Iron metabolism in the veal calf. The availability of different iron compounds*

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(*Received* 21 *April* 1972 – *Accepted* 9 *August* 1972)

1. The haematological status of Ayrshire bull calves reared on fat-supplemented skim milk from about 17 d of age has been examined. The calves were moderately anaemic after an 11-week experiment and it was shown that their performance was related to their blood haemoglobin concentration.

2. The relative availabilities of iron from $FeSO_4$, ferric citrate, ferric-ethylenediaminetetra-acetate and iron phytate were compared at a supplementary dietary concentration of 30 μg Fe/g. No significant differences were noted between the three soluble Fe sources but the Fe of the insoluble iron phytate was less available.

3. The decreases in blood haemoglobin concentrations in all calves were greatest in the early weeks of the experiment. In calves given supplementary Fe, however, there was generally an improvement in haematological status after 6 weeks. Plasma Fe concentrations fell to 0·20 μg/ml in the most deficient animals. Total Fe binding capacities averaged about 10 μg Fe/ml in the later stages of the experiment.

4. Some of the calves were copper-deficient, and in some animals the effects of dietary Cu supplementation on both Cu and Fe metabolism were studied. About 50% of the supplementary Cu was retained in the livers of the calves.

5. A dietary intake of 40 μg Fe/g milk powder appears to be sufficient to prevent all but a very mild anaemia, provided the Fe is presented in soluble form.

In the Brambell Report (Brambell, 1965) it was recommended that in the intensive production of veal calves 'the diet should be such as to ensure normal levels of haemoglobin . . .' and that 'the diet should be so reinforced with iron in a suitable form as to ensure that on a normal intake the animal is in no wise deficient in this element'. Implementation of these recommendations has been complicated by the absence of knowledge of what constitutes 'normal' haemoglobin (Hb) levels, of what constitutes a 'suitable' form of Fe and of the criteria by which a state of deficiency should be assessed.

The rearing of young calves for veal solely on milk-based rations can result in the development of a state of anaemia. The anaemia can be readily prevented by supplementing the ration with Fe, given by mouth or parenterally, and some assessment has been made in the past of the Fe requirements of such calves (Blaxter, Sharman & MacDonald, 1957; Matrone, Conley, Wise & Waugh, 1957; Roy, Gaston, Shillam, Thompson, Stobo & Greatorex, 1964). Most of these assessments have been made on calves maintained on whole milk, and it is uncertain how applicable these estimates are to present commercial practice where the diets generally consist of fat-supplemented skim-milk preparations. Such diets generally produce greater weight gains than whole milk in calves and therefore the absolute requirements for Fe will also be greater (Roy *et al.* 1964).

* A preliminary report of this work was published in *Proc. Nutr. Soc.* (1972), **31**, 75A.

Table 1. *Composition of milk-substitute powder (g/kg)*

Oil	240
Protein	265
Fibre	0
Ash	70

Supplements

Vitamin A (μg retinol equivalent/kg)	4800
Cholecalciferol (μg/kg)	100
Vitamin E (mg α-tocopherol equivalent/kg)	40
Thiamin (mg/kg)	3
Riboflavin (mg/kg)	10
Pyridoxine (mg/kg)	3
Pantothenic acid (mg/kg)	10
Nicotinic acid (mg/kg)	20
Ascorbic acid (mg/kg)	250
Cyanocobalamin (μg/kg)	30
Magnesium (mg/kg)	571
Manganese (mg/kg)	28
Zinc (mg/kg)	30

In this paper, the availability of various Fe sources to calves maintained under conditions resembling those in commercial practice is assessed by comparison of the haematological status and tissue Fe concentrations of the animals. In addition, as commercial milk preparations frequently contain no supplementary copper, the Cu status of the calves was determined. In some animals the effects on both Cu and Fe metabolism of supplementation of the ration with Cu was examined.

EXPERIMENTAL

Diets and supplements

The diet, obtained by spray-drying an emulsion of skim milk and fat, was manufactured to specification by Volac Ltd (Wendy, Royston, Herts.). The composition of the diet is described in Table 1. The Fe and Cu contents of the milk powder were found to be 9·8 and 0·8 μg/g respectively. The milk powder was reconstituted with deionized water at 37°. The feed intake of the calves at the start was 284 g milk powder/feed. This was increased by about 56 g/feed twice each week so that the final feed intake was 1535 g/feed. The concentration of solids in the milk was 120 g/l initially and was increased by about 6 g/l every week over the first 6 weeks until it was 177 g/l. It was kept at this figure after that. The calves were fed twice a day, from plastic buckets.

The diet was supplemented with one of four Fe compounds: $FeSO_4$, ferric citrate, ferric-ethylenediaminetetra-acetate (Fe–EDTA) (provided by ABM Industrial Products Ltd, Stockport, Cheshire) and iron phytate. The iron phytate was prepared from aqueous solutions of $FeSO_4$ and sodium phytate (Na salt of inositol hexaphosphoric acid). The resultant precipitate was collected by centrifugation, washed with water and ethanol, and then dried under reduced pressure at 40°. The Fe content was 177 g/kg.

The Fe compounds were dissolved (or dispersed, for iron phytate) in distilled water and were added to the reconstituted milk at a level of 30 μg Fe/g dry powder, making the total concentration 40 μg Fe/g dry powder. In some instances the milk was further supplemented by the addition of 15 mg Cu (as an aqueous solution of $CuSO_4$) per feed.

Experimental design

The experiment was of a randomized block design and consisted of five treatments in each of four blocks of calves. The treatments were:

Treatment	Fe supplement
1	None
2	$FeSO_4$
3	Ferric citrate
4	Fe-EDTA
5	Iron phytate

It was realized during the experiment that the diet being used was low in Cu; consequently one calf in each treatment group was given the Cu supplement from the beginning of week 7 onwards (*c.* 53 d of age). The calves were kept on experiment for 11 weeks.

Calves

The Ayrshire bull calves were bought from farms in the neighbourhood of Aberdeen at about 5 d of age. They had all received colostrum and were maintained on unsupplemented whole milk until the start of the experiment, when they were generally about 17 d old. They were then housed in a heated, well-ventilated building in wooden pens, fitted with slatted floors. The small amount of external metal in the pens was covered with polyurethane paint.

The calves were weighed weekly. In the event of severe diarrhoea or of excessive food refusals the food intake was reduced for a short period until the faeces were normal or the appetite was restored. If this had no effect the animals were dosed with sulphadimidine (14 g) and streptomycin (0·7 g) over 3 d.

Blood samples were collected, fortnightly or, in some instances, weekly, into acid-washed heparinized tubes from the jugular veins of the calves at about 2 h after the morning feed.

Analytical methods

Plasma Fe concentrations were determined by the automated colorimetric method of Young & Hicks (1965). Total Fe-binding capacities were determined by the method of Birdsall, Kok & Wild (1965), except that the amount of ionic Fe added to the plasma was double the recommended quantities and the final Fe analyses were by the above method. Blood Cu concentrations were measured by atomic absorption spectroscopy after deproteinization by precipitation with an equal volume of 100 g trichloroacetic acid/l. Ceruloplasmin levels were determined by the method of Houchin (1958), using the standardization procedure of Rice (1962).

Fe and Cu concentrations in freeze-dried tissues were determined after digestion with HNO_3–$HClO_4$–H_2SO_4 by the method of Young & Hicks (1965) and by atomic

Table 2. *Effect of different iron supplements on performance of calves over an 11-week period on treatments* 1–5

(Mean values for four calves/treatment)

Treatment No.	Supplement	Initial wt (kg)	Weight gain (kg)	Feed* intake (kg)	Feed conversion ratio (kg milk powder intake: kg weight gain)
1	None	43·8	85·8	130·3	1·52
2	$FeSO_4$	38·8	87·5	126·1	1·45
3	Ferric citrate	37·3	87·5	130·4	1·49
4	Fe-EDTA	41·4	91·6	135·1	1·48
5	Iron phytate	38·6	85·0	123·6	1·46
SE of difference between means	—	—	6·8	5·9	0·064

* Weight of milk powder.

absorption spectroscopy respectively. Storage of Fe in liver was measured by the method of Drysdale & Ramsay (1965).

Statistical analysis of results was by analysis of variance unless otherwise indicated.

RESULTS

Live-weight gain and efficiency of food conversion

The effects on live-weight gain and feed conversion ratio of supplementing the milk with the various Fe compounds are shown in Table 2. There were no significant differences between the different treatments. The performance of the animals, with an average weight gain of 1·14 kg/d, was probably fairly typical for Ayrshire calves maintained for veal production. The calves usually consumed almost all the milk offered and total milk consumption over 11 weeks varied between 127 and 140 kg. Some calves suffered from anorexia throughout the experiment (two calves consumed less than 110 kg milk) and in most calves food intake was more irregular towards the end of the experiment. Food intake was not significantly related to dietary treatment, although there was a possible positive trend in its relationship with blood Hb concentration.

The performance of the calves was related to their final blood Hb concentrations. The combined relationships for all calves, on all treatments, were expressed by the following regression equations, with the SE of the regression coefficient in parentheses:

$$Y = 0{\cdot}826 + 0{\cdot}0039x\ (\pm 0{\cdot}014),$$

where Y = weight gain (kg/d) and x = Hb concentration (g/l);

and

$$Z = 1{\cdot}70 - 0{\cdot}00269x\ (\pm 0{\cdot}0115),$$

where Z = feed conversion ratio and x = Hb concentration (g/l).

Haematological measurements

The changes in Hb concentration and packed cell volume (PCV) over the experimental period are shown in Fig. 1 for the control calves, those given soluble Fe salts

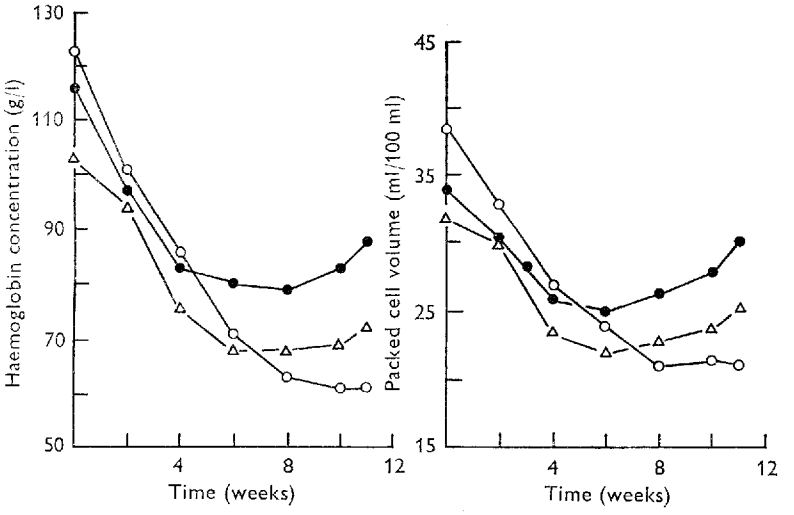

Fig. 1. Changes in blood haemoglobin concentrations and packed cell volumes during the experiment for control calves (○), for those given soluble iron (●) and for those given iron phytate (△).

and those given iron phytate. With all treatments there was a fairly rapid fall in Hb concentrations for about 6–8 weeks, following which the Hb concentrations in the calves given soluble salts increased to a final mean value of 88 g/l. Hb concentrations increased by only 4 g/l in calves given iron phytate and decreased by only a further 2 g/l in the control calves.

When allowance was made by covariance techniques for the greater initial Hb concentrations in the control calves, there were significant differences ($P<0.05$) in the Hb concentrations in the control and Fe-supplemented calves within two weeks (Table 3). The magnitude and statistical significance of these differences increased as the experiment proceeded. There were also differences in the apparent availability of the various Fe supplements. Hb concentrations in calves given Fe–EDTA were significantly greater ($P<0.05$) than those in other calves at weeks 4 and 6, whereas in calves given iron phytate the concentrations were significantly less ($P<0.05$ and <0.01) than in other supplemented calves from week 6 onwards.

The effects of treatment on PCV were generally similar to those on Hb concentration (Table 3 and Fig. 1). From week 8 onwards PCV was significantly least in the control calves and from week 10 onwards PCV in the calves given iron phytate was significantly less than in other Fe-supplemented calves.

Although there was a general decrease in erythrocyte count, mean corpuscular volume and mean corpuscular Hb concentration over the experimental period (Table 3), there were no statistically significant differences between treatments in the final values attained. The decreases during the experimental period in erythrocyte count over all treatments from 9·17 to 7·86 (10^{-6}/mm^3) (SE ± 0·44) ($P<0.01$) and in mean corpuscular Hb concentration from 319 to 292 g/l (SE ± 10·3) ($P<0.05$) were significant, whereas the decrease in mean corpuscular volume from 38·3 to 34·9 μm^3 (SE ±

Table 3. *Effect of different iron supplements on haematological status of calves during an* 11*-week experimental period*

(Mean values of four calves/treatment)

Treatment no.	Supplement / Week...	Haemoglobin concentration† (g/l) 0	2	4	6	8	10	11	PCV (ml/100 ml) 0	11	RCC (10^{-6}/mm^3) 0	11	MCV (μm^3) 0	11	MCHC (g/l) 0	11	Plasma Fe (μg/ml) 0	11
1	No supplement	109	89	74	60	55	59	58	38·5	21·0	9·51	6·90	40·6	31·6	319	294	1·45	0·20
2	$FeSO_4$	105	93	81	81	80	86	88	31·5	28·8	9·76	8·49	34·7	34·0	344	305	1·29	0·54
3	Ferric citrate	113	96	77	71	77	82	90	36·3	30·5	9·28	8·83	39·1	34·4	312	299	1·13	0·68
4	Fe-EDTA	102	99	89	87	79	81	86	34·0	31·3	9·14	7·92	37·7	39·8	298	278	0·94	0·56
5	Iron phytate	103	98	78	70	69	69	72	31·8	25·3	8·12	7·18	39·6	34·9	324	285	0·87	0·43
SE of differences between means		11	2·8	4·3	4·6	6·2	4·5	8·0	3·9	3·0	1·06	0·80	5·9	4·5	29	15	0·40	0·26
Residual regression coefficient of covariate (±SE)			0·93 (0·073)	0·69 (0·11)	0·68 (0·12)	0·36 (0·16)	0·12 (0·12)	0·12 (0·21)	—	—	—	—	—	—	—	—	—	—
Significance of treatment effects	1 *v.* 2, 3, 4, 5		*		**	**	***	**		**	—	—	—	—	—	—	—	—
	4 *v.* 2, 3, 5			*	*						—	—	—	—	—	—	—	—
	5 *v.* 2, 3, 4				*		**	*		*	—	—	—	—	—	—	—	—

* Significant at $P<0.05$. ** Significant at $P<0.01$. *** Significant at $P<0.001$.

† Concentrations from week 2 onwards are corrected by analysis of covariance to allow for differences in treatment groups at the start of the experiment. The haemoglobin concentration in one calf in treatment 1 was abnormally high at the start of the experiment (156 g/l) and has been replaced by a substituted value throughout.

PCV, packed cell volume; RCC, red cell count; MCV, mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration.

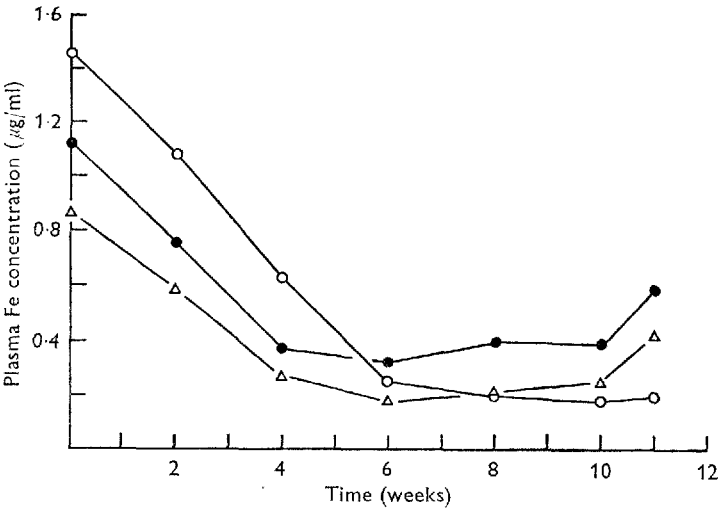

Fig. 2. Changes in plasma iron concentration during the experiment for control calves (○), for those given soluble Fe (●) and for those given iron phytate (△).

Table 4. *Effects of different iron supplements on tissue Fe and spleen copper concentrations (μg/g dry matter) in calves on treatments* 1–5

(Mean values for four calves/treatment)

Treatment no.	Supplement	Liver	Liver (non-haem Fe)	Spleen	Kidney	Muscle	Spleen Cu
1	No supplement	59·7	24·9	309	64·9	12·2	5·89
2	$FeSO_4$	55·7	19·5	238	100·8	18·1	4·27
3	Ferric citrate	54·3	21·8	274	83·6	19·6	4·98
4	Fe-EDTA	58·0	19·9	289	72·9	17·6	5·86
5	Iron phytate	52·0	21·9	256	62·7	14·5	3·99
SE of difference between means	—	5·9	2·0	77†	10·4	4·1‡	0·59
Significance of differences between treatments 1 *v.* 2, 3, 4, 5	—	—	*	—	—	—	*

* Significant at $P < 0{\cdot}05$.

† Approximate value only. One calf in treatment 4 had an Fe concentration of 1213 μg/g DM and a substituted value, obtained by statistical techniques, was used in calculation of the mean.

‡ Only three calves/treatment.

2·29) was not. Plasma Fe concentrations were greatly reduced over the experimental period; values initially averaged 1·4 μg Fe/ml but fell rapidly (Fig. 2), minimum values for the different treatments being attained usually within 6 weeks. No significant differences were noted between treatments, although the control calves generally had the lowest average concentrations (0·2 μg/ml) over much of the experimental period. Measurements of total Fe-binding capacity were made at the beginning and again in the later stage of the experiment; no significant differences between treatments

Table 5. *Effects of a dietary Cu treatment on tissue Fe concentrations in calves*

Treatment	No. of calves	Liver dry matter (g/kg)	Tissue Fe (μg/g dry matter)				
			Liver	Liver (non-haem Fe)	Kidney	Spleen	Muscle
No Cu supplement	15	277	56·7	23·7	78·7	258	22·3
+Cu	5	315	53·7	15·4	72·6	318	23·3
SE of differences between means	—	7·5	5·3	1·8	9·3	77	3·2†
Significance of differences	—	***	—	***	—	—	—

† Only ten calves in group receiving no Cu supplement.
*** Significant at $P < 0.001$.

were noted. Average values (± SD) at weeks 10 and 11 were 9·46 ± 1·31 and 10·24 ± 0·88 μg Fe/ml respectively, compared with the mean initial values of 5·77 ± 0·49 μg Fe/ml.

Tissue concentrations of Fe

The mean concentrations of Fe in liver, spleen, kidney and muscle for the different treatments are shown in Table 4. No treatment effects were evident in most instances, but the storage Fe reserves in the livers of the control calves were paradoxically greater than for the other calves ($P < 0.05$). There were no treatment effects on the dry matter (DM) content or total weight of the tissues.

Effects of Cu supplement on Fe and Cu concentrations in tissues

In Table 5 the effect of adding a daily supplement of 30 mg Cu (as $CuSO_4$) to the rations of one calf from each treatment from week 7 onwards are shown. There was a very highly significant reduction in the concentrations of non-haem Fe in the liver of the Cu-supplemented calves ($P < 0.001$), but no change in other tissues. There was also an increase in the liver DM content in the supplemented calves ($P < 0.001$). The Cu concentrations in the spleens of calves in Fe treatments 1 and 4 were significantly greater ($P < 0.05$) than those in treatments 2 and 5 (Table 4). However, in general, Fe treatments had no effect on tissue Cu concentrations; consequently in Tables 6 and 7 the mean results for the combined Fe treatments are reported.

In these tables the effects of Cu supplementation on blood Cu, plasma Fe and ceruloplasmin concentrations and on tissue Cu concentrations are shown. At no stage in the experiment were mean blood Cu concentrations indicative of the existence of a stage of hypocupraemia (< 0·6 μg/ml; Underwood, 1971), but in five of the calves blood Cu concentrations were in the range 0·6–0·85 μg Cu/ml and in these calves the possible development of marginal Cu deficiency might be suspected. Mean ceruloplasmin concentrations in these calves were 13·5 i.u./l, compared with an average of 20·6 i.u./l for all calves which were not given supplementary Cu. The concentrations of Cu in the livers of calves not given supplementary Cu were low, the mean value being 25·3 μg/g DM. Indeed, the liver Cu concentrations in about 50% of these

Table 6. *Effects of a dietary copper supplement on blood Cu, plasma iron and ceruloplasmin concentrations in calves*

(Mean values with no. of samples in parentheses; Cu supplementation was commenced at the end of the 6th week)

	Blood Cu (μg/ml)				Plasma Fe (μg/ml)				Ceruloplasmin (i.u./ml)			
Time (weeks)	No Cu supplement	+Cu	SE of difference between means	Significance of difference	No Cu supplement	+Cu	SE of difference between means	Significance of difference	No Cu supplement	+Cu	SE of difference between means	Significance of difference
6	1·28 (5)	—	0·08†	—	0·25 (20)	—	0·02†	—	—	—	—	—
7	—	1·57‡ (5)	0·04†	—	—	—	—	—	16·6 (5)	24·9 (5)	2·4	*
8	1·12 (5)	1·69 (5)	0·13	*	0·26 (15)	0·51 (5)	0·10	*	12·8 (15)	32·8 (5)	2·9	**
9	1·12 (15)	1·32 (5)	0·14	—	—	—	—	—	16·1 (15)	32·1 (5)	3·5	**
10	—	—	—	—	0·29 (15)	0·43 (5)	0·11	—	23·7 (5)	24·5 (5)	5·3	—
11	1·15 (15)	1·36 (5)	0·20	—	0·42 (15)	0·66 (5)	0·19	—	20·6 (15)	22·2 (5)	5·2	—

† SEM.

‡ Significantly greater ($P<0·05$) than the value at 6 weeks for the calves given no Cu supplement.

* Significant at $P<0·05$. ** Significant at $P<0·01$.

Table 7. *Effects of a dietary copper supplement on tissue Cu concentrations (μg/g dry matter) in calves*

Treatment	No. of calves	Liver	Spleen	Kidney	Muscle
No Cu supplement	15	25·3	4·50	19·9	2·22
+Cu	5	452	6·51	26·4	2·33
SE of difference between means	—	39·5	0·53	2·7	0·45†
Significance of differences	—	***	***	**	—

† Only ten calves in group receiving no Cu supplement.
** Significant at $P<0.01$. *** Significant at $P<0.001$.

animals were <12.5 μg/g DM and these animals could therefore be described as Cu-deficient. In Table 6 it can be seen that there were significant effects of dietary Cu supplementation on blood Cu and plasma Fe and ceruloplasmin concentrations. The increases in concentrations were statistically significant only in the period 2–3 weeks after Cu administration was commenced. The increased dietary intake of Cu caused a dramatic increase in liver Cu concentration. The mean value for the Cu-supplemented calves was 452 μg/g DM and it can be calculated from the liver weights of the calves that about 50% of the supplementary Cu was retained in the livers of these calves. There were also increases, though less pronounced, in the Cu concentrations in spleen and kidney ($P<0.001$ and <0.01 respectively), but not in muscle.

DISCUSSION

The experiment has shown that when calves are maintained on rations based upon milk products without supplementary Fe a state of anaemia rapidly develops. Within 2 weeks the calves showed signs of an increased rate of decline in Hb concentrations, when compared with Fe-supplemented controls. Final concentrations were 61 g/l after 11 weeks on experiment and, using the criterion of Holman (1956) for conventionally reared calves, this could be classified as a state of moderate anaemia. The average daily fall in Hb concentration in control calves over the experimental period was 0·66 g/l, but most of this fall occurred in the early stage of the experiment. Over the first 6 weeks the average daily fall was 1·17 and over the final 5 weeks 0·057 g/l.

These average daily falls in Hb concentration are similar to those reported by Blaxter *et al.* (1957) (0·65 g/l) and Roy *et al.* (1964) (0·82 g/l) for calves maintained on whole milk, which generally has an Fe concentration of around 3 μg/g DM, compared with 10 μg Fe/g in the basal diet used here. However, their calves gained weight over the comparable experimental period at only 0·42 and 0·76 kg/d, in contrast with the average weight gain of 1·14 kg/d for the calves in this experiment. It is probable that the apparent anomalies between Fe intake and change in Hb concentration in the different studies are associated with the differences in growth rate.

Although the mean weight gains of the calves given supplementary Fe in the form of $FeSO_4$, ferric citrate and Fe–EDTA were greater than for the other calves, the differences were not significant. This contrasts with the findings of Eeckhout, Casteels & Buysse (1969) and of Roy *et al.* (1964), who reported improved performance in

calves given supplementary Fe either orally or by injection. The failure to detect significant differences in performance in the present study is probably a joint consequence of the relatively small number of animals which could be allocated to each treatment and of the large variation in the Fe stores of individual calves at the start of the experiment. Hb concentrations initially ranged from 82 to 156 g/l. Roy *et al.* (1964) have also commented on the large variations in Fe status of calves at birth.

Despite this, it was noted that there was a significant correlation between the weight gain of the calves and the final Hb concentration and also a significant negative correlation between the feed conversion ratio and final Hb concentration. Roy *et al.* claimed that the beneficial effect of supplementary Fe on weight gain was mainly evident when Hb concentrations fell below 70 g/l and that the main nutritional benefit of Fe was to decrease anorexia. However, the present results did not reveal the existence of a significant correlation between feed intake and Hb concentration.

When supplementary Fe was given (at a total Fe intake of 40 μg/g DM), there was still a fall in Hb concentrations for about 8 weeks. The dietary intake of Fe may have been less than the calves' normal requirement, but nevertheless it is not certain that the fall in Hb concentration over the first weeks of life is a consequence solely of nutritional insufficiency. A similar phenomenon has been observed in human infants, with a decrease in Hb concentrations from 180 g/l to 120 g/l recorded over the first 3–4 months of life (Walsh, Kaldor, Brading & George, 1955). It is possible that this change is associated with the change from the relatively anoxic intra-uterine conditions and the destruction of foetal Hb. Changes in Hb concentrations in calves similar to those found here have been reported by several groups of workers, even in calves given apparently adequate supplies of Fe (Owen, Voelker, Jacobson & Allen, 1955; Wing, Jacobson & Allen, 1955; Eeckhout *et al.* 1969).

Lanz (1956) has reported that plasma Fe concentrations in calves decreased in the period immediately after birth from 1·60 to 0·50 μg Fe/ml and did not increase until vegetable fodder was consumed, with concomitant increase in dietary Fe intake. Bremner (1966) has claimed, however, that milk-fed calves which had access to dry fodder generally showed a transient increase in plasma Fe concentrations between 1 and 3 weeks after birth, normal adult levels of around 1·4 μg Fe/ml being maintained after that. In the present study, which commenced when the calves were 2–3 weeks old, there was a gradual fall in plasma Fe concentrations in all animals, regardless of Fe intake, from about 1·14 to 0·28 μg/ml over 6 weeks but in animals given supplementary Fe, the values then increased to about 0·55 μg/ml. These changes tended to precede the similar changes described earlier in blood Hb concentrations.

As is usual in Fe-deficiency anaemia the low plasma Fe concentrations were associated with high plasma total Fe-binding capacities. Initial values were fairly normal at 5·77 ± 0·49 μg Fe/ml but at the end of the experiment they had risen to the exceptionally high value of over 10 μg Fe/ml. Bremner (1966) has noted values of up to 8 μg Fe/ml in conventionally reared calves within a few weeks of birth, but these had fallen to about 4 μg Fe/ml by the time the calves were a few months old. Kolb (1963) has reported that values are higher in calves than in adult cattle. The transferrin in the plasma of the Fe-deficient calves in the present study was only 2% saturated.

Another notable feature of the haematological picture in veal calves is that there is frequently no hypochromia of the erythrocytes, although the calves are suffering from what would appear to be an uncomplicated Fe-deficiency anaemia. Blaxter *et al.* (1957) and Eeckhout *et al.* (1969) have reported previously that in Fe-deficient calves the anaemia is of a microcytic, normochromic nature, although Roy *et al.* (1964) have suggested that when the calves become severely anaemic, hypochromia develops. Unfortunately, it is not clear what constitutes 'normal' corpuscular Hb concentrations in calves and some of the control calves used for comparisons have themselves been partly anaemic. In most published reports there has been a tendency for mean corpuscular Hb concentrations to be less (although not necessarily statistically different) for calves receiving no Fe supplement and for these values to diminish as the animals grow.

The main aim of this work was to gain some information on the relative availabilities of different Fe compounds to calves. As the effectiveness of any therapeutic Fe compounds must be related eventually to their ability to prevent the development of anaemia, the comparison of the Fe sources was based on an examination of the haematological status of the calves. There have been several reports on differences in the availability of Fe in the compounds examined here to rats and to man (e.g. Brise & Hallberg, 1962; Turnbull, Cleton & Finch, 1962; Hopping & Ruliffson, 1963), but no information has been produced on their relative availabilities to calves. Indeed, very little is known of the relative availabilities of any Fe compounds to calves. It has been shown that there are no significant differences in the availability of ^{59}Fe-labelled $FeSO_4$, $FeCl_3$ and $FeCO_3$, as measured by tissue deposition of ^{59}Fe, although measurements of ^{59}Fe in serum implied that some differences may occur between $FeSO_4$ and $FeCO_3$; it was found that the Fe in Fe_2O_3 was significantly less available than these other sources (Ammerman, Wing, Dunavant, Robertson, Feaster & Arrington, 1967). These differences between soluble and insoluble Fe salts were also found in the present study, where $FeSO_4$, ferric citrate and Fe–EDTA were more available than was iron-phytate. The differences were found only after the calves had been on experiment for several weeks and were evident in the different Hb concentrations and PCV values of the calves.

The calves given the soluble Fe salts were improving in haematological status towards the end of the experiment and in all probability if the animals had been maintained until normal slaughter weight their final Hb concentrations would have been greater than the mean recorded value of 88 g/l. Thus, although a dietary intake of 40 μg Fe/g DM is not sufficient to prevent a rapid fall in Hb concentration in the early stages of growth (to a mean value of 79 g/l), nevertheless, in the long term it does prevent the occurrence of all but a very mild anaemic state. This level of Fe supplementation is higher than we believe is normally used by manufacturers of milk substitutes. The calves given iron phytate as Fe source were suffering from a moderate anaemia at the end of the experiment, with a mean Hb concentration of 72 g/l and with no signs of a significant upward trend.

Leslie & Kaldor (1971*a*, *b*) have demonstrated that the storage (non-haem) Fe in the liver and other organs of neonatal rats makes only a small contribution to the

animals' Fe requirements for Hb synthesis and that much of this Fe has been utilized by the time an animal has doubled its birth weight. It seems reasonable, therefore, to assume that in the latter part at least of the present experiment the main Fe source for haemoglobin synthesis was of dietary origin. By comparison of Fe intake with the increase in circulating Fe over that period it is possible, therefore, to obtain a rough estimate of the availability of the dietary Fe. Dalton & Fisher (1961) showed that the blood volume of young calves up to a few weeks old (29–38 kg) was 110 ± 20 ml/kg body-weight compared with $63 \pm 8{\cdot}3$ ml/kg body-weight for adult cows and heifers (weighing 200–600 kg). If the mean of these values is arbitrarily taken to represent the blood volume/kg body-weight of the veal calves in the 6–11 week period, and the Fe concentration of haemoglobin to be 3·4 g/l, then the amount of circulating Fe (mg) is given by:

$$\frac{\text{body-weight (kg)} \times 87 \times \text{Hb concentration (g/l)} \times 3{\cdot}4}{10}.$$

The % Fe utilization is then calculated to be 72, 43 and 33% for the control calves and those given soluble and insoluble Fe salts respectively. These estimates are necessarily approximate, but they do agree reasonably well with those of Matrone *et al.* (1957) who reported 60% utilization of Fe by milk-fed calves given 30 mg Fe/d (as $FeCl_3$) and 30% utilization by calves given 60 mg Fe/d. It has been noted in other species that the suckling animal is able to absorb a very high proportion of dietary Fe. Tarvydas, Jordan & Morgan (1968) reported, for example, that virtually all ingested Fe in milk or NaCl solution is retained by the suckling rabbit.

Fe supplementation had little effect on the concentration of Fe in any of the tissues analysed. Liver Fe concentrations were similar to those recorded by Roy *et al.* (1964) and Eeckhout *et al.* (1969) but less than those reported by Blaxter *et al.* (1957) (118 μg/g DM for Fe-deficient calves). As about 40% of the Fe in the liver was in the form of non-haem Fe, a total of about 25 mg Fe would be stored in the liver in this form. Total spleen Fe was generally around 30 mg; so Fe reserves in these organs are quite inadequate for Hb synthesis, considering that around 300 mg Fe would be necessary to increase blood Hb concentration by 10 g/l. Concentrations of Fe in *longissimus dorsi* muscle were around 16 μg/g DM and it has been shown that much of this Fe is present in the form of myoglobin and Hb (MacDougall, Bremner & Dalgarno, to be published).

It is not customary for the milk substitutes used in veal production to be supplemented with additional Cu. As the dietary Cu concentration is then frequently < 1 μg/g, the calves are very dependent on Cu stores, mainly in the liver, to satisfy their Cu requirements. These reserves were so severely depleted by the end of the present experiment that the animals could reasonably be classified as being Cu-deficient, although blood Cu and ceruloplasmin concentrations in most calves were not low. This is a normal progression in the development of Cu deficiency. Anke (1966) has also reported signs of Cu deficiency in intensively reared milk-fed calves with a daily intake of 2·2 mg Cu. Liver Cu concentrations were about 17·6 μg/g DM at 140 d of age, and he suggested that a daily intake of 7·7 mg Cu was sufficient to meet the Cu requirement of the calves.

The lack of any effect of dietary supplementation with Cu on performance or blood Hb concentrations confirms the conclusions of Matrone *et al.* (1957) that the anaemic condition of milk-fed calves was not a consequence of Cu inadequacy. However, there were some effects of Cu supplementation on Fe metabolism, as there were transient increases in plasma Fe concentrations and decreases of 35% in liver storage Fe reserves in the treated calves. These changes seemed to be associated with an increase in ceruloplasmin activity, which is in accordance with the view that in Cu deficiency the impairment of release of Fe from the reticuloendothelial system and hepatic parenchymal cells (Lee, Nacht, Lukens & Cartwright, 1968) is at least partly a consequence of the reduction in the level of plasma ceruloplasmin and, therefore, of ferroxidase activity (Osaki, Johnson & Frieden, 1966). It is noteworthy that in one calf which received the Cu supplement (in treatment 1) there was little change in the concentration of plasma Fe, ceruloplasmin or of liver storage Fe.

It is, perhaps, surprising that the concentrations of ceruloplasmin in the untreated calves were sufficiently low to affect the mobilization of Fe, as it has been suggested that in pig plasma, at least, there is generally about a tenfold excess of ceruloplasmin available for Fe oxidation (Ragan, Nacht, Lee, Bishop & Cartwright, 1969). This may not be so in calf plasma but it has been shown (Bremner, unpublished observations) that a normal amount of ferroxidase activity is associated with the ceruloplasmin.

The most pronounced effect of Cu supplementation was to increase liver Cu. Liver Cu concentrations were about 450 μg/g DM, and it can be calculated that about 50% of the dietary Cu was retained in the liver. Such a high retention of Cu is of considerable interest as it is probably related to the known susceptibility of the young calf to Cu poisoning (Shand & Lewis, 1957).

In conclusion, this experiment has shown that there are no apparent differences in the availability of $FeSO_4$, ferric citrate and Fe–EDTA to veal calves but that these Fe sources are all more available than is iron-phytate. The Fe requirements of calves maintained on fat-supplemented skim milk may be different from those of calves maintained on whole milk, but a dietary intake of 40 μg/g soluble Fe is sufficient to prevent the development of other than a very mild anaemia. Veal calves probably suffer from Cu deficiency if the diet contains no supplementary Cu and this results in less complete utilization of liver Fe stores. More information is required on what constitutes optimum or 'normal' haematological status in veal calves, on their Fe and Cu requirements and on the effect of anaemia on the 'well-being' of the animals. These subjects will be considered in later papers.

The authors are grateful to Mr J. Mathieson for the iron analyses, Mr G. A. M. Sharman for the haematological analyses, Mr I. MacDonald for the statistical analysis and Mr R. B. Marshall for technical assistance.

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Printed in Great Britain