

## Iron-deficiency anaemia in calves

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It is well known that milk is a poor source of dietary iron for almost all species. Experiments with calves restricted to a ration of whole milk have shown that anaemia develops after a time and can be prevented by the addition of Fe to the ration (Cannon, 1931; Knoop, Krauss & Washburn, 1935; Herman, 1936). Haematological studies of calves in two herds at Beltsville, Maryland, showed that low concentrations of haemoglobin in the blood occurred under reasonably normal systems of calf management in which milk, concentrates and unlimited alfalfa (lucerne) hay were given (Thomas, Okamoto, Jacobsen & Moore, 1954). Supplements of Fe given to moderately anaemic calves caused an increase in the blood haemoglobin. It may be emphasized that these calves had access to both grain and roughage: they were not restricted to milk alone.

Our experiments have been carried out to find whether in the herds of beef cattle of north Scotland anaemia, induced by Fe deficiency, was a practical problem. The husbandry of calves in this area has already been described (Sharman, 1954). The rearing system is characterized by a large daily intake of milk, and the intake of solid food is usually small. Further, the pens and stalls in which these calves are kept are usually of wood: there is little adventitious Fe the calves could ingest by licking or chewing their surroundings. Our experiments were of two types. Firstly, a small group of calves was reared at the laboratory on milk as the sole food, the development of anaemia being studied in detail to provide information on what to expect in mild anaemia and in severe anaemia. Secondly, extensive experiments were carried out on commercial farms in the north of Scotland.

### EXPERIMENTAL

#### *Laboratory experiment*

*Animals and housing.* Six Ayrshire bull calves were housed in wooden pens with wooden slatted floors, and all screws and nails in construction countersunk. No bedding was allowed, and the pens were washed each day with tap water. Clinical records, including pulse rates, were taken daily. Each animal was killed after at least 18 weeks of experiment and was examined *post mortem*.

*Ration.* Cows in late lactation were milked by machine and the milk was transferred, without cooling, into acid-washed glass bottles. The calves were given this milk as the sole food in plastic (Polythene) buckets, which were washed with distilled water after use. Values for the contamination of the milk by Fe when these precautions were used are given later. The diet of milk was supplemented daily with 25 mg  $\alpha$ -tocopheryl acetate and 5 ml. of a solution of  $MgCl_2$  supplying 200 mg magnesium. Each calf was given 25 mg Cu as sulphate once monthly. Two calves (nos. 264 and 266) were given a daily supplement of 20 mg Fe as ferric citrate in aqueous solution. Four were given no Fe supplement; two of these (nos. 262 and 263) were given daily 2.5 g  $CaCO_3$  as a supplement, since the findings of Greig (1952) indicated that in mice the addition of  $CaCO_3$  to the diet led to anaemia that could be cured by Fe administration. No such effect of calcium supplementation was found in the calves. One calf given the Fe supplement (no. 266) was also given additional  $CaCO_3$ . The milk intake was increased with age from 4 to 12 l. daily. Records of refusals of milk were kept. After 134 days on the deficient ration, calf no. 262 was given 50 mg Fe daily in a curative test. The tissues of all the calves were analysed for Fe. Bone marrow and spleen were examined histologically.

*Analytical methods.* Fe was determined with the  $\alpha$ - $\alpha'$ -dipyridyl reagent. The methods for whole blood and serum were those of Ramsay (1952, 1953). The Fe content of milk and colostrum was determined on ashed material. Haemoglobin was determined as carboxyhaemoglobin in dilute ammonia solution by measuring its absorption at 540  $m\mu$  spectrophotometrically. The Fe content of the whole blood was simultaneously determined by the method of Ramsay (1952, 1953).

When the spectrophotometer reading was plotted against the Fe content of the whole blood, there was complete concordance over the range 14–48 mg Fe/100 ml. which is equivalent to 4–14 g haemoglobin/100 ml. The Fe content of crystalline calf haemoglobin was found to be 0.34%, and so both the Fe determinations and the spectrophotometric readings could be converted into g haemoglobin/100 ml. blood. Calcium and magnesium were determined by the method of Godden (1935). Potassium was determined in the red blood cells, after washing them with dilute sucrose solution, by the method of Barry & Rowland (1953).

*Haematological methods.* Red cells and white cells were counted in a haemocytometer. The packed-cell volume was determined in haematocrit tubes centrifuged for 1.5 h at 3000 r.p.m. (2200 g). Blood films were fixed and stained with Leishmann's stain.

#### *Field experiment*

Fifteen farms co-operated in the field experiment and provided ninety-two calves for experimental purposes. On each farm the calves, which were Aberdeen Angus or Aberdeen Angus crosses, were allocated at birth, either to receive no treatment or to receive 35 mg ferrous iron daily supplied as ferrous succinate. Late-born calves that had received treatment for less than 14 days were not included in the final analysis of results. Blood samples were taken from each calf at least twice in the course of the experiment, and the haemoglobin content, erythrocyte count, packed-cell volume and plasma protein were determined. The plasma protein was determined by the copper

sulphate-specific gravity technique (Hawk, Oser & Summerson, 1947) and the results were used to find if haemoconcentration or haemodilution had taken place. Heparin was used as anticoagulant. Samples of blood were taken from a limited number of pairs of calves at 3-week intervals throughout the experiment.

## RESULTS

*Laboratory experiment*

*The iron content of milk and colostrum.* In order to find what precautions had to be taken in the laboratory experiment to minimize contamination of milk with Fe, milk samples were collected at various stages of the handling process. Typical results are given in Table 1.

Table 1. *Contamination of milk with iron at various stages of milk handling*

History of sample	Fe content of milk (mg/l.)	
	Cow no. 1	Cow no. 2
(1) By hand milking after washing the udder and hands with distilled water	0.347	0.251
(2) After machine milking, normal method	0.409	0.289
(3) After cooling (2) over a tinned-copper surface cooler	0.439	0.334
(4) After storing (3) in a tinned milk churn	0.614	0.483
(5) After leaving (3) in a 1 gal. galvanized pail for 1 h	0.485	0.378

It appeared that machine milking with a milking plant constructed mainly of aluminium gave rise in this instance to a 15% increase in the amount of Fe in the milk. A series of seven tests gave for the Fe in hand-drawn milk mean values of 0.237 and in machine-drawn milk of 0.290 mg/l. The mean difference of  $0.053 \pm 0.010$  mg/l., representing an increase in Fe content of 22%, was highly significant statistically. The process of cooling the milk caused a further increase, and churn storage increased the Fe content by 80%. The normal process of calf-feeding in machine-milked herds in which milk is placed in a bucket with or without cooling would provide the calf with about 50% more Fe than its dam does.

It was noted that colostrum contained considerably more Fe than did milk from cows in mid to late lactation. Individual samples of uncontaminated colostrum contained as much as 0.86 mg Fe/l., the mean for ten samples from five cows being 0.51 mg/l. Uncontaminated mid-lactation milk contained from 0.17 to 0.30 mg/l. The high concentration of Fe in colostrum was partly due to the presence of cells. The deposit from centrifuging colostrum had invariably a high Fe content, even though no red blood cells were present. Red blood cells were present, however, in the sample containing 0.86 mg/l.

The precautions observed in taking milk for the experiment were based on these results. Some Fe additional to that produced by the cow—about 25%—was clearly present, but occasional analysis of the milk showed that the Fe content was seldom above 0.45 mg/l. More usually the content was about 0.3 mg/l. The value for normal

milk obtained in these experiments was somewhat lower than those quoted in the literature for similar samples (Johnston, Gellman & Strom, 1948). It was distinctly lower than the values given in food tables, 0.7 mg/kg (United States Department of Agriculture, 1945) or 0.8 mg/kg (McCance & Widdowson, 1946). Clearly considerable Fe contamination must have taken place in the commercial milk samples quoted in these food tables.

*Clinical observations.* The daily gains in body-weight of the six calves are given in Table 2.

Table 2. *Gains (kg/day) in weight of the calves in the laboratory experiment*

Period	Calves given Fe		Calves not given Fe			
	No. 264	No. 266	No. 260	No. 261	No. 262	No. 263
Birth-6 weeks	0.31	0.38	0.34	0.35	0.30	0.40
6-12 weeks	0.66	0.69	0.49	0.57	0.45	0.42
12-18 weeks	0.72	0.59	0.10	0.07	0.34	0.16
18-24 weeks	Killed	0.59	Killed	Killed	Curative test	0.19
					Killed	Killed

During the first 6 weeks the calves given Fe and those given none grew normally and at the same rate of 0.34 and 0.35 kg/day respectively. In the second 6-week period the control calves gained an average of 0.67 kg/day, and the deficient calves 0.49 kg; in the third 6-week period the controls gained 0.66 kg and the deficient calves only 0.17 kg. One control animal was killed at 18 weeks of age. The other was given the supplemented diet of whole milk for 30 weeks when the experiment was terminated, the calf then weighing 137 kg. The calves given no Fe were killed when severe clinical signs had developed. One calf (no. 261) was killed when 16 weeks of age, one (no. 260) at 19 weeks, one (no. 263) at 23 weeks and one (no. 262) at 27 weeks.

The earliest sign of abnormality was a disinclination to drink the milk diet. Refusal of part of the day's allowance was common and clearly contributed to the slow gain in weight. Later, scours developed that were often refractory to treatment with antibiotics. Acidification of the milk with dilute hydrochloric acid was effective in reducing the diarrhoea. The mucous membranes of the mouth were very pale indeed at this stage, and the papillae of the tongue tended to be smooth compared with those of the control calves. The conjunctiva was markedly pale. Small crater-like ulcers appeared on the tongues and gums of two of the calves (nos. 261 and 260). Pulse rates taken at rest were the same in control and deficient calves. Attempts were made to produce similar circulatory stress in the calves by controlled exercise. This proved difficult, since the Fe-deficient calves were reluctant to move, whereas the calves receiving Fe were extremely boisterous. When the deficient calves were exercised successfully frothing at the mouth occurred, the pulse rate was increased to over 250/min and did not return to normal within 40 min. Under similar conditions, the pulse rate of control calves did not exceed 200/min and had returned to the pre-exercise value in 20 min. The final stage of the disease was a progressive anorexia: the animals were then killed.

*Haematological studies.* The results of the haematological studies are summarized in Table 3.

Table 3. *Erythrocyte count, packed-cell volume and haemoglobin content of the blood of calves in the laboratory experiment*

Age (weeks)	Calves given Fe		Calves not given Fe				Normal calves (Holman, 1956)
	No. 264	No. 266	No. 260	No. 261	No. 262	No. 263	
Erythrocyte count ( $\times 10^6$ mm <sup>3</sup> )							
At birth	10.24	10.05	9.41	8.28	9.34	10.88	9.8
6	9.69	9.74	9.36	7.55	8.95	10.45	9.4
12	8.64	10.02	9.12	7.08	8.67	8.24	9.5
18	9.01	10.70	6.68	6.89	7.24*	6.60	—
23-24	—	9.20	—	—	8.82	6.35	8.04
27	—	—	—	—	11.67	—	—
Packed-cell volume (%)							
At birth	38.4	36.7	36.0	33.8	38.2	44.8	42.5
6	32.3	33.1	26.8	25.3	26.7	36.2	29.0
12	28.4	27.1	15.8	18.0	19.9	23.1	34.1
18	31.8	27.5	12.9	16.2	14.1*	17.5	—
23-24	—	27.8	14.3	—	26.0	13.3	36.0
27	—	—	—	—	29.3	—	—
Haemoglobin (g/100 ml.)							
At birth	11.43	9.79	10.37	9.59	11.73	12.69	12.9
6 weeks	9.72	9.35	8.84	9.43	7.73	9.64	10.5
12	8.05	7.72	4.38	5.59	6.01	6.63	11.7
18	8.41	7.90	3.47	4.54	3.99*	4.38	—
23-24	—	7.80	3.52	—	6.99	4.17	10.6
27	—	—	—	—	8.91	—	—

\* Given 50 mg Fe daily after 18 weeks.

The table shows that a severe anaemia was produced in the calves given no Fe, and the single test with calf no. 262 showed that iron cured the anaemia. The packed-cell volume and haemoglobin content of the blood of the control calves fell with age, most of the fall occurring in the first 8 weeks of life. This fall has been observed by others and has been regarded as normal (Greator, 1954; Holman, 1956). The haemoglobin content of the blood of the control calves was, however, rather lower than expected from the results of Thomas *et al.* (1954), who found that in calves given 140 mg Fe daily the haemoglobin content of the blood was in the region of 10.0 g/100 ml. Values of 8.2-9.0 g/100 ml. have been recorded by Holman (1956) in apparently healthy calves. There was little doubt, however, that the calves receiving no Fe supplement were grossly anaemic. The type of anaemia may be inferred from Table 4. The volume of individual erythrocytes dropped by 50% in the deficient animals, but there was no fall in the concentration of haemoglobin in each corpuscle. The anaemia was thus of the microcytic, normochromic type, with a reduction in the number of blood corpuscles. Fe-deficiency anaemias in man are usually microcytic and hypochromic.

In Herman's (1936) experiments, in which three calves developed anaemia when given a ration of whole milk for 300-500 days, the haematocrit and haemoglobin values also suggest that the anaemia was normochromic. The packed-cell volumes

obtained by Herman were, however, considerably greater than ours (40–60% in normal calves and 25–40% in calves given whole milk).

Table 4. *Mean erythrocyte volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration of normal and anaemic calves at 18–24 weeks in the laboratory experiment*

Calf no.	Mean erythrocyte volume ( $\mu^3$ )	Mean corpuscular haemoglobin ( $\mu\mu\text{g}$ )	Mean corpuscular haemoglobin concentration (g/100 ml. cells)
Calves given Fe			
264	35.2	0.93	26.4
266	30.2	0.85	28.1
262*	25.1	0.76	30.4
Calves not given Fe			
260	19.3	0.53	24.6
261	23.5	0.65	27.8
262	19.4	0.55	28.2
263	20.9	0.66	31.3

\* After curative test of 6 weeks.

In Fig. 1 are compared the haemoglobin content, cell count and erythrocyte volume of the blood of the calves given no Fe, of the calves given 20 mg Fe and of nine calves in the field experiment given 35 mg Fe daily. Blood was taken from this last group only once every 3 weeks. It will be noted that the haemoglobin content of the blood of calves in the field given iron remained in the region of 10 g/100 ml., whereas the values for the control calves were stabilized at about 8 g. These results suggest that 20 mg Fe was insufficient for calves given virtually uncontaminated milk, growing rapidly and subject to weekly removal of about 25 ml. blood (equivalent to 1.0 mg Fe/day).

From Fig. 1, it appears that Fe-deficiency anaemia in growing calves takes place in two stages. The initial fall in the haemoglobin content of the blood appears to be due to an increase in the proportion of microcytes. After 11–12 weeks the total number of erythrocytes per  $\text{mm}^3$  blood begins to decline. At no stage does there appear to be a reduction of haemoglobin concentration in the erythrocytes. It will be noted that, even in the healthy normal calves given 35 mg Fe in the field experiment, a decline in the volume of the erythrocytes took place during the first 7 weeks. This slow fall presumably reflects the death rate of large erythrocytes present in the calf at birth and their replacement by erythrocytes of normal size. This is no doubt the so-called physiological change noted by Holman (1956).

The microcytes seen in the later stages of the anaemia, that is when the haemoglobin concentration was below 5 g/100 ml., showed marked poikilocytosis. Considerable care was taken to avoid fixation artifacts in making the films, and wet diluted mounts confirmed these findings. Cells of very bizarre shape were seen, a large number of very small cells and many with markedly uneven crenation. The number of leucocytes in the blood of all calves ranged from 7000 to 15,000/ $\text{mm}^3$ . There were no differences that could be ascribed to an effect of treatment.

*Chemical composition of blood.* The total Fe content of the blood was markedly reduced in the anaemic animals. The changes were exactly similar to those found for haemoglobin and are not reported further. There was no indication at any time of any significant difference between the two methods of assessing the amount of pigment. Serum Fe was initially high and fell in all calves to quantities too small to estimate with accuracy without taking larger quantities of serum than was thought advisable. The determinations were then discontinued. The concentration of Ca in the serum was unaffected by either the  $\text{CaCO}_3$  or the Fe supplement, but all calves given  $\text{CaCO}_3$

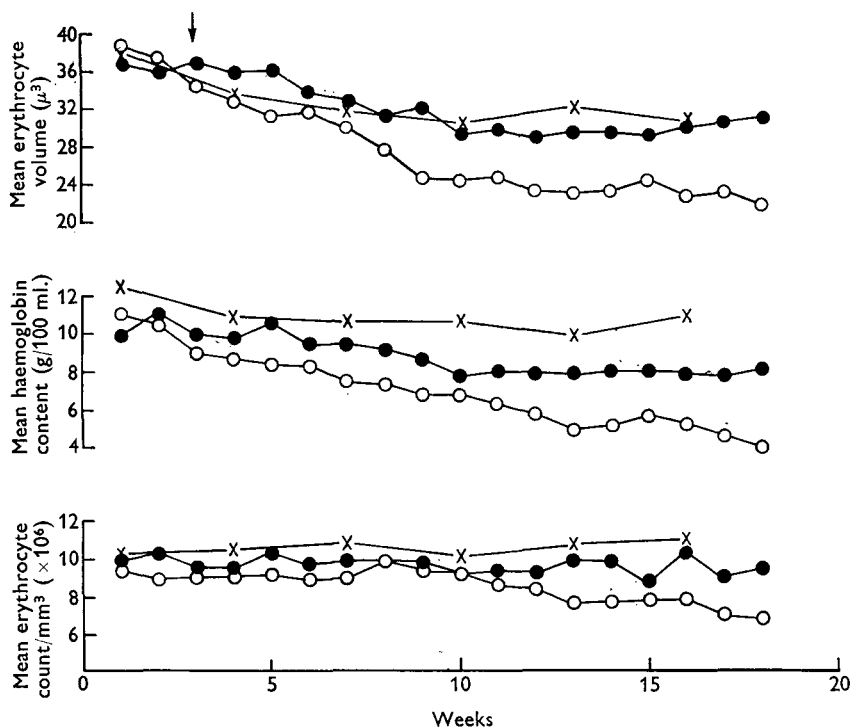


Fig. 1. Mean erythrocyte volume ( $\mu^3$ ), mean haemoglobin content (g/100 ml.) and mean erythrocyte count (count  $\times 10^6/\text{mm}^3$ ) in the blood of calves.  $\times-\times$ , nine calves in the field experiment given a supplement of 35 mg Fe daily;  $\bullet-\bullet$ , two calves in the laboratory experiment given a supplement of 35 mg Fe daily;  $\circ-\circ$ , four calves in the laboratory experiment given no supplement.

showed a depression in the Mg content of the serum. Thus, at 12 weeks, in the three calves given no supplement of  $\text{CaCO}_3$  the serum-Mg concentrations were 1.93, 2.55 and 2.01 mg/100 ml. In the three calves given  $\text{CaCO}_3$ , the concentrations were 1.40, 1.46 and 1.21 mg/100 ml. The Mg concentration in the serum of these calves was restored by doubling the amount of supplementary Mg given.

*Post-mortem findings.* Nothing abnormal other than pallor of the muscles and organs was noted in any of the calves at post-mortem. Even in those dosed with Fe there was no indication that the muscles were darker in colour. This was particularly evident in the calf allowed to survive for 30 weeks; it still retained the muscle coloration characteristic of very young animals. The Fe contents of the tissues are given in

Table 5. The normal values refer to Elvehjem & Peterson's (1927) analyses. The calves given no Fe supplement had lower concentrations of Fe in the liver, kidney and heart, but there was no obvious gross depletion of the Fe in the muscles or in the spleen. The spleen Fe represents in part the Fe moiety of erythrocytes undergoing destruction, and gross depletion would hardly be expected in this organ. It will be noted that the iron contents of the liver and kidneys of the calves given additional Fe were lower than the values given as normal by Elvehjem & Peterson (1927). This finding further suggests that the supplement of 20 mg Fe daily was not sufficient to remedy the Fe deficiency of cow's whole milk for the calf.

Table 5. *Concentration (mg/100 g dry matter) of iron in the tissues of calves in the laboratory experiment*

Tissue	Calves given 20 mg Fe		Calves not given Fe			Normal values for cattle (Elvehjem & Peterson, 1927)
	No. 264	No. 266	No. 260	No. 261	No. 262	
Liver	15.4	18.3	11.6	12.0	11.8	22.0
Kidney	24.3	24.2	17.1	14.7	14.7	29.0
Spleen	41.4	36.5	37.2	27.1	42.6	38.3
Heart	40.6	31.6	23.6	20.3	22.8	46.0
Longissimus dorsi	4.7	10.0	5.9	4.0	5.1	9-12
Rectus femoris	4.8	6.0	4.9	5.1	5.8	
Supraspinatus	11.0	10.1	7.5	5.9	6.4	
Infraspinatus	10.9	9.7	10.4	8.1	7.1	

*Histological findings.* In the control calves, the bone marrow showed a normoblastic reaction and normal cellularity. No free Fe was found in the spleen. In the Fe-deficient calf no. 260, there was a slight normoblastic reaction of the marrow but no free Fe was present. In the other Fe-deficient calves, nos. 261 and 263, the findings were similar. These results suggest that there were no abnormalities in the cellular reaction of the bone marrow, and certainly there was no cytological deficiency in the erythropoietic tissue. In calf no. 262, given additional Fe, the marrow showed a good normoblastic reaction. The spleen contained large amounts of free Fe but none at all was present in the bone marrow. This finding suggests that the anaemia has some aspects in common with one occurring after haemolysis of the erythrocytes *in vivo*, since free Fe would then be found in the spleen.

#### *Field experiment*

The results of the experiments are summarized in Table 6.

Statistical analysis of the results showed that in the first age group the difference between the haemoglobin contents of the blood of calves given Fe and of those given none was significant at odds of 14:1. In the age group 40-80 days, the difference was significant at odds of only 4:1. It may therefore be concluded that a mild anaemia was present in the calves that responded to iron administration, being particularly apparent during the 40-80th days of life. The extent of the anaemia was a 10% depression of the blood haemoglobin.

The other figures in Table 6 show that the anaemia, though mild, was similar to the



more severe anaemia produced by experimental Fe deficiency. Thus, the Fe supplement was without significant effect on the haemoglobin concentration in the erythrocytes, but had a statistically significant effect on the mean volume of the erythrocytes, so that the anaemia was microcytic and normochromic.

Table 6. *Effect of 35 mg Fe/day on the haemoglobin content of blood, the haemoglobin concentration in the erythrocytes and the mean volume of the erythrocytes of calves in the farm experiment*

Age of calves*	No. of pairs of calves	Hb content of blood (g/100 ml.)		Mean erythrocyte volume ( $\mu^3$ )		Hb concentration in erythrocytes (g/100 ml.)	
		No supplement	Fe supplement	No supplement	Fe supplement	No supplement	Fe supplement
		20-40 days	16	10.11	10.98	32.72	34.30
40-80 days	27	9.28	10.24	30.23	33.50	29.71	29.55
Over 80 days	8	11.00	11.27	30.83	32.95	29.95	29.60

\* Some pairs of calves occur in each group, when aged 20-40 days, and again when aged 40-80 or over 80 days.

More detailed analysis of the results, however, indicated that there were large differences in the haemoglobin concentrations in the blood of different calves even when they were given additional Fe. This is shown in Fig. 2 where the results for thirty-seven calves aged 40-80 days given iron are compared with those for forty-one calves given no supplement. The results are not paired: on some farms the pairs diverged in age by more than 20 days and in the results presented in Table 6 these were discarded in making the statistical analysis. The results indicate, however, that, although Fe administration reduced the proportion of calves with haemoglobin concentrations of less than 8.0 g/100 ml. from 44 to 13%, some calves, even when given supplements of 35 mg Fe daily, failed to maintain haemoglobin concentrations of 8.0 g/100 ml. blood. This peculiarity was clearly associated with particular farms. Thus, on one farm the haemoglobin content of supplemented calves aged 40-80 days averaged 14.1, whereas on another it averaged 8.8 g/100 ml. The analysis of variance given in Table 7 shows that differences between farms were highly significant, but there was no indication from the results as a whole that the animals on farms with

Table 7. *Analysis of variance of the haemoglobin content (g/100 ml.) of blood of calves in the farm experiment*

Component	Degrees of freedom	Estimated variance	$e^{2z}$
Total	53	—	—
Between farms	11	10.478	} 3.90**
Between pairs on farms	15	2.684	
Between total pairs	26	5.982	—
Effect of Fe dosage	1	12.317	} 6.05*
Interaction, farms $\times$ dosage	11	2.262	
Error term	15	2.034	1.11

\* Statistically significant when  $0.05 > P > 0.01$ .

\*\* Statistically significant when  $P < 0.01$ .

the more anaemic calves responded more to Fe therapy. In Fig. 3, the mean cell volume of untreated calves is plotted against the haemoglobin content of the blood, both being measured between the 40th and 60th day, and farm means being used for this purpose. On the same graph terminal values for deficient calves in the laboratory

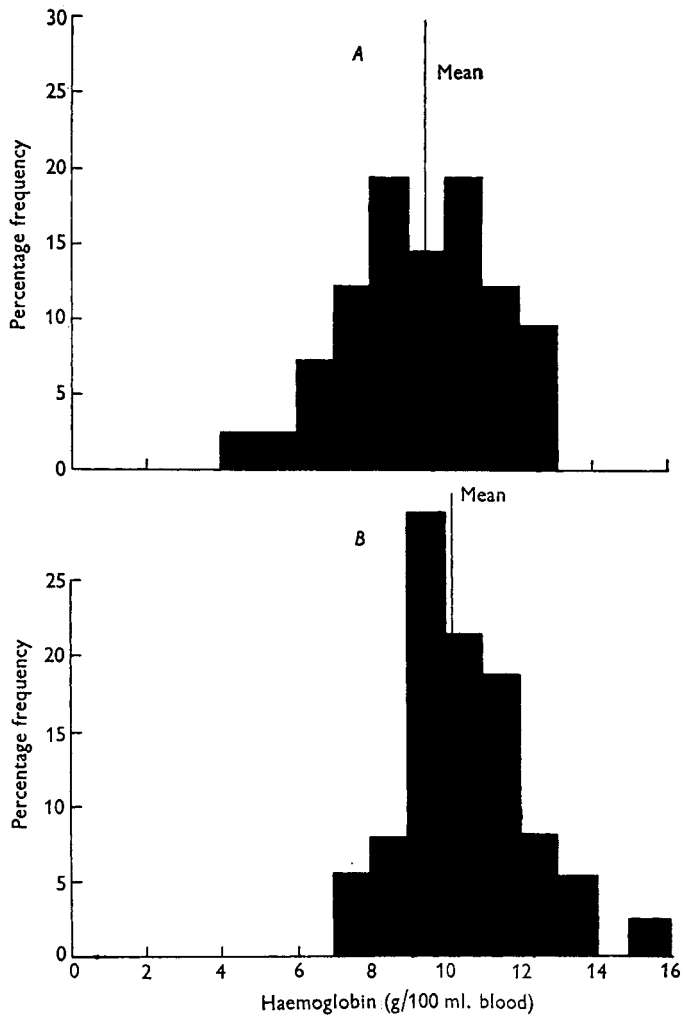


Fig. 2. Percentage frequency distribution of the haemoglobin content of the blood of (A) forty-one calves aged 40–80 days given no supplement, and (B) thirty-seven similar calves given an iron supplement.

experiment are plotted. It appears that the animals on farms with calves having a low blood haemoglobin content also had a low mean cell volume, which suggests that the mild anaemia on these farms was probably of the type that responds to iron dosage. In view of the fact that 20 mg Fe were not sufficient to maintain cell volume and haemoglobin concentration in the blood of calves in the laboratory experiment, it is possible that calves on many of these farms required more Fe than 35 mg daily.

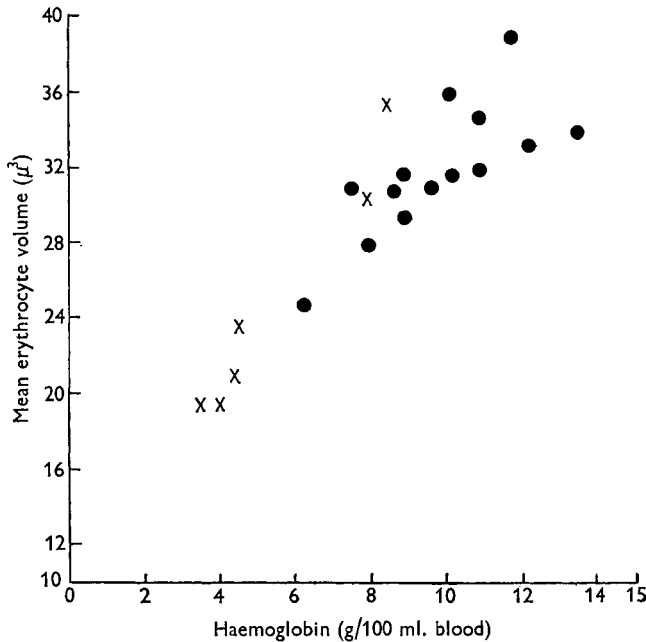


Fig. 3. Relation between the haemoglobin content of the blood and the mean volume of the erythrocytes of calves. ●, mean values for untreated calves on the fourteen farms; ×, values for individual calves in the laboratory experiment.

#### DISCUSSION

There is little doubt that Fe-deficiency anaemia was produced in the experimental calves at the laboratory, and that a mild Fe-deficiency anaemia exists under the normal conditions of calf rearing on farms in the north of Scotland. The anaemia is microcytic, normochromic and poikilocytic, it is associated with a normoblastic bone marrow and it responds to Fe therapy. The evidence suggests that 20 mg Fe daily for experimental calves or 35 mg Fe daily for calves on commercial farms is not sufficient to maintain normal haemoglobin levels. It is remarkable that under experimental conditions, when the unsupplemented calves received only 2.0–4.0 mg Fe in their daily ration and were subject once weekly to blood sampling and the removal of 25 ml. blood containing approximately 7–10 mg Fe, or 1 mg Fe daily, anaemia should have taken so long to develop. Nothing grossly abnormal could be detected during the first 6 weeks of life, and it was only after 2 months of experiment that differences between control calves and deficient calves became obvious. During this period, the calves had increased in body-weight by nearly 80%.

Bianca (1956), working with calves kept under conditions similar to ours, has shown that the total erythrocyte mass of the body is approximately 1.85% of body-weight. On the assumption that the normal haemoglobin content of the erythrocyte is 30% and the Fe content of haemoglobin 0.34%, the requirement of Fe for haemoglobin synthesis per kg gain in body-weight would be 19 mg. To this must be added the Fe contained in Fe-containing enzymes in the tissue cells and in the myoglobin of muscle.

This Fe may be estimated at approximately 20 mg/kg body-weight. The total requirement of a calf gaining 1 kg daily, apart from any need to store Fe in the liver or extra-hepatic tissue, is thus approximately 40 mg/day. If normal storage of the element is included, and the Fe content of tissue assessed at 15–20 mg/100 g dry matter, the requirement for Fe needs to be increased to 50–60 mg daily.

The calves given milk alone and growing at the rate of about 400 g/day thus required about 16–24 mg Fe and received 2–4 mg. The calves given an Fe supplement growing at a higher rate of 500 g daily required 20–30 mg and received 22–24 mg daily. The absorption and retention of dietary Fe can hardly be complete. Thus, in human babies, the maximal retention of dietary Fe noted by Stearns & Stinger (1937) was only 24%. If the same is true of the calf, requirements are probably in the region of 100 mg Fe daily.

It appears from the above calculations that the animals given Fe supplements in the laboratory experiment did not receive sufficient to meet their requirements and that the fall in cell volume and haemoglobin content of their blood may be referred to a dietary lack of Fe. The same is very likely the reason for the low haemoglobin concentrations in calves on some farms in the field, even when they were given 35 mg Fe daily. The fact that anaemia takes so long to develop in calves given diets low in Fe, compared with piglets, in which anaemia rapidly develops (Venn, McCance & Widdowson, 1947), must reflect both the smaller liver stores of the element in the pig, as suggested by McCance & Widdowson (1951), and the fact that the relative growth of the suckling pig is much greater than that of the calf. In 2 months, the weight of a piglet increases tenfold: a calf will normally have increased in weight by only 60–80% in that time (Brody, 1945; Braude, 1954).

#### SUMMARY

1. Iron-deficiency anaemia was produced in four calves under laboratory conditions on a milk diet supplemented with magnesium, copper and tocopherol. Two calves acted as controls, and received daily a supplement of 20 mg Fe as the citrate.
2. The anaemia was microcytic and normochromic and was associated with poikilocytosis and a normoblastic reaction of the bone marrow. Clinically, the anaemic calves showed poor gains of weight, an inability to withstand circulatory strain, atrophy of the papillae of the tongue and loss of appetite. The anaemia responded to Fe administration in the one calf to which the treatment was given.
3. A field experiment involving fifteen farms and forty-six pairs of suckling beef calves showed that daily dosage with 35 mg of Fe as the succinate resulted in a significant increase in the haemoglobin content of the blood and an increase in the mean cell volume.
4. It was shown that on different farms there exist different haemoglobin contents in the blood of calves and that calves with low haemoglobin concentrations usually have smaller mean cell volumes. It is shown that this 'physiological anaemia' is of the same type as Fe-deficiency anaemia.
5. Evidence is presented to show that a supplement of 20 mg Fe daily is not

sufficient for a calf aged 1-4 months receiving milk as the sole diet, and it is suggested that the requirement is nearer 100 mg daily. The daily iron intake in milk is usually 2-4 mg.

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