Folate nutrition in the kid

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1. A study was made on the folate content of goat's milk in relation to stage of lactation, and on the interrelationship between blood and milk folate concentrations in the dam and in the kid.

2. In seven goats the folate concentration in the colostrum at parturition ranged from 136 to 300 ng/ml, and averaged 205 ng/ml. The concentration fell sharply during the early days of lactation and by day 14 it averaged only 9·5 ng/ml. This pattern of rapid decline in milk folate concentration with advancing lactation was little affected by daily provision of folic acid parenterally in relatively large amounts.

3. The colostrum and milk contained a minor whey protein that combined strongly with folate, and presumably acts as a trapping mechanism to accumulate the vitamin from the plasma into the milk. The milk folate concentration is determined by the rate of milk secretion in relation to the availability of free folate in the blood plasma.

4. At parturition the plasma folate concentration in the kids was very low – about 1 ng/ml – but by day 2 it had increased to about 28 ng/ml. This folate was protein-bound and accompanied by an excess of free binder protein. It appeared that the folate-protein complex from the colostrum was transmitted intact into the kids' blood circulation.

5. The possible importance of the folate-binding protein in the regulation of folate metabolism is discussed.

Goat's milk is a comparatively poor source of folate. It contains about 6 ng/ml as against 50 ng/ml in human milk and cow's milk, and its prolonged consumption as the sole diet by suckling infants may cause 'goat's milk anaemia', which is now recognized as being a megaloblastic anaemia symptomatic of folate deficiency (Becroft & Holland, 1966). In man the metabolic turnover of folate is rapid and any severe restriction of dietary intake is quickly followed by a fall in serum folate and a slower progressive fall in red cell folate (Herbert, 1962). These observations prompted speculation on the folate nutrition of the newborn kid, and the present paper reports on the folate content of goat's milk in relation to stage of lactation, and on some interrelationships between blood and milk folate concentrations in the dam and in the kid.

EXPERIMENTAL

Pedigree British Saanen goats of the Institute herd were used. They were maintained on clover hay and concentrates and given daily access to pasture. They were brought indoors each evening and housed on concrete in individual pens. On the expected day of parturition and for about a week thereafter the goats were kept indoors. The kids remained in the pens for 3 weeks, during which time they were suckled by their dams and also had access to hay and concentrates.

Some of the goats were milked *pre-partum*, as deemed necessary by the goatherd,

to relieve painful distension of the udder and as a prophylactic measure against mastitis.

For the measurement of blood and milk folate concentrations during the first 4 weeks of lactation, three primiparous and three multiparous goats were selected. Samples of blood and milk were taken at parturition and at 2, 7, 14, 21 and 28 d thereafter. Further samples were taken from some of the animals at intervals before parturition. None of these animals was milked *pre-partum*. Blood samples were taken from the newly born kids before suckling, and again at 2, 7, 14, 21 and 28 d.

In a parallel experiment, blood and milk samples were taken, as described below, from four multiparous goats that had been milked *pre-partum*.

Sampling procedure

Blood samples (10–20 ml) were taken by venepuncture from the jugular vein, into 28·4 ml McCartney bottles to which had been added 1 drop of heparin (supplying approximately 20 i.u./ml blood) and 0·1 ml of tris–ascorbate buffer (pH 7), containing 4 mg ascorbic acid. Two subsamples were transferred to Wintrobe haematocrit tubes for determination of the packed cell volume (PCV). A further 2 ml portion was diluted to 10 ml by addition of 1 % (w/v) aqueous solution of ascorbic acid, as recommended by Hoffbrand, Newcombe & Mollin (1966) for the assay of whole blood folate. The remainder was centrifuged at 10 000 g for 10 min and the plasma layer transferred by pipette into a 7 ml 'bijou' bottle.

The milk samples – about 20 ml – were also taken into 28·4 ml McCartney bottles containing 0·1 ml of the tris–ascorbate buffer.

All the samples were stored at $-30°$ until required for folate assay.

Administration of iron-dextran to the kids

Preliminary experiments showed that the PCV values for the kid's blood fell from about 40 % at parturition to a minimum around 20 % at about 14 d after parturition, and then began to recover. The decline was prevented by intramuscular injection of 200 mg iron as iron-dextran complex (Imposil 200; Fisons Ltd), and all the kids used in the experiments now reported were given this treatment as a prophylactic measure.

Influence of parenteral folic acid on milk and blood plasma folate concentrations

From a primiparous goat in the 3rd month of lactation and yielding about 4·6 kg milk daily, samples of milk and blood were taken over an 8 d period, in accordance with the following schedule:

Day ...	1		2		3		4		5		6		7		8	
	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm
Milk sample	√	√	√	√	√	√	√	√	√	√	√	.	√	.	√	.
Blood sample	√	√	√	√	√	√	√	√	√	√	√	.	√	.	√	.
Folate injection	.	.	√	.	√	.	√	.	√	.	.	.	.	.	.	.

Folic acid (2 ml of solution containing 1 mg/ml in 0·9 % saline) was given by intramuscular injection on the morning of days 2–5, immediately after the samples of

blood and milk had been taken. The blood samples were centrifuged to provide plasma for assay.

Whole body folate in the kid

The aim in this experiment was to compare whole body folate in twin male kids, one of which was to be killed at parturition and the other at 3 weeks of age. In the event no suitable twin pairs became available and instead four unrelated male kids were selected. Two were killed at parturition before suckling and two after suckling for 3 weeks. The animals were skinned and the livers and guts removed. Each liver was weighed and homogenized with three times its weight of phosphate–ascorbate buffer at pH 7·8 (see below). The gut contents were washed out with phosphate–ascorbate buffer and homogenized. The empty gut was combined with the carcass and the whole weighed and then minced. A portion of the mince was homogenized with phosphate–ascorbate buffer. Samples of the various homogenates were stored at $-30°$ until required for folate assay. Test extracts were prepared essentially as described for the milk samples.

The skins were discarded and their folate content was not taken into account.

Folate assays

Folic acid activity was assayed microbiologically with *Lactobacillus casei* by the procedure of Herbert (1961).

Preparation of chicken pancreas enzyme. To 2·5 g of desiccated chicken pancreas (Difco Laboratories Inc., Detroit, USA) were added 25 ml of ice-cold buffer solution, made by dissolving 1 g ascorbic acid in 100 ml of $0·1 \text{M-Na}_2\text{HPO}_4$ solution and adding 4M-NaOH solution to pH 7·8. The mixture was homogenized gently in a test-tube with a close-fitting pestle of polytetrafluoroethylene, and centrifuged for 15 min at $15\,000g$. The supernatant fluid was filtered through Whatman no. 42 paper, and its content of free folate removed by fractional filtration in a column of Sephadex gel G 25; the folate conjugase was eluted from the column with the protein front, at near the void volume. The protein-containing fractions were combined and diluted to 50 ml with the buffer, and further diluted fivefold before use.

Preparation of the test extracts. Extracts of milk and colostrum were made as follows. To 2 ml samples in 15×150 mm test-tubes was added 1 ml of the buffer solution. The tubes were heated in flowing steam for 2 min and cooled in cold water, and to each was added 1 ml of the extract of chicken pancreas. The tubes were incubated for 2 h in a water-bath at 45°. The contents were then acidified to pH 4·8 by addition of $0·1 \text{M-HCl}$, diluted with water to 50 ml and filtered through Whatman no. 42 paper. The filtrates were adjusted to pH 6·8 and further diluted as needed for test.

Extracts of whole blood were prepared as described for human blood by Hoffbrand *et al.* (1966), using a smaller final dilution to compensate for the lower folate content in goat's blood. The plasma samples were extracted by a modification of the method of Waters & Mollin (1961). To 2 ml samples in 20×150 mm test-tubes were added 8 ml $0·1 \text{M-phosphate}$ buffer of pH 6·1 containing 200 mg ascorbic acid/100 ml. The tubes were autoclaved for 2·5 min at 115° and cooled in a water-bath. The contents
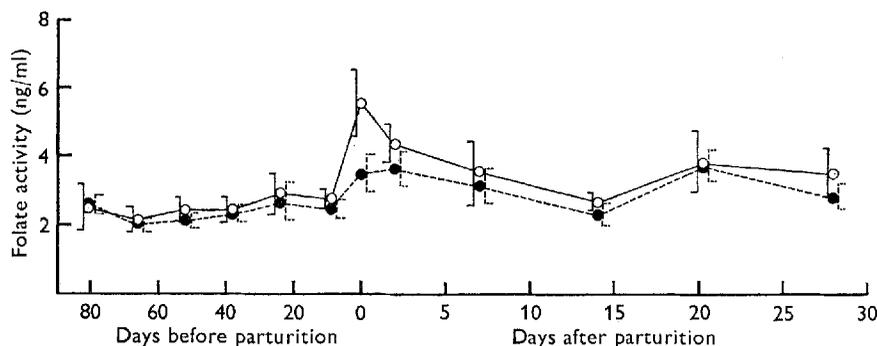
Fig. 1. Folate concentration in blood (O——O) and blood plasma (●-----●) of nine goats. Bars indicate standard errors of means. Haematocrit values range from 31 to 34%.

were adjusted to pH 4·6, diluted to 30 ml with water, and filtered through Whatman no. 42 paper. The filtrate was then adjusted to pH 6·8.

The concentration of folate in the red blood cells was calculated by subtracting the folate content of the plasma in the samples from that of the whole blood, and then correcting for the haematocrit value.

Measurement of folic acid-binding capacity of milk and blood plasma

Both in the human and in the cow, the accumulation of folate into the milk seems to be connected with the presence in the milk of a minor whey protein to which folate binds strongly (Ford, Salter & Scott, 1969; Ghitis, Mandelbaum-Shavit & Grosso-wicz, 1969). It seemed possible therefore that the low-folate content of mature goat's milk might reflect a comparatively low level of folate binding activity.

Samples of blood and milk were taken from a goat at parturition and again on the 7th and the 30th day of lactation. The blood samples were centrifuged to provide plasma for test.

To eight 1 ml portions of each sample of plasma and milk were added 2 ml buffer solution of pH 7·2 containing 0·15 M-NaCl, 0·02 M-sodium phosphate and 0·001 M-ascorbic acid, and graded amounts of folic acid dissolved in 1 ml buffer. A 2 ml sample of each mixture was then transferred to a sac of 8 mm cellulose dialysis tubing and dialysed for 48 h at 2° against eight successive 100 ml portions of buffer. The residual folate activity in the sacs was then assayed.

RESULTS AND DISCUSSION

Folate content of goat's blood

Fig. 1 shows the folate concentrations in the whole blood and plasma of six goats. They showed no large change over the period of the experiment, but there was a small increase at parturition. Preliminary experiments (Ford & Scott, 1969) had shown an average blood folate concentration of 7 ng/ml at parturition, which declined during the following 28 d to 2·3 ng/ml. It is not certain that this increase in the blood folate concentration at parturition was directly connected with the large secretion of folate
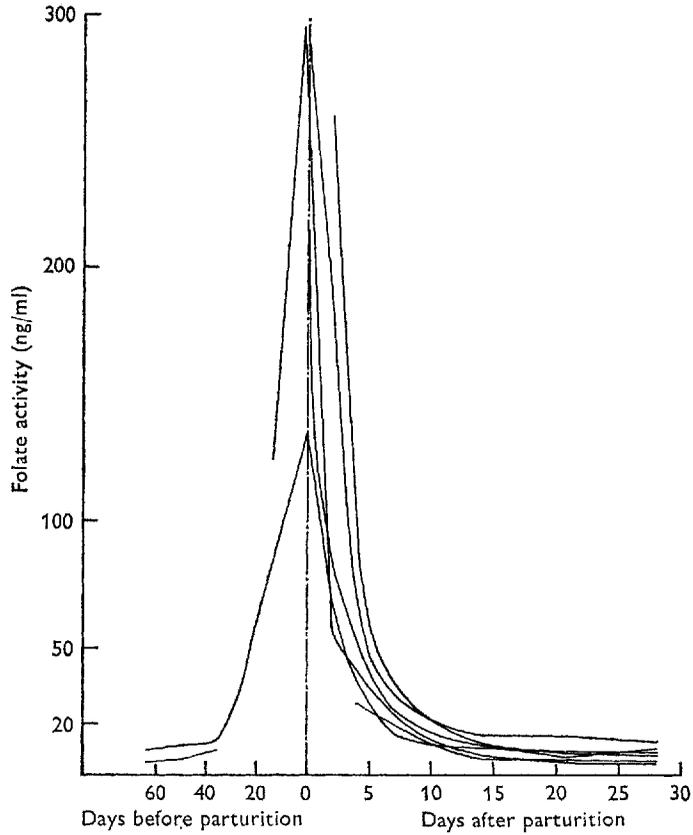
Fig. 2. Folate concentrations in samples of milk from six goats, in relation to stage of lactation.
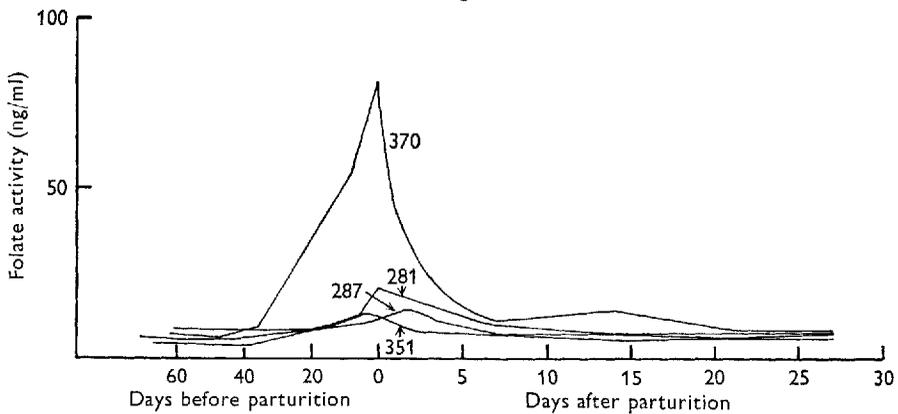
Fig. 3. Folate concentrations in samples of milk from four multiparous goats that had been milked *pre-partum*. The animals were milked as follows: goat 351, every 3 d until 2 d before parturition and then twice daily; goat 281, every 2 d until 4 d before parturition; goat 287, daily until 5 d before parturition; goat 370, every 3 d until 2 d before parturition.
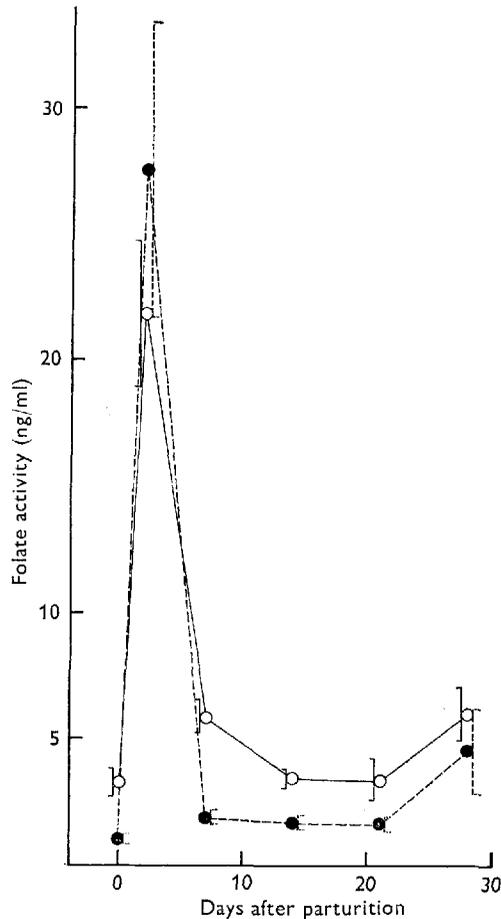
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Fig. 4. Folate concentration in blood (O———O) and blood plasma (●– – – –●) of nine kids. Bars indicate standard errors of means. Haematocrit values ranged from 31 to 38 %

in the colostrum: it might have had less to do with the physiology of milk folate secretion than with the change in feeding and management (see p. 571). It is possible, however, that the increase reflected a mobilization of folate from the liver and other tissues.

Folate content of milk in relation to stage of lactation

Fig. 2 shows the folate concentration in samples of colostrum and milk taken from six goats during the first 4 weeks of lactation, and from three of the animals at intervals before parturition.

At parturition the folate concentration in the colostrum was comparatively high and ranged up to about 300 ng/ml. It fell sharply during the early days of lactation and by day 14 averaged only 9·5 ng/ml. This pattern is qualitatively broadly similar to that reported for the dairy cow (Karlin, 1967) but in the goat the contrast between the folate concentrations in colostrum and mature milk was more extreme. In the human the pattern is normally different; the folate concentration is low in colostrum
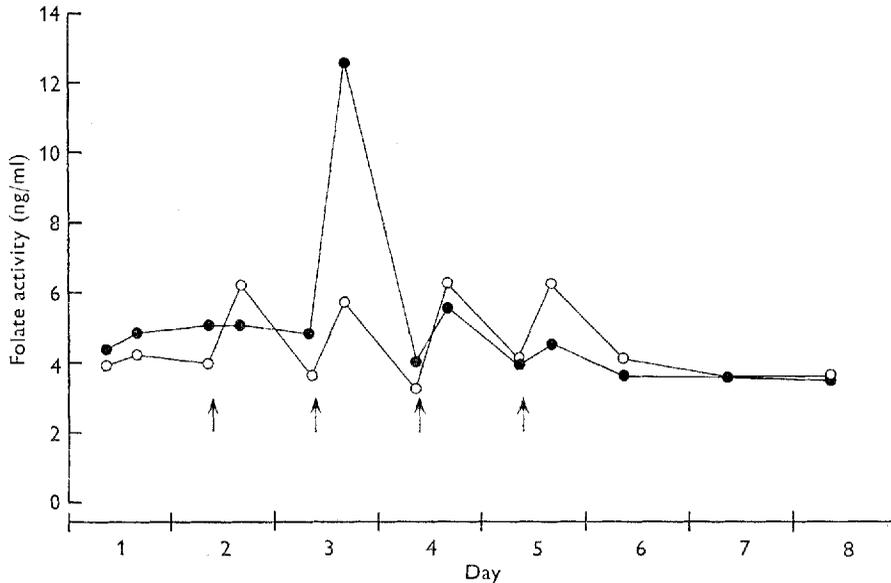
Fig. 5. Influence of parenteral folic acid on the folate activity in milk (●——●) and blood plasma (○——○) of a goat. Folic acid (2 mg) was injected intramuscularly on days 2–5, immediately after the morning samples of blood and milk had been taken, as indicated by the arrows.

and immature milk and increases to a sustained high level around 50 ng/ml in mature milk (Karlin, 1967).

The results shown in Fig. 2 are for goats that had not been milked *pre-partum*. Fig. 3 shows, for comparison, the results obtained with four multiparous goats that had been milked-out up to near the date of parturition. Two of the animals, 370 and 281, were milked daily until 4 weeks before parturition, and then two or three times weekly. Goat 351 was milked daily until 1 week before parturition, and twice daily for the 2 d before parturition. For three of these four goats the folate concentrations in the colostrum were little higher than those in the mature milk. For the remaining goat, no. 370, the peak in the folate content at parturition was more pronounced, but still low by comparison with the 'normal' values shown in Fig. 2. It seems reasonable to conclude that the different pattern of folate secretion in these four animals was caused by the pre-partum milking, and that the high folate concentration in normal colostrum is not determined by the event of parturition.

Folate content of the kid's blood

The folate in human milk is efficiently absorbed from the gut (Ghitis & Tripathy, 1970) and the results set out in Fig. 4 indicate that the same is true for goat's milk. The figure shows the pattern of changes in the folate concentration in the blood of six kids of the goats whose milk folate values are illustrated in Fig. 2. At parturition the plasma folate concentration was very low – about 1 ng/ml – but by day 2 it had increased to about 28 ng/ml. This increase was only transitory and by day 7 the plasma folate had

Table 1. *Influence of parenteral folic acid on the*
folate activity of blood and milk

(20 mg folic acid were injected intramuscularly at parturition, and again at 7, 14 and 21 d.
Samples of milk and blood were taken before each injection)

	Folate activity (ng/ml)				
	Day 1	Day 2	Day 7	Day 14	Day 21
Goat J-257; primiparous, not milked *pre-partum*					
Milk	136	78	10	5·5	2·3
Blood	4·4	6·0	4·7	4·7	4·9
Blood plasma	5·2	6·8	3·6	3·7	3·7
Goat K-294; multiparous, milked *pre-partum*					
Milk	4·0	4·5	5·1	5·2	4·0
Blood	4·0	5·5	5·5	4·5	5·3
Blood plasma	2·4	3·4	4·5	3·2	3·8

fallen again, to about 2 ng/ml. At day 28 the value again increased, to about 5 ng/ml. This same general pattern was also found in several kids that were not included in the present experiment, and the small rise in blood folate at day 28 was clearly significant. It presumably reflected the transition to a solid diet and the establishment of the rumen function.

Influence of parenteral folic acid on milk and blood plasma folate activity

Metz, Zalusky & Herbert (1968) observed that in lactating women with folate deficiency severe enough to provoke megaloblastic anaemia, orally administered folic acid was taken up by the milk in preference even to the haemopoietic system. It seemed unlikely, therefore, that the low folate content of mature goat's milk could be explained in terms of depletion of the maternal stores of available folate, and adverse equilibrium between uptake of folate from the gut and physiological demand. However, to test the possibility, 2 mg folic acid were administered by intramuscular injection on each of 4 successive days, and the effects on blood and milk folate concentrations determined. The results of the experiment are shown in Fig. 5. The injected folic acid had only a fleeting influence on the plasma folate concentration. After 6 h the plasma concentrations had increased by about 2 ng folate/ml, equivalent in the whole body plasma to less than 0·5 % of the injected dose. After a further 17 h the concentration had fallen again to the initial value. This same sequence was observed after each of the four injections. The red cell folate was unchanged during the experiment.

The effects of repeated doses of folic acid on the milk folate concentration were less uniform. The first injection caused no increase in milk folate at the afternoon milking. This recalls the finding by Metz et al. (1968) that it takes up to 48 h for parenterally injected folic acid to appear in human breast-milk, suggesting that the vitamin is first incorporated in the mammary columnar epithelial cells and then becomes part of the apocrine secretion. The second injection was followed by a sharp increase, from 4·6
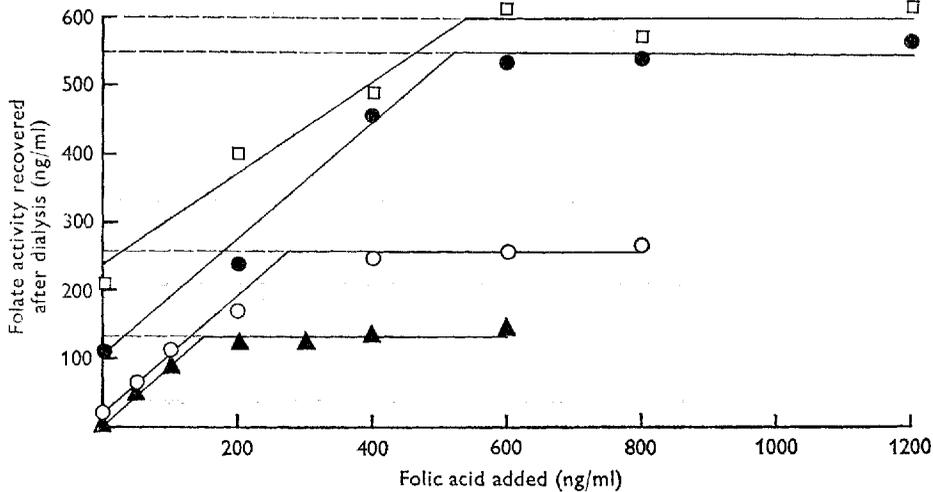
Fig. 6. Binding of folic acid added to goat's colostrum and milk. Goat 380. □, colostrum, day 1; ●, milk, day 2; ○, milk, day 7; ▲, milk, day 30. The intercepts of the dotted lines on the vertical axis show the amounts of folic acid activity retained per ml of sample after prolonged dialysis.

to 12·6 ng/ml, representing in the afternoon milk yield about 1 % of the injected dose. The subsequent injections caused smaller increases in the milk folate concentrations; there was no indication of a sustained increase. The effects of the injections were transitory, as they were on the plasma folate concentration.

In a further experiment, 20 mg folic acid were injected intramuscularly into two goats, at parturition and again at 7, 14 and 21 d. Samples of milk and blood were taken before each injection and assayed for folate activity. The results are shown in Table 1.

In goat J-257 the milk folate concentration showed the typical precipitous fall during the early days of lactation, despite the massive injections of folic acid, levelling off at a concentration broadly similar to that in the blood plasma.

Goat K-294 had been regularly milked-out up to the day of parturition, and so the folate concentration in its colostrum and milk was uniformly low. As with goat J-257, the injected folic acid had little influence on the milk folate concentration, which again was broadly similar to that in the blood plasma. From these results it is clear that the normal pattern of decline in milk folate concentration with advancing lactation was little affected by the provision of folic acid parenterally in relatively large amounts. Johns, Sperti & Burgen (1961) demonstrated that the body tissues extract folic acid from the blood plasma with great avidity, and this high capacity of the tissues to accumulate and bind folic acid probably explains the relative ineffectiveness of parenteral folic acid in increasing the milk folate level.

It may be that larger doses of folic acid would have caused a sustained increase in the plasma and milk folate concentrations. The test doses were chosen on the assumption that the daily folate requirement of the goat is about 200 μg, a 'guesstimate' which assumes that the requirement per kg body-weight is similar to that in a lactating woman. On this basis the injections of 2 mg were perhaps already
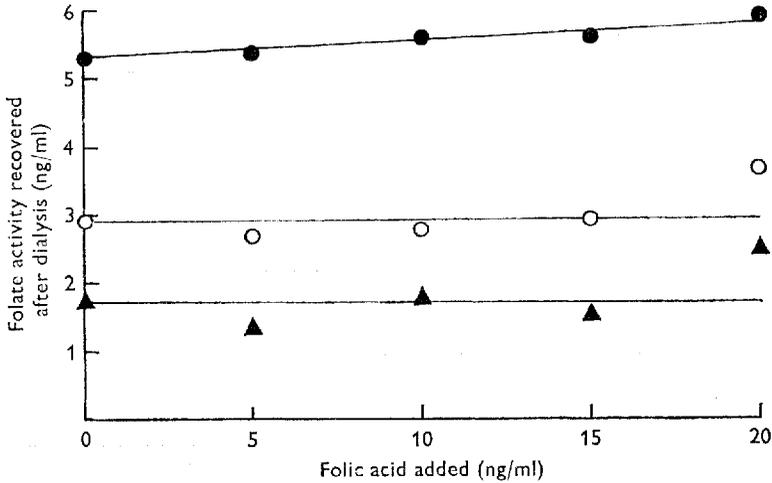
Fig. 7. Absence of folic acid-binding activity in samples of goat's blood plasma, taken at intervals after parturition. Goat 380, ●, day 1; ○, day 7; ▲, day 30.

on the high side, and the 20 mg injections could be regarded as being grossly unphysiological. It is planned to repeat the experiment, but with the folic acid given by continuous intravenous irrigation at about 50 μg/h.

Folic acid binding capacity of milk and blood plasma

A further possible explanation for the rapid fall in the folate content of the milk during early lactation is that there is a corresponding fall in the content of folate-binding protein in the milk, and therefore in the capacity of the milk to accumulate folate from the blood plasma against a concentration gradient (cf. Ford *et al.* 1969). Fig. 6 shows the capacity of colostrum taken at parturition, and of milk taken at intervals up to the 30th day of lactation, to bind added folic acid.

The colostrum had the capacity to bind about 600 ng folate activity per ml, and folic acid added in excess of this threshold concentration was not retained against dialysis. The natural folate in the colostrum was also bound, and wholly retained in the dialysed sample. Thus, the colostrum contained a high concentration of bound folate, and also a considerable excess of folate-binding protein. With advancing lactation the folate content of the milk declines, as did also the capacity of the milk to bind added folic acid. By day 30 the natural folate concentration had fallen from 210 to 5 ng/ml, and the folate-binding capacity from 600 to 130 ng/ml. However, the mature milk still contained more folate binder than did cow's milk, and so the relatively very low folate content was not wholly explained simply by the lower folate-binding capacity.

The presumption that the physiological role of the folate binder in milk is to trap folate from the blood plasma implies that some or all of the plasma folate occurs in the free form, and is not already firmly bound in protein. Fig. 7 shows that the plasma, unlike the colostrum and milk, had no capacity to bind added folic acid. However, the plasma folate was largely protein-bound, like that in the milk. The total folate activity

Table 2. *Folate activity in the blood plasma and mature milk of cows and goats*

	Plasma folate (ng/ml)		Milk folate (ng/ml)	
	Range	Mean	Range	Mean
Goats (9)	0·9–6·8	3·1	5·5–13·0	9·0
Cows* (9)	16–39	26	30–80	55

Figures in parentheses are the numbers of animals.
* Ford and Scott (unpublished).

Table 3. *Free and bound folate, and folate-binding capacity, in samples of blood plasma of kids taken at parturition before suckling, and at 2 and 21 d*

	Plasma folate concentration (ng/ml)		Added folate
Time of sampling	Total	Bound*	bound (ng/ml)
At parturition	1·9	1·3	0
2 d	58	58	24
21 d	2·4	2·1	0

* Measured after prolonged dialysis of the plasma sample (see p. 574).

in the day 1 sample was 5·5 ng/ml, and this was not significantly reduced after 2 d dialysis. With the day 7 sample, dialysis reduced the folate activity from 7·1 to 3·0 ng/ ml, and with the day 30 sample from 3·4 to 1·8 ng/ml. The content of folate binder in the plasma, like that in the milk, declined with advancing lactation, but there was no evident relationship between the concentration of free folate in the plasma and the folate content of the milk, or between the proportions of free and bound plasma folate and stage of lactation.

These findings provide no support for the concept that folate binder acts as a trapping mechanism that accumulates free folate from the plasma into the milk. However, it must also be taken into account that the colostrum is secreted comparatively slowly, and it may be supposed that there would be ample time for its folate binder to accumulate a high concentration of folate, even though 1 l of colostrum would clear the free folate from several hundreds of litres of blood plasma.

The rapid fall in the milk folate concentration during the few days after parturition may be largely attributable to dilution of the initial folate content. After parturition the milk is secreted at a faster rate and its folate content might be limited by the availability of free folate in the plasma, or by the rate at which plasma folate taken up into the milk is replenished from the body stores. In this connexion, it is of interest to compare the folate content of the blood plasma and mature milk of cows (Ford and Scott, unpublished) with the corresponding values for the goats (Table 2). The differences in the milk concentrations were matched by corresponding differences in the plasma folate concentrations. Furthermore, the comparatively high concentration in the cow's blood plasma was very largely present as free folate. For three of the cows the plasma folate concentrations were 22, 17 and 16 ng/ml; after dialysis of the plasma the corresponding values were 3, 4 and 5 ng/ml. Thus, from these findings it seems

Table 4. *Whole body and liver folate in four male kids, two of which had been killed at parturition before suckling, and two after suckling for 3 weeks*

| | | | Folate (μg) in | | | | |
| | | | Body* | | Liver | | Gut |
Kid no.	Time of killing	Live body-weight (kg)	Per g	Total	Per g	Total	contents, total
397	At birth	4·23	0·11	357	2·05	231	—
430	At birth	4·55	0·11	400	1·85	228	—
261	At 3 weeks	10·90	0·08	604	1·08	412	133
297	At 3 weeks	8·90	0·07	454	0·96	237	259

* Sum of folate in liver and skinned carcass, minus folate in gut contents.

that the milk folate concentration is primarily determined by the rate of secretion in relation to the availability of free folate in the blood plasma, and that the milk's content of folate binder does not limit the milk folate concentration. It also seems probable that the binder protein in colostrum and milk is of local origin within the mammary gland. Alternatively, it might conceivably be concentrated from the blood plasma, like the immune globulins, but if this were so, then the high degree of unsaturation with folate would remain to be explained.

Intestinal absorption of bound folate in the newborn kid

During a period lasting only a few hours from birth, the gut of the newborn ruminant transmits intact protein freely and non-selectively, and it is believed that the young animal receives into its circulation all the proteins that occur in the colostrum. The folate–protein is clearly no exception, since the high concentration of folate in blood plasma of 2-d-old kids was protein-bound and wholly retained in the plasma during prolonged dialysis. Table 3 shows the content of bound folate, and the folate-binding capacity, in samples of blood plasma taken from a kid at parturition before suckling, and at 2 and 21 d. The high concentration of bound folate at day 2 was accompanied, as in the milk, by a high concentration of free folate binder. At day 21 the plasma, unlike the milk (see Fig. 6), contained no free binder, and it seems that the period during which the intact binder is taken up from the gut is limited, though whether by 'cut off' in absorption or by destruction of the binder by the growing population of rumen micro-organisms is not evident from this experiment.

Whole body folate in the kid

Table 4 shows the whole body and liver folate in four male kids, two of which had been killed at parturition before suckling and two after suckling for 3 weeks. At birth the total liver folate averaged 230 μg, and comprised about 60 % of the total content of the body. The liver content at 3 weeks averaged 325 μg, but the concentration had fallen from 1·95 to 1·02 μg/g. For comparison, the folate concentration in the livers of six castrated adult male goats ranged from 5·5 to 9·8 μg/g, and averaged 6·9 μg/g.

The whole body folate content increased from 379 to 529 μg during the 3-week period. Some part of this increase was no doubt attributable to the unexpectedly

high level of ruminal synthesis in the 3-week-old kids, but the intake of folate with the colostrum and early milk could have been more than enough to account for the increase. If we assume an average milk yield of 2·2 kg/d during the 1st week of lactation and a folate concentration of 70 μg/kg, then the total secretion of folate during this week was 1080 μg. Since each of the kids was one of twins it would have received about 500 μg – rather more than the whole body folate at birth.

The above estimates are only approximate, but the comparatively small average net increase of only 150 μg in the whole body folate during the 3-week period in which their weight had more than doubled, and the sharp fall in liver folate concentration, suggest that folate balance in the kid is precarious until the rumen function is established. Any marginal deficiency in folate would be aggravated by pre-partum milking, whose effect is to deprive the kid of most of the folate that it would normally receive with the colostrum and early milk.

General

The folate binding in milk exemplifies a type of passive trapping process that is already familiar from the literature on the protein binding of vitamin B_{12} in milk (Gregory, 1954; Gregory & Holdsworth, 1955) and of steroid hormones in blood (cf. Jensen & Jacobson, 1962; Briggs & Brotherton, 1970). It may be that folate binder occurs in all the body tissues and constitutes the mechanism of folate homoeostasis. Similarly, in the gut, the uptake of folate may be mediated through the agency of folate binder, and the relative avidities of different natural folates for the binder could determine the differences (cf. Butterworth, 1968) in their biological availability. These questions remain to be answered; the possible importance of folate binder in the regulation of folate metabolism and in nutrition will no doubt encourage further research.

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