluble vitamins. In these deficiencies inefficient mammary transfer or greater sensitivity of the suckling young may be concerned. It is known that limited placental transfer of the fat-soluble vitamins results in new-born young with very low vitamin stores. Pathological changes reported in suckling rats subjected to different dietary deficiencies include the following: hyperexcitability and convulsions (magnesium), anaemias (iron and copper), skeletal abnormalities and ataxia (manganese), embryonic thyroid (iodine), loss of fur (riboflavin), convulsions and epileptiform fits (vitamin B_6), loss of fur and spasticity (biotin), kidney hypertrophy and uraemia (vitamin B_{12}), xerophthalmia, dental and bone abnormalities and hydrocephalus (vitamin A), muscular dystrophy (vitamin E), and dermatosis (essential fatty acids).

At the present time there is not sufficient information to do more than propose tentative approximations of the dietary requirements for lactation in the rat (Table XIII). This table shows that few lactation studies have been carried out with diets complete according to present nutritional knowledge. Much remains to be discovered about the interrelations of nutritive constituents during lactation, and the highly important field of nutritional-endocrine interrelations during the period has barely been explored.

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CHAPTER 17

The Composition of Milk and the Nutritive Value of its Components

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I. Introduction

Milk is the natural food of the new-born mammal and must be well balanced and complete to serve for a time as the sole nourishment. The nutritive value of the milk of various species for their young is discussed in Chapter 19 in which also the relationship between the composition of the milk of different species and the development of the young is considered. In this Chapter we are concerned with the chemistry of milk and with the value in nutrition of its various components.

Broadly speaking, the nutritive value of milk depends on its composition, and we have to deal therefore with those factors affecting the composition of milk as secreted. Under natural conditions milk passes directly from mother to offspring, but before it is used as an article of human diet it usually undergoes one or more stages of processing, each of which may cause chemical or physical changes which in turn may affect the nutritive value of the product. As a discussion of milk technology is outside the scope of this book, we do not consider the effects of any such procedures.

At the time of its secretion milk contains two liquid phases, fat and water, between which are partitioned at least forty chemical compounds. Dissolved in the fat, or held at the fat globule surface are numerous compounds such as phospholipids, sterols, carotenoids and fat-soluble vitamins. The aqueous phase holds in solution lactose, the water-soluble vitamins, and some of the minerals; it also carries in the colloidal condition proteins and the remainder of the minerals. Three physical states, solution, emulsion and colloidal suspension, are therefore in very intimate association; so intimate that changes in any one are not without effect on one or both of the others.

THE MAIN GROUPS OF MILK CONSTITUENTS

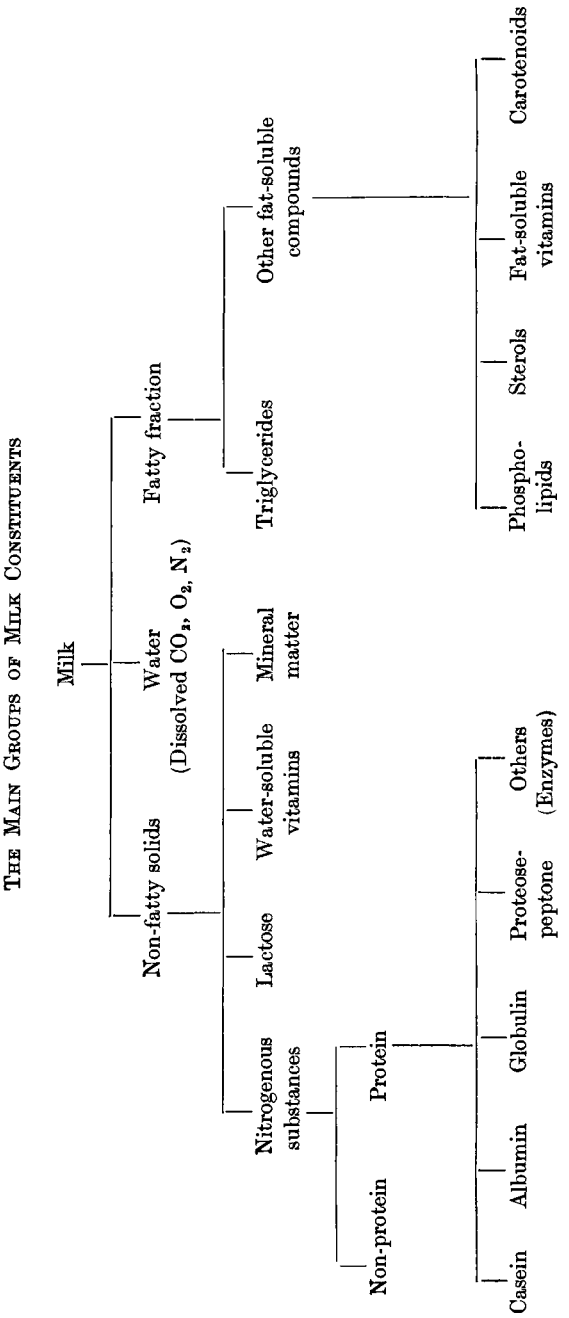


TABLE I

REPRESENTATIVE VALUES FOR SOME MAJOR CONSTITUENTS OF GOOD-QUALITY MILK OF DIFFERENT SPECIES

Species[1]	Fat (g/100 g)	Non-fatty solids (g/100 g)	Protein (N × 6·38) (g/100 g)	Casein (g/100 g)	Lactose (anhydrous) (g/100 g)	Ash (g/100 g)	Physiological energy (kcal/100 g)
Ass	1·5	8·6	2·1	—	6·2	0·40	46
Buffalo, Indian	7·45	9·32	3·78	3·2	4·90	0·78	100
Camel	4·2	8·7	3·7	—	4·1	0·75	70
Ox							
Friesian	3·50	8·60	3·25	2·6	4·60	0·75	62
Guernsey	4·65	9·10	3·65	2·85	4·70	0·77	75
Sheep	7·5	10·9	5·6	4·2	4·4	0·87	105
Goat	4·5	8·7	3·3	2·5	4·4	0·80	71
Llama	3·2	10·8	3·9	—	5·3	—	65
Horse	1·6	8·5	2·2	1·0	6·0	0·40	47
Reindeer	22·5	14·2	10·3	—	2·4	—	250
Pig	8·5	11·6	5·8	3·9	4·8	0·94	120
Yak	7·0	10·9	5·2	—	4·6	—	100

A dash denotes lack of information or unreliable information.

[1]Values for other species will be found in Chapter 19.

TABLE II

REPRESENTATIVE VALUES FOR SOME VITAMINS IN GOOD-QUALITY MILK OF DIFFERENT SPECIES

Species[1]	Vitamin A	Carotenoids[2]	Vitamin A activity[3]	Vitamin D	Thiamine	Riboflavin	Nicotinic acid	Pantothenic acid	Vitamin B_6	Folic acid	Biotin	Vitamin B_{12}	Ascorbic acid
	(μg/g fat)	(μg/g fat)	(i.u./100 g)	(i.u./100 g)	(μg/100 g)	(μg/100 g)	(μg/100 g)	(μg/100 g)	(μg/100 g)	(μg/100 g)	(μg/100 g)	(μg/100 g)	(mg/100 g)
Ass	—	—[4]	—	—	60	30	90	—	—	—	—	—	10·0
Buffalo, Indian	8	Trace	200	—	50	100	80	—	—	—	—	0·3	2·5
Ox													
Friesian	8	7·0	125	1·8	40	150	80	350	35	0·1	2·0	0·5	2·0
Guernsey	6	16·0	190	2·3	40	200	80	350	35	0·1	2·0	0·5	2·0
Camel	—	—	—	—	—	—	—	—	—	—	—	—	6·0
Sheep	8	0·4	200	—	70	500	500	350	—	0·2	9·0	0·3	3·0
Goat	8	Trace	120	2·3	50	120	200	350	—	0·2	1·5	0·1	2·0
Llama	—	—	—	—	—	—	—	—	—	—	—	—	—
Horse	8	3·0[5]	45[6]	—	30	20	50	300	30	0·1	—	0·3	10·0
Reindeer	—	—	—	2?	—	—	—	—	—	—	—	—	—
Pig	4	Trace	100	4·7	68	140	850	400	20	—	1·5	0·2	13·0
Yak	—	—[7]	—	—	—	—	—	—	—	—	—	—	—

A dash denotes lack of information or unreliable information.

[1]Values for other species will be found in Chapter 19.

[2]Active carotenes taken as four-fifths of total carotenoids.

[3]Vitamin A activity: 1 μg vitamin A = 3·33 i.u.
1 μg carotene = 1·67 i.u.

[4]Fat, orange-yellow.

[5]Values up to ten times higher have been reported for Kirgiz mares.

[6]According to some authorities the activity is similar to that of cow's milk.

[7]Milk, rich yellow.

Although some minor constituents may be present in both the fat and aqueous phases, it is convenient to consider milk as a mixture of water, fatty and non-fatty constituents. In the diagram on p. 197 are shown the main subdivisions of each group.

Human milk is considered separately in Chapter 18. Of the other milks, by far the greatest amount of information at present available is on the milk of the cow, but whenever possible the discussion will include the milk of other species. Each constituent will be considered separately, with the important fact in mind that the properties of any one may be extensively modified by the presence in milk of the others.[1]

For a broad comparison of the chemical and nutritional characteristics of the milk of different species the available information on their composition is presented in two tables of which Table I deals with the major components and Table II with vitamins.

II. Lactose and Other Carbohydrates

A. Lactose

1. *Form and Occurrence*

The characteristic carbohydrate of milk is the disaccharide, lactose, specifically elaborated in the mammary gland through the synthetic paths described in Chapter 11. Lactose exists in two stereoisomeric forms, α and β. Each is composed of one molecule of D-galactose and one of D-glucose.

In milk, as in aqueous solutions, both forms are present, the equilibrium mixture consisting of 1 part of α to 1·65 parts of β.

There is some uncertainty about published values for the lactose content of milk. In many publications the percentage of lactose is not given from direct measurement but calculated as the difference 100 — (% fat + % protein + % ash + % water) whereby the combined errors of four determinations are involved. Moreover, some authors give values in terms of the anhydrous substance, others in terms of the hydrated, without necessarily stating which. The values for lactose in Table I are those directly determined, taken from various publications and expressed as anhydrous lactose.

The osmotic pressure of milk is sensibly the same as that of blood and is maintained by the balance of concentration of lactose and of soluble mineral matter (mainly chlorides). Thus any variation in lactose concentration is counterbalanced by one in chloride concentration in the opposite direction. The smaller molecular weight of sodium chloride and its complete dissociation mean that a slight increase in the concentration of the chloride ion counter-

[1]As vitamins in milk are either closely related to the diet or affected by stage of lactation they will appropriately be discussed after the Section dealing with milk composition.

acts a much greater decrease in that of lactose. Thus, as in mastitis (see p. 243), a milk of normal osmotic pressure may have an abnormally low content of non-fatty solids (customarily abbreviated as S.n.F., solids-not-fat).

The more lactose a milk contains, the sweeter its taste, which is particularly noticeable, for example, in mare's and ass's milk. Cow's milk with 4·6 % lactose tastes faintly sweet. As the lactose content slowly declines with advancing lactation, towards the end of lactation, when the lactose : chloride ratio is low, it is quite common for individual samples to have a distinctly salty taste.

Lactose in milk is very readily fermented by lactic acid bacteria and the changes in taste that accompany the conversion of lactose into lactic acid are normally associated with the characteristic odour of souring milk, which, however, is due not to lactic acid, but to various by-products.

The aldehyde group of lactose may couple with free amino groups of proteins when milk is heated.

2. *Lactose in Nutrition*

Lactose, in common with other disaccharides, is normally not absorbed intact, but must be broken down to the simple sugars glucose and galactose before passage into the blood-stream and utilization in the body. Lactase (β-galactosidase), the specific enzyme responsible for the hydrolysis of lactose to D-glucose and D-galactose, occurs in the intestinal mucosa of mammals. It is evident from recent studies (Heilskov, 1956; Kitts *et al.*, 1956; de Groot & Hoogendoorn, 1957; Walker, 1957) that in many species lactase activity is highest during the first days of life and that it declines rapidly thereafter. In the pig, for instance, the activity of the intestinal mucosa has fallen by 8 weeks of age to one-tenth of its initial value (see also Chapter 19).

It seems probable that lactase activity in man also declines with age, though the evidence is conflicting. Thus Koehler *et al.* (1935) found no increase in blood glucose in normal adults after oral doses of 1·5 g lactose per kg body weight whereas Folin & Berglund (1922) and Winter (1931) found galactose in the urine of adults receiving smaller doses of lactose.

Contrariwise, the activities of sucrase and maltase increase during early life (Bailey *et al.*, 1956; Walker, 1957). Thus, whereas the suckling animal can utilize lactose, the growing and adult are less well equipped for a diet high in lactose than for one high in sucrose or products of starch degradation such as maltose. There is no satisfactory proof that continued consumption of lactose after weaning increases or maintains the production of lactase.

Even in young animals the hydrolysis and absorption of lactose appear to proceed somewhat more slowly than those of other disaccharides, since a proportion of the ingested lactose usually passes through the small intestine and reaches the colon.

The problems asociated with the utilization of lactose are not fully elucidated, but interest in its role in nutrition centres on certain characteristic effects not shared by other sugars; some of these are due to galactose liberated on hydrolysis, others are specific to the lactose molecule itself and derive from its less rapid and efficient digestion. The physiological effects of lactose were reviewed by Duncan (1955) and its effects on gastro-intestinal motility by Fischer & Sutton (1949).

Galactose occurs in plant and animal tissues and in certain oligosaccharides such as melibiose and raffinose. In animal tissue it is a constituent of brain cerebrosides. Galactose is metabolized after conversion into glucose which is itself readily convertible into galactose, so that galactose is not an essential constituent of the diet. The stepwise metabolism of galactose is summarized by Kalckar (1957) (see also Chapter 11); briefly the steps are:

1. α-D-galactose + ATP $\overset{\text{galactose kinase}}{\rightleftharpoons}$ α-D-galactose 1-phosphate + ADP

2. α-D-galactose 1-phosphate + UDPG $\overset{\text{galactose 1-phosphate uridyl transferase}}{\rightleftharpoons}$ glucose 1-phosphate + UDP-galactose
(UDPG = uridine diphosphoglucose)

3. UDP-galactose $\overset{\text{uridine diphosphogalactose 4′-epimerase}}{\rightleftharpoons}$ UDPG

In a rare human disease, congenital galactosaemia, infants are unable to metabolize galactose. Kalckar and his co-workers (cf. Kalckar, 1957) have recently shown that the biochemical lesion in galactosaemia is a lack of galactose 1-phosphate uridyl transferase. The condition can be detected by studying the metabolism of galactose by red blood cells taken from cord blood at birth (Schwarz *et al.*, 1958) and, if impaired metabolism is found, the child is given a diet free of lactose and galactose.

High levels of lactose or galactose in the diet of rats and of galactose in the diet of chicks give rise to toxic signs that include the development of cataracts (Mitchell & Dodge, 1935) and paralysis of the hind limbs (Ershoff, 1946) in rats, and nervous disorders in chicks (Dam, 1944). These effects do not become manifest unless the diet contains at least 40 % of lactose. Thus, whereas mineralized liquid skim milk supplemented with vitamins A and D given for prolonged periods to rats caused the development of cataract (Cashell & Kon, 1939), no such effects have been observed with whole milk. In general, in animal studies galactose as such is much more toxic than as present in the equivalent amount of lactose, probably because the slow and incomplete hydrolysis of lactose does not quantitatively liberate galactose for absorption. The exact causes of these disturbances are not yet understood, though Handler (1947) considered that galactose interferes with normal carbohydrate meta-

bolism. Recently Hansen *et al.* (1956) have shown that chicks fed on diets containing 15 % of galactose accumulate hexose-containing uridine nucleotides in the liver and that, whereas glucose is more abundant in UDP-hexose isolated from normal tissue, in chicks showing galactose toxicity the concentration of UDP-galactose is markedly increased. Chicks given diets containing lactose show poor growth and diarrhoea (Rutter *et al.*, 1953), but no signs of galactose toxicity, probably because the lactose is not digested: Hamilton & Mitchell (1924) found little lactase in the crop and none in the intestines of chicks.

The effects deriving from the presence of unabsorbed lactose in the intestinal tract may be either beneficial or frankly detrimental according to the amount present, which is, in turn, related to the level in the diet. Fischer & Sutton (1949) in their comprehensive review of the relation of lactose intake to diarrhoea concluded that young animals are more susceptible to digestive disturbance than older ones. In general, all mammals can tolerate lactose in the diet at concentrations similar to those in milk, but higher levels cause diarrhoea, though not dehydration of the body tissues. It thus seems probable that unabsorbed lactose in the gut, by exerting its osmotic pressure, interferes with the absorption of water. Furthermore, Roccuzzo (1945) showed that solutions of lactose given by mouth, whether hypertonic, isotonic or hypotonic, always led to excretion of less urine than did an equal volume of water.

The major part of the beneficial effect of lactose manifested in many species derives from its well-known role in favouring the establishment and maintenance of a lactobacillus flora and a resulting low pH in the lower gut. Possibly associated with the lowered pH in the gut of rats or chicks receiving lactose is the improvement in the retention of dietary calcium noted by several workers (cf. Duncan, 1955), though Fournier (1955) suggests that the effect is not in the gut, but that galactose formed by hydrolysis of lactose participates in the metabolism of the bone cell.

B. Other Carbohydrates

1. *General*

Milk may contain, in addition to lactose, traces of monosaccharides, and small amounts of oligosaccharides and hexosamines.

Chromatographic examination showed that in cow's milk (Roberts *et al.*, 1954; Sasaki & Taniguchi, 1956) and in sow's milk (Roberts *et al.*, 1954) the trace monosaccharides consist of glucose and galactose, and in human milk of glucose only; rat milk contains neither glucose nor galactose (Roberts *et al.*, 1954).

The oligosaccharides of milk were reviewed by Bell (1955). Much of the interest in these compounds arises from the fact that many of them,

particularly those of human milk, possess Bifidus Factor activity (see below). Deproteinized human milk contains about a dozen oligosaccharides, all of which contain galactose and glucose and most of them fucose and *N*-acetylglucosamine as well (Montreuil, 1957; Malpress & Hytten, 1957). At least three of these compounds are fucose derivatives of lacto-*N*-tetraose (Kuhn, 1957). Deproteinized cow's milk contains a series of oligosaccharides that differ from those of human milk and none of which contains fucose, but mannose is present in two of them (Trucco *et al.*, 1954).

Several investigators have reported the presence of carbohydrates in the milk proteins (cf. Johansson & Svennerholm, 1956). It is not clear whether they are combined with the protein or merely adsorbed on it during precipitation.

2. *Significance in Nutrition*

Oligosaccharides possessing growth-factor activity for *Lactobacillus bifidus* var Penn. are present in human milk and in the milk of other non-ruminants, but are almost absent from the milk of ruminants (György, 1955).

The significance of the Bifidus Factor in nutrition is not yet firmly established, but, as György suggests, it seems possible that the presence of it in human milk may facilitate the establishment of the bifidus flora early in the neonatal period (cf. Chapter 18). It should be emphasized, however, that many strains of *L. bifidus* do not require the Bifidus Factor for growth.

III. Milk Proteins and Other Nitrogenous Constituents

A. Distribution of Nitrogen

Normal cow's milk contains about 0·5 % combined nitrogen which is distributed as shown in the diagram on p. 205.

B. Proteins

The properties of milk proteins are discussed in great detail by McMeekin (1954). The total protein content (N × 6·38) of the milks of different species is given in Table I.

1. *Nomenclature*

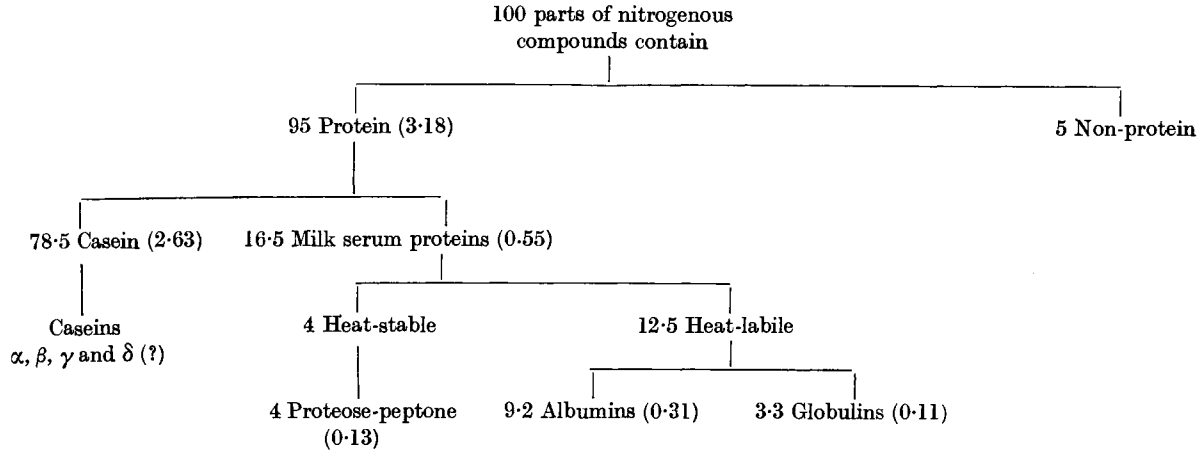
The true proteins of milk may be defined as follows.

Casein. The protein which precipitates when milk is brought to a pH of 4·6, leaving the milk serum proteins in solution.

Milk serum proteins. The fraction soluble at pH 4·6.

Heat-labile proteins. Those which after boiling of milk for 20 minutes are co-precipitated with casein when the pH is brought to 4·6.

THE NITROGENOUS COMPOUNDS OF MILK



Values in parentheses are the percentage concentrations in cow's milk of the protein fractions (Rowland, 1938),

Globulins. The fraction insoluble in half-saturated ammonium sulphate solution.

Albumins. The fraction soluble in half-saturated ammonium sulphate solution.

Proteose-peptone. The fraction not rendered precipitable at pH 4·6 by boiling of milk for 20 minutes.

2. *Casein*

As the principal protein of milk, casein has received a great deal of attention. Although the elementary composition of acid-precipitated casein from cow's milk is invariably constant, the protein is undoubtedly heterogeneous as revealed by study of solubility, sedimentation and electrophoretic behaviour. Mellander (1939) demonstrated that casein was composed of three electrophoretic components, designated α, β and γ in order of decreasing mobility. Casein from cow's milk contains 75 % α-, 22 % β- and 3 % γ-component (Hipp *et al.*, 1952). Cherbuliez & Baudet (1950) claim to have separated a fourth component, δ (probably a proteose). The caseins elaborated by different mammals are closely related though differences are found in the proportions of the fractions. Thus, Dovey & Campbell (1952) showed that goat's milk casein is composed of equal parts of α and β with a small amount of γ. The differences in the amino acid composition of the three main casein fractions are shown in Table III. The fractions also differ in their content of phosphorus and in their isoelectric points:

	α	β	γ	δ
% P	0·98	0·55	0·11	0·55
Isoelectric point	4·7	4·9	5·8–6·0	

Casein is one of the relatively few naturally occurring proteins that contain phosphorus. The biological function of phosphoproteins is unknown, though it is probable that they act as reservoirs of metabolizable phosphorus. Phosphorus can be bound in phosphoproteins as either —O—P— or —N—P— esters. Although phosphorylated derivatives of several amino-acids can be prepared, the only ones so far isolated from casein are the monoesters, *O*-phosphorylserine (Lippmann, 1933a,b) and *O*-phosphoryl-threonine (de Verdier, 1953). It has long been known that casein can be completely dephosphorylated by incubation at 37°C with 1 % sodium hydroxide (Plimmer & Bayliss, 1906), though with some disruption of the protein (Macara & Plimmer, 1940). Enzymic dephosphorylation of intact casein proved more difficult. However, partial tryptic digests of casein yield phosphopeptones that can be readily dephosphorylated by phosphatase (Rimington & Kay, 1926).

Perlmann (1955) in a review of the nature of phosphorus linkages in phosphoproteins, discusses her own work with phosphatases which has

demonstrated that phosphorus occurs also in the form of diesters and pyrophosphates. Thus α-casein contains 40 % of its phosphorus as monoester, 40 % as diester and 20 % as pyrophosphate. In β-casein all the phosphorus is present in the form of diester linkages between peptide chains. A breakage of the cross linkages leads to splitting of both α- and β-casein into smaller sub-units.

Sundararajan & Sarma (1957) recently prepared from ox spleen a protein phosphatase free from proteolytic activity and showed that the enzymic dephosphorylation of unfractionated casein is accompanied by the liberation of a small amount of acid-soluble nitrogen, that is a mixture of peptides probably derived from the hydrolysis of phosphate bridges cross-linking peptides in the intact protein. Dephosphorylated casein has approximately the same amino-acid composition as casein.

Casein prepared by acid precipitation is, apart from the phosphorus forming a structural part of the protein, completely demineralized and possesses sufficient acid groups to release CO_2 from $CaCO_3$. In milk, however, casein exists as calcium caseinate, and the question arises whether all the acid groups have been utilized in binding this base. So far as the milk of the cow is concerned it seems probable that about one-third of the total acid groups is still free in the calcium caseinate complex, which, for this reason, contributes significantly to the titratable acidity of freshly drawn milk (Ling, 1937). In addition to the phosphoric acid which forms part of the casein molecule and which is not extracted by treatment with dilute acid, there are in cow's milk approximately 0·03 % P and 0·06 % Ca closely associated with casein, and easily soluble in dilute acids. This ratio approximates to that in tricalcium phosphate, but as it is by no means constant it is at least possible that dicalcium phosphate is also present. The subject has received a great deal of attention from Pyne (1932, 1934), Ling (1936, 1937), ter Horst (1947), van der Burg (1947), Pyne & Ryan (1950), Evenhuis & de Vries (1955, 1956a,b,c, 1957a,b), Verma & Sommer (1957, 1958) and White & Davies (1958a,b,c,d).

The coagulation of milk by the stomach enzyme, rennin, is of interest not only in its relation to cheese making but also in ensuring that the major protein of milk remains in the stomach for a sufficient length of time to enable gastric digestion to proceed. The calcium caseinate–calcium phosphate complex of cow's milk is converted by rennin under slightly acid conditions to calcium paracaseinate–calcium phosphate, and a soluble proteose is detached. This newly formed complex gives, in the presence of calcium ions, the familiar cheese curd in which a coagulum of fibres enmeshes the fat and much of the whey. Thus, at the moment of clotting, the liquid milk suddenly assumes a solid gel-like condition. The physical nature of the curd varies considerably between "elastic" or somewhat "tough" to "soft" or "open." Though for

cheese making the latter types are not desirable, they are sometimes in demand for infant and invalid diets. Since the curd firmness is largely dependent upon the presence of calcium ions, a convenient household method of softening the curd is to add fruit juices or citric acid to the milk, whereby some of the calcium ions are sequestered.

The behaviour towards rennin of the various fractions of casein is under investigation now in several laboratories and there is evidence of unchanged β- and γ-caseins in the rennet curd, whereas α-casein is found as α_1- and α_2-paracasein (Cherbuliez & Baudet, 1950).

The casein content of the milk of various mammals is shown in Table I. It is noteworthy that milk of the ruminants, buffalo, cow, ewe and goat, which contains at least 2·5 % casein, gives the usual cheese type of curd with rennet, whereas human milk, mare's and ass's milk, low in casein and ash, do not.

3. *Milk-serum Proteins*

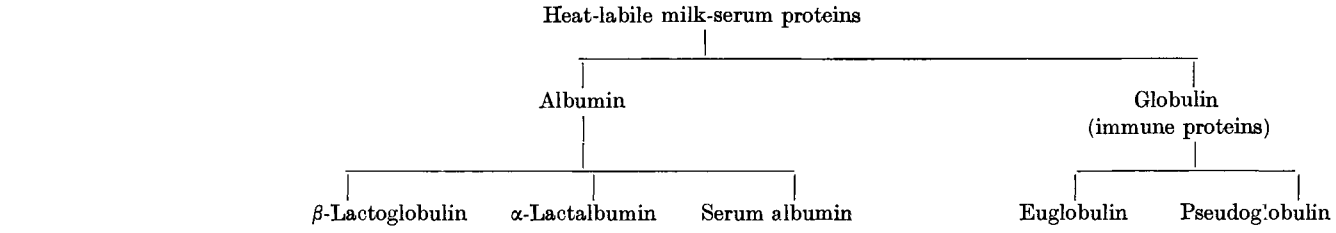
a. *General.* When whey is boiled the traditional albumin and globulin fractions are first denatured and finally precipitated. Heat denaturation of proteins is considered to involve a progressive weakening and ultimate rupture of some of the cross linkages of the polypeptide chains. As a result many reactive groups formerly tied in making the cross links are now exposed; the free polypeptide chains regroup themselves into new structures which usually display an increased sensitivity to precipitation; furthermore, some calcium ions may be sequestered by complex formation with the newly liberated polypeptides. Though it is true to say that all the milk proteins are subject to heat denaturation, casein requires the most drastic heat treatment (in cow's milk more than 30 minutes' heating under pressure at 130°C) before heat coagulation takes place.

When milk is boiled, the milk-serum proteins are denatured, but no precipitation takes place until the pH is brought to 4·6 when they co-precipitate with casein. Published work shows considerable differences in the extent of heat denaturation of the milk-serum proteins. For example, Harland *et al.* (1952) found 32 to 95 % denaturation at 74°C for 30 minutes, and Rowland (1937) observed 80 % denaturation under the same conditions. Some causes of the observed discrepancies are the heterogeneous nature of the milk-serum proteins, and variations in pH and ionic balance between different milk samples.

The components of the traditional albumin and globulin fractions of cow's milk are shown in the diagram on p. 209 and the amino acid composition of the individual proteins is given in Table III.

Each of these fractions has a different sensitivity towards heat denaturation. The immune globulins are the most sensitive, α-lactalbumin the least sensitive

COMPONENTS OF THE TRADITIONAL ALBUMIN AND GLOBULIN OF MILK SERUM



	β-Lactoglobulin	α-Lactalbumin	Serum albumin	Euglobulin	Pseudoglobulin
Quantity in milk (g/100 ml)[1]	0·2–0·4	0·07–0·15	0·02–0·05	0·05	0·11
Isoelectric point	5·18	4·1–4·8	4·7	6·0	5·6
Molecular weight	35 500	15 100	65 000	180 000	
Remarks	Source of R-SH groups in heated milk	High tryptophan content	Identical with blood-serum albumin	Source of milk antibodies	Contain hexoses and hexosamines

[1]Rolleri *et al.* (1955) quoted by Jenness *et al.* (1956).

TABLE III

VALUES FOR THE AMINO ACID CONTENT (g/16 g NITROGEN, I.E. PER 100 g PROTEIN) OF INDIVIDUAL AND TOTAL MILK PROTEINS AND OF WHOLE EGG PROTEIN

As the values for individual proteins are from different sources; recalculation of the values for the total proteins from them will not necessarily tally with the value quoted.

Amino acid	Total milk proteins[1]	Casein[1]	α-Casein[2]	β-Casein[2]	γ-Casein[3]	Milk-serum proteins (lact-albumin)[1]	α-Lact-albumin[4]	β-Lacto-globulin[1]	Immune globulins[5]	Serum albu-min[6]	Mem-brane protein[7]	Whole egg protein[1]
Alanine	3·6	3·1	3·8	1·8	2·4	7·0	2·2	6·8	4·8	6·3	—	—
Arginine	3·5	4·2	4·4	3·5	2·0	3·1	1·2	2·9	4·1	5·9	9·3	6·7
Aspartic acid	7·5	6·5	8·7	5·1	4·2	11·1	18·8	11·7	9·4	10·9	6·3	8·2
Cystine	0·9	0·4	0·4	0·1	0·0	2·7	6·6	3·0	3·2	6·5	1·4	2·3
Glutamic acid	21·7	23·6	23·2	24·1	23·8	17·7	13·0	19·8	12·3	16·5	—	12·6
Glycine	2·1	2·1	2·9	2·5	1·6	2·5	3·2	1·7	5·2	1·8	4·0	3·6
Histidine	2·7	3·0	3·0	3·2	3·8	1·8	2·9	1·7	2·1	4·0	2·7	2·4
Isoleucine	6·5	6·6	6·6	5·7	4·6	6·7	6·9	7·4	3·0	2·6	5·7	6·9
Leucine	9·9	10·1	8·2	12·1	12·5	12·0	11·6	15·0	9·6	12·3	11·5	9·4
Lysine	8·0	8·2	9·2	6·8	6·4	9·7	11·6	11·9	6·8	12·8	9·7	6·9
Methionine	2·4	3·3	2·6	3·5	4·3	1·9	1·0	3·3	0·9	0·8	2·7	3·3
Phenylalanine	5·1	5·8	4·8	6·0	6·0	4·0	4·5	3·8	3·9	6·6	8·0	5·8
Proline	9·2	12·3	8·5	16·6	17·7	4·7	1·5	5·2	10·0	4·8	6·2	4·5
Serine	5·2	6·3	6·5	7·1	5·7	4·8	4·8	4·3	11·5	4·2	6·2	7·8
Threonine	4·7	4·5	5·1	5·3	4·6	5·2	5·6	5·2	10·5	5·8	11·5	5·0
Tryptophan	1·3	1·5	1·7	0·7	1·2	1·8	7·1	2·3	2·7	0·6	1·2	1·6
Tyrosine	4·9	6·3	8·4	3·3	3·8	3·2	5·4	4·0	6·7	5·1	4·1	4·1
Valine	6·7	7·4	6·5	10·6	10·9	5·3	4·8	5·8	9·6	5·9	8·0	7·4

[1]Mean values derived by Block & Weiss (1956).
[2]Gordon *et al.* (1949).
[3]Gordon *et al.* (1953).
[4]Gordon & Ziegler (1955).
[5]Hansen & Carlson (1956).
[6]Stein & Moore (1949).
[7]Hare *et al.* (1952).

and β-lactoglobulin and serum albumin occupy intermediate positions. Heating of milk for 30 minutes at 77°C caused complete denaturation of the immune globulins, but only 66 % of β-lactoglobulin and 50 % of α-lactalbumin were denatured (Larson & Rolleri, 1955).

b. *Albumin fraction.* (i) β-Lactoglobulin. This protein constitutes the major portion of the milk-serum proteins. It may be isolated and crystallized from the conventional albumin fraction (see p. 206) subjected to prolonged dialysis at pH 5·2. It is of special interest as being the main source of sulphydryl groups which are liberated when milk is heated. They appear when milk is momentarily heated to about 75°C, causing a lowering of the oxidation–reduction potential and the simultaneous appearance of a cooked flavour.

There is now strong evidence to support the view that β-lactoglobulin is not homogeneous, but consists of two components, A and B, the former showing greater migration velocity in paper electrophoresis at pH 8·6. Both may be crystallized, but whereas the form of A crystals corresponds to that of the original crystalline β-lactoglobulin first prepared from bulk milk by Palmer (1934), that of the B crystals is different (Aschaffenburg & Drewry, 1955). These latter authors have established that some cows produce only A, others only B, and some produce both, and that the phenomenon is genetically controlled. A similar instance of genetic determination of protein differences has been observed by Cabannes & Serain (1955) in the haemoglobin of cow's blood. It is too early yet to assess the significance of the findings, about β-lactoglobulin but it is clear that the origin of the milk samples, i.e. whether individual or bulk, may determine the degree of heterogeneity of the β-lactoglobulin fraction, and that earlier work must be re-examined in the light of this discovery. Also, one is prompted to inquire whether similar conditions govern the relative proportions of the casein fractions.

(ii) α-Lactalbumin. Historically the following steps led to the recognition of this protein. Pedersen (1936) found in whey three components which he called α, β, γ. Sørensen & Sørensen (1939) isolated a crystalline protein from whey. Later Gordon & Semmett (1952, 1953) using a similar method prepared this protein in a state in which it was homogeneous in the ultracentrifuge, and with a sedimentation constant corresponding to Pedersen's α-component. The name α-lactalbumin is now accepted for this protein. For a milk protein it has an unusually high tryptophan content of about 7 %.

(iii) Serum albumin. Milk contains a small amount of blood-serum albumin, probably by direct infiltration from the blood.

c. *Globulin fraction.* The proteins of this fraction are of importance as carriers of immune antibodies. They occur in only small amounts in normal milk but in much higher concentration in colostrum. Their immunological significance is discussed in Chapter 20.

d. *Proteose-peptone.* The proteose-peptone fraction, as its name implies, consists of material of smaller molecular size than true proteins. Little is known about it.

4. *Fat-globule Membrane Proteins and Associated Enzymes*

It has been known for a long time that the globules of fat finely dispersed in milk are surrounded by a membrane. The properties of this fat-globule membrane, which consists essentially of protein and phospholipids, are fully discussed by King (1955) and are considered on p. 220. Milk fat contains 0·4 to 0·8 g of membrane protein per 100 g, approximately 2 molecules of protein being linked to one molecule of phospholipid (Jenness & Palmer, 1945). The membrane protein is probably a globulin though it is not identical with any of the other milk proteins. Its amino acid composition is shown in Table III from which it is evident that it contains more arginine, glycine and phenylalanine and less aspartic acid, glutamic acid and leucine than the other milk proteins (Hare *et al.*, 1952; Brunner *et al.*, 1953).

The membrane proteins are very sensitive to heat and liberate sulphydryl groups when milk is heated momentarily to 70°–75°C.

It is considered probable that many of the metallic trace elements of milk are concentrated as metallic proteinates in the membrane.

Several enzymes are also associated with the globule-membrane protein, amongst them being the Schardinger enzyme (xanthine oxidase), phosphatase, and an aldolase. The first, which catalyses the oxidation of xanthine to uric acid and of a number of aldehydes to acids, contains iron and molybdenum and some of the riboflavin of milk in its prosthetic group, flavineadenine-dinucleotide (see also p. 251). The absence of xanthine oxidase from human milk serves as the basis of a test to distinguish it from cow's milk (Rodkey & Ball, 1946). Alkaline phosphatase, present in the milk serum and on the globule surface, is closely associated with lipoprotein. Since the temperature of inactivation of alkaline phosphatase is slightly above that of the thermal death of pathogenic organisms, the efficiency of pasteurization of cow's milk may be judged by the degree of inactivation of this enzyme (Kay & Graham, 1935; Tramer & Wright, 1952). The phosphatase test is now widely used for this purpose.

5. *Other Enzymes*

Other enzymes found in milk include lactoperoxidase, catalase, lipase, amylase, protease and an acid phosphatase.

These enzymes, as those of the membrane, are probably adventitiously present in milk through passage from blood or from the breakdown of mammary cells. The substrates on which several of the milk enzymes act are not present in it, and as the enzymes are largely destroyed in the process of

digestion they are of no nutritional significance. In raw milk, lipase may at times cause slight hydrolysis of fat giving rise to a bitter flavour.

Bacteria growing in milk may add their own enzymes to those originally present.

6. *Nutritive Value*

The literature on the nutritive value of milk proteins is voluminous; it has lately been reviewed by Henry (1957).

The nutritive value of a protein depends on its composition and on its digestibility. Of the twenty or so amino acids known to be present in proteins, eight are essential for man at all stages of life, i.e. cannot be synthesized by the body. They are: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (cf. Rose, 1957). The rat and the chick require also histidine and arginine, and the chick needs the further addition of glutamic acid and glycine. Some of the non-essential amino acids can partly replace certain essential ones. Thus cystine may spare methionine, tyrosine may spare phenylalanine, and serine may spare glycine (for the chick). The nutritive value of a protein depends, therefore, very largely on its content of essential amino acids. An "ideal" protein would contain all the essential amino acids in the right proportions as judged by the needs of the body as a whole. The protein most nearly satisfying these requirements, and the one that has been widely used as a standard of comparison, is egg protein. The nutritive value of other proteins is assessed by comparison with egg protein in biological tests, or by direct comparison of the amino acid composition of the protein with that of egg protein. The latter method takes no account of digestibility and may, therefore, be no more than a guide to the value of a protein as a food.

The amino acid analyses of the protein components of cow's milk are given in Table III. Values for the essential amino acids in total milk protein, whole casein and whole egg protein are included for comparison. It will be seen from the table, that relative to egg proteins those of milk are low in cystine and methionine but somewhat richer in lysine. The extent of the methionine deficiency is similar in the total protein and its individual fractions; but the cystine deficiency is much more marked in casein than in the total proteins, whereas in α-lactalbumin and β-lactoglobulin the concentration of cystine is not only higher than in milk but also than in egg. α-Lactalbumin is slightly, and β-lactoglobulin markedly, richer in lysine than casein or the total milk proteins. These differences in amino acid composition are reflected in the results of biological tests.

The most widely used biological methods for assessing the nutritive value of proteins with rats are the growth method of Osborne *et al.* (1919) measuring the protein efficiency ratio (gain in weight in grams per gram protein intake),

and the nitrogen balance method of Mitchell (1923–24) and Mitchell & Carman (1926) in which the biological value (the percentage of absorbed, or digestible, nitrogen retained by the body) is measured. Milk proteins, individual and total, are almost completely digestible, but with less well digested proteins the results of determinations of biological value may be conveniently expressed as the "net protein utilization" which is the biological value multiplied by the digestibility (expressed as a fraction of unity).

Table IV gives mean values for the biological values and protein efficiency ratios of milk proteins. It is clear from the table that casein has a lower nutritive value for the rat than total milk proteins or milk-serum proteins (lactalbumin).[1] The addition of methionine improves the biological value of total milk protein and of casein for rats (Henry & Kon, 1953, 1956). Cox *et al.* (1947) showed, however, that whereas the addition of methionine to casein improved growth in the rat and nitrogen retention in the dog, it was without effect on N retention in human adults and infants. They concluded that the nutritive value of casein and milk-serum proteins should be the same for man, which they confirmed experimentally (Mueller & Cox, 1947).

TABLE IV

MEAN BIOLOGICAL VALUES OF MILK PROTEINS DETERMINED ON SEVERAL SPECIES AND MEAN VALUES FOR PROTEIN EFFICIENCY RATIOS DETERMINED ON GROWING RATS

	Biological value	Protein efficiency ratio
Raw milk	85	—
Casein	76	2·26
Milk-serum proteins (lactalbumin)	88	3·13

(Mean values derived by Henry, 1957.)

The relative nutritive value of casein and milk-serum proteins has been further compared in a number of studies with rats and dogs (cf. Henry, 1957). Since casein contains about the same amount of methionine but considerably less cystine than milk-serum proteins, the deficiency of total sulphur amino acids is definitely greater in the former. It has long been established that methionine can fully replace cystine in the diet, but that cystine can only

[1]Milk-serum (whey) proteins are frequently called lactalbumin in nutritional papers.

spare part of the methionine (cf. Henry & Kon, 1953). Thus for particular functions where methionine is specifically required, casein may be superior to milk-serum proteins, but for general tissue building the latter with their higher total content of sulphur amino acids are the more valuable for most animals. In fact, the higher biological value for growth and maintenance of the total milk proteins over casein reflects the mutual supplementary effects of proteins having different amino acid compositions.

Much of the value of milk proteins in human nutrition, apart from their obvious importance during infancy when milk is the sole food, lies in their supplementary role in mixed diets. For in such diets amino acid deficiencies in the protein of one food are made good by relative surpluses in another. Thus, for example, with their high lysine content, milk proteins can often make good the deficiency of this acid in cereals. In general, it is possible to predict supplementary effects from amino acid analyses of individual proteins, but confirmation by biological experiments is necessary as some amino acids may be present in foods in a form that resists digestion. Henry & Kon (1946), in experiments with rats, observed marked supplementary relationships between the proteins of milk and potato, and between those of bread and cheese, when the members of each pair supplied equal amounts of protein and when they were given simultaneously. No such supplementary effects were evident when a 24-hour interval separated the consumption of the individual protein foods. Similarly in experiments with young women Leverton & Gram (1949) found better nitrogen retention from a diet supplying 63 g protein daily when the milk (about 22 g protein) was distributed equally between three meals than if the breakfast portion was taken with the mid-day meal. Taking the mid-day portion in the evening did not affect nitrogen retention, probably because of the shorter interval between the morning and evening than between the evening and mid-day meals (Leverton *et al.*, 1951).

C. Non-protein Constituents

Most natural foods contain nitrogenous compounds which are not proteins, but milk contains comparatively small amounts. These include free amino acids, urea, creatine, creatinine, uric acid, ammonia, phospholipids and certain vitamins, the concentration of most being subject to large variations. For example urea was found to vary between 6·13 and 20·4 mg N per 100 ml in the individual samples of cow's milk examined by Shanhani & Sommer (1951a,b).

The non-protein compounds are blood constituents that appear to have filtered into the milk. Their level in milk is raised considerably in diseases of the mammary gland that change its permeability. Phospholipids and vitamins are considered on pages 225 and 244.

IV. Minerals

A. Major Constituents

Table V shows that both in percentage composition and in the balance of individual mineral constituents the milks of various species differ markedly.

There is, however, a common pattern of distribution between the soluble and colloidal phases. Calcium and magnesium are present in both, being linked with casein, phosphoric and citric acids, whereas by far the greater proportion of potassium and sodium is in the soluble phase as chlorides and to a lesser degree as phosphates and citrates; very small amounts of these last two elements are protein-bound. It is important to note that one of the acids, citric acid, is completely lost during the process of ashing. In milk ash, the

TABLE V

Representative Values for the Mineral Constituents of the Milks of Various Species

Species	K g/100 g	Na g/100 g	Ca g/100 g	Mg g/100 g	Cl g/100 g	P g/100 g	Inorganic P / Total P
Ass	—	—	0·09	—	0·04	0·05	0·46
Buffalo	—	—	0·18	—	0·06	0·12	0·50
Ox	0·15	0·05	0·12	0·01	0·11	0·10	0·70
Sheep	0·19	—	0·19	—	0·14	0·15	—
Goat	0·17	—	0·14	0·02	0·15	0·12	—
Horse	0·07	—	0·10	0·01	0·02	0·06	—
Pig	—	—	0·27	—	0·09	0·16	—

(Mean values compiled from numerous sources.)

basic elements are therefore in excess of the acidic radicals. Cow's milk contains about 0·17 % of citric acid but, owing to the high molecular weight of the acid, this concentration only represents 0·01 molarity as against 0·03 molarity for chloride and phosphate. The presence of citric acid in cow's milk ensures that much of the soluble calcium is sequestered. Of the total calcium of cow's milk, two-thirds is in the colloidal form as calcium caseinate, phosphate and citrate, and of the remaining third, 55 % is bound by citric acid, 10 % by phosphoric acid, and 35 % exists in the ionic form (Smeets, 1955). Only in this manner is it possible for milk to contain so high a concentration of calcium and at the same time maintain the normal osmotic equilibrium with the blood.

On occasion, the calcium-ion concentration of cow's milk reaches an abnormally high level. The milk then manifests extreme sensitivity to heat, although the acidity is quite normal. Known as the "Utrecht" abnormality, this condition causes casein to come out of solution when the milk is heated. Such milk can be stabilized by addition of sodium citrate through its sequestering effect on calcium ions (Seekles & Smeets, 1947).

Of the phosphoric acid, approximately one-half is colloidal in cow's milk. Organically and inorganically bound forms are present in both soluble and colloidal phases.

It is in the calcium and phosphorus distribution that differences in mineral composition of the milk of different species are most apparent. Buffalo's milk, for instance, has a higher proportion of colloidal calcium and a lower proportion of inorganic phosphorus than cow's milk. In ass's milk calcium is evenly divided between the soluble and colloidal phases, and the proportion of inorganic phosphorus is even lower than in buffalo's milk.

Under the influence of heat, the equilibrium between the soluble and colloidal phases of calcium and phosphorus is disturbed in the direction of the colloidal phase. Heating for 30 minutes at 65°C brings about a 20 % reduction of soluble calcium and 1 hour's boiling causes a 40 % reduction.

In a study of the mineral elements characteristic of the soluble phase, Barry & Rowland (1953) found that in cow's milk a linear relationship exists between sodium and chloride, sodium and potassium, potassium and chloride, and between each of these elements and lactose.

B. Trace Elements

The trace elements present in cow's milk are listed in Table VI.

A considerable portion of the metallic trace elements is attached to proteins of the fat-globule membrane. Some are constituents of enzymes, iron in peroxidase and with molybdenum in xanthine oxidase, and cobalt in vitamin B_{12}. The very low concentration of iron increases appreciably by adventitious contamination during the normal handling and processing of milk. If at any time milk comes in contact with metallic copper, the copper content is greatly increased. In whole milk, copper becomes fixed to the fat globule membrane as a copper proteinate—and it is thus liable to stimulate fat oxidation. Oxidized taints in milk, tallowy and fishy taints in butter or whole milk powders are always more prone to develop whenever the cream or milk has taken up copper during handling or processing.

C. Minerals in Nutrition

Milk contains in varying quantities all the mineral elements of nutritional significance. Only those of particular interest in connexion with milk as food

need be discussed here. They are considered in Chapter 19 in relation to the requirement of the young of different species. The milks of milch animals are characterized by a high calcium content, three or four times that of human milk and, in common with all milks, by a very low content of iron.

The richness in easily available calcium makes milk an important source of this mineral in mixed dietaries. There is evidence that the association of

TABLE VI

TRACE ELEMENT CONTENT OF COW'S MILK
(parts per million)

		Reference
Al	less than 0·008	1
Ag	0·015–0·037	1
B	0·5–1·0	2 and 3
Br	0·18–0·24	4
Co	0·0002–0·0011	5
	0·0004–0·0007	6
Cu	0·05–0·14	7
	0·25–0·45	8
	0·37	1
F	0·227	9
	0·2	10
I	0·0007–0·021 (goitrous area)	11
	0·0138–0·0321 (transitional area)	11
	0·0211–0·0488 (non-goitrous area)	11
	0·056–0·082	12
Fe	0·15–0·37	1
	0·67	13
Mn	0·037–0·37	1
Mo	0·075–0·15	1
	0·048	14
Si	less than 1	15
Sr	0·0075–0·075	1
Zn	0·225–2·25	1
	3·0–5·0	16

[1]Gehrke *et al.*, 1954.
[2]Hove *et al.*, 1939.
[3]Owen, 1944.
[4]Casini, 1946.
[5]Archibald, 1947.
[6]Paulais, 1946.
[7]Itzerott, 1942.
[8]Ryś, 1953.
[9]Gabovich, 1951.
[10]Evans and Phillips, 1939.
[11]Binnerts, 1954.
[12]Davidov and Anisimova, 1953.
[13]Schäfer *et al.*, 1955.
[14]Teresi *et al.*, 1942.
[15]Strohecker *et al.*, 1955.
[16]Berfenstam, 1952.

calcium with lactose in milk may enhance its retention (cf. p. 203) and that a high protein intake facilitates the absorption of calcium through the formation of soluble co-ordination compounds with the amino acids (McCance *et al.*, 1942; Wasserman *et al.*, 1956).

The higher the intake of calcium the greater, up to a point, is its absolute absorption and retention. Cow's milk contains more calcium and phosphorus than human milk, and the relative amount of phosphorus in cow's milk is higher, so that the Ca : P ratio in cow's milk is 1·27 whereas in human milk it is 2·26. It is perhaps surprising, therefore that tetany due to low serum calcium may occur in babies fed on cow's milk during the first week or two after birth. The explanation lies in the inability of the kidney of the young baby to excrete the greater amounts of phosphorus absorbed from cow's milk, so that the serum phosphorus increases with a concomitant fall in serum calcium (cf. Widdowson, 1956). Human nutritional requirements of calcium and iron are reviewed by Widdowson (1956) who discusses *inter alia* the interesting question of adaptation to low calcium intakes.

All milks are low in iron, and in the young of some animals, such as man, haemoglobin is formed from the liver reserves laid down in the foetus; in others, the pig for example, the liver reserves are too low for the rate of growth and an additional source of iron is necessary to prevent anaemia. Sucking pigs reared outdoors obtain iron from rooting in the soil, but pigs reared indoors must be dosed with it.

Little is known about the requirements for the trace elements in nutrition. In general, the level in milk of trace metals that occur as cations, such as manganese, copper and cobalt, is under physiological control and is raised little or not at all through increased intake. The level of the physiologically important halides, iodine and fluorine, is more easily affected by their content in the diet.

The metabolism of strontium closely resembles that of calcium and it is well known that it is deposited in the bones in small quantities. As such these quantities are harmless. Normally in cow's milk the ratio of strontium to calcium is about one-tenth of that in the fodder of the cow. However, when herbage takes up radioactive Sr from the fall-out of products of nuclear fission some of the ^{90}Sr gets into the milk. In consequence a small rise in the content of ^{90}Sr in the bones of young children has lately been noted. Fortunately the levels are still well below the accepted tolerance (Bryant *et al.*, 1956, 1957).

V. Milk Fat

A. General

The fat content of milk varies more than that of any other major constituent. As the fatty fraction of milk tends to separate from the aqueous phase,

in most species the fat content varies throughout the act of milking or suckling, generally increasing as the gland is emptied. Apart from these sampling differences, the fat content in all species is influenced by the stage of lactation, the plane of nutrition and, in ruminants, by the quantity and physical condition of the roughage in the diet. It is clear therefore, that the values in Table I are only likely approximations.

B. Fat Globules

The fat in milk is present in the form of minute globules ranging in diameter from 0·1 to 10 μ. For cow's milk the mean is 3 μ and for goat's milk 2 μ. In different breeds the size of the globules tends to vary with the fat content of the milk; thus the mean for Holstein milk is 2·5 μ and for that of Channel Island cattle 3·5 μ (Schultz & Chandler, 1921; Gamble *et al.*, 1939). Ayrshires are an exception: though their milk is higher in fat that that of the Holstein, the size of the globules is the same. As lactation advances the fat globules tend to become smaller.

Each globule is surrounded by the fat-globule membrane, a hydrophilic surface layer that maintains the fat in an emulsified state. The membrane is composed of a complex mixture of phospholipid, protein, vitamin A, carotenoids, cholestrol, a high melting point glyceride, and various enzymes. The sequence is probably fat—phospholipid—protein—water. The long fatty-acid chains of the phospholipid are buried in the fat and the hydrophilic part of the molecule is directed outwards. It is likely that the carotenoids, vitamin A, and cholesterol associated with the membrane are in the phospholipid layer. The nature of the globule surface is such that at ordinary temperatures the globules are grouped together in the form of clusters which play an important part in the rising of cream. The globule surface affords some protection against the fat-splitting activities of the enzyme, lipase. If, however, milk is homogenized, the enormous increase in globule surface area necessitates a substitute layer for the great majority of newly formed globules, as the quantity of phospholipid is now insufficient. Lipase is now able to act with little hindrance, hence the increased sensitivity to lipase action of homogenized milk. The constitution of the membrane, its break-up in the churning of butter, and associated phenomena are fully discussed by King (1955).

That milk fat is a complex mixture is at once manifest in its physical properties. Cow's milk fat begins to melt at 28°C but is not completely liquid until the temperature has reached 33°C. The liquid fat sets over a similar but lower temperature range (24° to 19°C). As secreted, the fat gobules are therefore liquid, but when milk is brought to ordinary temperatures the adjustment of the physical state to the new conditions is prolonged and may take 24 hours to reach completion. One reason is that the phenomenon is one of

crystallization from solution rather than solidification. Another is that, the liquid fat being in the disperse phase, there is no opportunity for the hastening effect of "seeding" which usually operates when the liquid is in a continuous phase. Variation in the composition of milk fat is responsible for slight differences from sample to sample in specific gravity, 0·936 to 0·946 at 15°C, and refractive index, 1·458 to 1·464 at 15°C. Although milk fat is soluble in fat solvents such as petroleum and ether it is not possible to extract fat from milk by merely shaking it with these solvents. For quantitative solvent extraction the fat globule membrane must be removed by the action of acid or alkali. A convenient way is to add ammonia solution followed by alcohol which acts as a common solvent enabling the fat solvents to reach the now unprotected fat globules.

C. True Fats

It is convenient to consider first the compounds constituting the bulk of the fat, namely the true fats which are triglycerides of numerous saturated and unsaturated fatty acids. Although information is available about the component fatty acids, the problem of their combination with glycerol still awaits full elucidation. The three hydroxyl groups of glycerol may be replaced by the same fatty acid or by two or by three different fatty acids. From the large number of fatty acids known to be present in milk fat it has been calculated that at least 4913 glycerides are possible. Much work has been done on the constitution of the component glycerides of milk fat which, in its complexity, is discussed by Hilditch (1956). It may be taken broadly that the tendency is for the appearance of as little as possible of simple triglycerides (containing three radicals of the same fatty acid), with a concomitant maximum appearance of mixed triglycerides with the fatty acids distributed not randomly but as widely as possible. The unravelling of the chemical identity of the component acids was a problem of great difficulty. The subject is covered minutely by Hilditch (1956) and by Shorland & Hansen (1957), workers outstanding in this field, from whose publications much of the information here given was taken.

The fatty acids of milk fat fall into well-defined groups. Table VII presents the approximate composition of the milk fat of different species. It has been compiled from data selected from Hilditch's (1956) book and various other sources. As many factors are known to modify the composition of milk fat, the figures are of necessity approximations.

1. *Saturated Fatty Acids*

Most of these in milk fat contain even numbers of carbon atoms ranging from C_4 (butyric acid) to C_{20} (arachidic acid). The major component of this group is palmitic acid or *n*-hexadecanoic acid, $C_{15}H_{31}COOH$. Until recently it

TABLE VII

COMPONENT FATTY ACIDS OF THE MILK OF VARIOUS SPECIES
(Percentages by weight, values derived from Hilditch, 1956)

Species	Saturated									Unsaturated							
										Monoethenoid					Diethenoid	Trieth-enoid	
	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{18}	C_{18}	Others
Buffalo	5	2	1	1	3	11	29	15	1	tr	tr	1	4	25	1	—	—
Camel	2	1	1	1	5	7	29	11	—	—	—	—	—	39	4	—	—
Ox	3	1	1	3	2	10	26	13	1	tr	tr	1	3	32	3	1	tr
Sheep	3	2	2	5	4	10	24	13	1	tr	tr	tr	2	26	5	—	2
Goat	3	2	3	10	6	12	28	6	1	tr	tr	1	3	21	4	—	tr
Man	tr	tr	tr	2	6	9	23	7	1	tr	tr	tr	3	37	8	tr	3
Horse	tr	1	3	5	6	7	16	3	tr	1	1	2	8	19	8	16	5
Pig	←			1	→	2	27	7	—	—	—	—	8	37	15	—	4

tr = trace, well below 1 %

was considered that milk fat, like most animal fats, contained only the straight chain, or normal, fatty acids with even numbers of carbon atoms. The work of Shorland and his colleagues in New Zealand, reviewed by Shorland & Hansen (1957), has demonstrated the presence of the following.

(a) Normal saturated acids with odd-numbered carbons, amongst which *n*-pentadecanoic and *n*-heptadecanoic acids predominate. The total of this group accounts for 2 % of all the fatty acids.

(b) methyl-branched-chain saturated acids with odd and even numbers of carbon atoms, the total members of this group accounting for some 2 % of all the fatty acids.

In the proportion of saturated acids the milk fat of ruminants differs sharply from that of other species:

Buffalo	Camel	Ox	Sheep	Goat	Man	Horse	Pig
%	%	%	%	%	%	%	%
67	57	60	64	71	48	42	36

The distinction between ruminants and non-ruminants is further emphasized by differences in component acids. Human milk fat has only traces of acids below decanoic. The presence of appreciable quantities of butyric acid is characteristic of the milk fat of ruminants for, although this acid is present in the fat of mare's milk, its concentration there is only one-tenth that in cow's milk fat; very small quantities of this acid are present in sow's milk. Large quantities of volatile acids, amongst which acetic acid predominates, are produced in the rumen by fermentation of cellulose, carbohydrate and protein. The synthesis of milk fat and the part played in it by these lower acids is considered in detail in Chapter 12. Despite these differences between ruminants and non-ruminants it is interesting to note that the proportion of palmitic acid tends to be fairly constant in the milk fat of all species except the horse, in which it is much lower.

2. *Unsaturated Acids*

Whereas the saturated acids above C_8 are solid at ordinary temperatures, the unsaturated ones are predominantly liquid, and the balance between the two determines many of the characteristic physical properties of milk fat such as hardness and melting point.

a. *Monoethenoid acids.* Oleic acid, $C_{17}H_{33}COOH$, is by far the most abundant acid of the monoethenoid type but others may be present, ranging from $C_9H_{17}COOH$ to $C_{15}H_{29}COOH$.

The contribution of the monoethenoid acids to the total acids of milk fat is as follows for different species:

Buffalo	Camel	Ox	Sheep	Goat	Man	Horse	Pig
%	%	%	%	%	%	%	%
30	39	37	29	25	41	31	45

In all the main monoethenoid acids of milk fat the double bond is in the 9 to 10 position. Thus in oleic acid it is in the centre of the chain

$$CH_3(CH_2)_7CH = CH(CH_2)_7COOH$$

but it is terminal in dec-9-enoic acid, $CH_2 = CH(CH_2)_7COOH$. Like oleic acid, the members of this group are *cis* acids.

Of recent years evidence has accumulated that milk fat contains other monoethenoid fatty acids, in which the molecules are of the *trans* configuration, or in which the double bond occupies other positions, or both.

The best known of these isomers is "vaccenic acid" now considered to be a mixture of *trans*-octadecenoic acids in which the Δ^{11} isomer predominates with lesser amounts of the Δ^{10} and probably also the Δ^{9} and Δ^{12} acids.

Vaccenic acid is solid at room temperature, whereas oleic acid is liquid. Earlier analyses (Geyer *et al.*, 1947) showed that the fat of cow's milk contained about 0·7 %, but more recent infra-red analysis gave much higher values, 6 to 8 % in summer and 3·5 % in winter (Cornwell *et al.*, 1953).

b. *Diethenoid acids.* The diethenoid acids of milk fat are mostly of the C_{18} series. Some are geometrical isomers of linoleic acid, octadeca-9, 12-dienoic acid, and others, so far uncharacterized, have a conjugated double bond structure.

In human milk fat a considerable portion of the linoleic acid is in the *cis-cis* form characteristic of the natural linoleic acid of vegetable fats. With the milk fat of ruminants the position is less clear in that *trans-trans* and *cis-trans* forms have been found together with the *cis-cis* form (Scott *et al.*, 1959). Natural linoleic acid is one of the "essential" fatty acids that are not synthesized in the body but come from the food. Its unnatural forms in the milk fat of ruminants are probably due to isomerization in the rumen.

In most species the C_{18} diethenoid acids constitute some 2 to 5 % of the milk fatty acids, but in human milk fat they amount to about 8 % and in sow's milk fat to about 14 %.

c. *Triethenoid acids.* Natural linolenic acid, *cis-cis-cis*-octadeca-9, 12, 15-trienoic acid, another of the "essential" fatty acids, has been identified as the main component of the C_{18} triethenoid acid fraction; traces of conjugated C_{18} triethenoid acids have also been found.

In cow's milk fat the amount of this acid is probably related to the nature of the food. Thus New Zealand butterfats from cows on pasture contain some 1 % of it, whereas in stall-fed cows the content may be much lower. The linolenic acid content of mare's milk is exceptionally high (Table VII).

d. *Polyethenoid acids.* Traces of polyethenoid acids of the C_{20} and C_{22} series have been detected in milk fat. The presence of arachidonic, eicosa-5, 8, 11, 14-tetraenoic acid, the third of the "essential" fatty acids has not been clearly demonstrated.

Various authors, using the reliable method of alkali isomerization and ultraviolet spectroscopy, obtained values for the content of polyethenoid acids in cow's milk fat which show the following ranges.

Non-conjugated %			Conjugated %		
Diene	Triene	Polyene	Diene	Triene	Polyene
0·2–2·7	0·35–2·0	0·27–0·60	0–2·8	Trace-0·05	0·008

D. Other Compounds Present in the Fat of Milk

These compounds are dissolved in the fat, or held on the globule membrane.

1. *Phospholipids*

The phospholipids of milk are concentrated in the fat-globule membrane and are also present in the aqueous phase. Cow's milk fat contains close on 1 %, lecithin and cephalin being the major components, with small amounts of sphingomyelin (and possibly also of the closely related cerebrosides). The fatty acid composition of lecithin and cephalin is markedly different from that of the true fats of milk. Thus no acid below myristic (C_{14}) is present but in addition to oleic and linoleic acids there are appreciable quantities of highly unsaturated C_{20}–C_{22} acids.

The phospholipids have both lipophilic and hydrophilic groups and as such are excellent emulsifying agents. As already noted, in combination with proteins they form part of the fat globule membrane and contribute to the stability of the emulsion of fat in milk.

Because of the notable proportions of highly unsaturated fatty acids the phospholipids of milk are prone to oxidation and are believed to be either responsible for or to initiate the changes leading to the development of oxidative taints in milk.

2. *Sterols*

a. *Cholesterol.* Cholesterol is an invariable component of milk, there being, however, marked variations in concentration between species. Cow's milk contains 0·010 to 0·014 %. The content in woman's, goat's, mare's and ewe's milk is rather higher and sow's milk is said to be ten times richer (Deuel, 1957). In spite of the predominance of vegetable sterols in the ration of the cow, there are none in milk fat, and upon this fact is based the phytosteryl-acetate test for the presence of vegetable oils in butterfat.

c. *Other sterols.* The vitamin D potency of milk can be markedly raised by ultraviolet irradiation through which vitamin D_3 is formed, and it follows that milk must contain 7-dehydrocholesterol, the precursor of vitamin D_3.

3. *Carotenoids*

These yellow or orange pigments which give the characteristic colour to the milk fat of some species are discussed in detail on page 245.

E. Milk Fat in Nutrition

We have pointed out in the preceding Sections that the milk fat of ruminants, the cow in particular, differs from that of other mammals and from animal depot fats and from vegetable fats in containing appreciable amounts of glycerides of short-chain fatty acids. There is no evidence that their presence affects the nutritive properties of the fat as a whole. As such, milk fat is, like other natural fats of similar melting points or like semi-hardened vegetable oils, almost completely digestible (95 % to 97 %).

In the scientific work of the last 30 years two phases can be distinguished in the attitude to the nutritive properties of milk fat. Until the late forties the question was whether the fat of milk possesses unique properties not shared by other fats. Evidence for the superiority of milk fat came mainly from the Wisconsin group of workers in the States and from Boer and his collaborators in Holland. Others, including Deuel and his group in the States and workers at the National Institute for Research in Dairying, in England, were unable to detect differences in favour of milk. In retrospect it would be fair to say that the bulk of experimental evidence seems to indicate that many vegetable oils are nutritionally equal to butter. The subject is fully reviewed by Deuel (1957) and also by Kon & Henry (1954).

One interesting aspect of these studies was the suggestion that "vaccenic acid" (see p. 224) was the component directly responsible for the superiority of milk fat. However, tests with the purest preparations available did not confirm this view (cf. Deuel, 1957; Kon & Henry, 1954).

With the growing realization that fat plays some part in atherosclerotic changes, the pendulum swung in the other direction and views are now being put forward (cf. Sinclair, 1956) that milk fat of ruminants may be particularly suspect because of the "unnatural" configuration of its essential fatty acids (see p. 224). It is established that linoleic, linolenic and arachidonic acids cannot be synthesized in the animal body though they can be interconverted. They are nutritionally indispensable in that they are involved in processes of growth, in the nutrition of the skin in maintaining the capillary pressure in subcutaneous blood vessels, and in that they are involved in normal cholesterol transport and metabolism. Linolenic acid is partly converted in the body to arachidonic acid which latter is on good evidence the most active biologically. The argument is that the *cis-trans* or *trans-cis* forms present in cow's milk fat cannot act as essential fatty acids and that the ruminant milk fat is thus very low in essential fatty acid activity and that

moreover those unnatural isomers may antagonize the essential fatty acids in the remainder of the diet. The whole problem has so far been so inadequately explored as to preclude any sensible assessment. The latest work of Scott *et al.* (1959) (see p. 224) casts some doubt on Sinclair's premises.

VI. Dissolved Gases of Milk

Freshly drawn cow's milk contains dissolved gases: carbon dioxide, nitrogen and oxygen, the volumes of which, measured at 0°C and 760 mm pressure are approximately 6·6 ml CO_2, 1·2 ml N_2 and 0·1 ml O_2 per 100 ml milk. Dissolved oxygen is important in fat oxidation and also in the oxidation of ascorbic acid (see p. 254). On exposure of milk to air, an extensive exchange of gases takes place, carbon dioxide being released into the atmosphere and oxygen being absorbed, the final equilibrium leaving milk with only about 4·5 ml CO_2, an increased oxygen content (about 0·5 ml) and nitrogen slightly increased to 1·3 ml, per 100 ml milk (cf. Noll & Supplee, 1941).

VII. Some Physical and Other Properties of Milk

A. pH and Milk Acidity

When freshly drawn, cow's milk has a pH in the region of 6·6. When such milk is titrated to the phenophthalein end-point, about 1·8 ml of 0·1 N-NaOH are required per 10 ml milk. This acidity is due to acid citrates and phosphates, CO_2 and casein, some of the acid groups of which are in the free condition. In milk and dairy products, the problem of distinguishing between natural acidity and that developed as lactic acid by bacteria is complicated by the variable degree of natural acidity exhibited by different milk samples. Owing to differences in protein concentration and mineral distribution, the natural acidity of the milk of various mammals shows wide variation.

B. Oxidation–Reduction Potential

It is clear that in milk, the co-existence of dissolved oxygen and reducing agents such as ascorbic acid and riboflavin establishes a measurable oxidation–reduction potential. Drawn under anaerobic conditions, cow's milk has an E_h of about + 0·13 volts. Exposure to air raises this value to + 0·2– + 0·3 volts. This value is lowered by bacterial action which may use up dissolved oxygen and produce bacterial metabolites with a powerful reducing action. It is because of these effects that dye-reduction tests are so useful in assessing the hygienic quality of milk. For example, methylene blue is not reduced until the E_h has fallen from the normal for raw milk of + 0·2– + 0·3 volts to the region of + 0·03– + 0·1 volts. The dye, resazurin, is

useful in so far as the colour changes through all intermediate shades from blue to pink and pink to colourless. It is thus possible to assess the extent of bacterial contamination, for the first reduction occurs at a higher E_h value than the final decolorization, and the intermediate shades reflect the E_h values in much the same manner as the familiar pH indicators. On heating milk, the E_h value is depressed from two causes (a) expulsion of oxygen and (b) the liberation of sulphydryl compounds, which are strong reducing agents. However, subsequent exposure to air will tend to restore the normal E_h value. The subject is discussed in detail by Saal & Heukelom (1947).

C. Colour, Odour and Flavour

The white opalescence of milk due to the colloids and the fat globules may be tinged with yellow according to the carotene content of the fat (see p. 245). Milks that do not contain carotene, such as goat's, ewe's, and Indian buffalo's milk, appear whiter than cow's milk, which in turn varies in colour according to the season of the year and the breed of the cow. Riboflavin imparts to whey its characteristic green-yellow colour which, however, is masked in milk itself.

Cool, freshly drawn milk has a bland slightly sweet odour and flavour. While still warm, milk has been said to have an odour faintly reminiscent of the animal. Normally the flavour of milk is determined largely by the ratio of chloride to lactose. Certain feeding-stuffs and wild plants, if taken in excess, are prone to alter the flavour of milk and impart taints, as for example the fishy taint associated with high intake of betaine from molassed sugar-beet pulp. Patton *et al.* (1956) have detected methyl sulphide in the breath of the cow and in the milk, where it contributes to the odour and flavour. Owing to the remarkable facility with which milk absorbs extraneous odours and flavours, it is frequently difficult to decide whether any particular off-flavour has been passed from the food to the milk or whether it has been absorbed from the atmosphere.

D. Specific Gravity

The specific gravity of cow's milk averages 1·031 to 1·032 at 60°F/60°F (15·6°C), corresponding to a density of 1·029 to 1·030 g per ml at 20°C, but varies widely in samples from individual animals owing to the different proportions of fat (sp. gr. 0·9), non-fatty solids (sp. gr. 1·6) and water present.

For a period of up to 24 hours after milking, the specific gravity of cow's milk gradually increases to the extent of 0·001 in all, cool conditions favouring the most rapid attainment of the maximum. This phenomenon, known under the name of its discoverer Recknagel, arises in part from the slow solidification and contraction of the fat globules. Separated milk shows a similar, but smaller tendency attributable to changes in the hydration of the milk proteins.

Certain it is that when milk or separated milk is kept cool there is an increase in bound water (cf. Pyenson & Dahle, 1938a,b,c). Of the, say, 87·5 % total water in milk, some 3 % is bound: 1·5 % by casein, 1 % by albumin and globulin and 0·5 % by the fat-globule membrane. In freshly drawn milk, however, the bound water is only about 2·7 % and it is significant that the increase of 0·3 % in bound water occurs simultaneously with the increase in specific gravity. The changes vary from sample to sample and the figures quoted are intended only to convey some idea of their magnitude. When milk is heated above 37°C, the fat liquefies, some bound water is released, and the initial lower specific gravity is quickly restored.

When milk is cooled to room temperature after this heating the specific gravity remains steady for some time and then increases slowly again. These considerations are of importance in the dairy laboratory in which formulas expressing the relationship between the specific gravity, fat and total solids contents are used for rapid commercial analysis of milk. Many such formulas have been proposed, and Herrmann (1956) has reviewed the relevant literature since 1879. Among the most widely known in different countries are the early and similar formulas of Fleischmann (1885), Babcock (1892) and Richmond (1895). Richmond's, for example, is: T.S. = 0·25 G + 1·2 F + 0·14, where T.S. = total solids %, G = 1000 × (sp. gr. at 60°F − 1), and F = fat %, the sp. gr. being at its maximum. Of recent years formulas have been developed relating to milk tested for its sp. gr. or density, when these values are at their minimum, i.e. the fat is in the liquid, and readily reproducible, state.

E. Osmotic Characteristics

As milk is in osmotic equilibrium with the blood, it follows that its osmotic pressure varies only slightly. The freezing point depression of milk (averaging 0·545°C) is in fact its most constant property—and the depression is used as a criterion of unwatered milk. As the development of acidity causes an increase in the freezing point depression, the determination is always coupled with measurement of acidity, and appropriate corrections are made, provided the sample is only slightly acid. In the British Isles, a freezing point depression of less than 0·530°C is taken to indicate intentional or accidental watering. Since milk fat makes no contribution to the osmotic pressure, it is impossible to detect by this method addition of separated milk or removal of fat.

F. Electrical Conductivity

For normal milk values for electrical conductivity range from 0·003 to 0·005 $\Omega^{-1}cm^{-1}$. Variation is directly related to the salt content, and on this

account the conductivity rises as lactation advances, and mastitis produces the same effect.

G. Viscosity and Rigidity

Milk is almost a true fluid, that is, it flows at a constant rate under constant pressure and, when the pressure changes, at a rate almost proportional to the pressure, provided there is no turbulence. The viscosity, which is given by the ratio of pressure to flow-rate, is for normal cow's milk about twice that of water. It falls in a rather complicated way as temperature is increased. Accurate measurements of viscosity (except for homogenized milk) are difficult because of creaming.

The viscosity of colostrum is sometimes very high, and that of prenatal mammary secretions can be even higher.

The viscosity and, later, rigidity of milk when curdled by acids or rennin are of importance in connexion with its processing and doubtless, though this is hard to test directly, with its digestion in the stomach.

Two fairly recent surveys of work on viscosity of milk may be consulted: Cox (1952) and Scott Blair (1953).

H. Surface Tension

Measurements of apparent surface tension of milk give slightly different values according to the method adopted. The average value for normal milk is about three-fifths that of water, and is influenced by several factors. A high concentration of inorganic salts increases the surface tension slightly, and a high fat content lowers it somewhat. The surface tension of milk is discussed by Watson (1956).

I. Specific Heat

The specific heat of milk is to some extent temperature-dependent, and is influenced by variations in the content of fat and non-fatty solids. It is generally only slightly less than that of water (Phipps, 1957).

VIII. Factors Influencing the Composition of Milk

A. Composition of the Milk of Different Species

From the consideration of the various milk constituents, it is clear that not only does the composition of milk vary between different species, but that within any one species composition varies markedly. In the following discus-

sion of various factors known to influence milk composition it is useful to visualize the mean values around which the variations occur. Table I is compiled for this purpose.

B. Effects of Stage of Lactation

1. *Colostrum*

Colostrum, the secretion of the first few days after parturition, differs markedly in composition from milk secreted in established lactation (Table VIII). In most species, colostrum is characterized by a much higher globulin

TABLE VIII

Representative Values for the Composition of the First 24 Hours' Colostrum of the Cow, Mare and Sow

Constituent		Cow[1]	Mare	Sow
Fat	(g/100 g)	3·6	2·5	4·4
Non-fatty solids	(g/100 g)	19·5	12·5	21·3
Protein	(g/100 g)	14·2	7·2	17·8
Lactose	(g/100 g)	3·1	4·7	3·5
Ash	(g/100 g)	1·0	0·6	0·6
Carotene	(μg/100 g)	150·0	25·0	1·0
Vitamin A	(μg/100 g)	140·0	33·0	95·0
Thiamine	(μg/100 g)	60·0	40·0	100·0
Riboflavin	(μg/100 g)	500·0	140·0	140·0
Nicotinic acid	(μg/100 g)	100·0	160·0	170·0
Pantothenic acid	(μg/100 g)	220·0	750·0	130·0
Vitamin B_6	(μg/100 g)	50·0	—	2·5
Biotin	(μg/100 g)	4·0	—	5·3
Folic acid	(μg/100 g)	2·0	—	—
Vitamin B_{12}	(μg/100 g)	1·0	—	0·15
Ascorbic acid	(mg/100 g)	2·5	6·0	30·0
Vitamin D	(i.u./100 g)	4·0	—	—

[1]See also Table IX

content with a corresponding increase in non-fatty solids, and by a lower lactose and a higher chloride concentration; the fat content is not appreciably different. In the colostrum of bovine animals (cow, Indian buffalo) some workers found the fat more unsaturated than in milk, though others detected no difference (cf. Hilditch, 1956). In the cow the calcium content of colostrum is 0·17 g per 100 ml which is rather higher than that of milk, but in the sow,

TABLE IX

PERCENTAGE COMPOSITION OF COLOSTRUM AND EARLY MILK OF HOLSTEIN COWS
(Mean values from Parrish *et al.*, 1950)

Milking	Specific gravity	Total solids	Fat	Non-fatty solids	Protein	Lactose	Ash
1st	1·056 (29)	23·9 (10)	6·7 (29)	16·7 (10)	14·0 (8)	2·7 (8)	1·11 (10)
2nd	1·040 (29)	17·9 (10)	5·4 (29)	12·2 (10)	8·4 (8)	3·9 (8)	0·95 (10)
3rd	1·035 (29)	14·1 (10)	3·9 (29)	9·8 (10)	5·1 (8)	4·4 (8)	0·87 (10)
4th	1·033 (29)	13·9 (10)	4·4 (29)	9·4 (10)	4·2 (8)	4·6 (8)	0·82 (10)
5th & 6th	1·033 (29)	13·6 (10)	4·3 (29)	9·5 (10)	4·1 (8)	4·7 (8)	0·81 (10)
7th & 8th	1·033 (27)	13·7 (9)	4·4 (27)	9·3 (9)	3·9 (7)	4·8 (7)	0·81 (9)
15th & 16th	1·032 (25)	13·6 (10)	4·3 (25)	9·1 (10)	3·4 (8)	4·9 (8)	0·78 (10)
27th & 28th	1·032 (5)	12·9 (5)	4·0 (5)	8·8 (5)	3·1 (6)	5·0 (6)	0·74 (5)

Figures in parentheses give the number of cows sampled.

for example, it is 0·05 g per 100 ml, much lower than in milk (see Table V). The content of fat-soluble vitamins is generally much higher in colostrum, but with the water-soluble vitamins the relationship between colostrum and milk is quite variable.

The main importance of colostrum is as a carrier of immune bodies, in which respect it is considered in Chapter 20. Typical values compiled from the work of Parrish *et al.* (1950), showing the transition in composition from colostrum to milk in Holstein cows, are shown in Table IX.

2. *Pre-partum Milking*

The practice of milking cows before they calve has increased of late. The concentrations of those constituents characteristically high in normal colostrum are even higher in the earliest pre-partum secretion. The following values of Rowland *et al.* (1953) for the secretion of eight Shorthorn cows on the 14th day pre-partum may be taken as an example: total solids 23 to 37 %, fat 0·5 to 4·2 %, non-fatty solids 19 to 36 %, lactose 0·4 to 2·1 %, protein 16 to 32 % and ash 0·5 to 1·0 %.

Some pre-milked cows respond readily with marked increase in yield. These cows even before calving produce a true milk of slightly high solids-not-fat content. Other cows that give a small or negligible response in yield produce a colostrum-like secretion until after calving.

3. *Milk of Established Lactation*

Once the colostral stage is past, the lactational effects follow fairly well-defined trends, fat, non-fatty solids, protein and casein falling to minimum values early in lactation and thereafter making a steady recovery. The precise stage of lactation at which the minima are reached is subject to some variation, but Waite *et al.* (1956) observed that pedigree Ayrshire herds reached minimum values for non-fatty solids and protein about the 6th to 7th week of lactation, but the minimum in fat percentage came later at about the 10th week. The lactose content tended to move with the yield, rising to a maximum about the 45th day, thereafter declining slowly, and after the 24th week declining more rapidly. Differences between maxima and minima amounted to: 0·6 % fat; 0·3 % non-fatty solids; 0·8 % crude protein; 0·5 % casein and 0·4 % lactose. The first lactation differed from succeeding ones only in so far as the general level of concentration of all constituents tended to be higher, and that the lactose content fell by only 0·2 % from its maximum value of 4·8 %. Earlier work by Bailey (1952) emphasized that the increases in protein and non-fatty solids in late lactation milk were much less pronounced with older cows and with barren cows.

Throughout the lactation period the proportion of volatile fatty acids of milk fat falls, and that of the unsaturated fatty acids declines to a minimum

about the 4th to 5th month of lactation after which it increases slightly (Bartley *et al.*, 1951). There is also a tendency for the fat globules to become smaller as lactation advances.

Of the mineral constituents, the concentration of chlorides falls to a minimum around the 45th day, after which it increases steadily until the last few weeks of lactation when it rises more sharply. After an early decline the content of calcium recovers as lactation advances, but that of phosphorus tends to decline throughout the lactation period.

TABLE X

PERCENTAGE COMPOSITION OF MILK OF PEDIGREE AYRSHIRE COWS OF VARIOUS AGES (Waite *et al.*, 1956)

Lactation	No. of cows	Yield in gallons	Fat	S.n.F.	Total protein	Casein	Lactose
1st	187	730	4·11	9·01	3·36	2·72	4·72
2nd	138	792	4·06	8·92	3·35	2·66	4·62
3rd	108	846	4·03	8·82	3·28	2·63	4·59
4th	102	907	4·02	8·84	3·30	2·61	4·57
5th	75	879	3·90	8·72	3·26	2·54	4·53
6th	65	887	3·91	8·74	3·30	2·62	4·48
7th	44	961	3·94	8·67	3·25	2·53	4·48
8th	45	885	3·82	8·65	3·23	2·50	4·44
9th and over	50	889	3·92	8·67	3·28	2·51	4·47
Mean[1] 3·8		835	4·01	8·84	3·30	2·63	4·59

[1]For 814 cows.

It is clear that, if a large proportion of the herd reaches the point of the lowest content of fat or non-fatty solids in the milk at a time when other disturbing factors are in operation, the combined effect may bring the composition below the accepted standards for fat or non-fatty solids. Waite *et al.* (1956) point out that cows which calve from mid-November to the end of February will reach this minimum period in the early months of the year when in Great Britain feeding difficulties tend to depress the content of non-fatty solids. Further, if these two factors are aggravated by the presence in the herd of cows in their fourth or later lactations, the content of non-fatty solids of the bulk milk of the herd may well fall below the minimum of 8·5 % required in the U.K.

C. Effect of Age

This effect is illustrated in Table X, which is compiled from the observations of Waite *et al.* (1956). It is noteworthy that up to the fourth lactation, the decline in content of non-fatty solids is almost entirely accounted for by the fall in lactose concentration. The observation by the Milk Marketing Board (1956) that a decline of 0·1 % S.n.F. may be expected in every succeeding lactation up to the third, and smaller effects thereafter, is in agreement with these findings. Griffiths & Featherstone (1957) in an investigation of herds giving milk low in non-fatty solids found that the 0·1 % decline per lactation was maintained up to the sixth or seventh lactation.

D. Variation in Composition During Milking

The fat content of cow's milk rises during milking and, contrary to the generally accepted view, it would appear from the work of Gardner *et al.* (1956) that the non-fatty solids are also affected:

	Fore milk	Middle portion	Hand strippings
Fat %	2·4	3·8	8·9
Non-fatty solids in fat-free milk %	8·3	8·6	8·2

With experimentally milked sows, Whittlestone & Perrin (1954) found no increase in the fat content of the milk during milking and observed that, unlike human and cow's milk, sow's milk does not cream at body temperatures. Since clustering of fat globules is a necessary preliminary to creaming, they advanced the theory that in human and cow's milk, clusters of fat globules are able to form in the gland and that during milking the smallest clusters pass out first, and the largest are retained in the strippings. The globule clusters, whether large or small, contain representative selections of globules of all sizes.

Whittlestone (1953) found no increase in the size of fat globules in cow's milk throughout milking.

E. The Influence of the Breed

Even within the same species, breeds differ in the composition of their milk. The main difference is in the content of fat, and that of non-fatty solids tends to vary in the same direction, but not to the same extent. Tables XI and XII illustrate these differences for the cow. The effect of breed on the vitamin content is considered in Section IX.

TABLE XI

PERCENTAGE COMPOSITION OF MILK OF DIFFERENT BREEDS
(Anonymous, 1949)

Constituent	Friesian	Shorthorn	Ayrshire	Guernsey
Fat	3·49	3·56	3·72	4·55
Non-fatty solids	8·59	8·71	8·78	9·01
Non-fatty solids in fat-free milk	8·90	9·03	9·12	9·45
Protein	3·28	3·32	3·38	3·57
Casein	2·47	2·52	2·56	2·74
Lactose	4·46	4·51	4·57	4·62
Ash	0·75	0·76	0·74	0·77

TABLE XII

PERCENTAGE COMPOSITION OF MILK OF DIFFERENT BREEDS IN MANITOBA, 1952–54
(Mean values)
(Reinart & Nesbitt, 1956a, b)

	Holstein	Jersey	Guernsey	Ayrshire	Red Poll	Brown Swiss
No. of samples	75	72	23	70	20	23
Total solids	11·91	14·15	13·69	12·69	13·28	12·69
Fat	3·56	4·97	4·58	3·97	4·24	3·80
Non-fatty solids	8·35	9·19	9·14	8·72	9·05	8·89
Non-fatty solids in fat-free milk	8·66	9·68	9·56	9·08	9·47	9·23
Lactose	4·61	4·70	4·78	4·63	4·77	4·80
Protein	3·05	3·66	3·50	3·25	3·40	3·18
Ash	0·73	0·77	0·75	0·72	0·72	0·72
Ca	0·117	0·143	0·137	0·120	0·126	0·129
Mg	0·015	0·015	0·016	0·015	0·016	0·015
P	0·091	0·102	0·104	0·092	0·096	0·097
Cl	0·110	0·098	0·088	0·101	0·084	0·092
	Nitrogen distribution as % of total N					
Casein	77·7	79·0	77·9	77·4	80·0	77·8
Albumin	9·0	9·2	9·1	8·4	7·9	8·8
Globulin	3·7	3·7	3·4	4·0	3·1	3·6
Proteose-peptone	4·0	3·7	4·1	4·9	3·9	3·8
Non-protein nitrogen	5·7	4·4	5·5	5·3	5·2	6·0

F. Milking Intervals

Though it is generally agreed that it is desirable, on the grounds of yield and composition, to milk cows at regular intervals, in practice uneven milking intervals are imposed by expediency. The procedure appears to affect fat content only. After a longer night interval the fat percentage in the morning's milk is lower than that of the previous evening's milking. The differences are approximately of the order:

Intervals:

15 h night 9 hr day, 1% more fat in evening milk.
14 h night 10 hr day, 0·7% ,, ,,
13 h night 11 hr day, 0·3% ,, ,,

Superficially, it may appear that the yield of non-fatty solids is also affected, but recalculation on a fat-free milk basis shows that it is not. For example: morning milk 3·22 % fat, 8·98 % S.n.F.; evening milk 4·46 % fat, 8·84 % S.n.F.; on a fat-free basis, the non-fatty solids are respectively morning and evening, 9·28 % and 9·25 %.

Even when the milking intervals are 12-hourly the fat percentage in the morning milk tends to be less than in the evening milk. From records for forty Holstein cows over a period of 4 years, Nicholson *et al.* (1957) obtained the following values:

	Yield	Fat	S.n.F.	Weight of fat	Weight of S.n.F.
	lb	%	%	lb	lb
Morning	18·56	3·66	8·60	0·68	1·60
Evening	16·47	3·76	8·59	0·62	1·42

G. Feed

In most animals not specialized for milk production the composition of milk is, apart from the vitamins, largely independent of the quality of the diet, any inadequacies or shortages being reflected by a diminished yield. In milch animals, however, and particularly in the ruminants, dietary effects may be marked. During the past 10 or 12 years a great deal of research in all the milk-producing areas has been concentrated on such effects of quantity and quality of the food on the composition of milk. Three main aspects have been studied:

1. Variation in energy and protein intake;
2. quantity and quality of roughage;
3. grass feeding.

1. *Variation in Energy and Protein Intake*

Wartime shortages of sources of energy and protein for milk production impressed upon workers in all countries the fact that inadequate feeding can give rise to poor-quality milk. Controlled experiments have since confirmed those impressions, and there is now ample evidence to support the view that insufficient energy intake leads to a fall in the content of non-fatty solids of the milk. The milk constituent chiefly affected is protein.

Although a low-protein ration has sometimes resulted in a low content of non-fatty solids, the effect is not so severe as with diets low in energy. Small variations above or below the accepted requirements for milk production are without effect on milk composition.

TABLE XIII

EFFECTS OF UNDERFEEDING ON THE CONTENT OF THE NON-FATTY SOLIDS IN MILK

Reference	Starch equivalent for production given above the maintenance level		S.n.F. percentage	
	lb per day	lb per 10 lb milk	Original	Change
Bartlett & Rowland, quoted by Burt (1957a)	1·9	1·0	8·40	
	5·9	2·7	8·69	+ 0·29
Dijkstra, 1942	6·9	1·8	8·32	
	9·6	2·3	8·52	+ 0·20
Riddet *et al.*, 1941-42	Production ration reduced 50%			− 0·3 to − 0·5

A further, and equally valid conclusion may be drawn from these investigations, namely that the feeding of balanced concentrates beyond the recognized standards of energy intake may cause the non-fatty solids content of milk to increase. The milk yield may also increase and thus it becomes a matter of economics to decide just how far this practice may be justified. The subject is fully discussed in a review by Burt (1957a). Typical examples of these effects are summarized in Tables XIII and XIV.

Underfeeding has its effect also on the composition of milk fat. The main changes are an increase in the proportion of unsaturated fatty acids with a corresponding decrease in the proportion of all fatty acids up to C_{14}. These

TABLE XIV

EFFECTS OF FEEDING BEYOND ACCEPTED REQUIREMENTS ON MILK YIELD AND COMPOSITION

Breed and reference	Starch equivalent for production given above the maintenance level		Yield per day per cow	Percentage composition of milk				
	lb per day	lb per 10 lb milk	lb	Non-fatty solids	Fat	Protein	Casein	Lactose
Ayrshire								
Holmes *et al.*, 1956	12·2	3·1	38·9	8·62	4·01	3·09	2·43	4·59
	13·8	3·4	40·6	8·75	3·02	3·22	2·55	4·58
	14·8	3·8	40·9	8·77	3·75	3·23	2·55	4·59
Holmes *et al.*, 1957	5·3	2·4	22·2	8·34	3·99	3·06		4·37
	7·2	3·0	23·8	8·40	3·94	3·09		4·36
	9·1	3·5	26·0	8·58	3·99	3·20		4·47
	11·5	4·2	27·0	8·60	4·09	3·27		4·44
Shorthorn								
Burt, 1957b	5·9	2·7	21·9	8·59	3·60			
	7·3	3·2	22·9	8·62	3·51			
	8·8	3·8	23·2	8·71	3·66			
Friesian								
Burt, 1957b	6·0	2·2	27·2	8·26	3·40			
	7·7	2·7	28·5	8·26	3·48			
	9·5	3·2	29·2	8·34	3·30			

effects have been noted by Riddet *et al.* (1941–42) and by Smith & Dastur (1938) as a result of withholding food for 12 days and may be explained by reduction in the formation of volatile fatty acids in the rumen.

2. *Quantity and Quality of Roughage*

The production of milk fat is partly dependent on the formation of acetic acid in the rumen (see Chapter 12). Thus any rations that depress the production of acetic acid will depress the fat content of milk. Such abnormalities may be brought about by insufficient roughage or by reducing the roughage to a fine state of division (Powell, 1938, 1939, 1941). Further work by Loosli *et al.* (1945), Stoddard *et al.* (1949), Tyznik & Allen (1951), Balch *et al.* (1953, 1954a,b,c, 1955a,b) and Emery *et al.* (1956), has shown that a reduction in fat content by 1 to 2 % may be expected when the quantity of hay is reduced from the normal 16–18 lb to 2–6 lb, the content of non-fatty solids being unaffected. The addition of sodium acetate to low-roughage diets brings about a partial recovery in the fat content of the milk. Neither sodium butyrate nor sodium propionate is effective. The effect of abnormal rumen conditions is greatest in early lactation, and the composition of the milk fat is modified in the same way and for essentially the same reasons as in underfeeding—there being less volatile acids and more oleic acid.

3. *Grass Feeding*

The change from stall feeding to grazing frequently, but not always, brings about a depression in fat content. Indeed, the sudden change from a fibrous winter ration to a lush herbage often has this effect. Furthermore, herbage contains substances possessing oestrogenic activity (Legg *et al.*, 1950; Pieterse & Andrew, 1956). These substances are present in greater quantity in spring grass and may have an effect on the production and composition of milk.

There is general agreement that the change from stall feeding to grazing causes an increase in the non-fatty solids of milk, but it is not known whether the effect is that of a higher plane of nutrition, of oestrogenic activity of the herbage or of some factor as yet undiscovered. In the opinion of Bartlett & Kay (1950) any cow that does not respond to the stimulus of the new grass in this manner may be considered a genetically poor producer, or may be suffering from disease.

The changeover from stall feeding to grazing and vice versa affects the the composition of milk fat. The lipids of grass are very rich in unsaturated C_{18} acids, particularly di- and tri-enoic. Shorland *et al.* (1955) have shown that in the rumen these acids may undergo hydrogenation to oleic, and perhaps stearic, acid. In fact, the proportion of oleic acid in milk fat increases when cows go to pasture to the extent of some 10 to 15 % and decreases when they return to stall feeding (Bartley *et al.* 1951).

Garton & Duncan (1956) found that the inclusion of grass silage in the ration of stall-fed cows brought about an increase in oleic acid content of the milk fat (from 21 to 29 %) on the molar basis. At the same time stearic acid increased from 5 to 9 % and palmitic acid decreased from 33 % to 28 %.

H. Seasonal Influences

It is only by examination of large bulks of milk that the true seasonal effects can be assessed for, as already seen, the composition of milk is subject to the operation of many factors, which in small bulks of milk would obscure the seasonal effect. Changes in the composition of the bulk milk of the Nottingham University herd in England throughout the year were far more closely related to changes in the "lactation composition" of the herd than to any of the factors normally associated with season (Ling, 1937). Data from the records of large dairies give a clear picture of the seasonal effect on fat content. In Great Britain it falls from a maximum in late autumn to a minimum in early summer, rising steadily thereafter to the autumn value. The content of non-fatty solids shows two minimum periods, one in March–April and the other in July–August. The extent of these effects is clearly shown in the figures from the Milk Marketing Board's creameries for the years 1948–52 (Milk Marketing Board, 1956).

	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Fat %	3·61	3·54	3·49	3·46	3·40	3·42	3·51	3·57	3·67	3·78	3·82	3·73
S.n.F. %	8·59	8·58	8·56	8·57	8·71	8·73	8·66	8·63	8·69	8·72	8·68	8·61

There are, however, significant regional differences in England and Wales: for example, the decline in the content of non-fatty solids during the early months of the year tends to be greater in the west than in the east, but the summer decline is much more marked in the east. One cause of the decline during the early months of the year is undoubtedly the feeding of rations low in energy, a situation which is likely to arise in countries that rely so much upon home-grown concentrates. In the western areas the early decline is always more marked after a bad hay harvest. The summer decline is in part due to a falling-off in the quantity of grass available and a tendency for its energy value to lessen as the season advances. These effects are likely to be greater in the eastern areas of lower rainfall than in the moister western districts. Conditions of summer drought have long been known to cause a serious reduction in content of non-fatty solids in milk.

That these are critical periods is illustrated by the Milk Marketing Board's records for 1947–53 (Provan, 1955). Of the total number of consignments failing to reach the required limits of 3 % fat and 8·5 % S.n.F., the majority

were deficient in non-fatty solids rather than fat. However, fat deficiencies did occur, particularly in the period April–June when the effects of the new grass were operative. The month of June was the least troublesome so far as the attainment of a satisfactory level of non-fatty solids was concerned. For every 100 samples below standard in June there were 231 in July, 257 in August, 162 in September, 148 in October, 184 in November, 262 in December, 313 in January, 339 in February, 377 in March, 377 in April and 191 in May.

I. Environmental Temperature

The general question of climatic conditions as related to milk production and composition is reviewed by Hancock (1954).

The yield and composition of milk are much the same within the temperature range 40° to 70°F. From 70° to 80°F the yield decreases slowly and the fat percentage is reduced, but beyond 80°F the decline in yield is much more marked and, whereas the fat percentage increases, the content of non-fatty solids is usually depressed. At these higher environmental temperatures, two factors come into operation: loss of appetite and the disturbing effect of high body temperature discussed on page 244. It is noteworthy that three of the European breeds have shown different degrees of tolerance to high environmental temperatures. The decline in yield has been observed earlier with the Holstein breed than with Jersey or Brown Swiss cattle. The low-yielding Brahman (zebu) cattle are very tolerant of temperatures up to 90°F. Below 40°F the yield falls and concomitantly fat and non-fatty solids increase.

J. Irregular Drinking

Irregular drinking may have peculiar effects on the composition of milk. In one of the recorded instances in Great Britain (Aschaffenburg & Rowland, 1950) healthy, well-fed cows were brought in at 4 p.m. and housed until 9 a.m. with no access to water. When turned out in the morning they drank at once excessively and not again during the day. This heavy intake of water was further increased by a large ration of mangolds eaten during the morning. As a consequence, the evening's milk showed all the characteristics of watering, and the morning's milk showed some signs of dehydration of the animals.

Evening milk: 8·26 % S.n.F., 0·526°C freezing point depression
Morning milk: 8·83 % S.n.F., 0·560°C freezing point depression

This abnormality disappeared when the cows went out to grass and water was available night and day.

K. The Decline in Milk Solids Observed in England and Wales

It may be of general interest to quote here a phenomenon, locally observed in England and Wales, in so far as it reflects the complicated physiological and nutritional basis of milk production.

A comparison of modern records with those of 20 or so years ago has shown that present-day values for non-fatty solids are about 0·2 % lower; the fat content is only slightly less. No such change has been observed in Scotland (Waite & White, 1956). No adequate explanation is forthcoming, but three possibilities are worthy of consideration:

(i) Emphasis on quantity rather than quality;
(ii) changes in breed composition of dairy herds;
(iii) increasing age of cows in milk.

(i) Whenever and wherever milk is bought and sold on a volume basis, with statutory requirements for content of fat and non-fatty solids below the mean values for milk, producers have tended to increase yields rather than raise the nutritive value of milk. Whether this tendency has increased unduly over the past 20 years must remain a matter of opinion.

(ii) A striking change in the breed composition of herds in England and Wales has taken place during this period (Davis, 1952). The numbers of the once popular Dairy Shorthorn are decreasing and those of the Friesian are increasing. Although the records show that the Friesian breed gives milk of only about 0·12 % less non-fatty solids than the Shorthorn, it is clear that this change may be expected to make some contribution to the general decline. In Scotland the breed composition has not changed so markedly.

(iii) Advances in animal husbandry have quite conceivably increased the dairy cow's expectation of life. As observed on p. 235, the yield may increase up to the sixth or seventh lactation, but the content of non-fatty solids declines from the first lactation. Thus any significant increase in the average age of cows in the herds must cause a decline in the content of non-fatty solids in their bulked milk.

L. Effects of Mastitis

It is true that the extensive use of antibiotics has diminished the incidence of mastitis, particularly streptococcal mastitis, among cows, but it would be unwise to ignore the effects of mastitis on the yield and composition of the milk of the infected quarters (cf. e.g., McDowall, 1945–46). Together with a drastic reduction in yield, the content of non-fatty solids falls. The fall may be slight (but, exceptionally, may reduce the content of non-fatty solids to 5 %), and is due mainly to an abnormally low lactose concentration. The casein concentration

also falls, but because of infiltration of blood protein the total nitrogen even increases slightly. As a result the ratio $\frac{\text{casein nitrogen}}{\text{total nitrogen}} \times 100$ decreases appreciably from the normal value of about 79 % (as little as 40 % having been observed in severe attacks). Indeed, this measure provides a useful laboratory test for detecting sub-clinical mastitis. It is well to bear in mind, however, that late lactation also causes a change in nitrogen distribution which lowers the casein ratio. Other characteristics of milk from cows affected with mastitis are:

(i) A high chloride content which corrects the effect of low lactose concentrations on the osmotic pressure;
(ii) a higher pH (up to 7);
(iii) a greatly increased enzyme content (cf. p. 212).

M. Effects of Other Diseases

It is difficult to find definite information on the effects of other diseases on milk composition, but King (1956) observes that illness that raises the body temperature above 102·4°F will depress the yield and non-fatty solids content of milk and that, though the effect on the fat percentage is not so clearly defined, there is a tendency for it to increase—at least during the initial stages. Conversely, illness that does not cause the body temperature to rise above 102·4°F is without effect on the milk composition—although the yield may suffer.

Seelemann (1957) has reviewed the effects of foot and mouth disease on the composition of milk. If the udder is not infected with organisms causing mastitis before the onset of the disease, the effects on milk composition are precisely those attributed to an increase of body temperature. If there are ruptured vesicles or teat sores, the yield declines because of difficult milking and retention of milk in the udder, and the milk acquires the characteristics of milk drawn very late in lactation, namely low concentrations of lactose and casein, and high concentrations of serum proteins and salt. Prior infection of the udder superposes the characteristic effects of mastitis on those of the foot and mouth disease. Inoculation with the foot and mouth vaccine has of itself no appreciable effect on the composition of milk.

IX. Vitamins

A. General

It is not easy to define what vitamins are, and possibly one of the clearest descriptions is that of Harris (1951) who considers them to be "substances

that (a) are distributed in foodstuffs in relatively minute quantities, that (b) are distinct from the main components of food (i.e. proteins, carbohydrates, fats, mineral salts and water), that (c) are needed for the normal nutrition of the animal organism, and (d) the absence of any one of which causes a corresponding specific deficiency disease."

Strictly speaking vitamins, which for the most part are fully characterized chemically, should be considered either in relation to their chemical composition or to their association with the different phases of milk. It is more convenient, however, to group them together as a body of trace compounds having in common their essentiality in nutrition.

The subdivision into water-soluble and fat-soluble vitamins is not only customarily convenient but, save for vitamin C, separates those vitamins known to be components of the prosthetic groups of enzymes and to be closely concerned with fundamental metabolic processes of living cells and ubiquitously distributed, from those that appear to have only highly specialized functions for certain tissues and cell groups and whose biochemical function is still obscure (Beerstecher, 1950).

When it comes to the milk of ruminants, the subdivision is particularly appropriate since they are able to synthesize through the agency of their ruminal microbial flora all the vitamins of the B complex, but are dependent on an exogenous supply of the fat-soluble ones. Vitamin C is a dietary essential only for man, the primates and the guinea-pig.

The vitamin content of the milk of different species is given in Table II.

Further reference to the occurrence of vitamins in cow's milk will be found in the reviews by Kon & Henry (1949, 1951, 1954) and by McGillivray & Porter (1956, 1958, 1960).

B. Fat-soluble Vitamins

1. *Carotene and Vitamin A*

Though vitamin A is an essential nutrient of great importance, its only well-defined function is as part of the visual pigment, rhodopsin, and as such it is essential for normal vision. Animals obtain vitamin A preformed from animal products in their diet, or from carotenes. The carotenes are yellow or orange pigments that occur in plants. Most of the carotenoids consist of two unsubstituted or substituted ionone rings linked by an unsaturated chain of the same nature but twice as long as the vitamin A side-chain. Goodwin (1952) discusses the comparative biochemistry of the carotenoids. To be active as a vitamin A precursor a carotenoid must have at least one unsubstituted β-ionone ring. From such carotenoids vitamin A (not found in plants) is formed in the animal body. β-Carotene with two β-ionone rings is the main

presursor of vitamin A for omnivorous and herbivorous animals; dietary α- and γ-carotene and cryptoxanthin may also contribute to the formation of vitamin A. Species vary widely in their ability to utilize carotenoids. Many animals, including the sheep, the goat, the Indian buffalo, the rat, the rabbit and the pig have almost completely white body fat and only traces of carotenoid pigments in their tissues, blood and milk. The main site of conversion into vitamin A of ingested carotene is the intestinal wall (Glover *et al.*, 1947; Mattson *et al.*, 1947a,b; Wiese *et al.*, 1947; Thompson *et al.*, 1947) so that the absence of carotenoids from the tissues of these animals suggests that in them, carotenoids are either not absorbed into the circulation or are rapidly degraded in the tissues into colourless products.

In other animals such as man, the cow and the horse, conversion of ingested carotene still takes place in the intestine, but substantial amounts of carotenoids are absorbed unchanged.

In the cow, the circulating carotenoids are mainly carotenes in which β-carotene predominates with, according to the nature of the diet, some xanthophylls and lycopene. Although the milk of all animals contains vitamin A, some 98 % of which is esterified, probably as the palmitate, and 2 % uncombined, only those animals having carotenoids circulating in the blood secrete these compounds into milk. Thus woman's, cow's and mare's milk contains both vitamin A and carotenoids, whereas that of the goat, ewe, Indian buffalo and pig contains only vitamin A. In cow's milk, active carotenes constitute about four-fifths of the total carotenoids, whereas in human milk they amount to only one-quarter.

In the mechanical separation of cream from milk some 0·1 % of "fat" remains in the separated milk. The concentration of carotene and vitamin A alcohol in this residual "fat" is markedly higher than in the cream lipids. Vitamin A in the ester form is not so partitioned, and as it constitutes 98 % of the total vitamin A the effect on the partition of whole vitamin A is negligible. For carotene, Kon *et al.* (1944) suggested its presence in the fat-globule membrane as an explanation of the phenomenon: the smaller globules in the separated milk having relatively more membrane than the larger globules in cream. Recently McGillivray (1957) confirmed these findings but suggested that they could be interpreted in a different way and that some of the vitamin A alcohol and carotene is present not in the fat phase but combined with the globulin fraction of the milk-serum proteins. This interpretation would in fact fit the original findings of Kon *et al.* (1944) since these authors found the differences in the fats extracted by solvents from the different fractions but to a much lesser extent in the fats obtained by churning.

It is likely that both explanations are partly true and that carotene, apart from that in the fat phase proper, is present both in the fat-globule membrane and as a protein complex.

The conversion of carotene into milk vitamin A is inefficient. Thus Baumann *et al.* (1934) calculated that cows on pasture put only 1·3 % of the ingested carotene into the milk as vitamin A. Furthermore, different breeds of cattle show marked differences in their utilization of carotene. Under the same feeding conditions the Channel Island breeds produce fat containing much more carotene and less vitamin A than that of other breeds such as the Holstein, which may be explained by greater uptake of carotene into the circulation and less efficient conversion of it into vitamin A in the gut (Table XV).

TABLE XV

TOCOPHEROL, CAROTENOID AND VITAMIN A CONTENT OF MILK FAT
(Krukovsky *et al.*, 1950)

Breed	Period	No. of cows	Tocopherols	Carotenoids	Vitamin A
			p.p.m.	p.p.m.	p.p.m.
Holstein-	1	18	22·5	5·1	5·5
Friesian	2	20	20·1	2·9	4·0
	3	13	24·9	7·7	9·1
	Average		22·2	4·9	5·8
Jersey	1	5	30·4	12·4	5·8
	2	4	19·1	3·4	3·0
	3	7	27·4	13·7	6·3
	Average		26·2	10·7	5·3
Guernsey	1	9	31·6	15·8	3·8
	2	7	23·3	7·7	3·1
	3	12	33·5	24·8	6·6
	Average		30·3	17·7	4·9

Period 1: October, towards the end of grazing season.
Period 2: March after 5 months' stall feeding.
Period 3: July after 3 months' grazing.

Cow's colostrum is up to ten times richer in carotene and vitamin A than milk (see Table VIII), but after the first few days the levels become steady and there are no further lactational trends.

Vitamin A is also several times higher in the colostrum of the goat (Chanda, 1953) and of the sheep (Pope *et al.*, 1949) than in their milk.

The greatest variations in the vitamin A activity of milk are due to variations in the carotene content of the cow's food. During summer, grazing cows

will ingest 3 to 5 g of carotene daily, but during the winter months the intake will be very much lower unless the diet contains a large proportion of silage or kale which may supply 0·5 to 1·0 g of carotene daily. All countries in the northern hemisphere show a similar seasonal fluctuation with higher values in the summer than in winter, depending on the carotene content of the feed.

It is interesting to note that in New Zealand, where the cows are on pasture throughout the year, seasonal differences in carotene and vitamin A are still manifest. Thus Barnicoat (1947) reported higher values in winter than in summer and McDowell & McDowall (1953) give the following figures:

	Vitamin A (i.u./g fat)	
	Winter	Summer
New Zealand	41·9[1]	33·8[2]
Great Britain	18·5	29·0
Sweden	15·0	24·2
Denmark	16·5	33·1

[1]April to August [2]September to March

McGillivray (1952) showed that the herbage in New Zealand provided an adequate carotene intake even in midsummer and suggested that these findings depict impaired utilization of the carotene from the summer sward, possibly associated with changes in the composition of the sward lipids (cf. McGillivray *et al.*, 1958).

Although the vitamin A potency of milk and butter is a reflection of the amount of carotene in the milch animal's feed, the potency derived from this source reaches a maximum at about the summer level. It is, however, possible to increase markedly the vitamin A content by giving preformed vitamin A. Thus Blaxter *et al.* (1946) showed that in the cow daily additions of 0·3 g vitamin A caused a three- to six-fold increase in the potency of the milk fat from around 7 μg per g. Such high quantities of vitamin A depress the concentration of carotene in the milk.

Unlike most other vitamins, vitamin A is stored in appreciable quantities in the liver. The relationship between the forms and levels of carotenoids and vitamin A in blood and milk and these liver stores is still uncertain. The free and esterified forms of vitamin A are present in both milk fat and blood plasma, the proportions in plasma being the reverse of those in milk, i.e., 98 % alcohol and 2 % ester. It seems probable that normally the vitamin A ester in milk fat is derived directly from that in the blood. Under conditions of low carotene intake, milk-fat vitamin A ester may be derived from the liver stores via the vitamin A alcohol in the plasma, for Chanda *et al.* (1955) showed

that the mammary gland can esterify plasma vitamin A alcohol. Carotenoids in the milk fat reflect very closely the changing levels of carotenoids in plasma.

However, McGillivray (1957) showed that when cows were transferred from pasture to a carotene-free diet the levels of carotene in the plasma did not change immediately although the milk-fat carotene decreased from 7·2 to 3·0 μg per g in 7 days. McGillivray & Thompson (1957) suggest that, by analogy with plasma vitamin A alcohol and ester, there may be two distinct forms of carotene in the blood plasma. One, corresponding to vitamin A alcohol and associated with plasma proteins, contributes little carotene to milk fat. The other, only present when the animals are receiving carotene in the diet, represents a transport form, probably associated with the vitamin A ester and dietary fat in the chylomicrons, which can be taken up directly by the mammary gland and secreted into the milk.

2. *Vitamin D*

Vitamin D, the antirachitic vitamin, is required by birds and mammals for efficient uptake and utilization of calcium and phosphorus.

In the milk of milch animals vitamin D is normally present both as vitamin D_2, a derivative of ergosterol, which stems mainly from hay, and as vitamin D_3, a derivative of 7-dehydrocholesterol, produced by the direct action of the ultraviolet rays of the sun on the animal. The concentration of vitamin D is directly related to its supply in the diet and to the direct exposure of the animal to sunlight. Young grass contains only traces of vitamin D, but during haymaking irradiation of plant sterols causes the production of vitamin D_2 so that hay provides a fair source of the vitamin during indoor feeding. None the less, the concentration of vitamin D in milk is usually greater during the summer months when the animals are outdoors. Even in summer, milk contributes negligible quantities of vitamin D to the human diet. The vitamin D level of milk may be increased by giving sources of the vitamin, by ultraviolet irradiation of milk or, most simply, by adding to milk a dispersible preparation of the vitamin. Other things being equal the vitamin D content of milk is proportional to its fat content, and milk of Channel Island breeds is rather higher in vitamin D than that of Holsteins. Colostrum is some two to three times richer in vitamin D than the later milk.

3. *Vitamin E*

The tocopherols comprise a family of substituted quinones condensed with the alcohol, phytol, and occur in most plants. These compounds have a special role as antioxidants in the animal body. Their vitamin nature is less defined than that of other vitamins since other antioxidants, such as methylene blue or *NN'*-diphenyl-*p*-phenylenediamine, are able either to carry out many biological functions of the tocopherols or spare them markedly in metabolism.

Cow's milk contains vitamin E in the form of α-tocopherol (Brown, 1952). The concentration of the vitamin in milk is related to that in the diet, the levels being about twice as high during summer grazing as during indoor feeding. The feeding of 2 g tocopherol per day to cows on winter rations restored the tocopherol content of the milk fat to summer levels (Jensen & Pedersen, 1954).

Apart from its vitamin function tocopherol may act as an antioxidant in milk fat but its role is still obscure.

4. *Vitamin K*

Two natural forms of the antihaemorrhagic vitamin K, both related to napthoquinone are known. Vitamin K_1 occurs widely in plant materials; vitamin K_2 is a bacterial product and is synthesized in the alimentary tract of most animals. Thus, though milk is a relatively poor source of vitamin K, the concentration of vitamin K in it, unlike that of other fat-soluble vitamins, is unaffected by the supply in the diet.

C. Water-soluble Vitamins

In ruminants all the vitamins of the B complex are synthesized in the rumen and ruminants are largely independent of an exogenous supply. For this reason the level of these vitamins in their milk varies much less than in that of simple-stomached animals, for example in human milk, and is determined largely not by the diet but by factors such as breed and stage of lactation. Examples of lactational trends in milk of Friesian and Shorthorn cows are shown in Fig. 1 taken from Gregory *et al.* (1958). As stated on page 254 the ascorbic acid content of the milks of most species is independent of the supply of the vitamin in the diet, human milk being a notable exception.

1. *Thiamine*

Thiamine pyrophosphate, cocarboxylase, is a coenzyme concerned in lactate and pyruvate metabolism and is essential for all forms of life.

Thiamine occurs in milk both free and phosphorylated, probably as the monophosphate and not as cocarboxylase (de Jong, 1942). The proportion of the phosphate shows a close negative correlation with the milk's content of phosphatase. Thus in the cow, early-lactation milks contain approximately 25 % of free thiamine and mid- and late-lactation milks 80 and 100 %, respectively (Houston *et al.*, 1940). In goat's and sow's milks, which throughout lactation contain less phosphatase, the combined form accounts for some four-fifths of the total (Hodson, 1945). A small proportion (10 %) of the thiamine in milk is combined with protein (Houston *et al.*, 1940).

In early lactation, cow's milk contains 45 to 55 μg per 100 ml; the content declines to a steady level of 30 to 36 μg per 100 ml and is unaffected by the nature of the diet or by other seasonal influences. In mastitis, milk from

infected quarters contains about a quarter less total vitamin B_1 than normal milk (Thompson, 1945). This change is associated with falls in the concentration of thiamine phosphate and protein-bound thiamine (Chanda, 1953).

As Table VIII shows cow's colostrum is richer in thiamine than milk; the opposite trend in human milk is discussed in Chapter 18.

2. *Riboflavin*

Riboflavin is a constituent of two different coenzymes: flavinmononucleotide (riboflavin-5′-phosphate), concerned in carbohydrate metabolism; and flavinadeninedinucleotide (FAD), present in diaphorase and xanthine oxidase.

The form in which riboflavin occurs in milk is known to vary with species (Davis *et al.*, 1950). For instance cow's milk was found to contain mainly free riboflavin whereas sow's milk contains principally flavinadeninedinucleotide (Gregory & Holdsworth, 1953). Recently the partition of riboflavin between free riboflavin, riboflavin-5′-phosphate and FAD in the milk of several species was further studied by Modi & Owen (1956), who found that the milk of cows, goats, ewes and rabbits contained mainly free riboflavin, whereas human, mare's and sow's milks contained only FAD. In a subsequent paper Manson & Modi (1957) investigated the occurrence and metabolism of FAD in the milk of the sow and cow. The free riboflavin in cow's milk and a proportion of the FAD in sow's milk were ultrafiltrable from unheated milk, but heating the milks at 95°C for 30 minutes denatured the milk proteins and liberated all the FAD in both milks. When FAD was added to fresh cow's milk it was rapidly destroyed with the formation of free riboflavin and riboflavin-5′-phosphate, but when it was added to pasteurized milk that showed no phosphatase activity the final product was riboflavin-5′-phosphate alone. There was no alteration of FAD added to sow's milk. In cow's milk some of the FAD is present as xanthine oxidase, but no xanthine-oxidase activity could be demonstrated in sow's milk and Manson & Modi suggested that sow's milk must contain a different combined form of FAD.

The level of xanthine oxidase activity in milk in relation to the molybdenum intake of the cow was studied by Kiermeier & Vogt (1956) and Kiermeier & Capellari (1957). The mean activity of the milk of cows receiving fodder containing 13 μg of molybdenum per 100 g was only about one-tenth of that of the milk of cows grazing grass containing 353 μg of molybdenum per 100 g dry matter. Preliminary findings indicated, however, that an inorganic molybdenum supplement does not cause such a marked increase.

Cow's colostrum is markedly richer in riboflavin than milk (see Table VIII). In milk riboflavin is largely unaffected by diet, season or stage of lactation (Fig. 1). Breed differences in the riboflavin content of milk are exemplified in Table II.

3. *Nicotinic Acid*

Nicotinamide is linked with adenine, ribose and phosphoric acid in coenzymes I and II that are dehydrogenases concerned in a variety of dehydrogenation reactions.

Most of the nicotinic-acid activity of milk appears to be due to nicotinamide (Krehl *et al.*, 1946). There is little evidence of a direct effect of the composition of the feed on the nicotinic acid content of milk. However, Lawrence *et al.* (1946) and Gregory *et al.* (1958) found progressive lowering of the level in winter and spring followed by a rise during the summer and autumn (shown in Fig. 2).

Milk is a relatively poor source of nicotinic acid. Two important properties, however, enhance its value as a source of this factor: one is that milk proteins are a good source of tryptophan from which nicotinic acid is formed in the body (Sarett & Goldsmith, 1949), the second is that nicotinic acid in milk is fully available whereas most of it in cereals is in a bound form in all probability not available to man (Kodicek *et al.*, 1956).

4. *Pantothenic Acid*

Pantothenic acid forms part of coenzyme A which is concerned with acylation.

Almost all the pantothenic acid in the milk of the cow, goat and sow is uncombined. The level in cow's milk rises to a peak (4 to 5 μg/ml) during the first week after calving and then gradually falls to a steady level as shown in Fig. 1. It is not affected by the diet or the season (Lawrence *et al.*, 1946; Pearson & Darnell, 1946; Gregory *et al.*, 1958).

5. *Vitamin* B_6

Vitamin B_6 occurs naturally in three forms, pyridoxine, pyridoxal and pyridoxamine. The phosphates of pyridoxal and pyridoxamine are the functional forms of the vitamin and act as coenzymes for several enzymes concerned with intermediary metabolism.

Rabinowitz & Snell (1948) showed that fresh cow's milk contains mainly pyridoxal and some pyridoxamine.

The vitamin B_6 content of cow's milk is at its highest during the first week of lactation, falling thereafter to the normal level unaffected by seasonal or dietary changes. Sunlight and ultraviolet irradiation of cow's milk cause destruction of vitamin B_6 (Morris *et al.*, 1949).

6. *Biotin*

Biotin is possibly the coenzyme of an enzyme concerned in carboxylation reactions. It is present in cow's milk in the uncombined form, but in sow's milk part of it is bound (Ford *et al.*, 1953). Lawrence *et al.* (1946) and Gregory

et al. (1958) found a great variability in the biotin content of milk from individual cows, and for any one cow the level varied considerably from day to day (Fig. 1). It is difficult, therefore, to assess the significance of possible seasonal and dietary effects, though these are probably small.

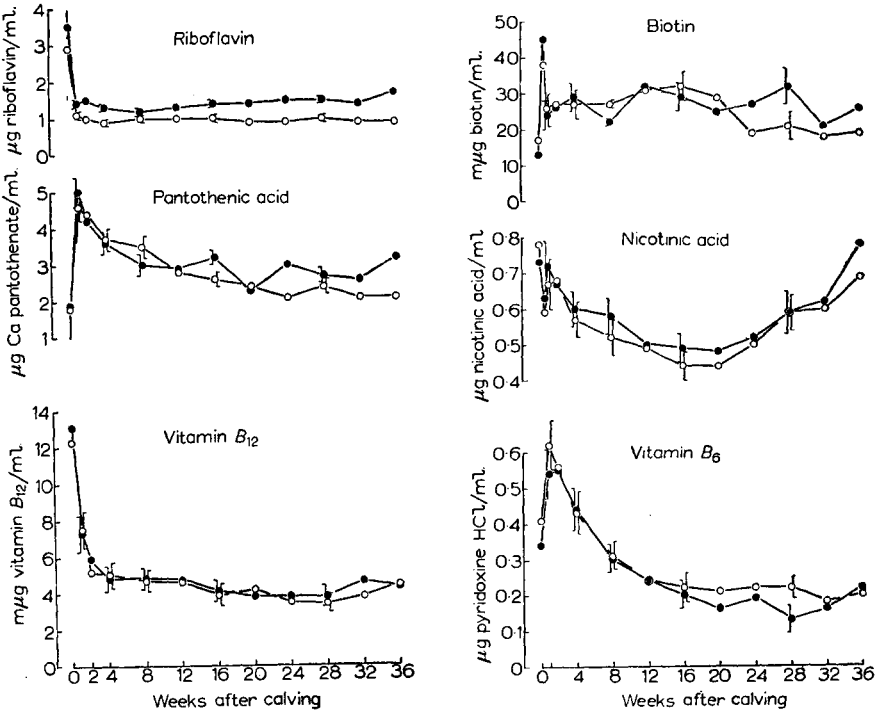


FIG. 1. Variation with stage of lactation in content of riboflavin, nicotinic acid, pantothenic acid, vitamin B_6, biotin and vitamin B_{12}, in the milk of Shorthorn (○—○) and Friesian (●—●) cows. The symbols ○ [ ] ● give the standard error of the means.

7. *Folic Acid*

The term folic acid includes several closely related compounds: pteroylglutamic acid, and the tri- and heptaglutamates. Folic acid, probably as folinic acid (5-formyl-5, 6, 7, 8-tetrahydropteroylglutamic acid), is required in birds and mammals for normal blood-cell development, and is concerned with the formation and metabolism of formate and other one-carbon fragments.

The concentration of folic acid in milk is low and its accurate determination has proved difficult. Present indications are that in cow's milk about 80 % is present as pteroylglutamic acid and the remainder as folinic acid (Collins *et al.*, 1953).

8. *Cyanocobalamin*

Vitamin B_{12} is of prime importance in man in red blood cell formation and hence in the prevention and cure of pernicious anaemia. Like folic acid, vitamin B_{12} takes part in protein utilization not only for haematopoiesis, but also for growth and development in all animals, and also in the formation and transfer of one-carbon intermediates.

The cyanocobalamin of the milk of the cow, goat, woman, sow, and ewe is bound (Gregory, 1954), that in cow's, goat's and ewe's milk being more easily released than that in sow's or woman's milk. The binding substance in sow's milk is a mucoprotein (Gregory & Holdsworth, 1955a,b).

As with biotin, there is considerable day-to-day variation in the concentration of cyanocobalamin in the milk of individual cows (Fig. 1). Provided the diet is not frankly deficient in cobalt, feed has little effect on the level in milk. It is also little affected by season or stage of lactation. Table II shows that goat's milk contains much less vitamin B_{12} than cow's milk. It is not known whether this low content is in any way connected with goat's milk anaemia sometimes observed in infants reared on goat's milk.

9. *Choline, Inositol,* p-*Aminobenzoic Acid*

It is a moot point whether or not these substances should be classed as true vitamins and it is sufficient to say that they are present in milk. Cow's milk contains, per 100 g, about 20 mg of choline, 18 mg of inositol and 10 μg of *p*-aminobenzoic acid.

10. *Ascorbic Acid*

Vitamin C, the anti-scurvy vitamin, exists in two biologically active forms: ascorbic acid, the more stable reduced form, and the reversibly oxidized form, dehydroascorbic acid which decomposes on heating in aqueous solution. Freshly drawn milk contains only reduced ascorbic acid but oxidation may occur on standing either in the light through a photocatalysed oxidation in which riboflavin acts as the energy acceptor, or in the dark through the presence of adventitious metals, particularly of copper. Subsequent heat treatment of the resulting mixture of ascorbic and dehydroascorbic acids results in the destruction of the latter, so that milk reaching the consumer usually contains only one-half or less of its original content.

In connexion with the photocatalysed oxidation it is interesting to note that the flavinadeninedinucleotide present in sow's milk does not act as an energy acceptor so that ascorbic acid in sow's milk is not oxidized in light unless free riboflavin is added (Coates *et al.*, 1950).

It is generally accepted that the vitamin C content of milk is little affected by the feed of the cow or by the season or the stage of lactation.

X. Milk in the Diet of Man

It is only in the artificial feeding of infants, considered in Chapter 18, that milk forms an almost exclusive human diet; otherwise in most of its dietary uses it forms part of a mixed diet. Its nutritive merits and significance can be gauged in two ways. The first is by the contribution of a customary intake to the daily requirements and the other by the proportion of nutrients it supplies in a normal mixed dietary.

TABLE XVI

APPROXIMATE PERCENTAGE CONTRIBUTION OF 0·5 l. GOOD-QUALITY FRIESIAN MILK TO THE DIETARY ALLOWANCES RECOMMENDED BY THE BRITISH MEDICAL ASSOCIATION: COMMITTEE ON NUTRITION (1950)

	2–6 year old child		Man (medium work)	
Nutrient	Recommended daily allowance	Percentage contribution by 0·5 l. milk	Recommended daily allowance	Percentage contribution by 0·5 l. milk
Calories	1500 kcal	20	3000 kcal	10
Protein	56·0 g	30	87·0 g	20
Iron	7·5 mg	2	12·0 mg	1
Calcium	1·0 g	60	0·8 g	75
Vitamin A activity	3000 i.u.	30	5000 i.u.	15
Vitamin D	400 i.u.	2	—	—
Thiamine	0·6 mg	35	1·2 mg	20
Nicotinic acid	6·0 mg	7	12·0 mg	3
Riboflavin	0·9 mg	85	1·8 mg	45
Vitamin C	15·0 mg	70	20·0	50
Iodine	0·15 mg	10	0·1 mg	15

The requirements cannot be easily defined and it is usual to express them in terms of recommended allowances which themselves vary with the opinion of the authority putting them forward. Table XVI lists the contribution of half a litre of milk as it comes from the udder (Tables I and II) to the allowances of specified nutrients put forward by the British Medical Association: Committee on Nutrition (1950). The list of the British Medical Association, as those of other authorities, is restricted to those of the many important nutrients for which the requirements and allowances are sufficiently known.

It will be seen that half a litre of milk will provide a child with almost all of the needed riboflavin, with close on two-thirds of the calcium and with one-third of the protein, vitamin A and thiamine. As it comes from the udder, milk supplies a large part of the needs for vitamin C as defined by the B.M.A. However, the great lability of ascorbic acid in milk may reduce the contribution. The shortcomings of milk in iron and vitamin D (see pages 219 and 249) are reflected in the smallness of its contribution to the allowances for these two nutrients. The contribution of nicotinic acid is small but, as stated on p. 252, the richness of the milk proteins in tryptophan and the complete availability of the nicotinic acid enhance the potential contribution of milk.

When milk is assessed not by the extent to which it can cover requirements or allowances but by its contribution of nutrients to a mixed diet, its significance will naturally depend on the composition of the remainder of the diet and varies markedly throughout the world with changes in dietary pattern (cf. Kon, 1959). Into these natural dietaries milk evidently goes either in the liquid form after suitable processing or in the shape of various milk products such as dried and condensed milk, cheese and so on. Though the effects of such treatments are outside the scope of this article it may be useful to consider in general terms the total nutrient contribution that milk in its various forms makes to mixed diets. Though in certain countries, for example in North America and Scandinavia, it provides up to 20 % of the calories in the national diet, its importance as a food is measured much more adequately by the contribution of important nutrients rather than that of energy. In the Western type of diet milk provides about one-quarter of the total protein and about one-half of the animal protein. Because of the marked supplementary relationships between the proteins of milk and those of cereals (see p. 215) it plays an important part in assuring a well-balanced intake of essential amino acids. In other countries the contribution of milk in this respect is much smaller either because local production and consumption are very low and economic difficulties make it more practicable to satisfy the protein needs in other ways, as for example in the Near and Far East, or because of high consumption of other sources of animal protein, as for example, meat in Argentina. None the less, dried skim milk has proved a most valuable means of balancing inadequate protein intake in many wanting countries. Those countries where milk products are a popular and important part of the diet rely on them to supply most of their dietary calcium. Thus in North America, Australia, Holland and the Scandinavian countries, three-quarters or even more of the calcium consumed comes from this source. In some other countries, however, the traditional dietary pattern contributes to the calcium needs from foods other than milk. In many countries where the milk intake is low the customary diet nevertheless remains unbalanced in calcium, and calcium deficiency is prevalent, as for example in many regions of the Indian sub-

continent. In such countries increase of milk consumption by development of dairying or importation of dried skim milk would be among the important measures in making calcium intake adequate.

Whole milk and whole milk products are among the valuable sources of vitamin A. However, most countries rely on green plants and vegetables to satisfy their vitamin A needs in the form of β-carotene, the provitamin, and even in countries with well-developed dairy industries vitaminized margarine competes with milk products as a source of the vitamin.

Information about the riboflavin content of national dietaries is scanty and comes mostly from adequately nourished countries where milk products are an established major component of the diet. In North America, Great Britain and Australia, for example, milk products provide nearly half of the total intake of riboflavin. Countries that consume little milk or animal products derive their riboflavin mainly from pulses, cereals and green vegetables, but in many places the intake is inadequate and there is no doubt that milk, and particularly imported dried skim milk, may play a determining part in increasing riboflavin intake.

XI. Conclusion

Milk is justly regarded as the most complete single food and, though it may be assessed on its important content of individual essential nutrients, its value in human nutrition is perhaps best judged by the way in which it complements and supplements many dietaries in different parts of the world.

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CHAPTER 18

Human Milk and Cow's Milk in Infant Nutrition

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I. Introduction

The great increase in knowledge of the fundamentals of nutrition in the past quarter of the century has focused renewed interest on the chemical, biological and nutritive aspects of breast feeding, human milk, and cow's milk as a substitute.

In an evaluation of the nutritional value of a food it is necessary to take into account its chemical and biochemical properties and the physiological status of the individual receiving it. This discussion is limited primarily to a general consideration of the physiological value of breast milk as a food for the normal full-term infant from birth to 6 months of age, with remarks regarding the value of cow's milk as a food for young infants. It is necessary also to refer briefly to many other factors that may play a role in the kind of milk an infant receives and his response to it.

A. Advisability of Breast Feeding

The infant's body must accumulate essential elements in varying proportions to provide for growth and development and the enlarging functions of individual organs, tissues and structures. These essentials are derived directly from the maternal blood during the foetal stage as well as from the milk the mother provides immediately after birth.

It is generally agreed that breast milk is the best food for young infants (Macy *et al.*, 1945; (U.S.A.) National Research Council, 1950a,b, 1953; Aldrich, 1947; Kon & Mawson, 1950). It contains adequate quantities of food elements for good nutrition in the early months of life, provided that it is sufficient in amount and that the mother eats a good diet during pregnancy and lactation. Breast feeding has definite therapeutic and preventive values, factors of importance especially to infants in the less-favoured economic classes, for whom adequate health measures may not be available. When the mother's supply is sufficient, breast milk is economically controlled to meet the needs and desires of the infant. It is the safest food for very young or premature infants who are able to nurse at the breast and is probably the best method of providing gratification and a sense of security to babies. Breast feeding is also a maturation point in the sequence of maternal development, which, according to some authorities, is important physiologically and psychologically to mothers. Since human milk represents a nutrient mixture that best meets the physiological requirements of the young infant, "it is believed that the assessment of the nutritional requirements of infants can be based on the composition of milk of well-nourished mothers" (Kon & Mawson, 1950).

B. Biochemical Individuality

1. *Infant*

The infant at birth has been influenced by environment and heredity. *In utero* it is known to be affected by the general nutritional and health status of the mother as well as by the mother's food intake during pregnancy, whether overabundant or limited, balanced or unbalanced in essential nutrients. It has experienced a comparatively long intra-uterine life and will have a relatively long infancy.

Full-term infants may vary considerably in nutritional endowment, in degree of maturity at birth, and in post-natal developmental age. Their bodies may vary in size, physique, and composition (National Research Council, 1950b). These important cumulative factors determine in large measure the food-handling capacity of the infant and must be a consideration in the assessment of the physiological value of human milk and cow's milk as a food.

The premature infant is at a disadvantage because of its immaturity of structure and function and its limited endowment of nutrients, even when the maternal food supply and milk are adequate. The greater the immaturity and the foetal tissue deficiency, the greater will be the nutritive needs and the nutritional reconditioning or conditioning necessary for tissue construction and function during post-natal feeding.

2. *Human Milk*

Milk secreted by different women varies widely in composition (Kon & Mawson, 1950; (U.S.A.) National Research Council, 1950a, 1953). It has been demonstrated that the quality and quantity of breast milk secreted by a mother may be altered by the following conditions: inherent biochemical, physiological, or psychological individuality; amount and kind of food consumed; environmental conditions such as fresh air and sunshine; amount and intensity of work, rest and exercise; disease; size and anatomical structure of the mammary glands; and heredity. Mammary glands vary in capacity and in the quality of milk secreted at different stages of lactation and the length of time that lactation persists under normal conditions, beyond the completeness of the emptying of the breast and stimulation of the nursing reflexes. In general, the quantity of milk secreted by the mammary gland adjusts itself to the nursing demand (Macy *et al.*, 1930). Human milk that has been expressed, pasteurized, analysed, or fed to a baby by bottle is subject to the above factors and in addition to the variations, losses, and deterioration characteristic of other formulas.

Table I presents mean, minimum and maximum values for various constituents of human milk ((U.S.A.) National Research Council, 1953). These values are based on samples of colostrum, transitional and mature milk taken under controlled conditions of collection and analysis from a group of women living in the same geographical area and known to have had an adequate dietary. Although in some instances the supply of milk was limited, thereby restricting the number of constituents that could be determined, it can be seen that considerable variation occurs.

C. Characteristics of the Biochemical and Physiological State

1. *At Birth*

The inherent physiological status of the full-term infant may be modified by (1) the traumatic birth experience; (2) the biochemical and metabolic adaptations required by its transition from an aquatic to a terrestrial environment; (3) the sudden transfer from an intimate and warm parasitic being

TABLE I

VARIABILITY OF THE CONSTITUENTS OF HUMAN COLOSTRUM, TRANSITIONAL AND MATURE MILK [1]
(Values per 100 ml whole milk)

Constituent	Colostrum 1-5 days				Transitional milk 6-10 days				Mature milk			
	No. of samples	Mean	Min.	Max.	No. of samples	Mean	Min.	Max.	No. of samples	Mean	Min.	Max.
Specific gravity					2	1·035	1·034	1·036	333	1·031	1·026	1·037
Energy, kcal	8	67	59	73	21	74	68	83	438	75	45	119
Total solids, g	29	12·8	10·0	16·7	46	13·3	10·5	15·6	610	12·9	10·3	17·5
Ash, g	5	0·31	0·25	0·35	16	0·27	0·23	0·34	390	0·20	0·16	0·27
Calcium, mg	28	48	24	66	46	46	23	63	628	34	17	61
Chlorine, mg	8	59	44	101	21	46	30	72	216	38	9	73
Magnesium, mg	28	4	3	8	44	4	2	5	302	4	2	6
Phosphorus, mg	28	16	8	25	46	20	10	32	628	14	7	27
Potassium, mg	11	74	66	87	25	64	53	77	18	51	37	64
Sodium, mg	28	50	26	136	44	29	19	54	302	17	6	44
Sulphur, mg	8	23	20	26	19	20	15	23	116	14	5	30
Copper, mg	3	0·04	0·02	0·05	12	0·05	0·04	0·07	67	0·03	0·01	0·07
Iron, mg	3	0·04	0·02	0·05	12	0·04	0·02	0·05	67	0·03	0·02	0·09
Zinc, mg	7	0·559	0·072	0·981	25	0·382	0·039	0·588	58	0·118	0·017	0·302
Lactose, g									313	6·8	5·0	9·2
Fat, g	5	3·0	2·5	3·2	16	3·5	2·7	5·2	408	4·5	1·3	8·3
Protein, g	33	2·3	1·5	6·8	44	1·6	1·3	1·9	583	1·1	0·7	2·0
Casein protein, g					2	0·5	0·4	0·6	166	0·37	0·14	0·68
Lactalbumin protein, g					2	0·8	0·7	0·8	163	0·36	0·14	0·60
Non-protein nitrogen, mg					2	48	42	53	151	32	17	60
Non-protein creatine, mg									41	3·1	0·0	12·4
Non-protein creatinine, mg									44	3·0	2·0	5·1
Non-protein urea, mg					1	23·3	23·3	23·3	52	37·8	26·7	49·4

Non-protein uric acid, mg									56	6·6	3·9	12·3
Non-protein amino acid nitrogen, mg					2	4·0	4·0	5·0	157	5·0	2·8	11·3
Total arginine, mg	13	126	62	516	28	64	48	89	147	45	28	64
Total histidine, mg	13	57	35	178	28	38	29	45	147	23	16	34
Total isoleucine, mg	13	121	88	291	28	97	73	121	147	68	46	102
Total leucine, mg	13	221	145	660	28	151	113	197	147	108	72	159
Total lysine, mg	12	162	95	509	28	113	86	148	147	76	53	104
Total methionine, mg	13	33	19	90	28	24	16	34	146	14	9	21
Total phenylalanine, mg	13	104	60	377	28	63	48	74	147	41	30	58
Total threonine, mg	13	147	75	630	28	79	61	96	147	54	40	76
Total tryptophan, mg	13	51	25	205	26	28	23	33	144	18	13	26
Total valine, mg	13	168	98	571	28	105	77	136	147	71	48	114
Vitamin A, μg	24	161	75	305	26	88	58	183	264	61	15	226
Carotenoids, μg	24	137	41	385	26	38	23	63	265	25	2	77
Ascorbic acid, mg	17	7·2	4·7	10·4	20	7·1	4·5	9·0	233	5·2	0·0	11·2
Biotin, total, μg	20	0·1	0·0	0·3	35	0·4	0·0	1·8	266	0·8	trace	4·2
Choline, total, mg									29	9	5	14
Choline, free, mg									3	2	2	2
Folic acid, μg									22	0·22	0·14	0·36
Inositol, total, mg									7	45	39	56
Inositol, free, mg									5	44	39	52
Nicotinic acid, μg	29	75	50·0	145	34	175	60	360	268	183	66	330
Pantothenic acid, μg	29	183	29	302	33	288	135	412	269	246	86	584
Pyridoxine, μg									3	18	10	22
Riboflavin, total, μg	25	30·2	12·0	45·3	35	36·9	27·5	49·0	272	37·3	19·8	79·0
Riboflavin, free, μg	19	19·0	7·7	27·0	33	24·0	16·6	40·8	256	24·2	10·5	61·8
Thiamine, total, μg	25	2	1	3	35	6	2	10	277	14	8	23
Thiamine, free, μg	19	0·4	0·1	0·9	35	0·8	0·2	1·9	258	5·3	1·2	15·8

[1]From (U.S.A.) National Research Council, 1953, Table I. For purposes of controlling methods of collection and analyses only data collected by the workers quoted in references 132, 252 and 253 of that publication are recorded.

dependent on predigested food obtained from the mother's blood supply, to an independent functioning individual organism in a cold world, required to digest, absorb and utilize an exogenous food supply through the use of its personal digestive, respiratory, and excretory systems; and (4) the specific inherent biochemical needs for continued rapid growth, development and physiological function of the separate organs and tissues. In addition to these requirements, nutritional reconditioning or conditioning may be necessary.

2. *During Infancy*

a. *Growth and development.* The value of a food is usually determined by measurements of height, weight, and general well-being of the infant. In recent years there has been an urge to develop children up to and beyond a theoretical average or so-called "normal" weight for age and height. Whether the bigger and more rapidly growing individual enjoys nutritional stability and a sound foundation for building good health through succeeding phases of the life cycle is a question of fundamental significance deserving of intense scientific study. For the present our goal is a high level of physical development for every child to permit the greatest possible mental development in a nutritionally stable body through adequate dietary measures and proper hygiene.

Growth involves more than increase in mass. It is accompanied by changes in organ function and adjustment in body composition, both of which may be reflected in the nutritional requirements. Growth in weight or linear dimensions does not proceed with uniformly accelerated velocity. On the contrary, each growth phase takes place relatively slowly at first, then more rapidly, and again more slowly, thus yielding an S-shaped curve designated as a growth cycle. The initial cycle of man has its inception during intrauterine growth subsequent to implantation of the embryo, is interrupted by birth when the cycle is not yet half completed, and finally culminates toward the end of the first year of post-natal life. The rate of weight gain is greatest in the first 6 months of infancy, and the greatest gain occurs in the first 2 to 12 weeks.

Scammon (Harris *et al.*, 1930) demonstrated the remarkable uniformity of pre-natal growth and the equally striking diversity of course of the post-natal increment. He demonstrated also that the most rapid general type of growth of the body as a whole (musculature, skeleton, respiratory and digestive organs, etc.), the lymphoid type of growth and the neural type of growth (the brain and its parts, spinal cord, etc.) takes place in infancy.

b. *Metabolic needs.* Nutritional needs are modified by type and intensity of growth. Evidence indicates that these needs are especially urgent for the repletion and strengthening of the new-born infant after the shock of birth,

dehydration and adjustment of body composition in the neonatal period. Breast milk apparently has unique properties that are physiologically advantageous to the adaptation, growth, and development of the human species, especially in the initial weeks of infancy. At this time the baby requires a food that (1) forms a soft and easily digestible curd in the stomach; (2) satisfies his high energy requirements and greater water requirements; and (3) meets his needs for essential amino acids, minerals, and vitamins for the enlarging demands of protoplasmic and skeletal growth and maturation.

c. *Body composition.* There are two ways of estimating the substances required for foetal nutrition: first, by assuming that the requirements of the foetus are similar to those of the new-born which is maintained by its mother's milk, and, second, by analysing the body of the foetus on the supposition that the substances found therein represent its requirement for growth (Slemons, 1919). It is important, therefore, to know how much material is laid down in the foetus at different periods of its development, not only to determine the requirements for foetal nutrition but also to appraise the nutritive drain on the maternal body at various stages of gestation and to learn something of the endowment of the new-born infant on which recommended dietary allowances may be based.

The use of the chemical composition of the human foetus as a basis of evaluating the nutritional requirements of the mother and her offspring dates back to a century ago when the first chemical analysis of a human foetus was made (see Widdowson, 1950). Since that time, the quantitative chemical make-up of the bodies of men, women, children, infants and foetuses in health and disease has been investigated ((U.S.A.) National Research Council, 1950b; Widdowson & Spray, 1951; Widdowson *et al.*, 1951; Friis-Hansen, 1957). Changes in composition of the whole body during growth may reflect (1) similar changes in composition of all tissues; (2) different alterations in different tissues; and (3) changes in the amount of fat in the body tissues. All these factors may be operating during growth and therefore affect the nutritive requirements. Growth of tissues is associated with a relative increase in the proportion of the cells and a resultant net decrease in the percentage water content of the body. From the standpoint of nutrition, the type of chemical and nutritional endowment obtained in the foetal phase of life influences and is basic to the establishment of dietary allowances for infancy. Indeed, this is true not only for infancy but also for later years inasmuch as what happens nutritionally in one phase may carry over into the next.

From their chemical analyses of the bodies of foetuses and stillborn babies, Widdowson & Spray (1951) emphasize the large increase in the proportion of fat from less than 1 % in the early foetal stages to over 28 % in one of the full-term infants (Fig. 1), a decrease in the proportion of water, and a

corresponding increase in the proportion of protein when expressed on a fat-free basis (Fig. 2). These investigators have compared the estimated percent-

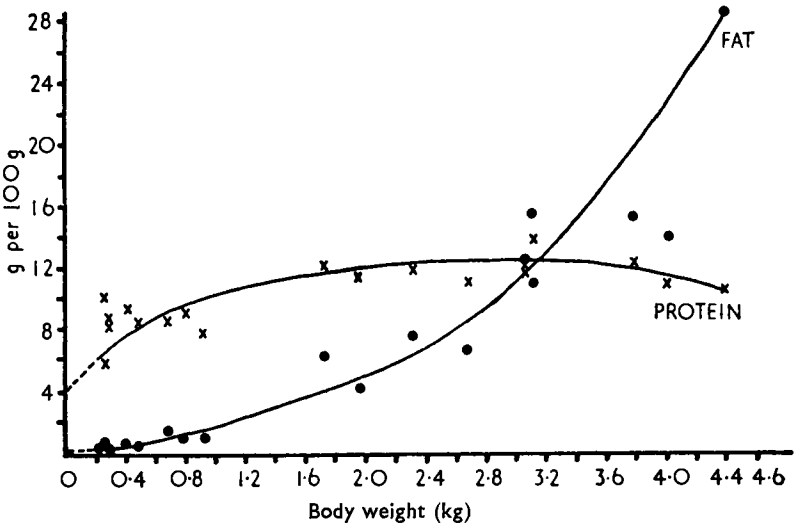

FIG. 1. Percentage of fat and protein in the foetal bodies. (Widdowson & Spray, 1951.)

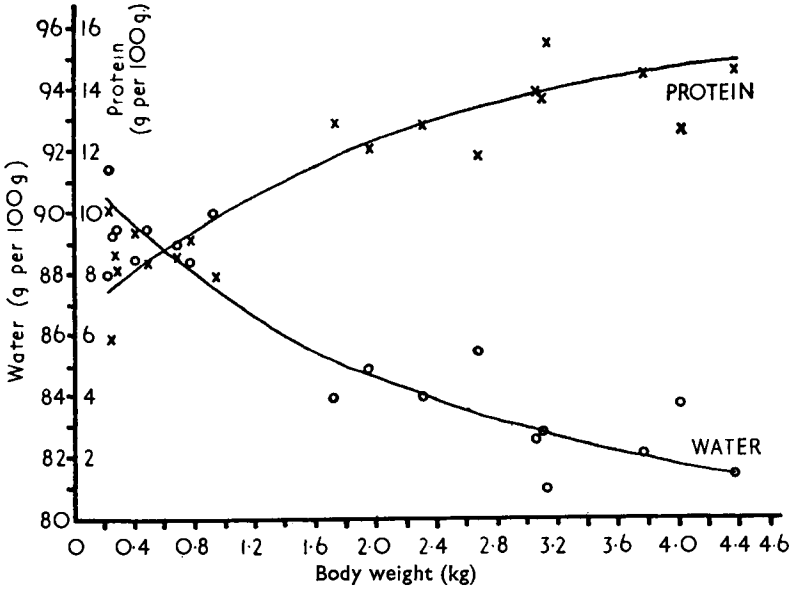

FIG. 2. Protein and water content of fat-free foetal tissue. (Widdowson & Spray, 1951.)

age composition of premature and full-term infants (Table II). The proportion of the fat is greater, the more mature the foetus.

According to Widdowson & Spray (1951), a foetus of 1500 g, or roughly 8 to 9 lunar months, has accumulated only 13 % as much fat as a full-term infant. In contrast, a foetus of 2500 g, approximately 9·5 lunar months, has 43 % as much fat as a full-term infant. The increase in fat during the last 1 to 2 pre-natal months is very rapid, the percentage rising from 3·5 % at 8 to 9 lunar

TABLE II

TOTAL AND PERCENTAGE AMOUNTS OF VARIOUS CONSTITUENTS IN THE BODIES OF PREMATURE AND FULL-TERM BABIES[1]

Constituent	Total amount			Per 100 g			Percentage of full-term	
	Premature 1	Premature 2	Full-term	Premature 1	Premature 2	Full-term	Premature 1	Premature 2
Weight, g	1500	2500	3500				43	72
Water, g				82·5	77·3	68·8		
Protein, g	160	290	415	11·6	12·4	12·0	39	70
Fat, g	60	200	470	3·5	7·6	16·2	13	43
Na, g	3·50	5·35	6·75	0·23	0·21	0·19	52	80
K, g	2·80	4·70	6·00	0·19	0·19	0·17	47	78
Ca, g	10·2	19·0	28·2	0·68	0·76	0·81	36	68
Mg, g	0·35	0·60	0·76	0·023	0·024	0·022	46	79
P, g	5·6	10·8	16·2	0·37	0·43	0·46	35	67
Fe, mg	106	184	262	7·1	7·4	7·5	41	70
Cu, mg	4·8	8·8	13·7	0·32	0·35	0·39	35	64
Zn, mg	26·0	40·0	53·0	1·7	1·6	1·5	49	76

[1]From Widdowson & Spray (1951).

months, to 7·6 % at 9·5 lunar months, to 16·2 % at birth, more than doubling its relative status each time. The increase in fat is more rapid at this later stage of pre-natal life than in the other constituents estimated. The other constituents have reached 64 to 80 % of the full-term value at approximately 9·5 lunar months, whereas fat has accumulated to the extent of only 43 %.

The percentages of calcium, phosphorus, iron, and copper display a steady but less spectacular rise. During the same interval, water, sodium, potassium,

and zinc are decreasing in percentage values. The percentages of protein and magnesium display no definite trends.

II. Constituents and Properties of Human Milk

The average values for the constituents of human milk, given in Table III, are taken from a summary compilation of the composition of milks published by the Food and Nutrition Board of the (U.S.A.) National Research Council (1953). As stated in this publication, the tabulation includes "data for a greater number of individual components than any summary published to this time and is believed to delineate adequately the chemical structure of these milks as reliably as is possible with existing knowledge. . . . Data have been compiled from sources selected from among more than 1500 reports of investigations of the composition of the mammary secretion of 'average', 'normal', or 'healthy' subjects."

A. Tables of Average Values

The values for the composition of human milk depend on a variety of factors—the methods employed, the accuracy and completeness of the milk collection, the timing and sampling procedures, the care maintained in preventing losses of nutrients, and the method of chemical analysis. Because of these and other possible sources of differences, there are hazards involved in compiling tables of average compositions of human milk collected from women of different races, countries, and economic status; yet this is the only procedure available at present to obtain information on the structure and constituents of human milk, the natural food for infants. Therefore judgment must be exercised in interpreting compiled average data, for the analysis of milk from an individual woman may not agree closely when compared with average figures. This does not mean that the compilation or reference standard has little value but rather that the biochemical individuality of the milk is such that it may fall in the range above or below the average. Therefore, the table serves as a guide or a yardstick, and the values should not be considered absolute.

III. Physiological Value of Human Milk

Nutrients are generally grouped into six major categories—proteins, fats, carbohydrates, water, minerals, and vitamins. This discussion will include the essential amino acids in milk proteins, the fatty acids in the fat, certain well-defined minerals, and the fat-soluble and water-soluble vitamins known to have significance in the nutrition of the infant.

TABLE III

CONSTITUENTS OF HUMAN COLOSTRUM, TRANSITIONAL AND MATURE MILK COMPARED WITH COW'S MILK[1]

(Average values per 100 ml whole milk)

Constituents	Colostrum 1–5 days	Transitional 6–10 days	Mature after 30 days	Cow's milk
General properties				
Fat, melting point, °C		32[2]	31	35
Gold number	2370	792		
Iodine number		60·4[2]	61·6	38·4
Index of refraction (at 40°C)			1·4570	1·4001
Polenske number		0·6[2]	1·2	1·5
Reichert-Meissl number		1·8[2]	0·8	28·8
Saponification number		205·1[2]	204·7	248·0
Specific gravity	1·034	1·035	1·031	1·032
Energy, kcal	58	74	71	69
Total solids, g	12·8	13·6	12·4	12·7
Fat, g	2·9	3·6	3·8	3·7
Lactose, g	5·3	6·6	7·0	4·8
Protein, g	2·7	1·6	1·2	3·3
Ash, g	0·33	0·24	0·21	0·72
Minerals				
Calcium, mg	31	34	33	125
Magnesium, mg	4	4	4	12
Potassium, mg	74	64	55	138
Sodium, mg	48	29	15	58
Total electropositive, m-equiv.	5·86	4·93	4·04	13·28
Chlorine, mg	91	54	43	103
Phosphorus, mg	14	17	15	96
Sulphur, mg	22	20	14	30
Total electronegative, m-equiv.	4·75	3·76	2·95	10·55
Excess positive, m-equiv.	1·11	1·17	1·09	2·93
Other minerals				
Iron, mg	0·09	0·04	0·15	0·10
Copper, mg	0·05	0·05	0·04	0·03
Zinc, mg	0·62	0·77	0·53	0·38
Iodine, mg	0·012	0·002	0·007	0·021
Nitrogen distribution				
Total nitrogen, g	0·515	0·317	0·227	0·550
Protein nitrogen, g	0·424	0·251	0·188	0·518
Non-protein nitrogen, g	0·091	0·066	0·039	0·032

TABLE III (contd.)

Constituents	Colostrum 1–5 days	Transitional 6–10 days	Mature after 30 days	Cow's milk
Protein distribution				
Casein, g	1·2	0·7	0·4	2·8
Lactalbumin, g		0·8	0·3	0·4
Lactoglobulin, g		0·5	0·2	0·2
Whey protein, g	1·7		0·6	0·6
Non-protein components				
Creatine, mg			3·3	3·1
Creatinine, mg			2·2	0·9
Urea, mg		23·3	32·2	15·1
Uric acid, mg			4·6	1·9
Amino acids				
Dispensable				
Alanine, mg			35	75
Aspartic acid, mg			116	166
Cystine, mg		55[2]	29	29
Glutamic acid, mg			230	680
Glycine, mg			0	11
Proline, mg			80	250
Serine, mg			69	160
Tyrosine, mg		125[2]	62	190
Indispensable				
Arginine, mg	126·	64	51	124
Histidine, mg	57·	38	23	80
Isoleucine, mg	121·	97	86	212
Leucine, mg	221·	151	161	356
Lysine, mg	163·	113	79	257
Methionine, mg	33·	24	23	87
Phenylalanine, mg	105·	63	64	173
Threonine, mg	148·	79	62	152
Tryptophan, mg	52·	28	22	50
Valine, mg	169·	105	90	228
Fat distribution				
Fat, total, mg	2900	3600	3800	3700
Lipid phosphorus, mg	2	3	4	4
Total cholesterol, mg	27	29	20	14
Lecithin, mg			78	57
Fatty acid distribution, g/100 g fat				
Saturated				
Butyric	0·2		0·4	3·1
Caproic	0·1		0·1	1·0
Caprylic	0·4		0·3	1·2
Capric	2·2	1·8[2]	1·7	2·6

TABLE III (contd.)

Constituents	Colostrum 1–5 days	Transitional 6–10 days	Mature after 30 days	Cow's milk
Lauric	1·8	5·6[2]	5·8	2·2
Myristic	3·8	9·4[2]	8·6	10·5
Palmitic	26·2	23·6[2]	22·6	26·3
Stearic	8·8	7·8[2]	7·7	13·2
As arachidic	3·8	1·4[2]	1·0	1·2
Unsaturated				
Decenoic	0·2	trace[2]	0·1	0·2
Dodecenoic	0·1	0·1[2]	0·1	0·2
Tetradecenoic	0·2	0·8[2]	0·6	1·1
Hexadecenoic	2·4	3·2[2]	2·9	3·1
Oleic	36·6	34·6[2]	36·4	32·2
Octadecadienoic	6·8	7·1[2]	8·3	1·6
Octadecatrienoic	0·3		0·4	
Octadecatetraenoic			0·2	
Arachidonic	1·7		0·8	1·0
As eicosadienoic	4·6		2·4	
C_{20} to C_{22}	10·2	4·6[2]	4·2	1·0
Vitamins				
Fat-soluble				
Vitamin A, μg	89	88	53	34
Carotenoids, μg	112	38	27	38
Vitamin D, U.S.P. units			0·42	2·36
Vitamin E, mg	1·28	1·32	0·56	0·06
Vitamin K, Dam–Glavind units			26	100
Water soluble				
Ascorbic acid, mg	4·4	5·4	4·3	1·6
Biotin, total, μg	0·1	0·4	0·4	3·5
Vitamin B_6 (pyridoxine), μg			11	48
Vitamin B_{12} (cobalamin), μg	0·045	0·036	trace	0·56
Choline, total, mg			9	13
Choline, free, mg			2	4
Folic acid, μg	0·05	0·02	0·18	0·23
Inositol, total, mg			39	13
Inositol, free, mg			44	6
Nicotinic acid, μg	75	175	172	85
Pantothenic acid, μg	183	288	196	350
Riboflavin, total, μg	29·6	33·2	42·6	157·0
Riboflavin, free, μg	19·0	24·0	24·2	
Thiamine, total, μg	15	6	16	42
Thiamine, free, μg	0·4	0·8	4·8	23·0

[1]From (U.S.A.) National Research Council, 1953, Table V. [2]Early lactation.

Some forty to sixty nutrients are found in the body and are known to be essential for the physiological processes of life, growth, and development. These indispensable constituents differ in chemical structure and in their digestibility, absorption, utilization and excretion in the body; they perform various specific functions in metabolism and in the construction and maintenance of the protoplasmic, skeletal and fluid tissues of the body; and they participate in diverse enzymic and biochemical systems and in homeostatic and neural functions and controls. Human milk produced by a healthy woman consuming an adequate diet possesses all these constituents in varying amounts and proportions, depending on the stage of lactation.

The effectiveness of mother's milk for an infant is determined by the capacity of the milk to yield nutrients in sufficient quantities and proper proportions to meet day-to-day physiological adjustments. Early and late milks vary in quality and quantity. Perhaps the most significant characteristics of human milk are its special physical properties that permit it to form soft and easily digestible curd in the alimentary tract of the infant; its high protein value for growth attributable to the perfect balance in proportions of the essential amino acids which it contains; the emulsified small fat globules and the proportion of unsaturated fatty acid of low molecular weight, which contribute to physiological well-being; the uniqueness of the carbohydrate, lactose and carbohydrate containing compounds; and the amount and distribution of the vitamins and minerals best suited for the synthesis of the necessary rich protoplasmic composition of the very young organism and its rapid growth and development and for satisfying its high energy requirements.

A. Colostrum and Transitional Milk

The residual mixture of material present in the mammary glands and ducts at parturition and immediately after is progressively diluted with newly secreted milk forming the colostrum and later the transitional milk. True colostrum is believed to last during the initial 5 or 6 days after parturition and is considered to have specific value and immunological properties for the newborn, who must clear its alimentary tract of meconium in preparation for the normal functioning of its own digestive, respiratory and urinary systems. The meconium is charged with an essential growth factor for a variant of *Lactobacillus bifidus* (György *et al.*, 1954a,b,c; Gauhe *et al.*, 1954; Zilliken *et al.*, 1956; Bigwood *et al.*, 1957) and is the first culture medium in the sterile intestinal lumen of the new-born infant.

From the standpoint of a food for a very young infant, it is important to study the secretion produced when the glands are changing from a quiescent state to an actively secreting one and containing maximum amounts of

tissue debris from the alveoli and milk ducts. In order to acquire information on the constituents found in the initial mammary gland fluid (Miller *et al.*, 1950), a composite sample (I) was obtained from approximately seventy women not less than 8 hours and not more than 24 hours after childbirth

TABLE IV

ESSENTIAL AMINO ACIDS IN "FIRST" AND EARLY COLOSTRUM[1]

Composite[2]	Total solids, g	Total nitrogen, mg	Leucine, mg	Isoleucine, mg	Valine, mg	Histidine, mg	Lysine, mg	Methionine, mg	Phenylalanine, mg	Arginine, mg	Threonine, mg	Tryptophan, mg
						Concentration per 100 ml						
I	17·7	1372	659·6	291·0	571·0	178·5	508·6	90·1	377·4	516·1	629·5	205·2
II	14·4	720	345·7	180·9	298·4	97·8	267·1	53·2	195·9	260·7	317·0	103·6
III	11·8	346	175·5	104·2	140·2	51·6	148·5	30·1	85·7	115·4	129·3	39·9
						Percentages of total nitrogen						
I			5·1	2·3	5·0	3·5	7·1	0·6	2·3	12·1	5·4	2·0
II			5·1	2·7	5·0	3·7	7·1	0·7	2·3	11·6	5·2	2·0
III			5·4	3·2	4·8	4·0	8·2	0·8	2·1	10·7	4·4	1·6

[1]From Miller *et al.*, 1950.

[2]Volumes of composites were: I, "first" colostrum, 133·6 ml; II, first day of lactation, 274·5 ml; III, second day of lactation, 794·9 ml.

and before any spontaneous or nursing loss had occurred; composites II and III were collected after composite I and represent the 1st and 2nd days *post partum*. The samples were collected by one nurse skilled in the technique of manual expression of the breasts (Davies, 1945) and were handled and analysed under controlled procedures. The values for total solids, total nitrogen and ten essential amino acids are given in Table IV.

The concentrations of total solids, total nitrogen and all essential amino acids in the initial colostral fluid exceed any values for human colostrum found in the literature. Inspection of these values shows a relatively consistent reduction by as much as one-half in the data for composite II and a further reduction of about the same magnitude in composite III. The amino acid nitrogen of the ten amino acids accounts for 45 % of the total nitrogen

in each of composites I, II, and III, a value considerably higher than the 38 % total nitrogen accounted for by the amino acids in samples representing the 2nd to 10th day post-partum (see Table V). The greater percentages of amino nitrogen in the composites, however, were accounted for by significantly higher values for arginine and threonine. As a matter of fact, in composites I, II, and III arginine accounted for 12·1 %, 11·6 % and 10·7 %, respectively, of the total nitrogen, and threonine accounted for 5·4 %, 5·2 % and 4·4 %.

In general, the amino acid concentrations per 100 ml of secretion varied with subject and day post-partum and did not parallel changes in volume of secretion or concentration of total solids. Total nitrogen and amino acid concentrations followed the same general pattern, with highest values occurring on the 5th to 8th day post-partum. The ratios between the nitrogen of the essential amino acids and the total nitrogen were not consistent, indicating the possibility of shifting proportions of the casein, albumin and globulin fractions of the total protein.

It is of considerable interest to speculate concerning the larger amounts of essential amino acids, especially arginine and threonine, occurring in the earliest mammary fluid after childbirth. The total body proteins of the parturient mother and of the physiologically unstable new-born infant are in steady flux; anabolism and catabolism of the tissue proteins are occurring simultaneously. During labour, parturition, and puerperium, the body substances of the maternal organism may be broken down in order to provide for the synthesis of milk proteins and to prepare the mammary glands for their secretory function. At the same time the enhanced supply of essential amino acids in the colostrum forms a pabulum best suited for the physiological readjustment and promotion of tissue regeneration after birth, for the growth and differentiation of the organ systems with their specific protein synthesis, and for the development of the organism as a whole at the threshold of the greatest growth phase in its life cycle.

Volumes of secretion per day at different intervals post-partum vary widely among different women, and concentrations of total solids and nitrogen seem not to be related to daily volume (Miller *et al.*, 1950). Table V presents, in mg per 100 ml of secretion, the concentrations of amino acids in each of thirty-eight samples of colostrum and transitional milk obtained from ten well-fed women, 2 to 10 days post-partum, with the corresponding volumes of secretion during 24 hours and the concentration of total solids and total nitrogen. The range for all determinations indicates general conformance to a pattern of composition, with total solids ranging from 11·5 to 14·4 g (averaging 13·1 g) and total nitrogen from 248 to 371 mg (averaging 305 mg) per 100 ml of secretion. The biological individuality of the women is shown by the ranges in data for the same mother on different days post-partum.

These ranges for the individual women are narrower, however, than the ranges for the group of women. Individual differences are also shown by the variations between levels of total solids and total nitrogen.

The data for the individual amino acids conform with the value for total nitrogen, indicating variability based on individual differences among women and among days post-partum. Mean values per 100 ml of secretion were: leucine, 155 mg; isoleucine, 98 mg; valine, 108 mg; histidine, 39 mg; lysine, 114 mg; methionine, 24 mg; phenylalanine, 65 mg; arginine, 67 mg; threonine, 80 mg; and tryptophan, 30 mg; differences between minima and maxima for the individual amino acids varied from 17 mg for histidine to 101 mg for leucine. In general, the greatest concentrations of each amino acid were found from the 5th to the 8th day post-partum, possibly reflecting physiological instability in the secretory mechanism and thus re-emphasizing the fact that during the second 5 days of lactation changes in the composition of the secretion from the human breast occur in the direction of mature milk production (Coryell *et al.*, 1945). Variations in the amino acid composition of the secretion from different women and from the same woman during different days post-partum are obscured in results from analysis of composite samples.

Although the content of total nitrogen or any amino acid in breast secretion per 24 hours is grossly related to the volume produced, the concentration in milligrams per 100 ml is not so related. At any time during the puerperium one mother may supply in a smaller daily volume of secretion an amino acid mixture equal or superior in value to that provided by another mother. The relative distribution of the nitrogen of the individual amino acids in each 100 ml of secretion varies for different women, indicating possible differences in the combination of proteins in their secretion, but shows no trend ascribed to interval in lactation.

It appears that in addition to alterations involved in the transition of the mammary gland to an actively secreting organ, the anomalous composition of colostrum may be, in part, a reflection of an abnormal blood picture which in turn is associated with the nitrogen metabolism of the puerperium indicative of bodily protein catabolism presumably associated with the involution of the uterus and recovery from childbirth. It is not known to what extent the non-protein nitrogen fractions of milk are used by the infant; however, drastic alterations in these fractions may indicate the presence of certain abnormal conditions and serve clinically as an index of infection of the mammary gland or other bodily disturbances as reflected in the blood.

Non-protein nitrogen compounds are usually included in the figures for protein; they are often disregarded, but they may have important significance both in the physiology of milk secretion and in the nourishment of the infant, even though they are present in little more than traces. Investigation has

TABLE V

ESSENTIAL AMINO ACIDS IN HUMAN COLOSTRUM AND TRANSITIONAL MILK[1]
(mg per 100 ml of secretion)

Woman	Post-partum day	24-hour volume ml	Total solids g	Total nitrogen	Leucine	Iso-leucine	Valine	Histi-dine	Lysine	Meth-ionine	Phenyl-alanine	Arginine	Threo-nine	Trypto-phan
L.F.	4	1122	13·0	306	145	94	100	35	100	26	62	64	76	30
	5	1441	12·9	313	148	92	98	35	95	23	60	62	75	27
	6	1501	13·0	312	155	96	100	38	106	22	61	63	77	27
	7	1596	13·0	321	139	103	113	40	119	26	65	69	85	30
	8	1638	13·3	318	128	105	106	38	118	26	64	67	83	27
	9	1676	13·2	318	137	97	107	39	119	24	65	70	88	28
	10	1872	13·5	314	119	94	98	34	95	24	58	64	83	28
V.G.	4	1413	11·5	292	147	88	100	40	104	20	63	64	75	30
	5	1471	12·8	286	155	95	109	42	111	20	65	70	80	31
	6	1782	13·1	281	167	104	110	44	112	25	71	71	85	30
	7	1630	13·5	295	169	105	111	44	123	28	69	67	79	30
	8	1895	13·4	284	156	98	96	41	117	22	63	55	75	27
	9	1828	13·0	266	149	94	94	39	107	21	59	60	80	27
	10	1770	13·2	266	137	86	90	37	99	22	54	56	72	27
V.L.	4	794	13·2	307	164	99	112	42	133	19	71	74	87	25
	5	844	13·5	313	157	104	109	44	139	25	74	72	86	31
	6	955	13·9	331	176	112	112	45	141	26	74	69	86	33
	8	1098	14·4	323	188	101	117	45	148	28	71	67	85	32
	9	1118	14·0	318	165	107	110	43	138	24	63	60	80	28
	10	1200	14·0	293	153	99	97	40	137	25	64	57	80	26

C.O.	3	335	11·6	371	214	115	149	46	118	36	84	96	104	41
	4	595	12·3	371	194	109	145	43	119	32	76	84	86	35
	6	821	13·0	322	133	105	122	40	135	25	71	89	96	
	7	798	12·9	322	174	99	115	41	103	30	66	70	77	31
	8	950	12·9	280	162	94	112	38	97	34	61	62	68	29
	9	931	12·8	280	149	88	104	34	91	23	58	56	65	27
J.M.	2	385	11·7	352	174	94	130	44	112	24	79	90	98	42
	5	1121	13·4	352	191	110	132	41		30	70	71	76	33
	6	1125	13·2	315	164	98	108	38	116	24	66	69	82	30
	7	1287	13·2	300	144	92	105	34	106	21	61	61	80	28
	10	1336	12·5	273	113	95	94	30	106	21	51	57	68	
V.S.	8	837	14·2	325	197	121	136	44	122	26	71	71	91	32
	9	932	14·2	317	168	108	117	44	124	26	66	66	86	29
	10	924	14·3	323	170	112	116	40	123	25	69	73	80	30
G.S.	7	1017	12·1	249	130	77	77	29	93	16	48	48	61	23
M.S.	7	880	12·3	261	124	74	86	34	86	18	53	58	70	27
E.L.	8	688	12·5	248	128	73	97	32	88	17	55	50	65	23
F.W.	7	953	14·2	260	123	79	91	35	101	19	60	62	78	25

[1]From Miller *et al.*, 1950.

indicated that some of these relatively minute components may reflect more specifically than the gross composition certain dietary changes and pathological conditions of the mammary gland itself (Erickson *et al.*, 1934). There appear to be two possible sources of the non-protein nitrogen constituents in

TABLE VI

NON-PROTEIN NITROGEN CONSTITUENTS IN BLOOD AND MILK[1]
(mg per 100 ml)

Subject	Lactation	Stage of lactation	Non-protein N		Urea N		Amino acid N		Uric acid	
			Blood	Milk	Blood	Milk	Blood	Milk	Blood	Milk
6	First	Colostrum	32·7	53·3	13·3	11·1	7·4	4·2		
		Transition	33·8	47·2	11·3		6·3	4·6		
		Early mature	37·0	41·0	11·4			6·4		
7	First	Colostrum	40·0	42·5	11·6		12·7	4·7		
		Transition	51·3	37·5	26·7		6·4	5·2		
		Early mature	36·4	28·0	18·9			4·3		
	Third	Colostrum	52·9	51·7	24·5	11·1		6·1	4·9	2·8
6	Second	6th month	35·0	30·3	17·3	16·7	5·6	6·3	3·2	1·8
		9th month	30·9	25·6	15·0	16·2	5·7	6·6	2·9	2·0
		14th month	28·4	30·5	13·2	17·8	6·2	6·1	3·2	2·9
7	Second	6th month	32·9	30·6	15·8	15·5	7·8	5·4	3·5	2·1
		8th month	30·3	27·3	17·3	16·0	8·0	7·2	3·2	1·5
8	Second	6th month	53·3	59·2	25·5	17·4	8·4	7·7	4·9	2·0
		10th month	53·8	30·2	26·2	20·4	5·5	5·8	3·9	2·3
		14th month	63·8		21·2	15·9	12·7	4·8	4·1	2·8

[1]From Erickson *et al.* (1934). Determinations were made on fasting samples of blood and 24-hour samples of milk.

the milk. They may be derived from the synthetic or even catabolic metabolism of nitrogenous compounds in the milk or in the gland itself; or they may filter from the blood directly into the milk, as indicated by observations which show that they normally occur in approximately the same concentration in milk as in blood (Table VI). Even though the individual non-protein nitrogen constituents may be derived directly from the blood, it is possible that they may either maintain equilibrium with the corresponding composition of the blood or vary in concentration, depending on the rapidity and the extent of

their utilization in the synthesis of milk protein and other nitrogenous compounds. Non-protein nitrogen is elevated in early lactation, when the gland is changing from the quiescent to an actively secreting organ. It is also elevated by pathological disturbances of the mammary gland.

The partition of non-protein nitrogen in colostrum is strikingly irregular, with an elevated total non-protein nitrogen, uric acid, creatine, and creatinine. These constituents are still somewhat augmented during the transitional milk period. In fact, some of the constituents even at the 7th week of lactation do not assume the concentration of mature lactation.

The foetus *in utero* receives antigens from bacteria and viruses, or antibodies from bacterial antitoxins and agglutinins by way of placental circulation, some of which may remain after birth for periods of a few weeks, but gradually decline in amount. Human colostrum and milk provide a carrier of antibodies and serve protection by ingestion. Smith (1951) surmises "that even minor concentrations of antibody absorbable from colostrum and milk might be serviceable to the infant because of the repeated opportunity for their ingestion." The availability of globulin or of globulin-forming amino acids is a factor of importance also in the production of antibodies (Cannon, 1942). Inasmuch as the globulin content of the serum is in large measure correlated with antibody production, it is evident that this process must in some way be curtailed in the new-born, who is handicapped with a low euglobulin level at birth. Studies reveal a tendency for the low total serum protein values at birth to result essentially from globulin deficiency (Smith, 1951).

Colostrum has unique and characteristic properties as a food, especially with relation to the absolute amounts and proportions of essential constituents and biological properties that appear best suited to satisfy the distinctive nutritive requirements of the new-born infant. The general consensus is that there exists at birth an adequate group of enzymes for digesting simple foods except starches; therefore the high protein and low fat content of colostrum are in keeping with the degree of digestibility and utilization of foods at this period. The reserves of the new-born infant with regard to minerals and vitamins are more or less dependent on the maternal diet and the avarice of the foetus. A marginal supply of vitamin C, iron, and amino acids is available at birth, since the blood levels of the infant exceed the blood levels of the mother. Colostrum is rich in fat-soluble vitamin A and carotenoids and in vitamin E. The average concentration of vitamin A on the 3rd day postpartum may be three times that in mature human milk, and the average concentration of carotenoids may be ten times that in mature milk. A concentration level equivalent to that of mature milk is attained by these nutrients by approximately the 10th day, and in some cases it may remain at a higher level for a longer period. The vitamin E content of colostrum may be

two or three times as great as in mature milk. The rich concentration of these vitamins in colostrum aids in the inauguration of the infant's digestive, respiratory and excretory functions, inasmuch as so many of the essential metabolic systems are dependent on the enzyme and protein complex systems for the digestion and assimilation of food and its synthesis into body tissues. Colostrum is a rich source of the factors that promote the removal of meconium and the establishment of the desirable bacterial flora in the digestive tract of the new-born (György *et al.*, 1954a,b,c; Gauhe *et al.*, 1954; Bigwood *et al.*, 1957).

The water-soluble vitamins of breast milk, on the other hand, increase rapidly in the first few days and by the 10th day characteristic mature milk levels are usually attained for nicotinic acid, pantothenic acid, riboflavin, thiamine, and vitamin C. Although the fat concentration of colostrum is low, it is important to new-born infants as a source of energy and essential fatty acids, especially octadecadienoic acid. In uniformly collected and timed samples, the percentage of phospholipid in colostrum is high (0·80 %) during the initial 2 days of lactation but decreases to a lower value (0·06 %) in later milk (Baldwin & Longenecker, 1944). There appears to be a regular increase of C_{12} and C_{14} acids and a significant decrease in stearic acid and all acids of greater molecular weight than C_{18} (see Table III). There is an absence of butyric acid and other fatty acids below decanoic acid (C_{10}). Nevertheless it is possible that these changes and adjustments in fatty acids and the initial high phospholipid in colostrum may have nutritional significance to the new-born, whose blood contains very low concentrations of lipid substances, when compared with that of the mother (Boyd, 1936). There is a two- to three-fold increase in most blood fats during the first 2 weeks of life (Senn & McNamara, 1937), which may be related to the respiratory and metabolic adjustments of the new-born.

B. Relation of Human Milk to Infant Nutrition

World Wars I and II impressed on the minds of the peoples of the world the fact that the health and vigour of nations depend on the health and nutrition of their infants and children. As a basis for evaluating the nutritional requirements of infants, new information has been amassed in many countries by scientists conducting carefully designed and executed investigations on the amounts and proportions of various components found in mother's milk from the beginning to the end of lactation and the factors that may alter these constituents and affect the nutrition of the infants receiving the milk.

Table III gives the average values for colostrum, transitional and mature milk and is of great value because the samples represent large groups of

women of many races and samples of milk collected and analysed in various laboratories. To study accurate trends in changing composition of breast milk, however, it is more accurate to observe successively the same women under standardized procedures from the beginning to end of lactation, as illustrated in Table I.

Human life studies demonstrate that each period in the life cycle has its own unique metabolic requirements and physiological and psychological characteristics. For this reason it seems justifiable to present data on specific infants' consumption of milk and protein as determined from their mothers' milk, and the records of the infants' concurrent growth and development. These records illustrate the biochemical individuality of human milk and the biological individuality of the infants consuming and metabolizing the nutrients in the milk. To illustrate the nutritional value of human milk for the young infant, we present information about three infant girls whose sole source of food during the first 6 months of life was their mothers' milk (Beach *et al.*, 1941). The infants had healthy nutritional histories, as judged from detailed physical, medical, and X-ray examinations of both infants and mothers during pregnancy and lactation. Breast milk and water were the only foods received during the period studied. Milk was expressed at 4-hour intervals and pooled in twenty-four hour samples by each mother, all of whom were experienced in the technique of manual expression of milk. A portion of the pooled milk was taken for total and non-protein nitrogen determinations, and the rest was stored for bottle-feeding the next day. In accordance with paediatric practice, the milk was fed at 4-hour intervals, but the records present the amount of milk voluntarily consumed by the infants. The growth rate of each child, the average daily amount of milk consumed, and the average daily nitrogen intake were determined, and the intake of seven essential amino acids was estimated.

Infants A, B, and C weighed 3·84 kg, 3·91 kg, and 3·06 kg, respectively, at birth. Table VII shows the weight gain, the breast milk and protein intake, and the estimated protein deposition of the three infants per kilogram of body weight per month. It also shows the wide difference in protein concentration of the three samples of human milk. The adaptability of human breast milk to the requirements of the infants is strikingly illustrated by the weight gains and the nitrogen deposition of the infants. If we determine by Rubner's factor (see Table VII) the monthly deposition of protein in body tissues per kilogram of body weight, and if from the protein nitrogen intake we estimate the protein intake per kilogram of body weight, the monthly protein intake, the deposition, and the milk volume intake of the three infants can be compared. There is a decrease in volume intake of breast milk per kilogram of body weight accompanied by decreasing protein intakes and deposition of protein in the body tissues. The values are much higher

during the 1st, 2nd and 3rd months than during the 4th and 5th months. The degree of effectiveness of mother's milk for an infant is determined by the capacity of the milk to yield nutrients to meet growth and development needs. The degree of efficiency of the three milks was calculated by dividing

TABLE VII

TOTAL WEIGHT GAINS, BREAST MILK AND PROTEIN INTAKES, AND ESTIMATED PROTEIN DEPOSITIONS OF THREE INFANTS[1]

(Values per kilogram of body weight per month)

Infant	Age months	Weight gain[2] kg	Intake			Estimated protein deposition[3]	
			Milk l.	Protein g	Protein g/l.	g	Percentage of intake
A	1	0·77	4·5	68·4	15·2	34·1	50
B	1	0·60	4·5	54·4	12·1	26·6	49
A	2	0·68	4·4	32·2	7·3	25·7	80
B	2	0·82	4·4	47·2	10·7	31·3	66
A	3	0·91	4·3	43·1	10·0	29·6	69
B	3	0·80	4·3	37·7	8·8	26·2	69
A	4	0·45	3·8	31·3	8·2	13·1	42
B	4	0·46	3·8	44·2	11·6	13·5	30
C	4	0·57	4·0	49·9	12·5	20·1	40
A	5	0·45	3·8	31·7	8·3	12·4	39
B	5	0·54	3·8	36·6	9·6	14·8	40
C	5	0·45	3·3	42·9	13·0	14·4	34

[1]From Beach *et al.* (1941).

[2]Gain in weight from the first to the last day of the month.

[3]Based on Rubner's estimate of 30 g of nitrogen per kilogram of body tissue.

the average daily gain, in 10-g units, by the number of ounces (28·35 g) of milk consumed (Poole *et al.*, 1937). Table VIII shows the efficiency of the three milks for infants A, B, and C. Infants A and B, although of approximately the same birth weight, possessed different patterns of physiological performance in regard to milk utilization in the formation of body tissue.

TABLE VIII

DAILY NITROGEN AND ESTIMATED AMINO ACID INTAKE IN THE FORM OF BREAST MILK PROTEIN BY THREE INFANTS DURING THEIR FIRST 5 MONTHS[1]

(Values per kg of body weight)

	1st month[2]		2nd month		3rd month		4th month			5th month		
	Infant A	Infant B	Infant A	Infant B	Infant A	Infant B	Infant A	Infant B	Infant C	Infant A	Infant B	Infant C
Average body weight, kg[3]	4·22	4·21	4·95	4·92	5·75	5·73	6·42	6·36	5·33	6·89	6·86	5·84
Gain in weight, kg	0·77	0·60	0·68	0·82	0·91	0·80	0·45	0·46	0·57	0·45	0·54	0·45
Degree of milk efficiency[4]	3·3	2·6	2·5	3·1	3·0	2·7	1·5	1·6	2·2	1·4	1·7	1·9
Average daily intake, ml breast milk	639	630	735	725	825	813	822	813	704	862	858	651
Average daily intake, mg/kg body weight:												
Protein nitrogen	365	290	172	252	230	201	167	236	266	169	195	229
Histidine	36	28	17	25	23	20	16	23	26	17	19	22
Arginine	116	92	55	81	73	64	53	75	85	54	62	73
Lysine	144	114	68	100	91	79	66	93	106	67	77	91
Tyrosine	144	114	68	100	91	79	66	93	105	66	77	90
Tryptophan	54	43	26	38	34	30	25	35	40	25	29	34
Cystine	57	45	27	40	36	31	26	37	42	26	31	36
Methionine	53	42	25	37	33	29	24	34	38	24	28	33
Cystine S + methionine S	27	21	12	18	17	15	12	17	19	12	14	17

[1]From Beach *et al.* (1941).

[2]The application of the analytical data upon the amino acid content of mature human milk in the calculation of the amino acid intakes during the 1st month, which includes the colostral and transitional periods of secretion, may have introduced some deviation from the true values.

[3]Birth weights: A, 3·84 kg; B, 3·91 kg; C, 3·06 kg.

[4]The degree of efficiency was calculated by dividing the average daily gain, in 10-g units, by the number of ounces (28·35 g) of milk consumed.

The peak of milk utilization was reached by infant A during the 1st month, whereas it was attained in the 2nd month by infant B. During the first 3 months of life, infants A and B utilized their mother's milk more completely, as shown by milk efficiencies of 2·5 to 3·3, than during the 4th and 5th months when the efficiency dropped to 1·4 to 1·7. Although infant C was of smaller birth weight, she used her mother's milk more efficiently (1·9 to 2·2) and grew more rapidly than infants A and B during the 4th and 5th months.

1. *Protein*

The nutritive value of protein resides in its component parts, the individual amino acids and possibly the peptide pattern after digestion (Mellander & Vahlquist, 1957). The proteins in breast milk, in body fluids, and in tissues are composed of numerous combinations of amino acids, varying in number and quantity. Some of the more than twenty amino acids can be synthesized in the body; others, known as "essential amino acids," must be furnished directly in the food. Table VIII shows the daily nitrogen and estimated amino acid intake, in the form of breast milk protein, by the three healthy infants during their first 5 months of post-natal life. Although the growth of an organism may be restricted by the supply of any single growth essential, whether it be mineral element, essential vitamin, or amino acid, it is ultimately limited, under conditions of optimal supply of nutrients, by the individual's inherent physiological capacity for growth. Whether conditions of greater protein supply would have enabled the individual infants in the present study to grow at more rapid rates than shown in their excellent records is a matter of speculation; the desirability of such excessive growth should also be considered from the standpoint of long-range health status. Only with a protein of nearly ideal amino acid composition, pattern, and nutrient mixture could one expect to find such efficient utilization by these biochemically different individualities represented by the infants and their mothers' breast milks.

The effectiveness of breast milk is best shown quantitatively by measuring the amounts of the nutrients and other chemical units taken into the body and their physiological progress through the digestive, absorptive, and excretory processes. Then the amount used for maintenance and nourishment of the body tissues and in the fabrication of the new units of growth may be determined. During the first few days of post-natal adjustment there is a large amount of tissue-building material in circulation which may represent a carry-over from foetal life or may result from the rapid chemical changes and adjustments of neo-natal life. The first urinary excretion is abnormally high in amino acids and other urinary substances including some of the vitamins (Smith, 1951; Hamil *et al.*, 1947a,b). After the infant's first week on breast milk, however, little nitrogen is found in the urinary excretion,

indicating that the nitrogen consumed in breast milk is in large measure utilized. The first few days after birth the infant is coping with problems of neo-natal haemoconcentration and immature kidney function. By the end of the second week, absorption of nitrogen from breast milk accounts for about 90 % of what is fed, and indicates a very large digestive capacity for protein as the infant enters on a rapid period of growth, a period when the body is composed of a relatively concentrated amount of protoplasmic tissue.

Proteins and their component amino acids are essential for the expansion of the muscle, neural, and glandular tissues, and for the multiplication of the number of cartilage cells in the area preceding the formation of new bone. Average breast milk provides approximately 2 g of protein per kilogram of body weight, but as solid foods are added to the infant's diet after a few weeks a higher level of 3 g per kilogram may be attained (Holt & McIntosh, 1953). The high concentrations of protein and other essential nutrients in the first milk the baby receives after birth may determine the extent to which certain essential elements are retained by the body. The infant born with conditioned malnutrition or severe stress owing to birth stress or exposure to lower environmental temperature may be inhibited from using certain nutrients until the enzymic and other chemical adjustments take place within the tissues to such a degree as to permit retention. Although biochemical individuality exists among infants and human milks, in general it may be assumed that when breast milk contains all essential amino acids, energy, vitamins and minerals in sufficient quantity and in proper balance, the rate of tissue synthesis and the efficiency of utilization of the diet for growth will approach a maximum. The protein of human milk is almost completely metabolized and the absorption is so efficient that the faeces of breast-fed infants contains only amino acids provided by the intestinal flora or intestinal secretion. Synergistic relationships are found to exist between amino acids such as cystine and methionine and between amino acids and vitamins such as phenylalanine and vitamin E and vitamin C.

2. *Carbohydrate*

Lactose is the characteristic carbohydrate of milk. Carbohydrate-splitting enzymes break lactose down into glucose and galactose in the process of digestion. From a nutritional standpoint, the presence of lactose in milk renders it much more important than galactose or glucose singly. Glucose in the body may arise from lactose, or it may be manufactured from protein and fat. Glucose is stored in the body as glycogen which is broken down and used as fuel. Carbohydrates may be associated with nucleoproteins, with glucoprotein and glucosamines, whereas galactose is associated with the formation of the galactolipids of nervous tissue. Lactose provides a beneficial medium for

intestinal activity regulating the bacterial flora, the hydrogen ion concentration of the alimentary tract, and the absorption of minerals, especially calcium for bone formation. Lactose is present in slightly lower amounts in colostrum (5 %) than in milk (6 %).

3. *Fat*

Fat serves primarily as a source of energy and is used in the formation of the nervous and osseous tissues, in providing fatty acids of desirable kinds and amounts, in sustaining body temperature and in regulating faecal elimination. When excess fat is deposited in the tissues there is a reciprocal relation with water. Although excess fat contributes to body mass, it does not contribute to true growth. A small essential fat moiety, approximately 10 to 15 %, is necessary for mediating the physiological processes of growth and metabolism. Fat in human milk is in the form of minute finely emulsified globules, each of which is surrounded by a protective coat of a mixture of protein and phospholipids.

Human milk fat solidifies at 20·2°C and has a specific gravity of 0·966 at 15°C. The percentage of fat is lower in early milk (3 %) than in mature milk (4 %) and may fluctuate during the day and during a single nursing. About 50 % of the fatty acids are unsaturated (Deuel, 1951). The initial mammary fluid of the first 3 days is lower in weight percentage (47 %) of saturated fatty acids than the mature milk (48 %), resulting in a higher percentage of unsaturated fatty acids (53 %) in the early secretion (Baldwin & Longenecker, 1944). Inasmuch as the young infant probably secretes little lipase, it has difficulty in handling and metabolizing fat; hence the low fat content of early milk is an advantage to the new-born. Lecithin and cholesterol have been found in respective amounts of 78 mg and 20 mg per 100 ml of human milk. Fat serves as the medium of the fat-soluble vitamins and is associated with their metabolism in the body.

4. *Energy*

There must be sufficient energy present in the food intake to meet the needs of activity, to maintain body temperature and basal heat production, and to permit full utilization of the essential amino acids for the synthesis of protoplasmic tissue in fulfilment of growth and development needs. If the diet does not provide enough energy, the protein intake cannot be fully utilized. Furthermore, the protein of the body tissues may be sacrificed and burned as fuel to provide for activity requirements. Human milk supplies about 75 kcal per 100 ml, or 20 kcal per ounce (Table I). The energy requirements of the new-born infant, in terms of body weight, are two to three times as great as those of the adult. The basal heat production of the infant is high owing to (1) the large proportion of protoplasmic tissue in his body and (2)

the relatively greater body surface which permits loss of heat. Mother's milk in sufficient quantity furnishes abundant energy for healthy growth in an active body of rich protoplasmic content but devoid of the handicap of excess fat. The breast-fed healthy infant has firm muscular tissue.

5. *Water*

Water plays a major role in cellular metabolism. All chemical reactions of life are conducted in an aqueous medium, and water is also an active participant in giving volume and form to the soft tissues. Water is the primary solvent, however, and serves as an inert vehicle for dissolving and emulsifying components in the human milk. Structurally, water composes approximately 80 % of the infant's body and 87 % of human milk (Table I). Water contributes also to the physiological role of the temperature-regulating mechanism of the body, and it is responsible for 25 % or more of the heat loss from the body by evaporation from the lungs and skin.

The total water in the body is distributed into three major fluid compartments: the intracellular fluid compartment, and the extracellular fluid compartment, which has been subdivided into extravascular (interstitial fluid) and intravascular (blood plasma). There is a wide range of values in the water distribution, due to the variation in the body fat content of the individual (Behnke, 1941–42; Friis-Hansen, 1957). In the diet of the infant, consideration must be given to the kinds and amounts of fats and proteins, to deficiencies or excesses of different minerals, and to the presence of water and vitamins to regulate enzyme and functional activities in the body. Human milk in sufficient quantity from healthy mothers provides the young infant with the pattern of those constituents best suited for its particular nutritive needs.

6. *Vitamins*

Vitamins are essential in many of the biological mechanisms having to do with growth, and in the enzymic systems necessary in energy, protein, fat, and carbohydrate metabolism. The vitamin content of colostrum and human milk has special significance in initiating the respiratory, digestive and excretory systems of the new-born infant. The level of each nutrient or its precursor in the blood passing through the mammary gland determines the amount of the nutrient the mother puts out in her milk. There are regulatory mechanisms in the mammary gland, however, to maintain the concentrations of some of the constituents within definite limits. Some women secrete a milk persistently low or high in one or more nutrients, which must also be considered in assessing the physiological value of human milk.

Although colostrum and mature human milk contain some digestive enzymes such as peptidase, amylase, invertase, catalase and lipase, the

efficiency of the milk in meeting the metabolic needs of the infant will depend on the efficiency of absorption and of enzyme production and hormonal factors. The growth hormone produced in the body of the growing individual aids in nitrogen conservation whenever dietary protein and carbohydrate are in short supply and may influence fatty acid and ketone metabolism (Russell, 1957). Available energy, vitamins, essential amino acids, and minerals along with the hormonal adaptation of the young organism provide sustenance for new and enlarged metabolic functions.

a. *Fat-soluble vitamins.* Vitamin A, carotenoids, vitamin D and vitamin E (α-tocopherol) are related to the fat content of milk. Vitamin A and carotenoids are inversely related to the fat content of milk, the concentration being less in fat when the fat content of the milk is high. Any conditions which interfere with fat absorption also interfere with the absorption of these vitamins. Carotene is converted into vitamin A in the body. This vitamin is necessary for protoplasmic growth, for proper vision, and for maintenance of the integrity of epithelial structures, the cartilage, and osseous growth. Vitamin A and its precursors, the carotenoids, play an important role in the epiphyseal cartilage sequences and endochondral bone growth (Mellanby, 1950) and in the shaping and moulding of bone. These substances also participate in many physiological functions in relation to endocrine activity and to metabolism. The serum vitamin A and carotenoid concentration in infants at birth is less than one-half the average concentration found in their mothers (Macy *et al.*, 1954). Premature infants appear to be less satisfactorily fortified with vitamin A, as judged by the blood levels, than full-term infants. Because of the interdependence of vitamin A and the carotenoids in metabolic and physiological activities, parallel low blood values would tend to intensify a deficient state. Table I indicates that vitamin A and carotenoid concentrations were higher in colostrum (average values of 161 μg and 137 μg per 100 ml of milk, respectively) than in mature milk (average 61 μg and 25 μg per 100 ml of milk). These high concentrations of vitamin A and its precursors in colostrum and transitional milk have special significance for the new-born who needs a generous supply of vitamin A for purposes of physiological adjustment and growth, and to increase the low vitamin A and carotenoid blood concentrations.

Vitamin D increases calcium and phosphorus absorption from the intestine and is related to calcium and alkaline phosphatase activity in bone formation. It is also associated with citric acid metabolism, probably because more than two-thirds of the body's store of citric acid resides in the skeleton and is associated with bone formation. Breast milk has a relatively low concentration of calcium (average 48 mg, 46 mg, and 34 mg of calcium per 100 ml of colostrum, transitional, and mature milk, respectively), yet breast-fed infants seldom develop rickets. The ratio of the average calcium-to-phos-

phorus concentrations and its relation to the protein concentration are equally important. Calcium in human milk, like proteins, is largely absorbed by the young infant. The high lactose concentration is alleged to enhance the absorption of the calcium by the breast-fed infant. In addition, parathyroid hormones act as regulators of calcium metabolism. Even without additional vitamin D, rickets is rare in breast-fed full-term infants except in the Negro; however, supplemental vitamin D is usually given after the 4th month. Premature infants usually receive supplements of vitamin D with breast milk.

Vitamin E concentration of the serum is low in the new-born infant. Cord blood has about one-fifth of the tocopherol content of mother's blood. The breast-fed infant soon overcomes the low vitamin E blood level with the high tocopherol concentration of colostrum. In breast-fed infants the serum tocopherol levels are about three times those in the serum of infants fed on cow's milk at the end of the first week.

b. *Water-soluble vitamins.* This group of vitamins is a large one; the human requirements for some are known with certainty, whereas others are in the exploratory stage. Thiamine is necessary for the utilization of carbohydrate, its prime function being its role in pyruvate metabolism, but human milk is a relatively poor source of this vitamin; however, adequately fed mothers produce milk that nourishes their infants satisfactorily. Mothers consuming limited diets of polished rice may produce breast milk of inferior quality which does not meet the infant's thiamine requirement. The total thiamine content of breast milk is 2 μg per 100 ml of colostrum; it increases to about 6 μg in transitional milk and to 14 μg per 100 ml of mature milk (Table I). Thiamine is necessary for synthesis of fat from carbohydrate in the body. Although the thiamine content of milk responds slowly to the amount of thiamine in the diet, it does not rise above 20 to 30 μg per 100 ml of milk.

Riboflavin serves as a component in the oxidative systems in the cells and is essential for protoplasmic growth. The total riboflavin content of breast milk increases from 30 μg per 100 ml in colostrum to about 37 μg in transitional and mature milk (Table I). The breast-fed infant is well fortified with both riboflavin and protein for its rapid protoplasmic growth.

The nicotinic acid requirement is conditioned by tryptophan-containing proteins. Human milk has on the average a nicotinic acid concentration of 75 μg per 100 ml in colostrum, and 175 μg and 183 μg in transitional and mature milk (Table I). On the other hand, tryptophan is high in colostrum (51 mg per 100 ml), as compared with 28 mg and 18 mg in transitional and mature milk. Nicotinamide has been shown to be an integral part of the pyridine nucleotide coenzymes that serve as a part of the intracellular respiratory mechanism of all cells.

Pantothenic acid is a fundamental part of coenzyme A, an important catalyst of acetylation reactions. This vitamin increases from 183 μg to 288 μg per 100 ml from colostrum to transitional milk and remains around that level thereafter.

Folic acid participates in many biological reactions in the body such as the conversion of glycine to serine, the methylation of homocystine to methionine, and the methylation of nicotinamide. Mature milk contains more folic acid than the earlier milks.

Vitamin C is involved in the activities of many enzyme and hormone systems. Highest concentrations of vitamin C are found in tissues of highest metabolic activity. The ascorbic acid content of the infant at birth averages 38 mg per 100 g of tissue (Ingalls, 1938), whereas during the first 4 months after birth it decreases to 10·8 mg. The content in infant blood at birth is higher than in the blood of its mother. Excretion of vitamin C in the urine of the new-born (Hamil *et al.*, 1947b) would indicate that there must be a plethora of vitamin C for foetal growth and development. It is a substance of great physiological importance and is probably involved in many intracellular chemical reactions.

The higher average concentration of vitamin C in colostrum (7·2 mg per 100 ml) varies with the diet of the mother and is probably influenced by the white blood cells contained in the colostrum, since they are known to possess much higher vitamin C concentration than blood plasma. Mature milk may possess 5 to 6 mg of vitamin C per 100 ml. The ascorbic acid concentration of human milk is above that of the maternal blood level. Even with supplementation, the degree to which the concentration can be increased is limited (Kon & Mawson, 1950; Pratt *et al.*, 1947). The maternal serum vitamin C level may drop precipitately immediately after complete emptying of the breast, at a time when milk synthesis by the mammary gland is at its peak (unpublished observations). Vitamin C is intimately related to protein metabolism and haemoglobin formation.

IV. Physiological Value of Cow's Milk in Infant Feeding

Nature provides a food best suited to fulfil growth and development needs of a particular species. It is to be expected, therefore, that the composition and relative proportions of nutrient constituents and the physical characteristics of cow's milk will vary from those of human colostrum and mature milk for the reason that the growth rate, the period of infancy, the digestive system, and the nutritive requirements of the calf and the human infant are so unlike.

Table III illustrates that the general properties of human and cow's milks differ in several respects, some of which are known to have significance in

infant feeding. Colostrum and mature human milk differ from average whole cow's milk in the following respects: iodine number, 60·4, 61·6 and 38·4, respectively; Reichert-Meissl number, 1·8, 0·8 and 28·8, respectively. Although the milks yield an equal number of calories per unit volume, modified cow's milk fed to a baby would furnish considerably more protein than an isocaloric quantity of human milk. Cow's milk fed whole or diluted is more concentrated in many major nutrients when compared with human milk, except that it contains less vitamin A, ascorbic acid, and lactose. In relative proportions of nutrients the artificial formulas may also differ from human milk.

According to some medical authorities, cow's milk is made more suitable for young infants by diluting it with appropriate amounts of water, usually half and half dilution; but even then it has a higher concentration than human colostrum or mature milk. Carbohydrate is added to provide the formula with an isocaloric mixture similar to breast milk. Artificial formulas are made safe by heat treatment. Pasteurization or boiling of formulas destroys the pathogenic bacteria and makes them safe for the young infant. These treatments, however, destroy much of the ascorbic acid which is already in short supply in cow's milk. Supplements of ascorbic acid and vitamin D contribute to the nutritional security of the infant until it is able to take a mixed dietary containing adequate essential nutrients.

A. Protein

The protein of milk is the essential tissue-producing substance. Nevertheless the protein of cow's milk has often been designated as one of the chief constituents causing digestive disturbances in infants. There is a distinct difference in the degree of denseness of the curd formed from human milk and from milk of different breeds of cattle. Heat or other suitable treatments of artificial formulas provide for the necessary physical adjustments of the curd formation in the alimentary tract of the infant through the adjustment of its isoelectric point. Either heat treatment or dilution of cow's milk will change the curd from a dense, hard mass, which is difficult to digest, to a fluid-like or flocculent consistency similar to that of human milk, which can be digested easily by the delicate system of the baby. The total protein content of cow's milk, human colostrum and mature human milk is 3·3 g, 2·3 g, and 1·1 g, per 100 ml, respectively. Cow's milk diluted with an equal volume of water still possesses a higher concentration of protein than breast milk.

Milk protein consists of a complex mixture containing relatively different concentrations of casein (the unique protein characteristic of milk) and its fractions, albumin and globulins of the whey fraction, and other poorly defined proteins ((U.S.A.) National Research Council, 1953). The relative proportions

of casein and the total whey protein are very different in the two kinds of milk, mature human milk containing on the average 0·4 % casein in contrast to 2·8 % in cow's milk. Under certain circumstances almost the whole quantity of protein of mother's milk may be retained by the infant (Stearns, 1939; Beach *et al.*, 1941; Stearns *et al.*, 1958). Infants fed on cow's milk store more protein but eliminate considerable amounts (Waterlow & Stephen, 1957). This comparison of the utilization of cow's and human milk by infants is further confirmation of the difference in physiological value of these two types of food. The degree of efficiency of utilization may be calculated by dividing the average daily body weight gain, in 10-g units, by the number of ounces (28·35 g) consumed. During the first three months of life, a group of infants fed on reconstituted cow's milk (diluted 1 : 1) as the sole food, after breast feeding had been discontinued within the first 2 weeks of post-natal life (Poole *et al.*, 1937), showed a degree of milk efficiency of 1·8 to 1·6. This is less than half the degree of efficiency of utilization of human milk, which was 3·3 to 2·5 in breast-fed infants in the same age range.

A study of the constitution of milk proteins is of interest because these nutrients are elaborated for the young at a time when the requirements for protoplasmic growth are at a maximum and may be expected to have the ideal pattern of amino acid distribution and other nutritive advantages for supporting growth and activity. The amino acid composition is not the only factor determining the biological value of proteins, but its importance cannot be minimized.

In the two milks the whey protein occurs in approximately equal concentration, but the casein content of cow's milk is seven times as great as that of human milk (Table III). Eighty-two per cent of the protein nitrogen of bovine milk is in the form of casein, whereas in human milk only 39 % is contained in the casein fraction (Beach *et al.*, 1941; (U.S.A.) National Research Council, 1953). The large difference in amino acid content of the casein and whey fractions of the milk has nutritive significance in infant feeding. The distribution of sulphur between cystine and methionine is worthy of note in view of the research on the nutritive value of the milks. Breast milk is superior to cow's milk in promoting sulphur retention (Blazso, 1939) and a higher retention ratio of sulphur to protein in infants (Ujsaghy, 1940). One of the differences of one type of milk protein from another would appear to be connected fundamentally with the distribution of sulphur and nitrogen among the nutritionally essential amino acids, the ability of the infant to make the conversion of methionine to cystine, and the cystine requirement of the infant.

Nitrogen and phosphorus are closely associated in the synthesis of protoplasm by the infant. In infant feeding, the relationship of nitrogen to phosphorus in the dietary intake and in metabolism has physiological

significance. Cow's milk contains 96 mg of phosphorus, in contrast to 16 mg in human colostrum and 14 mg in mature human milk. In a comparison of these milks, the nitrogen-to-phosphorus ratios are markedly different, namely, 5·7, 22·6 and 12·4 for cow's milk, human colostrum and mature milk, respectively.

More subtle differences between human and cow's milk are reflected in the enzymic digestion of the total protein in milk (Mellander & Vahlquist, 1957). The pronounced difference between the digestibility of the bovine and human caseins depends on the formation of enzyme-resistant phosphorylated peptides. The proteins of cow's milk are broken down more readily than the proteins of human milk, owing partly to the fact that the casein of human milk is more resistant to hydrolysis than is the casein of cow's milk. The resistance of the casein peptides toward the proteolytic enzymes apparently depends on the presence of the phosphoric acid in the casein molecule. In addition, more phosphorus is contained in the casein from cow's milk than in that from human milk.

Mellander (Scheinberg, 1956) has demonstrated that the linkage between the phosphoric acid and the peptide is easily broken by alkaline phosphatase in digestion and that the phosphopeptides form soluble salts with calcium, iron and other minerals, and aid in the absorption of these minerals. This investigator and his associates believe that, in addition to the amino acid pattern, the peptide pattern after digestion must be taken into consideration in infant feeding. Phosphorylation plays an important role in many metabolic and nutritional processes in the body, in which the metabolic function of the peptides of casein is significant.

B. Carbohydrates

Carbohydrates are important factors that influence protein needs and protein utilization. Cow's milk contains on the average 4·8 g per 100 ml, in contrast to 7 g in mature human milk, and this accounts for the practice of adding carbohydrate to cow's milk formulas for infants. Not only does the carbohydrate, lactose, which is characteristic of the mammary secretion, appear in greater concentration in human milk, but some investigators allege that lactose has special significance over forms of other carbohydrates in protein utilization (Cornely *et al.*, 1957). There are different polysaccharide compounds (György *et al.*, 1954a,b,c; Gauhe *et al.*, 1954; Bigwood *et al.*, 1957) which vary in concentration and proportion in cow's and human milk and are important factors in the conditioning and initiation of the young infant's digestive, metabolic, and excretory systems to exogenous food supply. They also influence the physiological value of milks used in early infant feeding.

C. Fats

Although the concentrations of fat in human and cow's milk are similar, the physical characteristics and fatty acid composition are significantly different (Baldwin & Longenecker, 1944; Hilditch, 1956; Hilditch & Meara, 1944). There is at present in many laboratories an active interest in the effect of saturated and unsaturated fatty acids on long-term health from infancy through adulthood in man.

D. Minerals

The mineral content of cow's milk is more than three times that of human milk. Even though cow's milk may be diluted (1 : 1 with water), it remains more concentrated than human colostrum or mature milk. Bovine milk contains 125 mg of calcium per 100 ml, whereas human milk contains 33 mg (Table III.) The respective phosphorus contents are 96 mg and 15 mg per 100 ml. The relation of calcium and phosphorus in bone formation is important. The calcium-to-phosphorus ratio is 1·3 in cow's milk, 3·0 in human colostrum, and 2·4 in mature human milk. Infants store more calcium on cow's milk feeding, but it remains to be demonstrated whether the excess of calcium and other minerals, with resultant rapid bone mineralization and growth, is an advantage or a disadvantage. If human milk is accepted as the desirable standard of reference for optimal health, cow's milk, whole or diluted, would appear to be too highly mineralized and to offer certain physiological disadvantages. Infants given artificial formulas without supplementation are notably susceptible to rickets.

E. Vitamins

The total and relative concentrations of the vitamins differ among cow's milk, human colostrum, and mature milk. Cow's milk contains less vitamin A, ascorbic acid, and vitamin E. When reconstituted cow's milk is fed, these vitamins become even more critical. Early supplemental feeding of other foods to the breast-fed and artificially fed infant is desirable as a safeguard against deficiencies and malnutrition.

V. Dietary Requirements of Infants

The nutritional endowment—i.e., size and body composition of the infant at birth (see Table II), type and intensity of physiological adjustment after birth, and potentiality for growth—is a basic factor that must be considered in the establishment of dietary allowances. In addition to basing these requirements on nutritional endowment, it is believed that they can be estimated from the composition of milk of well-nourished mothers. Nutrient

allowances that have been arrived at on this basis are recognized as approximate values formulated for guidance in planning food supply for the infant rather than for detailed application. When applied to individuals or groups of babies, however, these values must be judged by the effects on the infants involved—i.e., their resultant health, activity, general well-being, growth and development.

TABLE IX

DAILY NUTRIENT INTAKE OF HEALTHY BREAST-FED INFANTS COMPARED WITH DAILY RECOMMENDED ALLOWANCES

	Quantity taken[1]				Recommended allowance[2]
Constituent	End of 1st week	During 2nd week	5th–13th week	4th–6th month	2nd–6th month
Breast milk, ml	400	475	765	935·0	
Energy, kcal	232	352	543	664·0	720
Protein, g	10·8	7·6	9·2	11·2	—
Calcium, g	0·12	0·16	0·25	0·31	0·6
Iron, mg	0·36	0·19	1·15	1·40	5
Vitamin A, μg	356	418	405·0	496	349
Thiamine, mg	0·06	0·03	0·12	0·15	0·4
Riboflavin, mg	0·12	0·16	0·32	0·40	0·5
Nicotinic acid, mg	0·30	0·83	1·32	1·61	6
Ascorbic acid, mg	17·6	25·6	32·9	40·2	30

[1]Quantities taken are the mid-points of the ranges given by Holt & McIntosh (1953).
[2]From (U.S.A.) National Research Council (1958).

Table IX illustrates the average daily nutrient intake of healthy breast-fed infants during the 1st and 2nd weeks after birth, during the 5th to 13th week, and during the 4th to 6th month. The nutrient intake is based on the mid-points of the ranges of volume intake given by Holt & McIntosh (1953). Comparisons of the nutrient intake in breast milk are made with the Recommended Dietary Allowances of the (U.S.A.) National Research Council (1958). With but two exceptions, vitamin A and ascorbic acid, the recommended allowances are much higher than the nutrients from breast milk intake. This is not surprising, since the optimal intake of essential dietary constituents remains largely speculative.

The recommended allowances are not requirements. They are considered to represent not merely the minimal level for the average person but also the

nutrient levels selected to cover individual variations of a substantial majority of the population, to provide for increased needs in times of stress, and to permit other potential benefits. The margin between minimal requirements and recommended allowances varies among the essential nutrients for the reason that there are variations in the body's capacity for storage, the extent of variations in individual needs for certain nutrients depending on nutritional and growth status (Hamil *et al.*, 1938). Calorie allowances for infants are adjusted arbitrarily according to age, size, activity, rate of growth and stage of development.

The protein requirement of infants is not known with certainty (Scheinberg, 1956). The breast-fed infant receives 2 g or more protein per kilogram of body weight per day. The fact that nitrogen retention is apparently not maximum in the breast-fed infant (Gordon *et al.*, 1937; Stearns, 1939) has stimulated some concern over protein allowances for babies. It is stated in the Recommended Allowances, however, that "there appears to be no convincing evidence either that the breast-fed infant is lacking in protein or that the higher level supplied by many conventional formulas should be decreased."

Breast milk furnishes a relatively low calcium intake, yet the calcium is used most effectively. Proof is given by the infrequent occurrence of rickets under human milk nutrition, except in the Negro race or in premature infants. Whether super-mineralization with excess calcium assimilation is beneficial under normal conditions of early rapid growth and development (Smith, 1951) is problematical. The older infant and prematurely born infants are generally given additional vitamin D. The approximate calcium-to-phosphorus ratio in colostrum is 3 g per g, and for transitional and mature milk about 2·3. The recommended allowances for iron exceed by several times the amount of iron received by the breast-fed baby. The allowances of vitamins A and C approximate the quantities received by the infant nursing at the breast, whereas those for thiamine, riboflavin, and nicotinic acid may be about double those actually consumed at the breast.

Biotin, vitamin K, pantothenic acid, vitamin B_6, vitamin B_{12}, folic acid, choline, inositol, and many other constituents present in human milk have vital roles in numerous metabolic systems, but the metabolic requirements of the infant have not been determined with any degree of certainty. In a consideration of the implications of breast feeding (Macy *et al.*, 1945) we stated that "the final test of any food for an infant, whether it be breast milk or modified cow's milk, is the type of growth it produces and whether it produces a nutritionally stable, healthy infant who is resistant to infections and unhampered in physical and mental growth and development. . . . The healthiest baby may not necessarily be the one that grows the fastest or the one that has the largest bones and lays down the most calcium in a given period. . . . No one knows whether the biggest baby, or the one who

stores the most calcium [or nitrogen], has a greater advantage throughout life than the infant fed breast milk, who may grow more slowly but with greater nutritional stability and may extend that steady growth farther into childhood. Indeed, studies of animals indicate that years may be added to the life span and life to the years by giving diets complete in all known dietary essentials which produce slow but steady growth. . . . Perhaps the type of growth inaugurated by good breast milk may be controlled by the vitamin, protein, and mineral mixture best suited to the infant." Much more information is needed before the final chapter on the physiological value of milks can be written.

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CHAPTER 19

Lactation and the Growth of the Young

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I. Introduction

Lactation cannot be regarded biologically as an isolated phenomenon: it is a phase of the reproductive process as a whole. Nor can the value of milk as a food necessary for the survival of the young be discussed without due consideration of those other aspects of the post-natal maternal contribution

necessary for their survival, such as the protection, warmth and education the dam provides. In this regard, the developmental stage of the new-born mammal varies markedly from species to species, from the helpless, naked, minute young of the marsupial to the active, fully-coated calves of deer and antelope which are capable of accompanying their dams within a few hours of birth. The needs of the young and the relative importance to be placed on the dam's post-natal contribution must vary considerably from species to species.

This Chapter is concerned with some of the problems of the post-natal phase of growth and development of the young, and the ways in which the amount and composition of the milk supply, and maternal instincts are so adapted to the needs of the offspring that they ensure that it can complete its development and live an independent life. Owing to the paucity of quantitative data for wild species much of the information relates of necessity to domesticated and laboratory animals and to man. There is, however, a wealth of anecdotal information relating to aspects of lactation in wild species and, where pertinent, some of it has been quoted. From the assembled data, some broad generalizations about the biological significance of lactation emerge. These are generalizations and there are, no doubt, exceptions to them.

II. The Biology of Lactation

A. Anatomical Aspects of Suckling and Nursing

A previous Chapter (Mayer & Klein, Chapter 2) dealt with the structure of the mammary gland, and gave some attention to comparative aspects. As far as the mother–offspring relationship is concerned, it is of some interest to consider the physical means by which the young animal obtains its milk. In the sub-class Prototheria as represented by the most primitive of mammals, the egg-laying mammals, the duck-billed platypus (*Ornithorhynchus*) and the anteaters of Australia (*Echidna*) and New Guinea (*Proechidna*) the teat is completely absent, and the young simply lap or lick off the drops of milk as they are secreted. At later stages in the development of the young of *Ornithorhynchus* the dam lies on her back so that the offspring can nurse to better advantage (Newman, 1920). Whether or not this is a specific reaction to a nursing stimulus is not known. Whittlestone *et al.* (1952) have, however, detected milk-ejection hormone in the pituitary gland of *Echidna* as well as in two species of marsupials. Mere presence of the hormone, however, does not indicate that it has a similar function to that in more highly developed mammals. Indeed, the swordfish pituitary contains milk-ejection hormone (Whittlestone *et al.*, 1952). Reproduction in the Australian anteater involves the development of a so-called incubatorium into the deeper posterior part of which the leathery-shelled egg is transferred by the dam. There it is

hatched, and the young again lap the milk secreted from a small mammary pouch on a more shallow anterior part of the abdominal wall surface of the incubatorium (see Bresslau, 1920). The time relationships of reproduction in these species show the importance of the lactation phase. Egg development takes 14 days, incubation takes 10 days, while the suckling period is 3 to 4 months.

In marsupials, pregnancy is very short and the young are transferred to the marsupium where they suckle from teats. In some genera, such as in some of the opossums, the pouch may be incomplete or even entirely absent. Hartman's (1923) studies of reproduction in the North American opossum (*Didelphys virginiana*) showed that pregnancy lasts for 11 days, when the young migrate from the vaginal orifice to the marsupium, find a teat and attach themselves to it for a period of about 2 months. Again, in this order of mammalia, as in the monotremes, the lactational phase of the reproductive process is of considerable importance. The young of the pouchless marsupial *Marmosa* when they are born at $12\frac{1}{2}$ days are extremely immature. They are hairless, blind and apparently deaf, yet they can cling to their mothers by means of grasping reflexes of foot and mouth and find the teat by means of successive trial-and-error grasps (Beach, 1939). This instinct is truly remarkable in view of the immaturity of the new-born animal. The marsupial young are, in fact, often called mammary foetuses, to indicate their incomplete development. They hang passively in the marsupium attached to the teat to which the mouth becomes firmly adherent. At this time they have what is termed an "incomplete intra-narial epiglottis" which is in some ways analogous to that found in the Cetacea. The epiglottis is produced upwards into the respiratory division of the pharynx behind the soft palate and appears to be associated with the capacity to ingest milk while breathing continues through the nostrils. The passage from the nasal cavities to the larynx is completely unobstructed. The teat itself in most marsupials is long and thin and it is thought, on the basis of anatomical study, that milk is actively expressed from the gland by the contraction of the cremaster muscle (Parker & Haswell, 1930). Despite the studies of Whittlestone *et al.* (1952), it is not known how far humoral mechanisms of milk ejection are concerned. A further factor concerned in suckling in marsupials is that the milk dentition of the young is poorly developed, only the third premolar being represented by a milk tooth (de Beer, 1936).

The placental mammals, the Eutheria, all have teats. There are very considerable variations in the location, relative size and number of teats both between the different orders and between the species within the orders. Thus in the Ungulata vera teats are usually few and their placement is usually in the groin, but the order includes, besides the horse, ox and goat, the pig with its multiple thoracic and abdominal glands and *Hyrax* with six teats, four in

the groin and two in the axillae. Again, in the goat the teats tend to be long, whereas in the ewe, an animal of the same size, they are short. In general, throughout the mammalia there is a parity between the number of young born and the number of teats, though within species there may be some disparity. The biological significance of this disparity is dealt with later. The range of variation in number of glands appears to be from one pair to a maximum of eleven pairs as found in *Centetes*. Similarly, there is considerable variation from species to species in the number of ducts in each teat. How far this latter variation has survival value for the species concerned is not known.

Similarly, there is considerable variation in the milk dentition of the young at birth. It ranges from absence of lacteal teeth, as in dogs and in human infants, where the upper lip is modified to form a sucking pad, to calves and lambs which have temporary incisors either erupted at birth or easily palpable below the soft gum. Considerable variation occurs in the extent of erruption of the temporary incisors at birth in these species. The central incisors of the foal which are erupted at birth are set far more obliquely in the mouth than they are in the adult, which may facilitate sucking. In this species, the temporary dentition is not complete until 7 to 9 months of age. The piglet is rather peculiar in that it is born with four temporary canines and four temporary corner incisors and these infant teeth are particularly sharp. No molars are present though they can be palpated below the gums. In farm practice the canines of each piglet are often removed at birth to prevent laceration of the teat of the sow and of the ears and faces of its litter-mates, for the piglets fight with one another for access to the udder, and use their canines to good effect. Even in cows and ewes, soreness of teats due to small transverse cuts made by the incisors of their calves or lambs is quite a common minor malady.

B. Sucking and Swallowing

Suckling is an active process. Apart from the hormonal release of milk during nursing, the young of mammals expend energy to obtain the milk by sucking. In the sow, as soon as the teats are erect in response to initial massage the piglets "hold the teats fully extended appearing to swallow at furious speed rather than to suck." When milk flow stops "the piglets then suck vigorously as if to draw the last drop" (Gill & Thomson, 1956). In this species, the full secretory phase of nursing appears to necessitate little but swallowing on the part of the young. In human infants, there is a very wide range in the strength and duration of the sucking and swallowing reflex, and in premature infants the reflex is poorly developed (MacKay *et al.*, 1945). Control over movement of the tongue, enabling the infant to pass food to the back of the mouth does not develop until about the 3rd month of age

(Aldrich, 1942). In contrast, the young calf can eat solid food at 2 weeks of age, and will often chew straw within a day of birth.

Benzie & Phillipson (1957) have published excellent radiological photographs that show very clearly the swallowing process in the calf and goat kid, and a similar cineradiographic study of lip and tongue movement of the goat kid has been made by Ardran *et al.* (1957, 1958). The kid exerts a considerable negative pressure in sucking, the pressure in the mouth at the tip of the teat varying from atmospheric to −150 to −200 mm mercury. Changes in the form of the goat's teat when in the kid's mouth during suckling are analogous to the change in the form of the human nipple in the baby's mouth. The process of sucking in all instances seems to involve compression of the teat between tongue and soft palate, obliterating its lumen and displacing the contents into the back of the mouth. Inefficient bottle-feeding in human babies is probably largely due to inability to obliterate the lumen of the nipple.

The subsequent passage of milk into the stomach is of some interest. In rats, Platt's (1954c) studies show that a stratification of the milk occurs in the stomach in which the most recently consumed meal is represented by a shell enclosing the remains of those meals ingested earlier. The milk coagulates in the stomach and the whey fraction, apart from that dispersed in the interstices of the clot, leaves the stomach more quickly (30 minutes) than does the curd (20 hours). This sequence of layering of milk which is the reverse of that which happens with solid food (Platt, 1954b) means that diffusion processes within the stomach must be of considerable importance in ensuring digestion of the curd in the pyloric antrum. Presumably similar events take place in other species. Certainly gastric emptying time and gastric digestion are influenced appreciably by the frequency and size of meals in human babies (Clements, 1949).

The nature of the clot formed in the stomach is of some importance in determining the passage of milk through the gut. Yorsten & Hytten (1957) have shown that some human babies develop a "3 month colic" in which the predominating clinical sign is very rapid development of hunger after a breast feed. Weaning to a cow's milk food abolished the syndrome, and it appeared that in these babies the fine curd of breast milk was passing through the stomach very quickly, whereas the firmer curd of cow's milk was not. Blaxter & Wood (1953) showed that in the calf many of the alimentary tract disturbances or "scours" could be referred to rapid passage of milk through the abomasum (true stomach) without extensive preliminary gastric digestion. By alteration of the calcium and sodium contents of artificial milks, thereby altering their ability to clot with rennet, Kastelic *et al.* (1950) were able to induce diarrhoea in calves at will. The several methods adopted in human infant nutrition to modify the hard tough curd produced in the stomach when undiluted untreated cow's milk is given, thereby making it more similar to

that of human milk, are well known (Clements, 1949). They indicate that there are probably fairly specific relationships between the type of protein and its dilution, the ionic composition of milk of particular species and the activity of the gastric mucosa in producing acid and proteolytic enzymes. This close association was commented on very early by Kiesel (1905).

In the ruminants, where only one of the fore-compartments of the digestive tract is homologous to the stomach of rat and man, milk is normally by-passed directly into this organ during the suckling period. The milk passes through a conduit from the terminal restriction of the oesophagus, "the cardia," to the abomasum and little enters the rumen and reticulum. This "oesophageal groove" is formed in response to the soluble proteins and salts of milk (Wester, 1930); it is more pronounced during bottle feeding than during pail feeding and appears to be a reflex closely related to a sucking pattern rather than to a thirst pattern of behaviour (Watson, 1944). Whether a similar by-pass mechanism operative during the suckling phase operates in other species with complex fore-stomachs—such as the Sirenia, the Marsupialia the Cetacea and the Hippopotimidae—is not known.

C. Nursing Position

Some mammals lie to nurse and others stand. Hediger (1955), on the basis of observations at the Zurich Zoo, points out the considerable difference in the behaviour of relatively closely related species. Thus the elk and roe deer, though ungulates, adopt the lying position, whereas the capybara, though a rodent suckles in the standing position. The dugong holds her young to her breast with her flippers while partially submerged. The hare sits on her haunches to nurse her litter, whereas the rabbit lies. Differences in behaviour are not simply due to size. The hippopotamus, like the closely related pig, lies to suckle its litter; indeed it gives birth and suckles its young under water in shallow pools!

D. Frequency and Duration of Suckling

There is a wide variation between species in both the frequency and duration of suckling. There are difficulties in making such measurements in small species that make nests. Bateman (1957), however, concluded on the basis that the mouse can rarely be removed from her nest without some of her young being found attached to her teats, that a large fraction of her time while in the nest is actually spent in nursing the young. On average, about 80 % of the female's time was spent in the nest during early lactation compared with 50 % towards the end. As lactation advanced, nursing periods became shorter. Weaning of the young coincided with visits to the nest becoming

more frequent. In the rabbit kept under semi-natural conditions, the frequency of nursing (Deutsch, 1957) appears to be once every 24 hours. At the end of each nursing the dam leaves the nest and carefully fills its entrance, disguising it most effectively. Nursing frequency in sows appears to be at 50 to 60 minute intervals throughout the 24 hours and shows only a slight decline with the age of litter (Barber *et al.*, 1955). In sheep, Munro (1956) has shown that the number of completed sucklings, the term "completed" being used to denote a successful attempt on the part of the lamb, varies very widely, on occasions forty successful suckling periods being observed in the 16 hours of daylight. At a later age, Barnicoat *et al.* (1949a,b) found that ewes nursed their lambs about six times daily. Under pasture-grazing conditions, cows nursed their calves three times daily, each nursing lasting about 15 minutes (Johnstone-Wallace & Kennedy, 1944). Slightly more frequent nursing is usual at earlier stages of lactation (Walker, 1950). It does not seem possible to draw any general conclusions from such diverse behaviour.

There are difficulties in the precise definition of the normal duration of lactation, particularly in domesticated species which have been subjected to selection for milk production, and in man where social habits often determine the duration of breast feeding (Douglas, 1950). Even so, approximations to within a few days prove useful. For comparative purposes species can be arranged in an order defined by the magnitude of the ratio, duration of lactation : duration of pregnancy. The animals that show the highest value of this ratio are, of course, the marsupials, and the lowest ratio is found in the guinea-pig and certain aquatic mammalia. As will be seen later, this ratio appears directly related to measures and assessments of the maturity of the species at birth, the least mature having the highest ratio. Some typical ratios are given in Table VIII and others may easily be calculated from the scattered literature. Some are of more than passing interest. Thus the whale, contrary to popular belief, is a fast-growing animal, its suckling period of 5 to 6 months being relatively short compared to its gestation period of about 12 months (Matthews, 1946). In another aquatic mammal, the grey seal, pregnancy lasts about 275 days and lactation only 17 days. Then the mother abandons her young completely to break her fast which lasts from birth to weaning (Amoroso *et al.*, 1951). This gives a ratio of $<0{\cdot}1$, lower than that of the guinea-pig (0·2). By contrast, in the Cape fur seal, lactation is often prolonged until the birth of the next pup, so that the ratio is well above 1·0 (Rand, 1955).

E. Maternal Care

It is only too easy to interpret the very obvious maternal solicitude seen in the higher mammals in anthropomorphic terms. It is also very clear that there

are aspects of the behaviour of the nursing mother that can only be interpreted as attempts to teach the young to live an independent life. Game-playing with the offspring is seen in almost all large mammals and many delightful popular accounts of it have been written. Thus the sand seal, which unlike the grey seal spends its short infancy in and out of the water and indeed suckles under water, is protected from battering and drowning in the clasp of its mother's fore-flippers, and the mother plays with and apparently teaches the offspring to swim and dive at the same time (Lockley, 1957). Chimpanzee mothers not only play with their young, but some aspects of their maternal behaviour have been interpreted as attempts to exercise their offspring and to teach it to walk (Yerkes, 1943). One of the more curious aspects of maternal behaviour is that of the koala bear. Minchin (1937) showed that during the weaning period, when the young change from a pure diet of milk to eucalyptus leaves, the mother produces "soft faeces." The marsupium faces backwards in this species and the young stretch out to feed on the soft faeces. This is presumably an extension of the coprophagous habits seen in many herbivora. It is not known whether such weaning behaviour occurs in the rabbit in which coprophagy is an established adult habit.

III. The Regulation of Lactation

A. Number of Young to be Suckled

Broadly speaking, the number of young produced at a single birth in a particular species of mammalia is inversely proportional to the average size of that species. A further generalization is that when gestation exceeds 6 months only one young is produced. These generalizations and elaborations of them, which are usually attributed to Spencer (1899), have exceptions. The common bat (*Pipistrella*) produces one young only though it is no larger than a mouse, whereas a large sow, weighing as much as 300 kg can produce litters of over twenty. Within a species there is a considerable variation in the number of young born, and hence in the potential number to be suckled by the dam. Frequency distributions for the numbers born of guinea-pigs, lambs, mice and pigs, show that the number of young is subject to considerable variation within any one species, species with high mean birth number having greater absolute variation than others. The many factors that determine the number of young born in any one species cannot be dealt with here, but it suffices to say that they include nutritional and other environmental effects operative before ovulation and during pregnancy, as well as factors associated with the age and previous reproductive history of the dam. Table I summarizes for a number of species the usual number of young born and the number of teats of the dam. In general, there are ample teats for the young, the exceptions

being found in species of marsupials and in the guinea-pig, where the usual number born exceeds the number of teats. When account is taken, however, of the potential variation in litter size and of teat number, a general parity of litter size with number of teats as shown in Table I, is not always to be found. In the mouse, for instance, it is rare to find more than ten teats, though Turner

TABLE I

THE MOST COMMON NUMBER OF TEATS IN DIFFERENT SPECIES AND THE MOST COMMON LITTER SIZE

Species	Most common number of young born	Most common number of teats
Marsupials		
Opossum	20	13
Australian native cat	16	6
Cetacea		
Whale	1	2
Ungulates		
Ox	1	4
Sheep	1	2
Pig	12	14
Rodents		
Mouse	8	10
Rat	10	12
Guinea-pig	4	2
Carnivores		
Dog	8	10
Cat	4	8

& Gomez (1933) in their survey found 1·9 % of mice to have eleven and 0·7 % to have twelve teats. Yet the number of young born can be as high as sixteen. The pig is an even more striking example. The potential variation in litter size is from one to twenty-four piglets born, and the variation in teat number in sows is such that sows with more than sixteen teats are very rare indeed. The correlation between teat number and litter size in the pig is positive but quite small. In the ewe as many as five young may be born, and though

there are some strains of ewe with four teats (Ritzman, 1933) the usual number is two.

B. Post-natal Survival

Not all the young survive to suckle. In some of the marsupials where disparity between teat number and number of young born is greatest, the losses of young are due to failure to find the pouch and attach themselves to the teat. Losses due to failure to attach can, in fact, reach 75 % (Hill & O'Donoghue, 1913). In the guinea-pig, where there is also a marked disparity between birth number and nipple number, death rate of the young increases with litter size (Rowlands, 1949, 1955). Much of this can be attributed to maternal neglect at parturition. Rowlands (1955) has described parturition in the guinea-pig. The young are expelled head first surrounded by an intact amnion, all members of the litter being expelled before placentae appear. The sow guinea-pig tears the membranes from the face of the young animal, which then begins to breathe, and by its struggles breaks the umbilical cord. Rowlands noted that when large litters were born, the sow appeared to be more concerned with disposing of the membrane of the previously born member of the litter than she was about the survival of her newest born. This lack of interest may reflect a poor development of maternal instinct on the part of the mother. It may also be due to physical exhaustion, the interval between births being about 7 minutes, and birth of a litter of nine thus involving a parturition of over an hour. In this regard, the initial survival of twin and triplet lambs is closely related to the stamina of the ewe at parturition. Where ewes are underfed during pregnancy there is usually a considerable delay between the birth of one twin and that of the other, a delay referable to uterine inertia. Furthermore, the initial interest the ewe displays in her lamb immediately after birth depends on its initial activity. Vigour of the lamb at birth is in turn related to the amount of food the dam has received during pregnancy, and is another factor that may contribute to survival of the young (Thomson & Thomson, 1948–49). In the sow, a large number of the deaths of the new-born is due to the sow lying upon the piglets. The amount of care she takes in lying down and the agility of the new-born pigs in getting out of her way in part determine the number she rears.

It is usual farm practice where large litters are born to sows to reduce the number of young to the number of teats available. Many animals reduce litter size by infanticide. The mouse regularly indulges in infanticide by killing and eating a proportion of her litter if she gives birth to more than she can suckle; indeed most litters suffer depletion in this way (Falconer, 1947; Crozier & Enzmann, 1935). Infanticide also occurs in later stages of infant development. Carpenter (1942) describes, for instance, what occurred during

the transport of rhesus monkeys from India to Puerto Rico. Not only did the mothers uniformly fight their infants for the limited food supply, but about 10 % of the mothers killed their own babies. There is indeed little evidence to support the contention that a behavioural maternal altruism is commonplace in animals.

Apart from these losses of young directly associated with parturition, neo-natal and peri-natal deaths due to other causes reduce the number of sucklings. In domesticated livestock, losses of life of the sucklings during the first two days of life can be very high. In cattle, the overall loss including deaths at or during parturition and in the first week of life is about 10 to 15 %, in piglets about 20 %, in lambs 10 to 20 % and in dogs up to 40 %. Some of the post-natal losses are due to communicable disease, some to nutritional deficiency during foetal life and some to accident and unknown cause. The variation in the number of young to be raised is thus considerable, and raises the question of the degree to which the dam regulates her milk supply to the size of her litter.

C. Size of the Individual Young to be Suckled

The birth weight of the total litter relative to maternal weight varies from species to species according to the duration of pregnancy and the number born. Most frequently the birth weight is about 10 % of the mother's weight; thus, a cow weighing 450 kg produces a calf weighing 45 kg, the ewe weighing 60 kg produces a lamb weighing 3 to 5 kg, the rat weighing 300 g produces a litter weighing 35 g and the mouse weighing 40 g produces a litter weighing about 8 g. At the extremes of this distribution are the guinea-pig which produces a litter of 3 or 4 weighing 300 g, herself weighing 800 g, and the woman weighing 60 kg who produces an infant weighing 3 kg. These extremes are from 40 % of the mother's weight to 5 %. Inclusion of marsupials would further widen this range at the lower extreme.

Within any one multitocous species the weight of the individuals within litters of different sizes falls as litter size increases. Table II gives data for a number of species.

Litter weight rises with increasing litter number, and the mean weight of individuals falls. It appears with some species that there is an upper limit of weight for the litter as a whole. Thus in Venge's (1953) rabbits, the litter weight of the large race did not increase much above about 670 g and in the small race above 280 g. The weights of the individuals are maximal for small numbers, decline slowly until maximal litter weight is achieved and then decline in direct proportion to litter size. However, the observations of Asdell *et al.* (1941) indicate that in the rat, with increasing parity litter number and litter weights are highly correlated and individual weight changes very little.

It has been suggested that the size of the offspring is related both to the size of the placenta (Barcroft, 1944) and to a competition between foetuses for available nutrients (Hammond, 1944). These hypotheses are not mutually

TABLE II

LITTER WEIGHTS IN RELATION TO NUMBER BORN IN SOME MULTITOCOUS SPECIES

No. of offspring born	Total litter weight				
	Pig	Sheep	Guinea-pig	Rabbit	
				Large race	Small race
	(Murray, 1934)	(Hammond, 1932)	(Eckstein *et al.*, 1955)	(Venge, 1953)	
	(kg)	(kg)	(g)	(g)	
1		3·2	111		53
2		4·5	220		87
3		6·1	272		135
4			342		164
5			349		198
6				360	232
7	10·2			443	280
8				481	280
9				554	280
10	13·2			610	
11				683	
12				661	
13	15·7			689	
14					
15					
16	16·5				
17					

exclusive. As Leitch (1957) has summarized when dealing with human pregnancy, "The environment the mother provides is more important than the genetic make-up of the child in determining birth weight, and the maternal environment is not constant over the reproductive period but changes probably with both age and parity."

For any particular litter number, the weight of the litter is certainly affected by the nutrition of the dam before birth. In sheep, for instance, the weight of singletons can vary from 5 to 13 lb and directly reflects the feeding level of the ewe during late pregnancy (Wallace, 1948; Thomson & Thomson, 1948–49). The same is true of cattle (Joubert, 1954–55; Bonsma, 1949). In man it is only when maternal undernutrition is severe as in the famines of Rotterdam and Leningrad that birth weights of infants are reduced (Smith, 1947; Antonov, 1947). Under normal nutritional circumstances any relationship is very small (Garry & Wood, 1946).

These overall effects of litter size relative to size and age of mother and of maternal nutrition on the size of the young at birth deal with effects on mean size. Within any one particular litter there may be a very wide range of

TABLE III

REGULATION OF MILK YIELD TO LITTER SIZE IN THE PIG. AVERAGE WEANING WEIGHTS OF PIGS AT EIGHT WEEKS FROM SMALL AND LARGE LITTERS
(From Murray, 1934)

Average litter size	Average weight/pig at 8 weeks (kg)	Total weight of litter (kg)	Total weight increase of litter (kg)
4·8	15·0	71	68
8·6	12·8	111	100
10·6	12·4	130	117
12·4	12·0	149	134

individual birth weights. This is well recognized in farm practice where large litters from sows contain members varying appreciably in weight, from so-called "runts" to normal litter-mates more than twice their size.

D. REGULATION OF LACTATION TO LITTER SIZE

The very large variation that exists within a species in the number of young to be suckled and their size, together with the variation within a litter in the size of individual offspring, raise questions about the regulation of lactation to the overall demands of the litter. There is ample evidence that it takes place, and one example of it in the pig is given in Table III. Despite a

variation in average litter number from 4·8 to 12·4, the average weights of the pigs at weaning differed by less than 20 %. If it is borne in mind that the smaller litters have the larger individual pigs at birth, it means that sows regulate their lactation according to the number of young they have to rear. The last column shows that their lactation can provide for growth of the litter by either 86 kg or 134 kg, and these figures are by no means extremes. An even

TABLE IV

REGULATION OF MILK YIELD TO LITTER SIZE IN THE MOUSE. MEAN WEIGHTS OF INDIVIDUAL MEMBERS OF MOUSE LITTERS AT 12 DAYS OF AGE IN RELATION TO THE NUMBER IN THE LITTER
(Falconer, 1947)

Number in litter	Mean weight of individual (g)	Total litter weight (g)
4	5·9	23·7
5	5·7	28·5
6	6·1	36·4
7	5·5	38·7
8	5·6	45·0
9	5·8	51·8
10	5·3	52·9
11	5·8	63·9
12	5·1	61·1

more striking example of regulation of output is given in Table IV, which shows that over a range from 4 young to 12 young—a three-fold difference in demand—the mouse can so regulate her lactation that weaning weights of individuals differ by not, on average, more than 10 %. A further example of the regulation of lactation to litter size is given by Wallace (1948), who showed that ewes suckling twins produce about 60 % more milk than those suckling singles.

1. *Ante-natal Regulation*

The first question that arises is whether or not the dam regulates the growth of her mammary gland to suit the size of her unborn litter. Cole (1933) found that the rate of development of the mammary gland of mice was not related to the number of embryos *in utero* and a similar conclusion was reached by

Hammond (1950). By adjusting all litters of mice to a standard number of 8 at birth irrespective of the number born, Bateman (1957) found that there was a small, but statistically significant, effect of the number born on the weaning weight of a standard litter of eight. This approach effectively standardizes the post-natal factors affecting lactation. The results suggest that there is a very small quantitative control exerted pre-natally over the output of the mammary glands although there are probably higher levels of luteal, foetal and placental hormones produced when there is a large, compared with a small, number of foetuses in the uterus. With twin lambs in ewes, the death of one twin does not, however, result in faster growth of the survivor reared as a singleton; indeed, the reverse happens (Hammond, 1932). This suggests very strongly that in this species there is no considerable augmentation of yield in response to number of young carried. Wallace's (1948) studies also indicate that the growth of the udder in late pregnancy is conditioned very little by the number of young carried. In general, the evidence suggests that regulation of the quantity of milk produced to the demands of the offspring is not determined ante-natally, but post-natally.

2. *Post-natal Regulation*

The fact that regulation of lactation in multitocous species to meet the total demand of the offspring is almost entirely post-natal, suggests either that the supply of milk is always superabundant in quantity and sufficient for each individual and lactation is reduced to accommodate smaller numbers, or that the amount secreted depends upon the effect of some cumulative stimulus exerted by each member of the litter on the secretory activity of a constant amount of mammary tissue. The former hypothesis would suggest that regression and involution of the mammary glands are inversely proportional to litter number, and the second that the secretory activity is directly proportional to litter number, and it is difficult to separate the two factors without very careful experimentation. Clearly, if no young survive and no need for lactation ensues, the mammary glands must involute completely. Bateman's (1957) studies in which reciprocal changes of litters were made between dams rearing large and small numbers of young showed that if the dam had been suckling a large number she could soon accommodate to a small number, but if she had been suckling a small number she could not increase her milk production to suit the needs of a larger number. The same relationship is found in the ewe, where lactation is regulated within the first days to the number of young suckled (Wallace, 1948). Bateman's (1957) studies with reciprocal changes of litter number indicate that reduction of the secretion to accommodate smaller numbers is not associated with engorgement of the gland. On this basis he suggests that a reflex production of prolactin is involved in the regulation. Benson & Folley's (1957) recent studies show that

oxytocin, which is released from the neural lobe of the hypophysis in response to the suckling stimulus, certainly retards involution of the mammary gland, and these authors conclude that oxytocin stimulates the release of prolactin and other galactopoietic hormones from the pituitary gland. It thus appears that the accommodation of lactation to variable numbers and demand is carried out by an alteration of the involution rate of the mammary gland, and in it an accumulative stimulus exerted by the cumulative demands of the litter is involved.

E. Accommodation of Litter Variation and Competition between Sucklings

The variation in the size of the young at birth in some multitocous species has already been mentioned. In the mouse, where there is relatively little variation in size of young within a litter, the apportionment of milk to them appears to be very even. If a variation in the size of individuals is deliberately induced by interchange of young, the larger ones grow more rapidly than the smaller, presumably because they get a greater share of milk. This is seen even in very small litters where one would think that initially there was sufficient milk for all. Bateman (1957) calls the cause of these differences in intake "passive competition" between the young, for it involves no active fighting on their part, and suggests "that the larger young get the greater share of milk because they feed faster and not because they oust their weaker sibs from the best glands or spend longer sucking them." It must be pointed out that in the mouse the individual young show no preference for individual glands. In the rat, there is some evidence that all young show a preference for the fourth pair of nipples, possibly because they are most accessible. There is no specific preference on the part of individuals in which one suckling habitually suckles one particular teat (Tsai, 1931).

Both lambs and piglets, unlike mice and rats, show preferences for particular glands. With twin lambs, one invariably suckles one side and one the other. Instances have been recorded where an abnormality of one half of the udder in a ewe has been associated with dissimilarity of the growth of the twins (Wallace, 1948). In the ewe, however, active inter-sib competition does play a part in apportioning the milk to litter-mates. Thus, when triplets compete for her two teats the weakest one usually suffers most. Similarly, when twins suckle one teat, the other being non-functional, the stronger lamb obtains more milk and grows faster than the weaker one. This type of competition is certainly an active one in contrast to the passive competition found in the mouse.

The distribution of the milk between the piglets in a litter is of considerable interest. Within 4 days of birth each piglet has assigned itself a particular

teat which it continues to suckle throughout lactation. This preference is not due to an appreciation of the individual's place within the order of piglets when they lie against the sow, for even when other members of the litter are removed, the piglet still, after a few attempts, finds its "correct" teat (Donald, 1937b). Some regional landmarks are necessary, however, for more "errors" are made by piglets suckling the central mammae on the abdomen than those suckling posterior or anterior ones, and, if the sow lies on a side other than her accustomed one in order to nurse her litter, mistakes are often made by the right-handed and left-handed members of the pair of pigs suckling a particular pair of glands.

The piglets show a distinct preference for the anterior teats; the heavier pigs are usually found sucking these teats and these pigs usually grow more quickly (Donald, 1937a). From this association the initial selection of teat by the piglets might be thought to be the result of instinctive appreciation by the larger piglets that the anterior glands produce the most milk. There is little to support this supposition, and it appears more likely that the instinct to seek anterior rather than posterior teats is related to the safe position of these teats, for they are further removed from the hind legs of the sow. In this competition the larger, stronger pigs would prevail. The initial selection does not result in a uniform gradation of piglets in terms of weight from the anterior to the posterior teats; Donald (1937b), in fact, gained the impression that the initial selection was largely due to chance. Similarly, the anterior-posterior gradient of milk production is not always clear-cut (Donald, 1937a). Nevertheless, there are very real differences in the amount of milk consumed by individual piglets. Some typical data obtained by Barber *et al.* (1955) are summarized in Table V, and show that the variation between individuals can be considerably more than two-fold. This could be due to appetite variation between individuals as suggested by Comstock *et al.* (1942), implying a superabundance of milk in all glands and a post-natal regulation of the output of individual glands by some process of early involution. Such a regulation would result in an anterior–posterior gradient of milk production of the glands, determined by the initial process of teat selection in which the initially strong piglets prevail. A somewhat similar hypothesis was advanced by Wohlbier (1928) who referred to the difficulty smaller, weaker pigs might have in obtaining milk during the relatively short period during which milk ejection takes place. That stronger pigs suckle anterior glands and thereby obtain more milk from them is not the sole cause of the anterior–posterior gradient of milk yield. There is evidence to suggest that the anterior glands still produce more milk, even when suckled by the lightest pigs. These observations suggest that in the pig an active competition for milk occurs in the initial stages of lactation, but that once lactation is fully established competition between sucklings for a limited supply of milk is relatively small.

These three examples of the way in which the available milk is apportioned within a litter, indicate that parity of intake between sibs is rarely achieved, and that it is usually the strongest and most vigorous member of a litter that obtains the most. This often results in marked disparity in growth. As Barber *et al.* (1955) have shown with pigs, those piglets that consume the most milk use the milk slightly less efficiently than those that grow the least, but this compensation is not sufficient to produce even growth throughout the litter.

TABLE V

THE VARIATION IN THE INTAKE OF MILK BY INDIVIDUAL SUCKLING PIGS FROM BIRTH

(From Barber *et al.*, 1955)

Period of lactation (days)	Intake of milk (kg/week)					
	Sow 1		Sow 2		Sow 3	
	Highest	Lowest	Highest	Lowest	Highest	Lowest
0–21	18·3	10·2	16·7	7·3	15·0	6·9
22–56	27·2	17·2	26·1	11·0	27·6	12·0

IV. The Maturity of the Young

A. COMPOSITION OF THE YOUNG AT BIRTH

A useful biological generalization was made by Moulton (1923) who, on the basis of extensive studies of the chemical composition of different animal species throughout their life, concluded that the composition of the fat-free bodies of mammals showed but very small differences between species. von Bezold (1857, 1858) had made a similar series of generalizations. This relative constancy of the chemical composition of the fat-free body is to be expected, since the chemical composition of the major tissues of the body shows very little inter-species variation. Variation between species would arise in the main as a result of differences in the ratio of skeletal weight to soft-tissue weight, for the skeleton differs so markedly in composition from the soft tissues, whereas differences in the chemical composition of the different soft tissues, though they exist, are rather minor. Some exceptions to

Moulton's generalization are to be expected. Thus, adult man has a heavy skeleton relative to his weight, and the ash content of the dry matter of different species does tend to increase slightly with body size over the 10 000-fold range of body weight represented by the mouse and ox. Table VI summarizes the data relating to the approximate composition of the adult animal.

TABLE VI

THE APPROXIMATE CHEMICAL COMPOSITION OF THE FAT-FREE TISSUES OF ADULT ANIMALS

Component	Average (per 100 g fat-free tissue)	Variation Those species with heavy skeletons tend to have
Water (g)	73	Less water (70 g)
Protein (g)	21	Less protein (9 g)
Ash (g)	6	More ash (10 g)
Na (mg)	75	More Na (200 mg)
K (mg)	300	Less K (280 mg)
Ca (mg)	1300	More Ca (2000 mg)
P (mg)	750	More P (1100 mg)

It was further shown by Moulton that, during foetal life and for a period after birth, the chemical composition of the fat-free body differs very markedly from that found at maturity. He developed the concept of chemical maturity to denote the attainment of that constancy of composition indicative of adult life, when the concentrations of water, protein and ash in the fat-free body become stationary. He showed that the rat reached chemical maturity at 50 days, the cat at about 100 days, the pig at about 300 days and the ox at 150 days. Spray & Widdowson (1950) have pointed out that the concentration of some constituents of the body goes on increasing after the concentration of others has ceased to do so, and that the term "chemical maturity" should really be applied to the body as a whole only when all constituents have reached a constant value. Potassium for instance, reaches its adult concentration more quickly than does calcium (Spray & Widdowson, 1950). Despite these difficulties, however, the assumptions that the chemical composition of the fat-free adult body differs but little between species, and that chemical maturity is a meaningful term, prove useful in making inter-species comparisons. In Table VII, some analyses of new-born of mammalian species are

given which show the very large variation in the gross composition of the young at birth. This table shows that judged by their chemical composition the bovine animal and the guinea-pig are relatively mature at birth, whereas the mouse, rat and rabbit are decidedly immature. Pig and man are intermediate. As shown in Table VIII, with the protein content of the fat-free body of the new-born as an index of maturity, there is a general relationship between maturity of the new-born and the length of the lactation period relative to the uterine period of development. The naked young of rats, rabbits and mice require long lactation periods to attain nutritional independence of their mothers, whereas in the more mature bovine animal and guinea-pig lactation is short.

TABLE VII

THE COMPOSITION OF THE FAT-FREE BODY AT BIRTH

(From data of Armsby & Moulton (1925), Widdowson (1950) and Spray & Widdowson (1950))

Species	Composition of fat-free body					
	Water (g/100 g)	Protein (g/100 g)	Sodium (mg/100 g)	Potassium (mg/100 g)	Calcium (mg/100 g)	Phosphorus (mg/100 g)
Mouse	85	12·8	226	274	340	343
Rat	87	10·9	236	208	306	356
Guinea-pig	79	16·5	173	250	1131	741
Rabbit	87	11·3	243	206	484	361
Cat	82	15·2	231	222	661	436
Pig	80 85	20·0 11·4	241	209	999	739 575
Ox	76	18·4	150	230	1180	
Man	82	14·1	226	205	955	558
Seal					1088	648
Adult (see Table VI)	73	21·0	75	300	1300	750

With increasing "chemical maturity" in the sense employed by Moulton (1923), the tissue enzyme levels also change markedly. Some enzymes, such as the D-amino-acid oxidase of the liver of the rat, increase in concentration six-fold (Kuriaki & Kensler, 1954) from birth to adult life. Knox *et al.* (1956) have summarized pertinent data, and have commented that the increase in

the concentration of specific enzyme proteins cannot be explained in terms of the increase of tissue dehydration or the increase in protein content of the tissues during post-natal growth. These results suggest that the enzymic machinery for food utilization is markedly under-developed in the young. Unfortunately, too few data are available to permit between-species comparisons, most information applying to the rat only.

TABLE VIII

CHEMICAL MATURITY OF THE NEW-BORN IN RELATION TO LENGTH OF LACTATION

(From data compiled by Kenneth (1943) and other sources)

Species	Protein content of fat-free dry matter (g/100 g)	Duration of pregnancy (P) (days)	Duration of normal lactation (L) (days)	Relative length of lactation (ratio, L:P)
Rat	10·9	22	28	1·3
Rabbit	11·3	30	30	1·0
Mouse	12·8	21	21	1·0
Man	14·1	280	180	0·6
Cat	15·2	56	50	0·9
Pig	16·0	115	56	0·5
Guinea-pig	16·5	65	12	0·2
Ox	18·4	278	60	0·2

B. FAT CONTENT OF THE NEW-BORN AND HOMEOTHERMY IN THE YOUNG

Karl Thomas (1911) directed attention to the large variability in the fat content of different species at birth, and suggested that those species that are born fat had well-developed homeothermic mechanisms, whereas those born with very little were poikilothermic at birth. Table IX is largely drawn from Widdowson's (1950) analyses and the earlier ones of Armsby & Moulton (1925).

The variation in fat content is considerable, from about 1 % in the rat to an average of 16 % in man. It is certain that the guinea-pig, containing a large proportion of fat, has a measure of control over its heat-loss mechanisms from birth and that the mouse and rat have none (Barbour, 1941). This would agree with Thomas's generalization. Similarly, since calves and lambs can survive under a wide range of environmental temperature conditions it is

legitimate to suppose that they have fairly highly developed homeothermic mechanisms. These species, however, contain very little fat. Furthermore, there is evidence that the human baby is partially poikilothermic at birth and that prematurity increases this disability (Gleiss & Weber, 1955). An ability to regulate body temperature is certainly not conferred on human babies by their high fat content.

In pigs too, a partial inability to maintain body temperature constant under cold conditions, and indeed an ability to withstand body cooling is apparent.

TABLE IX

FAT CONTENT OF DIFFERENT SPECIES AT BIRTH

(From Armsby & Moulton (1925) and Widdowson (1950))

Species	Fat (g/100 g body weight)	Development of homeothermy
Rat	1·1	Negligible
Pig	1·5	Partial
Cat	1·8	Partial
Rabbit	2·0	Partial
Mouse	2·1	Negligible
Sheep	3·0	Good
Ox	3·5	Good
Seal	9·0	Presumably good
Guinea-pig	10·1	Good
Man	16·1	Partial

Recent studies by Holub *et al.* (1957), for instance, show that up to 6 days of age the baby pig is little able to respond to cold by increasing its heat production, and that thermogenesis in response to cold is not fully developed until 20 days of age. Considerable variation between piglets in this ability to regulate body temperature is apparent, and the heavier piglets are not necessarily the best regulators. However, the piglet is in other respects and by other criteria a relatively mature animal at birth despite the fact that it contains little fat. Thus Thomas's supposition that fat content and homeothermy are closely related does not appear to be generally true.

In aquatic mammalia where the young are in the water the whole time, as in whales and dolphins, or are left on exposed beaches during their early life, as in seals and sea elephants, a subcutaneous fat layer to provide insulation must be necessary for their survival. It appears that Widdowson's (1950)

suggestion that there is a connexion between the relative length of pregnancy and the fat content of the new-born is a likely one. It would explain the apparent association of homeothermy with fat content.

It is of some interest in dealing with the development of homeothermy that nest-building activity of the dam appears to be closely related to the needs of the young. The mother guinea-pig producing homeothermous young for instance, builds no nest, whereas the rat producing what are effectively poikilothermous young not only builds a nest but builds it more loosely when it is warm and more compactly when it is cold. The rat also retrieves pups that wander from the nest and are thus exposed to cold, and this activity wanes as the pups grow older (Wiesner & Sheard, 1933). The rabbit producing young that are partially homeothermic makes an underground nest, depilates her belly thereby exposing her nipples, and lines the nest with the fur she obtains. Contrary to the rat and mouse, she does not appear to have any desire to retrieve her young if they wander away (Deutsch, 1957). In monkeys, the furry body of the mother takes the place of a nest and the prehensile young thus spends all its early life in a warm local environment. Indeed, in all species that do not build nests or build poor nests, the very proximity of the mother must provide a local warm environment for the young. A further point arising from the incomplete homeothermy of many infant animals concerns the development of infant-type hair coats. These are peculiarly fluffy as in seals and lambs, have considerable depth, and hence provide maximal insulation for minimal fibre density. Since wool production is of great economic importance, the early stages of follicle development in sheep have been studied in some detail (see reviews by Carter, 1955; Turner, 1956). What emerges from such studies is that apart from her genetic contribution the maternal contribution to the weight of fleece produced in adult life is very considerable. Some handicaps, such as being born to a small dam or an undernourished dam, or being born as a twin, have long-term effects on the number of follicles and subsequent fleece production.

C. Growth and Development of the Enzyme Systems of the Digestive Tract

The digestive tracts of adult animals of different species show a very wide morphological variation. Some generalizations can be made. Thus, insectivores and carnivores have short intestines whereas rodents and hoofed animals have long ones. Diverticula of the digestive tract also show very large between-species differences as instanced by the presence of complex stomachs and caeca in many herbivores. There is similarly a considerable variation in the microscopic structure of the homologous parts of the digestive tract as between different adult species. Some detailed studies of the development of

the digestive tract have been made, and the instance of the post-natal development of the ruminant stomach may be cited (Khalilov, 1955). In the domesticated ruminants the proportions of the digestive tract and the development of its muscular and mucous coats at birth differ markedly from those in the adult, adult proportions certainly not being reached by the end of the suckling stage (Lagerlof, 1929). A continued diet of milk in ruminants does not lead to normal development of the digestive tract (Blaxter *et al.* 1952; Brownlee, 1956): solid food appears to be essential both for the development of the size of compartments and for the development of the papillae and epithelium of the rumen walls. This is not due solely to a mechanical stimulus by solid food (Warner *et al.*, 1956) but appears related to a specific stimulating effect of the lower steam-volatile fatty acids which are the normal end-products of carbohydrate dissimilation in adult life. This "vitamin-like effect" of the lower steam-volatile fatty acids on development of the ruminant stomach is not present during the normal suckling phase. Suckling in this instance could be regarded as retarding this normal developmental process.

1. *Proteolytic Enzymes*

A similar association of a major developmental change in the young animal with the cessation of milk feeding, and conversely the retardation of development by protraction of the suckling phase can be seen in the results of Berridge *et al.* (1942–44). The pepsin activity of the abomasal secretion in fistulated calves increased markedly only when solid food was given. The results of Kvasnitskiĭ & Bakeeva (1940) which showed that pepsin activity in the stomach of baby pigs was only apparent at 59 days of age is probably a comparable observation, for it could be related to ingestion of solid food by the piglets at this time. In the infant phase, proteolysis in the infant pig is due to rennin activity. No rennin is present in the stomach in adult mammalian life (Tauber & Kleiner, 1934).

The acidity of the stomach of the new-born pig, calf and human infant is usually low, but increases during the suckling period (Ebers *et al.*, 1956). There is, however, very little information on the changes with age of the proteolytic enzyme complex of the stomach to indicate the range of species variability. Tryptic activity appears to be well established both in human infants (Klumpp & Neale, 1930) and in pigs (Catron, 1955) and it shows but little change with age.

2. *Lactase Activity*

It has been known for a long while that lactase is present in the digestive tract of mammals. Plimmer (1906) summarized the observations made up to that time by the generalization that omnivores and carnivores have lactase present in the mucous membranes of their intestine the whole of their lives,

but that it is present in herbivores only when they are young. Lactase activity of the intestinal wall has been studied by several workers since Plimmer made his summary, and it appears that the generalizations which he made are not entirely correct. The most recent studies by Heilskov (1951) and de Groot & Hoogendoorn (1957) show that there are wide species differences in the occurrence of the enzyme at different ages. Table X summarizes most recent information. It will be seen that in all species studied, with one exception, lactase is present at birth in higher concentrations than those found in the adult, or even at the end of the suckling period. The exception is the guinea-pig in which the differences are not significant. This is in accord with the relatively late stage of growth and development which the guinea-pig has

TABLE X

THE APPROXIMATE LACTASE ACTIVITY OF THE MUCOSA OF THE INTESTINE IN RELATION TO THE SUCKLING PHASE OF GROWTH. UNITS OF LACTASE ACTIVITY/g MATERIAL

Phase	(a) Rat	(a) Pig	(b) Calf	(b) Rabbit	(a) Guinea-pig	(a) Cat	(a) Dog
At birth	18·0	18·0	50·0	14·0	5·0	4·0	6·0
At end of suckling period	2·0	2·0	11·0	5·0	4·0		
Adult	2·0	~ 1·0	~ 0·2	0·3	4·0	0·5	0·7

(a) de Groot & Hoogendoorn (1957).
(b) Heilskov (1951).

reached at birth. No studies on foetal lactase activity in the guinea-pig have, however, been made, but it is possible that intestinal lactase may be present at high concentrations at a foetal age corresponding to the conceptual age at birth of those related species that have high enzyme concentrations at this time. de Groot & Hoogendoorn's studies with the rat show that the lactase activity of the tract as a whole declines during this initial period; it is not simply a fall in activity per unit weight of mucosa. Evidence for the disappearance of lactase activity with increasing age in human infants is particularly meagre (Duncan, 1955) but lactase is certainly present at birth in concentrations of the same order as those found in cats and dogs. Furthermore, it is present in the foetus in increasing amounts up to term (Heilskov, 1951).

The question arises whether the decline in lactase activity might not be due to an adaptation of the enzymes of the alimentary tract in response to the

type of carbohydrate ingested. This is not supported by experiment for, contrary to the claims of Fischer & Sutton (1953) and Fischer (1955), Heilskov (1951) and de Groot & Hoogendoorn (1957) have found that prolongation of the period in which lactose is given does not halt the decline in lactase activity of the mucosa either in the rabbit or the rat.

Further indirect evidence showing that lactase activity declines with age is the observation of Becker & Terrill (1954) who showed that the suckling pig could tolerate 50 % concentrations of lactose in its diet but that at weaning (8 weeks) it could not. Human infants too are able to tolerate dietary concentrations of lactose that in adults lead to diarrhoea and abdominal distension (Fischer & Sutton, 1949; Duncan, 1955).

3. *Intestinal and Pancreatic Carbohydrases*

It appears that all young animals can hydrolyse lactose to some degree. They can also assimilate simple sugars such as galactose and glucose. There is accumulating evidence, however, that the digestive tracts of young animals are not well equipped at birth with saccharolytic enzymes other than lactase, and that their digestive enzyme complexes undergo gradual quantitative shifts with age. The evidence pointing in this direction is both direct and indirect. The indirect evidence consists of observations made when artificial diets in which sugars other than lactose are given. Thus, the new-born pig is unable to survive if given a milk substitute in which sucrose or D-fructose serves as a source of dietary carbohydrate. At 2 weeks of age sucrose can be tolerated to some degree, and survival is ensured on such artificial diets if the dietary carbohydrate is D-glucose or invert sugar (Becker *et al.*, 1954a,b). By following changes in the level of reducing sugars in the blood after test meals, Dollar & Porter (1957) and Dollar *et al.* (1957) have shown that the young calf cannot utilize maltose for the first 4 to 5 weeks of life and sucrose for at least 7 weeks. The young pig develops the ability to digest sucrose and maltose during the first 10 days of life. The more direct approach adopted by Bailey *et al.* (1956) involved measurement of the sucrase and maltase activity of excised portions of the small intestine of piglets. Sucrase and maltase activities at birth were negligible and increased to maximal values at an age of about 25 days. This increase coincided with a fall in lactase activity.

There is a similar inability of these two species to utilize the more complex sugars. The calf cannot hydrolyse dextrins or starch for the first month of life, and the piglet cannot utilize dextrin until about 10 days of age (Dollar & Porter, 1957). Similarly, the amylolytic activity of pancreatic extracts from piglets is negligible at birth, increases markedly with age and does not appear completely developed by 37 days of age (Kitts *et al.*, 1956). Examination of duodenal contents of new-born human infants shows that amylolytic activity

can be demonstrated in about a quarter of them (Hess, 1912), and this activity increases very slowly during the first 2 years of life (Klumpp & Neale, 1930).

These age changes in the relative importance of intestinal and pancreatic carbohydrases in different species all point to a closely integrated biological system, in which the natural duration of lactation is closely related to the rate of development of the ability of the animal to utilize the complex carbohydrates of an adult diet. At the same time, the persistence of the enzyme, lactase, in the intestinal mucosa is also related to the natural duration of the lactational phase of development. Whether the rate of post-natal development of the "adult carbohydrases" can be modified by dietary means during the suckling period, whether their development is dependent on age or related to aspects of size is not known. It is, however, pertinent to note that in premature infants the ability to digest carbohydrate is poorly developed.

4. *Lipase*

In marked contrast to amylolytic activity, the pancreatic lipolytic activity of animals appears to be of a high order at birth, and remains high as growth proceeds. This has been shown in pigs by Kitts *et al.* (1956). Martin's (1956) histochemical studies of the distribution of the enzyme in the tissues of dogs, cats, rabbits, guinea-pigs, rats and mice did not show any quantitative changes that could be referred to age. In view of the importance of fat in the diet of the suckling, this adequacy of lipolytic enzyme activity during early life is only to be expected.

D. Other Aspects of Physiological Maturity

The above Sections show that the newly born animal of a species is by no means evenly underdeveloped, some tissues and some physiological functions being relatively less developed than others. There is also a marked variation between species in their general and specific maturity. Further aspects of infantile immaturity can be mentioned. Thus the kidneys of infant animals are not able to concentrate excretory products to the same extent as can the adult kidney (McCance, 1948), and the myoglobin content of muscle is very low at birth compared with its content in adult life (Lawrie, 1950). In this regard, adult aquatic mammalia that have diving habits have about five times as much of this pigment in their muscles as have active land mammals (Robinson, 1939). This suggests very large differential rates of development of function as between species, and in view of the iron content of the myoglobin predicates a further reason for species differences in iron requirement during suckling. The ability of young animals to see is always poor, and indeed some are born blind. Liver reserves of fat-soluble and water-soluble vitamins,

which may be regarded as an insurance against dietary lack, are usually poor in the young, but here once more there is a wide variation between species (Moore, 1957; Goodwin, 1952). The high zinc content of hair (Eggleton, 1939) suggests that those animals that are born naked and grow hair during suckling must have much greater extra-uterine requirements for the element than those born fully coated. Spray & Widdowson (1950) have, in fact, shown that the rat, born naked, gains more zinc during growth than the cat which is born with fur. Zinc is a component of carbonic anhydrase and new-born mammalia all appear to exhibit low carbonic anhydrase activity in their erythrocytes (Vallee & Altschule, 1949). This also predicates markedly different needs for the element on the part of the young compared with the adult.

These isolated examples of the relative immaturity of the young could be multiplied almost indefinitely. What emerges from such considerations is that the young of different species at birth exhibit wide ranges of physiological maturity, and that simple assessment of the developmental stage of a species at birth in terms of its weight at birth relative to maternal weight, or its subsequent rate of increase in weight, tends to obscure many of the very real differences in developmental patterns which exist as between different mammalian species.

V. Milk as Food for the Young

From the preceding Sections it is clear that milk production by the dams of different species has to cater for a very wide range of requirements on the part of their offspring. Not only are there wide species differences in the physiological maturity of the young at birth, in the number that are born and reared and their relative size, but there are wide anatomical differences too. The mammalian class embraces a range of size which includes the pigmy shrew and the whale—a million-fold range in weight—and a range of size (weight) of the new-born that is even greater, from the young of the primitive marsupials measuring only a few mm in length to the 8 m baby of the blue whale. Equivalent variations in lactational performance must be present. On dimensional grounds, since the metabolism of the young is proportional to their surface area, the lactational performance of the dams as between species is also likely to be proportional to surface area. Per unit of weight the small animal should produce the most milk energy. This would apply only if all young were born at the same physiological stage of maturity. Brody *et al.* (1938) have shown, for instance, that though the gross energetic efficiency of lactation is much the same in the cow, goat, woman and rat, the lactational performance of the average rat is superior to that of the average cow. As previously indicated, the young of the rat are much less physiologically mature at birth than is the calf.

It is usually accepted that the milk of one species is specifically adapted to the growth of the young of that particular species (Lusk, 1928). Bunge (1874) and Aberhalden (1898) are those whose names are usually coupled with this generalization. Both, however, were more interested in relating the chemical composition of the milk to the growth of the young of different species than to the quantity of milk consumed. Any consideration of specific adaptation must, however, include questions of quantitative adequacy as well as qualitative adequacy. A further assumption that tends to universal acceptance is that the presence of a substance or nutrient in milk is indicative of a requirement on the part of the young, and that a quantitative measure of a nutrient in milk is a quantitative measure of the requirement of the nutrient by the young. In this regard, one finds such statements as, "the composition of breast milk is of interest primarily because it is a recipe of the most satisfactory baby food" (Gunther, 1952), very commonly in articles dealing with lactation.

It is difficult to separate fact from teleological argument in most of these statements about inter-species variation in lactation, or indeed in most inter-species generalizations of a comparable type. Certainly, many of the so-called natural laws of early post-natal growth, such as Rubner's (1908) "law of constant energy expenditure" or Flourens (1856) "law of longevity", based on a limited number of between-species comparisons, do not have the degree of universality that is usually ascribed to biological generalizations. Attempts to condense a mass of factual evidence concerning lactational performance in such a wide range of species into a few simple statements of relationship must necessarily include considerable approximation, and exclude other biologically important aspects of reproductive performance.

A. Optimal Growth and the Quantitative Aspect of Lactation

There is every reason to believe that in many species the amount of milk produced by the mother even under ideal nutritional and environmental circumstances does not allow for maximal growth of the young. Thus Parkes (1929) found that if infant mice were nursed by rats, they reached a weight at 21 days that was double that obtained if they suckled their dams. Further evidence obtained by MacDowell *et al.* (1930) points in the same direction. By removing young mice from litters at chosen intervals these workers were able to increase very considerably the growth rate of the single young mouse that remained. In this type of experiment, the normal regulation of lactation to litter number by the suckling reflex was manipulated by periodic removal of young to provide maximal growth of one young. Wilson's (1902) early work with baby pigs showed that growth could be enhanced by the addition of lactose to a diet of skimmed cow's milk, and that the normal time required

to double birth weight was thereby reduced. More recently it has been shown in many experiments (see Braude, 1954; Bellis, 1957) that the growth of baby pigs can be considerably increased by using artificial substitutes for sow's milk. The milk yield of the sow certainly does not cater for maximal growth of the litter, and the growing popularity of the use of artificial milk in the farm rearing of this species reflects recognition of this limitation of lactational capacity. A recent report (Anonymous, 1957) describes the production of a litter of eighteen pigs averaging 63 lb at weaning. This was obtained only by liberal use of supplementary sow's milk substitute, and indicates that under normal circumstances the inherent capacity of pigs to grow is restricted by the supply of milk. The same lactational inadequacy is true of the ewe that nurses twins. The growth of twins is never as great as of singletons, and the growth of triplets is less than that of twins (Hammond, 1932). Some compensation does take place for different litter number but this, even under the best of circumstances, does not appear adequate. A further example of a normal quantitative inadequacy of lactation occurs in the guinea-pig. Widdowson & McCance (1955) have shown that the infant guinea-pig normally undergoes semi-starvation during the first week of life. It gains in weight, but loses body fat in considerable amounts, and the changes in the chemical composition of its body are quite similar to those that occur in starvation. The milk it obtains from its dam is certainly quantitatively incompatible with maximal growth. In this regard, the human infant during the first days of life may be in a similar situation to the guinea-pig. Human lactation is not fully established for several days after birth (Morrison, 1952), and during this period the infant loses weight considerably. Platt (1954a) has pointed out that to give the infant supplementary food during this time may, in satisfying its hunger, reduce its contribution to the initiation of lactation by reducing the amount of fluid it removes from the breast. This point of view gives emphasis to the difficulties involved in attempting to separate the parent-offspring relationship in any simple way.

These examples of the fact that in some species, even under normal circumstances, the quantity of milk produced is insufficient to allow for maximal growth, raises problems concerned with assessment of the optimal growth rate. It can be argued that maximal growth is not optimal growth. For instance, it can be argued on teleological grounds that the undernutrition of the human baby and of the guinea-pig during their very early lives is in some unspecified manner desirable for their subsequent development or, as Platt (1954a) has shown, for the well-being of the mother. The delineation of what is an optimal growth rate is probably an impossible task owing to limited knowledge of the sequelae to deviations from such optima or, indeed, the criteria by which optima should be judged. As an example one can consider calcification of the skeleton of the human infant. Stearns (1939) has pointed

out that "the percentage of calcium in the body decreases rapidly during the rapid growth of early infancy and then remains constant at a value approximately equal to that of the sixth to seventh foetal month until the infant is 6 months of age or older." If the calcium content of the milk is increased, or if cow's milk having a higher calcium content is given, calcification of the skeleton is increased (Jeans *et al.*, 1936). From these and other studies Holmes (1945) concludes, "if one adheres to the thesis that the body which is saturated with the essential nutrients is in a better state of health than is one which contains barely enough of these nutrients to forestall the development of dietary deficiency disease, then one must conclude that the diet of the average infant has been too low in calcium." Holmes then suggests that maximal saturation of the skeleton with calcium is indicative of an optimal supply, but she further states, "there is also a need for determining whether or not the poor skeletal calcification of the breast-fed infant represents a handicap, temporarily or permanently." With respect to gain in weight in human babies Graham (1952) has asserted that "good nutrition cannot be determined merely by a gain in weight." With this no one would disagree: it is in the exact description of good nutrition that opinion takes precedence over fact.

So far, however, the examples have dealt with average lactational performance under feral or normal environmental circumstances. There is considerable evidence that within a species, the growth of the young varies almost directly with the amount of milk it consumes. This was established for the human infant many years ago, and in more recent years this aspect has been studied in domesticated livestock in some detail. Examples are taken from the lactational performance of the ewe. Experiments such as those of Wallace (1948), Barnicoat *et al.* (1949a,b), Coop (1950) and Thomson & Thomson (1948–49, 1953) have shown that the nutritional level of the dam both before and after parturition affects the milk production of the ewe. The range of milk production obtained by dietary manipulation in initially similar animals is well over two-fold. Typical figures are given in Table XI.

Similar information for cattle also shows that the nutritional environment of the dam before and after parturition exerts considerable effect on her lactational performance (see summary by Blaxter, 1957). With dairy breeds in which the milk yield is usually in excess of the requirements of the calf, such nutritional effects are rarely so extreme as to jeopardize survival of the calf. In beef breeds, however, kept under marginal conditions, the survival of the young depends on the nutritional level of the mother, for the milk yields vary widely and can be very low (Joubert, 1954–55). Surveys of the milk production of women in underdeveloped communities further show that undernutrition reduces both the yield of milk and the duration of lactation (Jeliffe, 1954).

In this regard it is as well to point out that short periods of starvation of the dam reduce milk production very considerably indeed. Thus, in the rat starvation of the dam for 4 days results in the death of her litter, her milk yield being reduced to zero. She herself recovers from starvation quite easily. Lactation is certainly not continued in the face of a severe environmental stress on the mother.

TABLE XI

MILK YIELDS OF EWES FED AT TWO DIFFERENT LEVELS BEFORE AND AFTER PARTURITION

	Average yield in gallons in 12 weeks			
	Thomson & Thomson (1953)	Barnicoat *et al.* (1949 a,b)	Wallace (1948) (singles)	Wallace (1948) (twins)
Low level	11	13	12	25
High level	20	26	28	40

B. GROSS COMPOSITION OF MILK AND THE GROWTH OF THE YOUNG

From Bunge's (1898) original table in which growth rate of the young was related to milk composition, it is quite evident that the species having young that grow slowly, produce milk containing little protein, whereas the milk of those that grow quickly contains considerably more. As reproduced here in Table XII, Bunge's results can be extended by inclusion of the rat which doubles its birth weight in 6 days and produces milk containing 12 % protein. The interpretation of Bunge's table is, however, not as simple as might first appear, for it ignores the quantitative aspect. It could be interpreted to show that fast-growing species produce less dilute milk than slow-growing species. It might also be concluded that, save for man who invariably declines to participate in biological generalization of this type, larger species produce the least concentrated milk. In this latter regard, it has already been pointed out that as far as the energy demand of a litter is concerned, total lactational performance in terms of calories milk energy per 24 hours is likely to vary with the surface area of the adult, implying that an animal weighing 1000 kg would produce not 1000 times as much milk as one weighing 1000 g but only 100 times as much. Conversely, per unit of weight, the smaller animal would have to produce ten times as much milk energy as the larger.

If the concentration of nutrients in the milk remained constant irrespective of size, this would mean that, per unit of weight, the young of the small species would have to consume ten times as much in volume as the large to meet its energy demands. It seems unlikely that stomach capacity would vary with body surface area rather than with body weight. Brody & Kibler (1941) have shown that, as between species, the weight of the digestive tract varies almost directly with body weight rather than with body surface. In Bunge's table, comprising an adult weight range from the mare weighing 800 kg to the cat weighing 2 kg, body surface area per kg weight varies by a factor of about 6.

TABLE XII

BUNGE'S (1898) ORIGINAL TABLE SHOWING THE RELATIONSHIP BETWEEN GROWTH AND MILK COMPOSITION

Species	Time in days for new-born animal to double its birth weight	100 parts of milk contain (parts)	
		Protein	Ash
Man	180	1·6	0·2
Horse	60	2·0	0·4
Ox	47	3·5	0·7
Goat	19	4·3	0·8
Pig	18	5·9	—
Sheep	10	6·5	0·9
Dog	8	7·1	1·3
Cat	7	9·5	—
Additional fast-growing species not included in Bunge's table			
Rabbit	6	14·0	2·2
Rat	6	12·0	2·0

The protein content of the milk given in the table varies in the same direction by a factor of 5. This suggests that the concentration of nutrients in milks of different species may be related to a nutrient demand that varies with surface area, and a physical capacity that varies with weight. Relative growth rates, even after the lactational phase is completed, increase as species size decreases. Thus part of the association noted by Bunge may be of more complex origin than a cursory examination of the table would suggest.

C. Protein Supply

It has already been shown that there are large between-species differences in the maturity of the young at birth. It is generally true in growing animals that, as growth proceeds, the protein requirement falls and the requirement for energy-yielding constituents rises. It is interesting, therefore, to relate the maturity of the young at birth to the proportion of protein calories present in the milk as shown in Table XIII. It must be pointed out that many of the analyses on which this table is based relate to one or at most a few samples only. In view of the wide variation in the composition of milk at different stages of lactation in those domesticated species in which lactation has been studied in detail, it is possible that errors arise in interpretation of isolated samples obtained from wild, or small laboratory, species. The table permits only the most flimsy generalizations. First, aquatic species of mammal tend to produce milks having a low protein content. This suggests that natural selection has placed a premium on secretion of milks containing high proportions of energy-yielding constituents, and it seems legitimate to suppose that this is related to the considerable heat losses in an aqueous environment due to the high thermal conductivity of water compared with air. The dolphin is anomalous: however, it frequents more tropical waters than do the other aquatic species listed.

Within the larger mammals, it might appear possible to conclude that those living in cold climates, notably the reindeer, tend to produce milks with lower protein contents than those, notably the llama, living in warm ones. The water buffalo, however, immediately appears an exception.

Rodents and carnivores tend, on average, to produce milks containing more protein than those of the large herbivores. The guinea-pig is worthy of mention in view of the maturity of its young. It produces a milk containing but 22 % of its calories as protein compared with 25 % in the rat. The young of the guinea-pig are very mature at birth, whereas those of the rat are extremely immature. The most immature of all mammals at birth are the young of marsupials and the Prototheria. Yet the milk of the anteater contains only 18 kcal protein per 100 kcal, and incomplete analyses of kangaroo milk do not suggest that it has a very high value for this ratio. More remarkable is the instance of man. Human milk on a calorie basis contains the lowest concentration of protein of all milks: but this does not appear to be generally true of the milk of other primates. The elephant also produces milk containing a relatively low proportion of protein. It is tempting to relate the long adolescent phase lasting 10 to 15 years of both man and elephant, or indeed their innate sagacity, to the concentration of protein in the milk, but what is more worthy of comment is that there does seem to be no very close general correlation between milk composition expressed in this way and the rate of

TABLE XIII

THE PROPORTION OF TOTAL CALORIES PRESENT IN MILK AS PROTEIN

Species	Protein kcal/ 100 kcal in milk	Remarks
Aquatic		
Fin Whale	18	Inhabit temperate and sub-polar waters
Blue Whale	15	
Seal	12	
Porpoise	12	
Dolphin	29	Semi-tropical species
Perissodactyla		
Horse	22	
Ass	19	
Artiodactyla		
Sheep	23	
Goat	21	
Ox	20	
Buffalo	23	
Water buffalo	16	
Reindeer	16	
Camel	23	
Pig	25	
Yak	26	
Subungulata		
Elephant	15	
Carnivora		
Dog	25	
Cat	27	
Fox	31	
Rodentia		
Rat	25	
Rabbit	27	
Guinea-pig	22	
Primata		
Man	9	
New world monkey	18	
Prototheria		
Anteater	19	

The information on which this table was based was obtained from a variety of sources. Summaries are given by Brody (1945) and by Davis & MacDonald (1953), and references to isolated analyses may be found in *Dairy Science Abstracts*. They are not quoted in full. The values for the more common milch animals are those given by Ling, Kon & Porter in Chapter 17.

development of the young. The seal, whose pup doubles its weight in 11 days, produces milk containing 12 % of its calories as protein, and the ewe whose lamb does the same in about the same time produces milk containing 23 % of its calories as protein. Similarly, the milk of the anteater contains the same amount of protein as that of the ass, though one produces an immature offspring which has to be fostered for long periods, whereas the other produces a foal which is capable of standing within hours of its birth.

D. Lactose and Fat

Table XIV summarizes published data relating to the relative importance of lactose and of fat in milk in providing non-protein calories for the young. Aquatic mammalia again form a separate group in which forty to fifty times as much energy is supplied by fat as is supplied by lactose. The rodents too produce milks containing considerably more energy as fat than as lactose. The Perissodactyla, as represented by the horse and ass, form the other extreme group in which almost twice as much energy is supplied by lactose as by fat. The remaining species fall within a range in which the ratio, fat calories : lactose calories, varies from 1 : 1 to 10 : 1. It might be permissible to attribute the higher values of this ratio within any one class of animals to those that inhabit the colder climates, but the data available for this type of conclusion are very meagre.

The variation in the ratio, fat calories : lactose calories, is predominantly the result of variation in fat content. Table XV, which summarizes data for species showing extreme values for this ratio, indicates that the lactose content of the fat-free milk varies between extreme species by about 50 %, from about 4 g per 100 ml in the dog and elephant to about 6 g in the Equidae and 7 g in man, whereas the fat content varies by over thirty-fold. This relative inter-species constancy of lactose secretion per unit volume of fat-free milk suggests that the enzymic processes leading to lactose synthesis are quantitatively and qualitatively very similar in species as diverse as the whale and the mouse.

The spatial configuration of the fourth carbon atom of the galactose half of the lactose molecule is found in the cerebrosides of brain and nervous tissue, and it has been suggested that lactose is of considerable importance in providing this configuration for the synthesis of cerebrosides and the normal post-natal myelination of nervous tissue (Sadhu, 1948; Platt, 1955). Galactose is also found as the constituent sugar in the muco-polysaccharides of the cornea (Woodin, 1952). This would ascribe to lactose a vitamin-like activity; indeed Platt (1955) has suggested that abnormal cerebrosides—glucocerebrosides rather than galacto-cerebrosides—would be formed when the dietary supply of lactose is low. He related this to the incidence of disorders

TABLE XIV

THE RELATIVE IMPORTANCE OF FAT AND LACTOSE, THE ENERGY-YIELDING CONSTITUENTS OF THE MILK OF DIFFERENT SPECIES

Species	Ratio, fat calories : lactose calories
Aquatic	
Whale	>40·0
Seal	>40·0
Porpoise	>50·0
Dolphin	>40·0
Perissodactyla	
Horse	0·6
Ass	0·5
Artiodactyla	
Sheep	3·8
Goat	2·3
Ox	2·0
Reindeer	21·0
Camel	2·3
Llama	1·4
Pig	4·0
Subungulata	
Elephant	10·0
Carnivora	
Dog	5·1
Cat	1·5
Fox	3·2
Rodentia	
Rat	26·5
Rabbit	39·5
Guinea-pig	17·5
Primata	
Man	1·5
Monkey	1·5
Prototheria	
Anteater	15·0

The information on which this table was based was obtained from a variety of sources. Summaries are given by Brody (1945) and by Davis & MacDonald (1953), and references to isolated analyses may be found in *Dairy Science Abstracts*. They are not quoted in full. The values for the more common milch animals are those given by Ling, Kon & Porter in Chapter 17.

TABLE XV

LACTOSE CONTENT OF FAT-FREE MILK[1]

Species	Fat content (%)	Total solids content (%)	Anhydrous lactose in fat-free milk (%)	Reference
Seal	53·2	67·7	5·5	Amoroso *et al.* (1951)
Whale	35·1	49·1	3·9[2]	White (1953)
Elephant	15·2	26·9	4·5	Krauze & Legatow (1949)
Dog	8·3	22·6	4·1	Davis & MacDonald (1953)
Pig	8·3	19·9	5·2	Braude *et al.* (1947)
Man	4·6	13·6	6·9	Kon (1959)
Ox	3·8	12·7	5·0	Davis & MacDonald (1953)
Horse	1·6	10·1	6·0	Ling *et al.* (Chapter 17)

[1]These results are based on fairly extensive analytical data thought to be reliable, relating to lactose determined directly.

[2]Probably low, owing to decomposition before analysis.

TABLE XVI

SADHU'S (1948) RESULTS IN WHICH THE GALACTOSE CONTENT OF THE BRAIN IS RELATED TO THE LACTOSE CONTENT OF THE MILK OF NINE SPECIES

Species	mg galactose/g dry brain	Lactose in mother's milk (%)
Mule	5·0	6·5
Horse	4·0	6·5
Pig	2·7	4·1
Mouse	2·5	3·0
Guinea-pig	2·2	3·0
Dog	1·9	4·0
Ox	1·8	4·8
Rabbit	1·8	2·0
Rat	1·7	3·0

in adult life associated with abnormalities of nerve, notably disseminated sclerosis (Lumsden, 1951) and the transverse myelitis which occurs in the West Indies. Platt did no more than suggest a possible relationship to account for the universal occurrence of lactose in milk, and indicate a possible locus of its action: no acceptable scientific proof of its essentiality as a nutrient, or of sequelae to its removal from the diet was adduced. The fact that glucose can be converted into galactose in the body (Kalckar, 1957) is evidence

TABLE XVII

CALCIUM, PHOSPHORUS, SODIUM AND POTASSIUM CONTENT OF THE MILK ASH OF DIFFERENT SPECIES[1]

Species	Percentage of ash			
	Ca	P	Na	K
Man	14·9	7·2	8·7	26·7
Horse	21·0	13·6	5·0	20·7
Ox	17·1	14·3	7·1	21·4
Sheep	22·6	16·5	8·2	10·4
Goat	17·7	15·6	5·8	13·5
Pig	26·5	17·6	7·0	9·5
Dog	23·6	15·7	9·6	10·8
Guinea-pig	21·6	15·7	6·5	7·8
Rabbit	25·1	17·2	5·8	8·2
Rat	26·1	23·2	—	—

[1]Compiled from a variety of sources, including Bunge (1874), Morrison (1952), Davies (1936), Abderhalden (1898), Luckey *et al.* (1955).

contrary to Platt's suggestion. Sadhu's (1948) results, obtained from examination of his graphs, are given in Table XVI.

A correlation is undoubtedly present, but whether it is fortuitous, to vanish when more species are included, is not known. Within Sadhu's table there is also present a statistically significant correlation with body size. This serves to illustrate the possibility of errors in its interpretation. No evidence has yet been provided to suggest that lactose is a specific and essential nutritional factor.

E. MINERAL CONTENT

Table XVII summarizes the composition of the ash of milk of different species. The total ash content of the milk in these species varies from over

2·5 % in the rabbit down to 0·2 % in man. Despite this large variation in quantity secreted per unit volume milk the composition of the ash fraction of milks of different species shows relatively little variation. Thus, apart from man, the phosphorus content of milk ash varies from about 14 to 23 % and the calcium content from 17 to 27 %. There is, however, one aspect in which species differences are large, namely in potassium content. The milk ash of man, ox and horse contains approximately twice as much K as does that of the milks of other species listed. The sodium contents of the milks of these species do not differ very markedly from those of the remainder.

Bunge (1874) in his classical studies of milk composition showed that the composition of the ash of milk was very similar to the composition of the ash of the embryo. This is generally true, though the ash of embryos and newborn animals usually contains considerably more phosphorus than does milk ash. A further generalization was that the alkali ratio in milk, that is the ratio, K_2O : Na_2O, was greatest in herbivores and lowest in carnivores and omnivores. This would be in accord with the fact that in fresh herbage there is an excess of K relative to Na. From Table XVII, it is clear that the analyses that have been made do not allow distinctions of this sort. Thus, man is an exception to the generalization in having a high alkali ratio though being omnivorous; so also are the sheep, the guinea-pig and the rabbit, for they have low ratios though they are herbivorous. The reasons for these large species differences in K content of the milk are not immediately apparent. From a comparison of Table XVII and Table XVIII there does not seem to be any clear-cut relationship between the composition of the ash of milk and the stage of development of the young at birth. Possibly the species less mature at birth, such as the rat, the rabbit and the dog, produce milk ash containing more bone-forming elements, but differences are small. The sow produces milk with an ash containing 26 % Ca for its piglet which is born well calcified, whereas the rabbit, which gives birth to a poorly calcified offspring, produces milk with an ash containing 25 % Ca, that is about the same concentration.

F. Iron Metabolism

Bunge (1889, 1892) was responsible for a further generalization about the nutritional adequacy of milk. On the basis of studies with rabbits and guinea-pigs he concluded that milk was a negligible source of iron, and he thought that those species of animal that depended on milk for long periods were born with a store of iron to tide them over the suckling period, whereas those in which the lactation phase was short were born with negligible stores of iron. If Bunge's theory were correct, then the total iron content of the body would show little if any increase over the suckling period. Fontès & Thivolle (1925a,b) attempted to extend Bunge's theory to the dog and cat, but found

that puppies in fact contained more iron at the end of suckling than at birth. Similar conclusions were reached by Smythe & Miller (1929) and by Huggett & Widdas (1949) with rats. Extension of investigation of the iron metabolism during suckling to include a wider range of species was undertaken by Lintzel *et al.* (1944) and by McCance & Widdowson (1951). Their results, on which this Section is largely based, show that Bunge's theory requires considerable modification if it is to be in accord with fact.

TABLE XVIII

THE IRON CONTENT OF NEW-BORN ANIMALS

(From Widdowson (1950), McCance & Widdowson (1951) and Fontés & Thivolle (1925a, b))

Species	Iron content of whole body (mg/100 g fat-free tissue)	Inorganic iron in liver at birth (mg/100 g)	Iron content of whole body at the end of suckling (mg/100 g fat-free tissue)
Mouse	6·7	16	4·6
Rat	5·9	15	2·9
Guinea-pig	6·7	4	6·1
Rabbit	13·5	84	4·5
Cat	5·5	23	5·1
Pig	2·9	9	~ 2·0
Seal	7·0	32	—
Man	9·4	22	—

The iron content of the body of different species at birth per 100 g fat-free tissue is given in Table XVIII. Clearly, there are large species differences, the rabbit representing one extreme and the pig another. A large part of this iron represents haemoglobin of the blood, but the third column of Table XVIII shows that the inorganic iron of the liver and spleen, the major store of non-haemoglobin iron, shows an even wider variation than does total iron. The rabbit is born with considerable iron in its liver, and the pig and guinea-pig with less than one-tenth this amount. The normal adult concentration of iron in the body appears to be about 6 to 7 mg per 100 g fat-free body tissue.

During the suckling period the concentration of iron in the fat-free body falls as shown in the final column of Table XVIII. This fall in concentration of

total iron is, however, in some measure due to depletion of the inorganic iron content of liver and spleen. Thus, in almost all species, the inorganic stores of iron fall far more than does the iron content of the body as a whole. An exception here is the rabbit, which according to Bunge is born with large iron stores to tide it over the lactation period. McCance & Widdowson's (1951) analyses showed that the iron content of the liver of the rabbit was 2·36 mg at birth and 2·32 mg at 15 days of age, that is virtually unchanged, though the iron concentration in the body as a whole had fallen during the period from 13·5 to 4·5 mg per 100 g fat-free tissue. By contrast, in the rat the total inorganic iron in the liver at birth was 56 μg and at 15 days of age 9 μg.

These marked falls in the organic iron concentration in the tissues, at a time when the mass of tissue cells in which iron is present as the prosthetic group of cytochromes, catalases and myoglobins is increasing, necessarily means that the haemoglobin content of the blood is particularly reduced. In this regard, some animals such as the cat, ox and man are born with a higher haemoglobin concentration in blood than they have in adult life. This could be regarded as a store of haemoglobin to be diluted during growth, which appears a reasonable explanation. Despite this initial haemoconcentration, however, suckling anaemia due to iron deficiency has been recorded in a number of species including pig, man, rat and ox.

In explaining the genesis of suckling anaemia in different species, a factor other than the iron stores of the young has to be considered, namely the iron supply relative to the supply of other nutrients. Milks of different species vary very markedly in iron content from over 700 μg per 100 ml in the rat to 30 μg per 100 ml in the cow. Lactation in the rat, however, permits the young to increase in weight each day by 25 % of their birth weight, whereas lactation in the cow permits the young to gain each day by 1 to 1·5 % of the birth weight. Absolute comparison of iron concentrations in milk is not meaningful unless referred to the supply of other dietary ingredients. The iron contents of milks of different species are possibly best compared in terms of mg Fe per 100 g protein, which makes the comparison less open to misinterpretation. This has been done in Table XIX which shows that there are large species differences that do not appear to lend themselves to any general statement. Thus the rat provides a considerable amount of Fe relative to protein and the rabbit does not. From Table XIX, one might expect suckling anaemia to be more common in calves than is noted in practice. Here haemoconcentration at birth may play some part since the calf is born with a packed cell volume very considerably greater than it has at maturity. Furthermore, the calf begins eating solid food long before it has doubled its birth weight, thereby obtaining additional iron. The piglet, however, increases in weight three- to four-fold before it eats solid food in appreciable amounts.

From the admittedly limited evidence on iron metabolism during suckling, several conclusions can be drawn. Firstly, Bunge's hypothesis that foetal liver stores of iron can be used for subsequent infant needs is not wholly tenable; secondly, species differ markedly one from another in the degree to which they are capable of meeting the iron needs of their offspring, and lastly a mild deficiency of iron, as judged by a depression of the iron content of the fat-free tissues of the body seems to be a normal accompaniment to suckling.

TABLE XIX

THE IRON CONTENT OF THE MILK OF DIFFERENT SPECIES

(From McCance & Widdowson (1951), Blaxter *et al.* (1957) and other sources)

Species	Fe content of milk (μg/100 ml)	Protein content of milk (g/100 ml)	Fe in milk (mg/100 g protein)
Man	100	1·6	6·2
Ox	30	3·5	0·9
Rabbit	120	14·0	0·9
Pig	180	5·8	3·1
Rat	700	12·0	5·8
Dog	900	7·1	12·6

G. THE BUNGE HYPOTHESES

The broad generalizations which Bunge and early German workers made when they reviewed gross analytical data for the milks produced by different species would appear from the above presentation to be open to criticism. What perhaps is more impressive than any specific evolutionary adaptation of milk composition to the growth of the young is the fact that variation in milk composition over the range of weight, functional and environmental adaptation found in the mammalia is so small. Thus, the lactose content of fat-free milk varies by about 50 % in the range of animals studied and, with the exception of the potassium content, variation in which must be reflected in compensatory variation in other minerals, the composition of the milk ash varies within but narrow limits, which appear poorly related to either the mineral composition of the young at birth or to the mineral accretion it must make during its subsequent growth. Some conclusions are possible,

notably the association of high energy content and consequently a relatively low protein content of milk with a habitat which is either cold or aquatic, but most generalizations seem to be subject to exceptions, even though the range of species coverage is sadly limited.

In making such comparisons between species, the major difficulty is that with most species there are no quantitative data relating to the amount of milk secreted by the dam and consumed by the young: the comparisons necessarily relate to the percentage composition of the milk rather than the amounts of each of the major nutrients supplied. A further difficulty is that very few analytical data are available for different mammals of approximately the same size but producing young with different developmental needs. Nor is there a common denominator for expressing these needs. To compare lactation in the rabbit, producing immature young, with lactation in the cow, producing relatively mature young, is to compare not simply differences due to maturity of the young. The comparison also involves two animals differing in size by a factor of well over 200. It has been pointed out that since many dietary requirements are proportional to energy metabolism, that is to surface area rather than to body weight, body size in itself must be regarded as a factor determining nutrient concentration in milk. How far this generalization applies to an accretionary growth, such as skeletal growth, is not known.

H. Vitamin B Complex

From a critical review of pertinent literature, Mitchell (1950) drew some broad generalizations relating nutrient requirements to body size. As between species and between different-sized individuals within species, some nutrients, notably vitamin A and ascorbic acid, are required in proportion to body weight, whereas others, such as protein, calcium, phosphorus and energy, are required in proportion to body surface area. The method adopted in the preceding Sections in dealing with the energy, protein and mineral supply from milk does, in fact, take cognisance of this broad relationship. Most of the B complex vitamins and those mineral elements that are trace components of enzyme systems are required in proportion to the total energy derived from dietary sources other than fat. For convenience, the requirements of adult animals for members of this latter group may be expressed as proportional to the dry food they consume. On this basis, for instance, the minimal requirement of all animals can be assessed per kg dry food, low in fat, as thiamine 1 mg, riboflavin 1·5 mg, pyridoxine 2 mg, pantothenic acid 5 mg, nicotinic acid 10 mg and copper 3 to 5 mg. How far this generalization applies to the suckling period is not known, but in view of the fact that most requirements have been assessed from results of experiments with young, rapidly growing

animals, it is probable that Mitchell's generalizations constitute a useful yardstick for comparative studies. The estimates would tend to be high ones since milk is much higher in fat content than the usual dry food animals consume in adult life. It follows that application of Mitchell's general requirements to the suckling phase is not likely to underestimate requirements.

Table XX summarizes some determinations of the vitamin content of the milk of a number of species. It will be noted that there is a very considerable variation between them. The thiamine contents of the milks show relatively small variation per kg total solids. Values range from 1·2 mg per kg for human milk to 6·0 mg per kg for ass's milk. Most values, however, are in the

TABLE XX

THIAMINE, RIBOFLAVIN, NICOTINIC ACID, PANTOTHENIC ACID, PYRIDOXINE AND VITAMIN B_{12} CONTENT (μg/100 ml) OF MILKS OF DIFFERENT SPECIES

Species	Thiamine	Riboflavin	Nicotinic acid	Pantothenic acid	Pyridoxine	Vitamin B_{12}
Man	17	40	170	200	10	0·03
Ox	40	150	80	350	35	0·5
Goat	50	120	200	350	7	0·1
Sheep	70	500	500	350		0·3
Pig	70	130	800	400	20	0·2
Ass	60	30	90			
Whale	140	150	2000	1300	110	0·9
Rat		400		900		1·2

Data are largely the representative values given by Ling, Kon & Porter in Chapter 17. Others are from Braude (1954), Gregory (1955), Gregory *et al.* (1955), Kon (1959), Pearson & Darnell (1946) and other sources.

region of 3·5 mg thiamine per kg total solids: the whale and the cow produce similar amounts of thiamine per kg milk solids. All the values with the exception of the figure for man are above the minimal requirement. In this regard, however, feeding with thiamine supplements will increase the thiamine content of human milk to about 20–25 μg per 100 ml (Slater & Rial, 1942), which would bring human milk more into agreement with the results for other species. A thiamine deficiency in breast-fed human babies which can be relieved by thiamine administration has been reported by Clements (1942), and can be common under conditions of suboptimal maternal intake of the vitamin in some parts of the world. In these instances noted in Australia where overt signs of deficiency were present, the thiamine content of the milk was about

one-third the normal value given in Table XX. As might be expected from the tabulated results, the disease does not occur when cow's milk is given. In general, it appears that over the range studied milks provide about two to three times the estimated minimal thiamine requirement. In the instance of the pig, further confirmation of this generalization may be found in studies in which the baby pigs were given artificial milks. Miller *et al.* (1954b, 1955) found the thiamine requirement to be 1·5 mg per kg dietary solids.

With riboflavin, the milks of woman, ass and whale all contain 2 to 3 mg per kg dry matter, which according to Mitchell's estimates appears to be an adequate margin of safety over the minimal requirement of 1·5 mg per kg. The milks of the cow, goat and ewe, however, contain 12, 9 and 27 mg riboflavin per kg dry matter. Braude *et al.* (1947) commented, "the low level of [46 μg per 100 ml] riboflavin in the milk of an animal [sow] whose young grow at a very rapid rate . . . is puzzling." Later analyses (Davis *et al.*, 1950) showed that in fact sow's milk contained plenty of riboflavin (130 μg per 100 ml). Even so, it seems perhaps strange that the riboflavin content of the milks of the cow, goat and ewe, the domesticated ruminants, is so high. It is possible that this high level of riboflavin secretion reflects massive bacterial synthesis of the vitamin in the rumen and its subsequent secretion. From American work (Miller *et al.*, 1954a; Terrill *et al.*, 1955; Anonymous, 1955) the lowest estimate of riboflavin requirement of the baby pig during the suckling phase is 1·5–2·0 mg per kg dry food. Sow's milk contains approximately 8·5 mg per kg total solids which would thus appear to be very adequate. Largely on the basis of analyses of milks of the ewe and the cow Pearson & Darnell (1946) concluded that there was a relationship between the B-complex vitamin concentration in milk and the growth rate of the young. In view of the previous discussion on Bunge's similar conclusion relating to the gross composition of milk, it would seem that such generalizations have to be considered very carefully. Pearson & Darnell's conclusion is certainly not true when analyses are extended to include the riboflavin content of milks of the sow and of the woman.

The nicotinic acid contents of milks when expressed on a dry matter basis show a much wider variation than do the contents of thiamine and riboflavin, and in this regard the cow appears to produce the least nicotinic acid. The concentration of 7 mg per kg is below the minimal one of 10 mg per kg dry matter. In this regard, attempts to produce nicotinic acid deficiency in calves by giving rations simulating milk but devoid of nicotinic acid failed to demonstrate any need for the vitamin on the part of the calf (Johnson *et al.*, 1947). When these experiments were repeated with rations devoid of those proteins containing appreciable amounts of tryptophan, however, a requirement for nicotinic acid could be demonstrated (Blaxter & Wood, 1952). There seems no adequate teleological explanation for the presence of nicotinic

acid in cow's milk where the proteins always supply sufficient tryptophan.

As far as pantothenic acid is concerned its concentration in milk ranges from 19 to 29 mg per kg dry matter with the exception of human milk where it is 15 mg per kg. It would appear to be always adequate in amount. With pyridoxine, however, the milks of the woman, the sow and the goat contain about 1 mg per kg solids whereas the milk of the cow contains three times this amount. The minimal requirement is assessed at 2 mg per kg. With due allowance for the fact that, for reasons already given, Mitchell's estimates may be rather high, one might nevertheless expect that pyridoxine deficiency would occur during the suckling phase of life in some mammalia. There is ample evidence to support this contention, especially where the maternal diet has been low or marginal in pyridoxine content. Thus, in rats pyridoxine deficiency in the dam results in the appearance of a convulsive syndrome in the young and death of the litter (Kline *et al.*, 1938; Daniel *et al.*, 1942; Patton *et al.*, 1944) or when the maternal deficiency is mild, in a form of thymic atrophy (Richards, 1949a,b). Richards's experiments are particularly interesting. The does were given a diet rich in thiamine containing 72 % extraction flour, and on this diet they could reproduce quite well but fits occurred in the litters, and thymic atrophy was generally present. By reciprocal interchange of litters between does supplemented and not supplemented with pyridoxine Richards was able to show that the milk of does on the unbalanced diet supplied insufficient pyridoxine. In the baby pig given artificial diets dietary deficiency of pyridoxine results in epileptiform fits, but whether deficiency of pyridoxine is of common occurrence under natural conditions in this species is not known.

In human babies reared on a heat-sterilized preparation of cow's milk overt signs of pyridoxine deficiency, characterized by convulsions, were noted and the findings were interpreted as indicating a marked loss of vitamin B_6 from milk during treatment. Subsequently it has become evident that a small minority of infants have an abnormally high requirement for this vitamin (cf. Hunt, 1957).

The variation between species in the vitamin B_{12} content of milk is extremely large and defies any attempt at rationalization. Ewe's milk, rat's milk and whale's milk all contain similar amounts of the order of 0·3 to 1·2 μg per 100 ml milk, whereas human milk usually contains less than 0·1 μg per 100 ml. Analytical difficulties, the presence of inhibitory substances, binding of the vitamin and the simultaneous presence of vitamin B_{12}-like compounds make interpretation of the data difficult (Gregory, 1954; Collins *et al.*, 1951). Especially is generalization difficult when it is noted that one sample of whale milk assayed by Gregory *et al.* (1955) contained 13 μg per 100 ml whereas two other samples assayed under comparable conditions contained 0·9 and 0·8 μg per 100 ml respectively.

The predominance of a *Lactobacillus bifidus* flora and the greater acidity of the faeces of breast-fed infants as contrasted with the more variable bacterial flora and higher pH of the faeces of infants fed artificially with diets based on cow's milk has been known for a long while. At various times this difference has been ascribed to the relatively high lactose content of human milk, a point which illustrates the many pitfalls inherent in the interpretation of species differences in milk composition. The establishment of the *L. bifidus* flora in the breast-fed infant may in part be due to the presence in human milk of a

TABLE XXI

RELATIVE BIFIDUS FACTOR (*L. Bifidus* VAR PENN GROWTH FACTOR) ACTIVITY OF MILKS OF DIFFERENT SPECIES. RECALCULATED FROM RESULTS OF GYÖRGY *et al.*, 1954

Species	Bifidus factor activity/ unit volume (Human milk = 100)
Man	100
Rat	60
Cat	50
Dog	20
Ass	11
Monkey	10
Pig	5
Horse	4
Rabbit	3
Goat	2
Ox	1
Sheep	1

specific factor or factors. One of these, a growth factor for *L. bifidus* var Penn has been studied in detail. It has been identified as a mixture of oligosaccharides containing N-acetyl glucosamine, glucose, galactose, fucose, neuraminic acid and N-acetyl chondrosamine (Bell, 1956). What is pertinent to this discussion is the wide species differences in the occurrence of this Bifidus factor in milks. This has been studied by György *et al.* (1954) and some of their assays are summarized in Table XXI. There seems to be no simple way in which such large species differences in the Bifidus factor activity of milk can be related to known species differences in growth and development of the young or to other attributes of their milks.

I. ASCORBIC ACID

Table XXII summarizes analytical data on the concentration of ascorbic acid in the milk of different species, a vitamin which, when required, is

needed in proportion to body weight rather than body surface (Mitchell, 1950). From this table it is seen that ascorbic acid is present in the milk of all the species listed whether the adults require the vitamin or not. It could be argued that the presence of the vitamin in milk and colostrum presupposes a need for it on the part of the young, but experiments with pigs (Braude *et al.*,

TABLE XXII

THE ASCORBIC ACID CONTENT OF MILK OF DIFFERENT SPECIES

(From Braude *et al.* (1950), from representative values given by Ling, Kon & Porter in Chapter 17, and other sources)

Species	Ascorbic acid in milk (mg/100 ml)
Species requiring an exogenous source of ascorbic acid	
Man	4
Guinea-pig	29
Species not requiring an exogenous source of ascorbic acid	
Pig	12
Horse	10
Sheep	3
Ox	2
Goat	2
Rat	0·4
Whale	7

1950) show that such a supposition is untenable. The baby pig admittedly metabolizes the vitamin at a high rate early in life, but synthesis of ascorbic acid in the tissues keeps pace with this demand and the piglet is able to synthesize the vitamin from birth. Teleological explanation of the presence of the vitamin in sow's milk, and presumably in the milk of species other than primates and guinea-pigs which require it in their food, though attractive, is hardly tenable. Yet, the experiments of Dugal & Thérien (1947) raise some doubt regarding such wholesale rejection of the teleological explanation. They found with rats, animals that do not need ascorbic acid in their food,

that those individuals that acclimatized themselves well to cold conditions had higher concentrations of ascorbic acid in their tissues than had those that acclimatized poorly. This association of tissue ascorbic acid concentration and homeothermy in an animal that does not require the vitamin in its diet raises questions relating to the possible role of the ascorbic acid content of milk in modifying the resistance of infant animals to cold. Is the high ascorbic acid content of whale's milk related to the high thermal demand of the environment in which it lives?

J. Fat-soluble Vitamins

Fat-soluble vitamins are transferred from mother to offspring both by passage through the placenta and by secretion in the colostrum and milk. Within a species, new-born animals have low and variable reserves of vitamin A in their livers at birth, a fact referable to the dietary supply of the dam. Species differ one from another with respect to placental transfer. Thus the vitamin A content of the liver of the new-born rat can rarely be increased to more than 10 i.u. per g although the liver of its dam can contain up to 20,000 i.u. (Henry *et al.*, 1949). In man, on the other hand, the concentration of vitamin A in the liver at birth can be as high as 154 i.u. per g, the mother's liver containing 285 i.u. per g only (Skurnik *et al.*, 1944). Part, but not all, of such species variation in the reserves of the new-born may be referred to the type of placentation in different mammalian classes. As far as colostrum is concerned its vitamin A activity shows wide between- and within-species variation. The latter variation is due almost entirely to variation in the supply of preformed vitamin A and of β-carotene in the diet of the mother. The normal milk of individual species shows similar wide variations in vitamin A content. Even so, Moore (1957) has shown that the vitamin A activity of milks of a number of normally nourished species varies little from a mean value of 140 i.u. vitamin A per 100 ml provided that the fat content of the milk varies little from a mean value of 4 %. Higher values are found in the smaller species producing milk with a higher fat content. This generalization seems reasonable: it would agree with Mitchell's (1950) contention that vitamin A requirements are proportional to body weight of the animal and thus, in the present context, to the volume of milk consumed.

There is, however, ample evidence that under some conditions the maternal supply of vitamin A to the offspring is not sufficient, and that a dietary lack of vitamin A on the part of the mother can seriously affect the survival of her young. The deficiency may be operative at any time during which the young are dependent on their dams for their nutrition, during pregnancy or during lactation. Thus, during pregnancy in pigs and rats a wide range of congenital

deformities has been produced by dietary lack of vitamin A (Hale, 1937; Warkany & Schraffenberger, 1946): at birth, blindness in calves due to optic stenosis can be referred to a maternal vitamin A deficiency (Moore *et al.*, 1935), and, finally, in piglets suckled by vitamin A-deficient sows, convulsive fits, nervous collapse and a cessation of growth occur (Foot *et al.*, 1939). The time at which defects in the young become apparent clearly depends on the time at which maternal reserves of vitamin A are depleted. Such depletion may take considerable time, as in the experiments of Braude *et al.* (1951) where three litters were born to and reared successfully by a sow given a diet deficient in vitamin A before signs of vitamin A deficiency appeared in the sucklings.

A similar difficulty arises in specifically attributing vitamin E deficiency in sucklings solely to a lack of vitamin E in the milk of the dam. Where diets low in vitamin E are given to pigs, signs of deficiency can be seen at birth (Adamstone *et al.*, 1949) whereas in cattle maternal deficiency of the vitamin usually results in a muscular dystrophy occurring during the suckling period (Blaxter & Brown, 1952). In the rat, vitamin E deficiency during pregnancy results in resorption of the foetuses.

Examples of a lack of dietary essentials in milk as exemplified by the appearance of particular deficiency syndromes in the sucklings could be multiplied considerably. Their occurrence does not necessarily indicate that the suckling phase of the reproductive cycle is a more vulnerable one than the foetal phase, but rather that a lack of some essential factors in the diet of the mother affects her reproductive performance as a whole. In her excellent review of the effect of diet on reproduction and the viability of the young of laboratory animals Russell (1948) states: "It is quite possible, where deficient diets have been fed to does from weaning or from the beginning of gestation that inability of the doe to rear her young may be due, not to inferior quantity or quality of the milk, but, as in manganese deficiency in rats, to congenital debility of the young resulting from faulty nutrition *in utero*." This statement implies a real difficulty in separating the uterine and the lactational phase of development as far as the genesis of nutritional deficiency in suckling animals is concerned, and it serves to emphasize the fact that the biological importance of lactation cannot be considered without due cognisance of earlier stages of development.

VI. Conclusions

In the above account of lactation in relation to growth of the young, a wide biological approach has been adopted, in an attempt to indicate the range of intra- and inter-species variation which occurs. Such variation can be seen in the anatomical means whereby sucklings obtain their milk, in the

wide differences within species in the potential and actual demand for milk as instanced by the number and size of the young, as well as in the very wide anatomical and physiological differences between the young of different species at birth.

Lactation and the maternal behaviour associated with it accommodate this variation with ease. In many instances, inter-species comparisons can be made to provide hypotheses which are of value in that they provide unifying concepts which condense masses of factual information. Such hypotheses and generalizations sometimes betray an anthropomorphic attitude or are purely teleological; often they are open to alternative interpretation, and in almost every instance the range of species included is far too limited in extent to warrant their general applicability. Many aspects of lactational performance and behaviour indeed often defy attempts to integrate them into some general principles. In this regard, a quotation from Baruch Spinoza's "Ethica More Geometrico Demonstrata" may perhaps be apt:

> "Whenever, then, anything in Nature seems to us ridiculous, absurd or evil, it is because we have but a partial knowledge of things, and are in the main ignorant of the order and coherence of nature as a whole, and because we want everything to be arranged according to dictates of our own reason. . . ."

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CHAPTER 20

Immunological Aspects of Colostrum

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I. Transfer of Immunity from Mother to Offspring in Different Animal Species

A. IMMUNITY AND THE FOETUS

Immunity is a misleading term and as Topley (1933) has said, resistance would be a better one. It is, however, used to "denote the resultant of two opposing systems, and this resultant can assume any value from zero to infinity." Immunity, therefore, plays a significant part in those mechanisms associated with infective disease, and mammals may display grades of active and of passive immunity. The use of the term "active" implies that the tissues of the individual take an active part in producing this increased resistance. This state of resistance may be transmitted to a normally susceptible animal by an injection of serum from the immune animal; the recipient then possesses an immunity of the passive kind since the tissues of the recipient play a relatively passive part. Immunity of this kind is sometimes transferred from a mother to her young. In some animals this occurs whilst the foetus is still within the uterus; in others the immunity is transmitted by the colostrum during early life.

It is not always an easy matter to measure the immunity or resistance of an individual to a particular infective agent. The measurement and demonstration of specific antibodies may sometimes furnish a reliable answer.

Antibodies may neutralize toxins, induce the clumping or agglutination of suspensions of particles such as cells, form precipitates with their appropriate antigens if in solution, or dissolve cells. These antibodies may, however, appear in the blood stream of normal animals and bear little relation to an increased resistance to a specific infection. These indirect methods of measuring resistance are therefore dependent upon the knowledge of a correlation between the presence and quantity of an antibody in a body fluid and the immunity enjoyed by the individual. These normal antibodies may be acquired by a natural, active immunization which is the result of the stimulus provided by clinical or even subclinical infection at some time during the life of an individual—man or beast. On the other hand, although this hypothesis may be true for an antitoxic immunity, it is doubtful if it is valid for those antibodies that sensitize bacteria: the agglutinins, the lysins and opsonins. In some cases these antibodies may be the result of the stimuli provided by the bacterium itself; in other cases it is possible that the stimuli have been provided by bacteria having similar chemical groupings. For example, some Salmonella bacteria share antigens with Pasteurella, and some pneumococci have an antigen in common with types of Klebsiella. There is little doubt that the normal antitoxins exert a protective effect and it is possible that a similar effect occurs with some, though not all, of the sensitizing antibodies. The new-born animal which has a mother possessing these antibodies, with or without the corresponding immunity, is endowed with these specific antibodies. The route may vary according to the species of animal, but there are mechanisms for the provision of these antibodies by the mother.

Young mammals depend on a passive immunity for their resistance to infectious diseases; the passive immunity may, in turn, be replaced by an active immunity provided by the tissues of the young animal itself. There are, however, immune responses that may be detrimental to the newly born; these may be associated with a genetic difference between mother and young.

1. *Passive Immunity and Infection*

Mumps, measles, diphtheria, scarlet fever and some other infections of man and of animals rarely attack the very young. This suggests that the mother has an active immunity against the specific infective agent and that this immunity is transmitted to the offspring. The immunity in the newly-born is of a passive nature and this sequence of events has been known for years. Young mammals are unable to produce antibodies themselves for some time after birth and they acquire these antibodies, which are associated with their immunity, from the mother either whilst in the uterus before birth or through the colostrum after birth. The relative importance of the route of transfer varies from species to species (Table I).

Orcutt & Howe (1922) and Little & Orcutt (1922) showed that agglutinins for *Brucella abortus* that they found in the blood serum of new-born calves had been derived from the colostrum of the mother. The blood of the new-born calf had no such agglutinins before it had received colostrum from its mother. If the colostrum was from a cow with a high content of agglutinins, then it could be shown that absorption was very rapid and agglutinins

TABLE I

CLASSIFICATION OF PLACENTATION ACCORDING TO THE INTERVENING TISSUES AND THE CORRELATION WITH TIME OF TRANSFER OF IMMUNITY FROM MOTHER TO OFFSPRING

Type of Placentation	Animal species	Uterine tissues			Foetal tissues			Time of antibody transmission	
		Endothelium	Connective tissue	Epithelium	Trophoblast	Mesenchyme	Endothelium	Before birth	After birth
Epitheliochorial	Horse Pig	+	+	+	+	+	+	−	+
Syndesmochorial	Ox Sheep Goat, etc.	+	+	−	+	+	+	−	+
Endotheliochorial	Dog Cat	+	−	−	+	+	+	±	+
Haemochorial	Man	−	−	−	+	+	+	+	−
Haemoendothelial[1]	Rat Mouse Rabbit Guinea-pig	−	−	−	−	−	+	±	±

[1]Although the rat and mouse have haemoendothelial placentation, it is stated by some authors (Mossman, 1937; McGirr, 1947) that transmission occurs mainly after birth.

appeared in the serum of the calf in a little over an hour after suckling. There is a relationship between the absorption of globulins and the absorption of agglutinins which are absorbed as late as 21 hours after birth by two calves under observation. The antibodies in the colostrum often exceed the titres of similar antibodies in the serum.

Other antibodies may be transferred in a similar manner; Mason *et al.* (1930) showed that a calf is able to absorb antibodies prepared in other animals such as sheep or horses.

2. *Incompatibility and Genetic Disorders*

The human blood group system ABO has been known for a long time, and mishaps that had occurred in blood transfusion were explained by the fact that the serum of some individuals was able to agglutinate the red cells of others. This agglutination of red cells by serum of the same species is called isoagglutination. Blood group O contains agglutinins against red cell antigens A and B. These agglutinins may be increased in the sera of women during pregnancy if they are carrying infants with red cells with A or B antigens which they themselves lack. Blood group incompatibility between mother and foetus received fresh attention when the significance of the Rhesus factor was appreciated. In brief it is clear that antibodies formed by an Rh negative mother in response to her Rh positive foetus may, under some conditions, destroy the red blood cells of the foetus and be responsible for the condition known as erythroblastosis foetalis or haemolytic disease of the new-born with signs of anaemia and jaundice. In the human subject the antibodies responsible for the condition may cross the placental barrier; fortunately not all Rh negative women may be sensitized by foetuses with Rh positive red cells.

Some domesticated animals may also suffer from haemolytic jaundice and the aetiology is somewhat similar. Blood groups of cattle, horses, sheep and pigs are being investigated and there is evidence of incompatibility by the occurrence of haemolytic disease in the new-born of many species. Haemolytic anaemia and jaundice known in new-born mules is due to iso-immunization of the mare. Jaundice in foals has also been reported and develops only after the first meal of colostrum. Should the foal be prevented from suckling its own mother, then it does not develop the disease; the colostrum and the serum of the mother contain agglutinins against the red cells of the jaundiced foals (Coombs *et al.*, 1948). Haemolytic disease of piglets is also known (Buxton *et al.*, 1955) and the signs vary within wide limits and depend upon the concentration of maternal antibody taken in by the young pig in the colostrum. The absorptive capacity of the digestive tract of the young pig is greatest during the first two days of its life, and it is during this period that it may absorb sufficient antibody to produce the disease.

B. Association between Placentation and Route of Transfer of Immunity

Most domesticated animals appear to acquire any immunity their mothers are able to give by way of the mammary secretions, and in this manner young animals are protected against the hazards they may meet in their early days. In general, we may say that whereas young rabbits, guinea-pigs and babies obtain their immunity whilst still in the uterus, the young of the

cow, sheep, goat, horse and pig derive theirs in the main from the colostrum. Dogs, rats and mice occupy an intermediate position, being provided with some immunity *in utero* and being able to absorb antibodies from the gut for a considerable time after birth.

The transmission of immune substances within the uterus can be correlated with the placental architecture (see Table I). Those animals that receive no maternal antibodies *in utero* are those to which colostrum is of the greatest immunological significance. These are the larger domesticated animals which have great economic importance throughout the world. Of these, the newly born lamb and calf in particular are prone to diseases that can cause a high mortality and for which colostrum can have a high prophylactic value. The immunological nature of this prophylaxis is discussed below.

II. Colostrum and the Farm Animal

A. Evidence for the Colostral Transmission of Specific Immune Substances

The concept of the transmission of immunity from a mother to her offspring is no new one. Sixty-eight years ago Paul Ehrlich (1892) investigated the maternal transference of immunity to young mice. Pregnant mice were given the toxalbumins ricin, robin and abrin and became immune to them. When the young were born, they were also immune. This immunity of the young mice was considered to be passive and the specific antibodies were transferred to the foetus across the placenta and through the milk immediately after birth. When the young from normal mothers were placed to suckle with immune mice, they too became immune, showing beyond doubt that immunity could be transferred in the milk. When, however, the young of immune mice were placed to suckle with non-immune mice, they still showed some immunity, though to a less degree than in the previous group. It appeared, therefore, that not all the immunity was being transmitted through the milk and that some was obtained by the young whilst still within the uterus.

The importance of colostrum for the new-born calf was investigated more than thirty years ago by Smith & Little (1922a) who described its protective effect against white scours in a small group of experimental animals. Of twelve new-born calves deprived of colostrum, eight died and a ninth was destroyed *in extremis*. All of ten calves that were allowed to receive colostrum survived. The authors commented that "the calf deprived of colostrum lacks something which permits intestinal bacteria to invade the body and multiply in the various organs." Whatever this something may have been, they were able to show that cow's serum might under some circumstances be a more or less successful replacement for it (Smith & Little, 1922b).

The colostral transmission of specific agglutinins, antitoxins and haemolysins occurs in many of the larger animals. A new-born calf has no specific *Escherichia coli* agglutinins in its blood serum until it has received colostrum containing them. If the animal is fed on milk instead, none appear (Nelson, 1924). There is similar evidence for the colostral transmission of agglutinins against *Brucella abortus*. None is found in the new-born calf until it has received colostrum containing them, after which they may appear in the blood as shortly as 1 to 3 hours after suckling (Little & Orcutt, 1922; McAlpine & Rettger, 1925; Thorp & Graham, 1933; McDiarmid, 1946). When a new-born calf takes colostrum containing agglutinins for *Trichomonas foetus*, its blood titre may reach that of the colostrum, whereas before suckling, these agglutinins are undetectable in serum dilutions as low as 1 : 6 (Kerr & Robertson, 1943). A calf will also absorb "foreign" antibodies in so far as a cow can be injected with diphtheria antitoxin which will appear in the colostrum and, after suckling, in the blood serum of the calf. Alternatively, lamb dysentery (sheep) antiserum and diphtheria (horse) antiserum may be given directly to the calf and be demonstrated in the blood some 24 hours later (Mason *et al.*, 1930).

Haemolysin appears in the colostrum of a goat if it is actively immunized against sheep red blood cells during gestation. After suckling, a kid will acquire a high level of serum haemolysin though none can be demonstrated in the blood serum of new-born kids that have not suckled (Famulener, 1912).

The colostral transmission of antitoxin from the ewe to her lamb has long been made the basis of an efficient and economic form of protection against lamb dysentery (Dalling *et al.*, 1926, 1927, 1928). The pregnant ewe can be actively immunized against the toxin of the causative organism (*Clostridium welchii*) and the specific antitoxins will appear in her colostrum. These later appear in the blood of the new-born lamb once it has suckled. Young lambs are also able to absorb tetanus antitoxin from the gut when it is given to them and this will appear in the blood stream in as little as 30 minutes, though antitoxins derived from colostrum are absorbed at a somewhat slower rate (Mason *et al.*, 1930).

Sows which show some resistance to the virus of swine influenza possess, in their colostrum, a substance capable of inhibiting haemagglutination by the normal swine influenza virus and by the Lee (human type B) influenza virus. The blood serum of new-born piglets is devoid of this haemagglutination-inhibiting antibody but it may be detected within 30 minutes of suckling (Young & Underdahl, 1949). Antibodies to swine fever and vaccinia virus are similarly transmitted (Manninger & Csontos, 1934; Nelson, 1932, 1934), as are agglutinins to *Brucella melitensis* (Hoerlein, 1952); these reach their maximum titre in the piglet's blood within a few hours of suckling.

It has been suggested that if a mare has a sufficiently high level of immunity, some maternal antibodies may pass across the placenta to the foetus (Lemétayer *et al.*, 1946a). There seems to be no doubt, however, that new-born foals are similar to the other large domesticated animals in deriving most, if not all, of their early immunity from the colostrum (Bruner *et al.*, 1948; Lemétayer *et al.*, 1946b).

B. The Association of Antibodies and Globulins and their Absorption from the Intestines of the New-born Animal

The absorption of large protein molecules unchanged from the gut of new-born animals is an accepted phenomenon. Egg-white given to kittens, puppies, kids and rabbits, of less than 8 days of age, can be demonstrated

TABLE II

Relation Between the Ingestion of Colostrum and the Composition of the Blood of New-born Calves. Results are Expressed as g per 100 ml of Blood

Age and state of calf	Total N	Euglobulin	Pseudo-globulin 1	Pseudo-globulin 2	Total globulin	Albumin	Non-protein N
5 hours after birth. No colostrum	0·596	0·023	0·004	0·140	0·167	0·376	0·053
6 hours after receiving colostrum, age 11 hours	0·838	0·173	0·235	0·087	0·505	0·280	0·053
1 day old	1·028	0·337	0·217	0·131	0·765	0·210	0·053
2 days old	0·962	0·271	0·310	0·075	0·656	0·253	0·053

(Howe, 1921b)

in the blood within a short time of feeding. None is absorbed if the young animals are more than 8 days old unless abnormally large quantities are fed or the intestinal wall is damaged (Ganghofner & Langers, 1904). The proteins of bovine colostrum are similarly absorbed by the new-born calf during its first day of life. Howe (1921a) precipitated serum proteins by the use of anhydrous sodium sulphate, and serum euglobulin was precipitated at a concentration of 13·5 % and pseudoglobulin at 17·5 %. Using these methods, Howe (1921b) demonstrated that these particular proteins are lacking in the blood of the new-born calf and that they appear suddenly after the ingestion of colostrum. The changes in serum protein, when a new-born calf is allowed to take colostrum, are given in Table II.

This work has since been confirmed by electrophoretic methods (Jameson *et al.*, 1942; Hansen & Phillips, 1947), the protein fraction which appears in the serum of a new-born calf after suckling being called the "γ" globulins.

TABLE III

CHANGES IN THE AGGLUTININ TITRE AND IN THE PROTEINS OF THE BLOOD OF CALVES AFTER THE INGESTION OF COLOSTRUM

Age of calf	Total N	Eu-globulin	Pseudo-globulin 1	Pseudo-globulin 2	Total globulin	Albumin	Non-protein N	Agglutination
				Calf of cow 'A'				
20 min	0·776	0·018	0·072	0·184	0·273	0·430	0·073	—
				Calf allowed to suckle dam				
4 hours	0·896	0·098	0·218	0·115	0·431	0·391	0·073	1 : 80
6 hours 10 min	1·066	0·162	0·303	0·183	0·648	0·354	0·064	1 : 160
14 hours 30 min	1·399	0·512	0·401	0·175	1·088	0·247	0·064	1 : 320
11 days	1·352	0·333	0·469	0·149	0·951	0·367	0·034	
				Calf of cow 'B'				
Dam's blood	1·135	0·090	0·286	0·119	0·495	0·606	0·034	—
35 min	0·759		0·068	0·154	0·222	0·460		—
				Calf allowed to suckle dam				
1 hour 50 min	0·700		0·073	0·149	0·222	0·422	0·077	1 : 40
3 hours 50 min	0·802	0·038	0·094	0·171	0·303	0·422		1 : 640
18 hours	0·925	0·085	0·290	0·153	0·443	0·354	0·043	1 : 2560

Results are expressed as g N per 100 ml blood, and as the highest dilution in which agglutination was positive. (Orcutt & Howe, 1922).

There is an immediate increase in the γ fraction if the calf is allowed to take colostrum within the first 24 hours of life but if it is withheld until after this time, the calf is no longer able to make use of it and there is no measurable increase in serum γ-globulins after feeding. If the animal is denied colostrum the blood protein levels do not begin to approach their normal values until the 8th week.

These animals are therefore able to absorb colostral antibodies and colostral globulins, the antibodies and globulins being associated in the same colostral fraction (Orcutt & Howe, 1922). It has already been mentioned that the new-born calf has no *Br. abortus* agglutinins in its blood until it has ingested colostrum containing them. The appearance of such agglutinins in the blood stream may be compared with the appearance of those protein fractions that are dependent on the ingestion of colostrum; a definite and significant relationship may thereby be established between them. In Table III, serum-antibody and serum-protein levels of a new-born calf are given side by side, determinations having been made before and at varying intervals after taking colostrum from an immune dam.

If the relative increases in the agglutinin titre and certain proteins in the first calf are considered, then it is seen that in the space of about 3 hours, serum euglobulin has increased from 0·018 g nitrogen per 100 ml blood to 0·098 g, and pseudoglobulin has increased from 0·072 g to 0·218 g; agglutinins specific for *Br. abortus* have appeared to a titre of 1 : 80. Readings taken some 2 hours later show further parallel increases. In the second calf, the absorption of globulins was not so rapid though the agglutinin titre was much higher. In both there was an increase in serum globulins associated with an increase in agglutinin titres.

If small quantities of colostrum or serum containing *Br. abortus* antibodies are fractionally precipitated with anhydrous Na_2SO_4 it can be shown that the removal of certain of the protein fractions entails the simultaneous removal of the antibodies. Table IV shows the results obtained when an immune serum is fractionated with various percentages of sodium sulphate.

As they are obtained, fractions are removed by filtration and washed with that concentration of sodium sulphate solution used for the precipitation. The precipitated protein is then re-dissolved in a known quantity of distilled water.

The first part of the table shows how a considerable part of the agglutinins can be removed by a concentration of 14·5 % sodium sulphate, and this, as we have already seen, is the critical level of euglobulin precipitation. That which remains can be brought down at a concentration of about 16·4 % which is well within the limit established for pseudoglobulins by Howe (1921a). The second part of the table gives the titres of the original filtrates as the various protein fractions are removed. These data are complementary to those in the first part.

The significance of these findings is clear. Agglutinins for *Br. abortus*, and presumably other antibodies as well, are associated with euglobulins and pseudoglobulins. It is these proteins that are absent from the blood of the new-born calf, but present in colostrum and absorbed unchanged from it when consumed soon enough after birth. Since there are similar changes in the blood proteins of new-born foals, kids, lambs and pigs when they receive

colostrum (Earle, 1935; Bauriedel *et al.*, 1954) it is highly probable that they too receive immune bodies in the globulin fractions.

An important question arising from these considerations is one concerning the actual route by which the colostral globulins leave the lumen of the

TABLE IV

THE AGGLUTININ TITRE OF PROTEIN FRACTIONS OF SERUM, SEPARATED WITH SODIUM SULPHATE AT DEFINITE CONCENTRATIONS, AND OF THE FILTRATES FROM THESE SEPARATIONS

Fraction	Dilution						Control
	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640	
Serum	C	C	++++	++	+	±	−
13·5*		++	+	±	−	−	−
14·2		++	+++	+	−	−	−
14·5		++++	++++	++	+	−	−
15·5		++	+++	+++	+	−	−
16·4		+++	+++	+++	++	−	−
17·4		++	+++	++	++	−	−
21·5		++	++++	++	+	−	−
			Filtrate				
13·5F		C	C	+++	+	−	−
14·2F		C	+++	+	−	−	−
14·5F		++++	++	−	−	−	−
15·5F		++++	+	−	−	−	−
16·4F		+	−	−	−	−	−
17·4F		−	−	−	−	−	−
21·5F		−	−	−	−	−	−

(Orcutt & Howe, 1922)

*Percentage of Na_2SO_4. C = complete agglutination.

intestine and reach the blood stream. There have been arguments in favour of an increased permeability of the intestinal wall of the young animal but few, if any, data have been advanced to support them. There is, however, evidence to show that absorption may take place through the lymphatics (Comline *et al.*, 1951). New-born calves that had been deprived of colostrum were anaesthetized and cannulas introduced into the duodenum, caecum and

the thoracic lymph duct. When colostral whey containing euglobulin, and pseudoglobulin 1 and agglutinin specific for *Br. abortus* was introduced into the small intestine, these substances could be detected in the lymph, but not in the blood, after an interval of 1 to 2 hours, the rise in agglutinin concentration being parallel to that of euglobulin and pseudogobulin 1. They did appear in the blood of control calves without cannulas. These proteins and agglutinins were also shown to pass into the lymph of new-born kids.

Absorption in the calf took place only in the small intestine. When the entry of colostrum was prevented by means of ligatures at the pylorus and the ileo-caecal junction, there was no absorption and no trace of colostral protein was found in either lymph or blood serum. Even in the small intestine, this absorption was limited to the first hours of life. If colostral whey was not given until about 65 hours after birth, there was a significant fall in the rate of absorption and the rate of increase of agglutinin titres which could not be compensated for by giving a more concentrated whey.

Why the ability of the young animal to absorb proteins unchanged should be restricted to the first few days of life is not clear, but this fact has been recorded on many occasions. Ganghofner & Langers (1904) have noted it with regard to the absorption of egg white in several species of animal, and Famulener (1912) with regard to colostral antibody in older kids. Ganghofner & Langers, however, quote 8 days as the period during which the young puppy, kitten, rabbit and kid have this ability, but this period is somewhat in excess of that demonstrable for foals, calves and lambs. A young foal is already too old to absorb at 5 days (Bruner *et al.*, 1948) and a calf probably begins to change as early as the 24th hour (Kerr & Robertson, 1946; Hansen & Phillips, 1947). A new-born lamb will have tetanus (horse) antitoxin in its blood within 30 minutes of feeding, but none passes in a 4-day-old lamb (Mason *et al.*, 1930).

C. Antibody Levels in the Colostrum and in the Young after Suckling

The most interesting fact deriving from immunological studies on colostrum is not so much that it may at times contain specific antibodies as that these antibodies are frequently at a much higher concentration than in the circulating blood. The ability of the cow to provide immune substances in her colostrum at a high level is restricted to the later phases of pregnancy; it probably occurs in other species as well. Modern techniques employing radioactive tracer elements have made it possible to study this process in some detail. Thus, bovine serum γ-globulins labelled with the radioactive isotope of iodine, ^{131}I, may be injected intravenously into a cow in different stages of pregnancy and the quantity incorporated into her colostrum is

estimated by a simple determination of its radioactivity. A heifer, in the later stages of pregnancy may concentrate this labelled globulin two to three times in her colostrum, whereas a multiparous cow 5 months pregnant is unable to emulate the younger animal and the colostral level of labelled globulin does not exceed that of the blood (Garner & Crawley, 1958). Yet at a period of about 3 weeks before calving, such a cow will concentrate labelled globulins in her colostrum to a level some thirteen times that of her circulating blood and this within 22 hours of injection (Blakemore & Garner, 1956). Simple immunological studies are in agreement with these findings. When pregnant cows and ewes are immunized with specific antigenic substances, the corresponding immune bodies which appear in the circulation are concentrated in the colostrum. This is true of lamb dysentery antitoxin in ewes (Mason *et al.*, 1930) and agglutinins for *E. coli*, *Trichomonas foetus*, and for staphylococcus antitoxin in cows (Nelson, 1924, Kerr & Robertson, 1943; Minett, 1937). Several of these authors record that the colostral titre of immune substances falls sharply at parturition. The sequence of events appears to be a sharp and significant rise after the intravenous injection of ^{131}I-labelled γ-globulin. This rise is followed by a drop in activity at parturition and though there is a simultaneous increase in colostrum volume, it alone is not sufficient to account for the drop in terms of dilution. It would appear that the first suck of colostrum will be of greater importance than subsequent ones to the new-born animal.

Once the young animal has suckled, colostral antibodies appear in the blood stream and probably reach their maximum within 24 hours. Thereafter they decline over periods ranging from weeks to months. Baby pigs lose most of their colostrally-acquired antibodies to *Br. melitensis* within 4 weeks (Hoerlein, 1952). Haemagglutination-inhibiting antibodies to swine influenza virus decline in about 8 weeks, the rate of loss being irrespective of the original titre (Young & Underdahl, 1949). Immunity to vaccinia virus is a little more persistent, beginning to decline in the 2nd month and being virtually absent by the end of the 3rd (Nelson, 1934).

Br. abortus agglutinins reach their maximum titre in the suckling calf in 24 hours, which is only to be expected since, as we have seen above, globulins cease to be absorbed soon after this time. Attainment of maximum titre is followed by a recession, apparently logarithmic, the duration of which may be weeks or months depending to a large extent on the titre of the colostrum (McDiarmid, 1946).

III. Colostrum and Disease

The discovery of the immunological properties of colostrum has led to their exploitation for prophylactic purposes in those animals which do not

acquire passive immunity whilst within the uterus. From an economic standpoint, the most important of these animals are calves and lambs, and it is with these that the greatest practical applications have been made.

A. Lamb Dysentery

In the first 2 weeks of life, young lambs are particularly susceptible to a condition known as lamb dysentery involving an enteritis with an extensive ulceration. The causative organism is *Cl. welchii* type B which establishes itself in the intestine of the young animal and then elaborates a toxin which may kill the host within a few hours. Fully grown sheep are not susceptible but may act as carriers; the teats of a ewe may become contaminated by soil or faeces. There are several predisposing factors: those lambs which suckle too much are particularly prone. However, protection against *Cl. welchii* gives a high level of protection against the disease. Ewes can be actively immunized by vaccination with a formalinized whole culture of the organism and the ensuing immunity will be passively transmitted in the colostrum to the young when they suckle. A measure of the efficiency of this technique may be gained from figures given by Dalling *et al.* (1926, 1927). 4724 ewes were inoculated twice, once in autumn and once in spring. Of 5760 lambs born of these ewes and permitted to suckle, 3·06 % died of lamb dysentery. A further 2248 control (uninoculated) ewes gave birth to 2509 lambs in the same season and the mortality from lamb dysentery was 16·06 %. In a subsequent season, the respective mortalities were 0·87 % of lambs born of inoculated ewes and 8·04 % of lambs from control ewes. This method of protection is now widely used in those areas where lamb dysentery is endemic.

B. Colibacillosis of Calves

For reasons which will be obvious later, the immunological properties of colostrum have not been applied to any distinct prophylactic techniques since the principal disease of young calves—colibacillosis—is not caused by any one particular serotype of *E. coli*. The clinical picture of the disease is one of profuse scouring, dehydration, high temperature, prostration and death: there may or may not be a septicaemia. Extensive research both experimentally and in the field has revealed those conditions under which colostrum may have the greatest protective effect, and the importance of careful animal husbandry; this work has necessitated consideration of the aetiology and epidemiology of colibacillosis and of the serology of *E. coli*, which are discussed below.

E. coli is generally accepted as the causative organism of colibacillosis of calves, commonly referred to as white scours. These bacteria have at least three important diagnostic antigens—somatic, surface and flagellar, of which 142, 86 and 41 respectively are known. There is no doubt that many more remain to be characterized and it will be seen that the coliform flora of any animal may be serologically very diverse. Surface antigens are thermolabile, whilst the somatic or body ones are thermostable and antisera may be prepared against them, and the different types distinguished according to methods described by Kauffmann (1954).

The examination of strains of *E. coli*, with and without surface antigens, has given rise to the opinion that the pathogenic activities of this bacterium are shared by the somatic and surface antigens. Smith & Bryant (1927) noted that strains of *E. coli* with surface antigens (in this instance they were represented by morphological capsules) were more virulent and less susceptible to phagocytosis than acapsular variants. Culture filtrates from both types of strain were equally toxic when injected intravenously into calves. A year later Smith (1928) recorded that "The results point to the capsular substance as the material carrying virulence or, expressed somewhat differently, the factor which protects the micro-organism in the host." More recent investigations have confirmed this. Sjöstedt (1946) found that phagocytosis of strains with surface antigens occurred only when type-specific antiserum was added. Furthermore, 25 % of strains with surface antigens were more resistant to the bactericidal effects of serum than those without. Ewertsen (1946), Wramby (1948) and Sjöstedt (1946) all made toxicity determinations and concluded that, irrespective of the type of surface antigen present, toxicity was linked with the somatic antigens and that some of these were more toxic than others. More recent evidence suggests that there is less variation in the toxicity of different somatic antigens than was previously supposed (Carne, 1958).

1. *The Degree of Protection Afforded by Colostrum*

The colostral transmission of specific *E. coli* antibodies to the calf, their association with γ-globulins and the protective effect of colostrum have already been mentioned. The exact nature and extent of this protection has been examined in considerable detail by Aschaffenburg *et al.* (1949, a,b, 1951). For the purposes of their experiments, new-born calves that had not suckled were brought into a series of experimental pens and fed on colostrum, on one of its fractions, or on a synthetic diet. To ensure that these animals had truly not suckled, it was necessary to demonstrate the absence of γ-globulins from the blood serum. This was done by means of a zinc sulphate precipitation test (Aschaffenburg, 1949) based upon the work of Howe (1921b) and Orcutt & Howe (1922) already discussed. Only calves negative

by this test were used. The standard of husbandry was "normal" and though there was no deliberate attempt to introduce infection into the calf community, spontaneous outbreaks of colibacillosis occurred.

In preliminary experiments, about 7 litres of the aqueous phase of colostrum were given to calves, but as little as 1 % of this amount contained as an admixture in a crude fatty fraction allowed calves to live and grow. Pure colostral fat was ineffective as was the colostrum substitute based on reconstituted dried skim milk, margarine and several vitamins in high concentration: calves receiving either of these in their first feeds were most likely to die of scouring (Aschaffenburg *et al.*, 1949a); the protective effect of the aqueous phase was subjected to further investigation. Four groups of six new-born calves were given in their first feeds either 400 ml, 200 ml, 80 ml or none of the aqueous phase. Five of the six deprived calves died; all the others survived despite severe scouring (Aschaffenburg *et al.*, 1949b). The results of this work showed that "only those calves whose initial diet contained the aqueous phase of colostrum made satisfactory progress" and that small quantities were able to protect calves from fatal scours even if they subsequently failed to gain weight normally.

2. *The Nature of the Protective Properties*

There was a strong suggestion in the work of Smith & Little (1922a) that the protective effect of colostrum might be of an immunological nature and that this protection might be directed against the surface antigen of the bacterium. More recently, Briggs (1951) has shown that mice can be protected against *E. coli* by inoculation of antisera containing agglutinins specific for the surface antigen of the strain in question. Since colostrum can also contain these specific agglutinins, the work on mice was repeated with colostral whey containing specific agglutinins substituted for the antisera (Briggs *et al.*, 1951). Again, protection against the surface antigen gave protection against the whole organism. Once this was known, the relationship between colostral immune bodies and survival in calves could be properly estimated. It was shown that when a calf died in an epidemic of white scours despite its having received small quantities of colostrum, that colostrum was deficient in agglutinins specific for the surface antigen of the strain which killed the calf. The following is a summary of the more recent investigations in this field (Ingram *et al.*, 1956):

"Agglutination tests with colostral wheys were made on more than 150 strains of *E. coli* that were associated with the death of (experimental) calves from white scour infections. Of fifty-nine colostrum fed calves that died, forty-five had received colostrum that was devoid of agglutinins against the strains associated with their deaths. Of ninety-four colostrum-deprived calves that died, sixty-six deaths were associated with strains against which

agglutinins could be demonstrated in the colostrum given to contemporary calves that survived. . . . On rare occasions only was a strain agglutinated markedly by one colostral whey and not by another."

3. *Animal Husbandry and the Epidemiology of Colibacillosis*

The serological techniques used in the classification of *E. coli* made it possible to trace the distribution of individual serotypes within herds of cattle. There is definite evidence for the transmission of individual serotypes from calf to calf both under experimental conditions and in the field (Briggs, 1951; Ingram *et al.*, 1953; Wood, 1955; Rees, 1958). Fig. 1 illustrates the distribution of serotypes in an enclosed community of experimental calves.

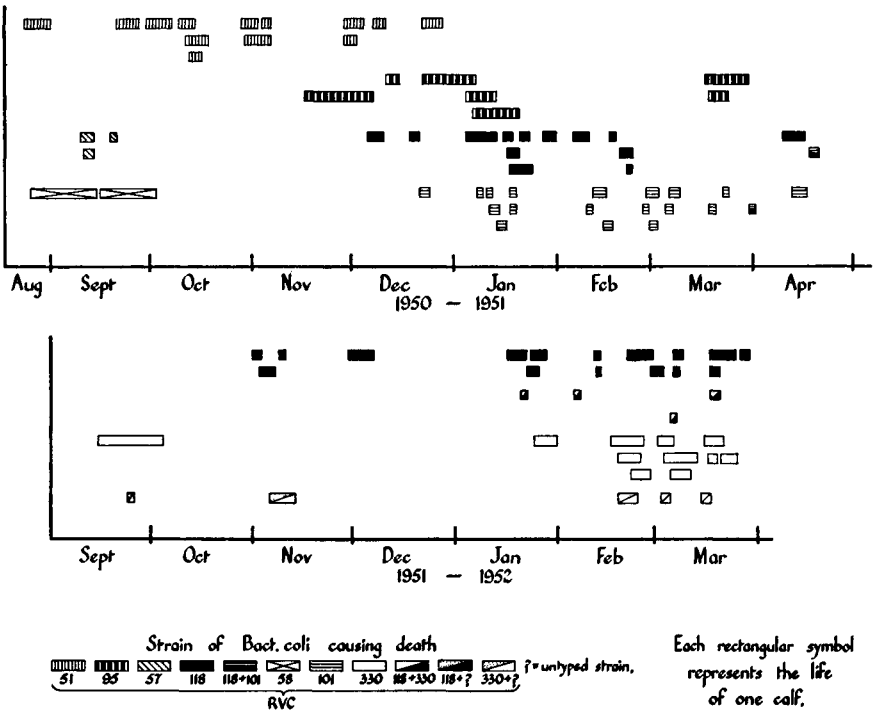

FIG. 1. Epidemiology of white scours. The persistence of individual serotypes of *E. coli* in an enclosed community of experimental calves. (Ingram *et al.*, 1953.)

Each rectangular block represents the life of a calf, its shading, the serotype or types of *E. coli* responsible for its death. It can be seen that (*a*) no particular serotype is dominant throughout the whole season, and (*b*) that two or more serotypes may be isolated from the same sick calf. Further serological work has shown that, despite the large number of serotypes of *E. coli*

already known, comparatively few are regularly associated with colibacillosis of calves, just as though some strains were more selectively pathogenic for calves than others. This idea was first put forward by Lovell (1937) and subsequent work has tended to confirm it. However, the number of these special "races" or serotypes is so large that it precludes specific immunization of the dam or the development of a suitable polyvalent antiserum.

The solution to the problem rests ultimately with the management of individual herds. Despite the fact that the coliform flora is dynamic, the dominant serotypes in the flora of a self-contained herd are liable to be shared by most or all of the animals in it and to persist long enough for colostral antibodies to develop against them before the calving season. For this reason, it is ill-advised to move the dam into a new environment just before the calf is due, or to move the calf soon after birth if the colostrum is to have its maximum protective effect. For similar reasons, new stock should not be introduced into a self-contained herd close to calving time. Finally, prepartum milking of cows and heifers should be avoided altogether since it changes the composition of the mammary secretion available to the newborn calf (Eaton *et al.*, 1949) and there is a decline in the level of globulins and agglutinins. Calves fed on this altered secretion do no better than those fed on ordinary heifer milk (Aschaffenburg *et al.*, 1951).

The evidence for the immunological significance of colostrum for the newborn calf is extensive and convincing, though it would be unwise to assume that *E. coli* agglutinins are the sole factor of importance from the protective standpoint. Nevertheless, this approach has its opponents, notably Fey (1957a,b) in Switzerland who is of the opinion that colibacillosis is an epizootic caused by a variety of factors and that, with the exception of one serotype, *E. coli* is only of secondary importance. This particular serotype, 078, K80, is found in many parts of the world and there seems to be universal agreement on its selective pathogenicity for new-born calves.

It seems, however, that most serotypes of *E. coli* possessed of a surface antigen can act as pathogens for young calves if given the opportunity. Some are better able to do this than others: the matter is one of a variable host–parasite relationship in which the immunological properties of colostrum can play an important rôle.

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