Lin, P. H. & Johnson, B. C. (1953). *J. Amer. chem. Soc.* **75**, 2974.
Mitchell, H. K. (1950). *Vitam. & Horm.* **8**, 127.
Oka, Y. (1954). *J. Biochem., Tokyo*, **41**, 89, 101.
Reddi, K. K. & Kodicek, E. (1953). *Biochem. J.* **53**, 286.
Roth, L. J., Leifer, E., Hogness, J. R. & Langham, W. H. (1948). *J. biol. Chem.* **176**, 249.
Schneider, W. C. (1948). *J. biol. Chem.* **176**, 259.
Sundaram, T. K. (1955). Ph.D. dissertation: University of Madras.
Sundaram, T. K., Radhakrishnamurty, R. & Sarma, P. S. (1954). *Curr. Sci.* **23**, 92.
Sundaram, T. K. & Sarma, P. S. (1954). *Curr. Sci.* **23**, 398.
Sundaram, T. K. & Sarma, P. S. (1956). *Biochim. biophys. Acta*, **22**, 547.
Yanofsky, C. & Bonner, D. M. (1951). *J. biol. Chem.* **190**, 211.

Calcium and Magnesium Metabolism in Calves

2. EFFECT OF DIETARY VITAMIN D AND ULTRAVIOLET IRRADIATION ON MILK-FED CALVES*

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It has previously been reported (Smith, 1957) that calves given a diet consisting almost exclusively of milk with no supplementary vitamin D showed, as they got older, a progressive decrease in the percentage of dietary calcium and magnesium utilized. The addition of sufficient vitamin D to the diet maintained calcium retention at a high level and prevented or cured the hypocalcaemia which frequently also occurred, but it appeared that abnormally large amounts of the vitamin were sometimes required to achieve this. Parr (1957) has also reported hypocalcaemia in milk-fed calves receiving vitamin D in amounts usually considered to be adequate. Part of the object of the present work therefore was to attempt to obtain general confirmation of these results and to examine further the effect of vitamin D levels on calcium utilization in milk-fed calves.

Our previous results (Smith, 1957) suggested that the fall which occurred in magnesium utilization with age and the accompanying hypomagnesaemia were not appreciably influenced by feeding with high levels of vitamin D. Other workers (e.g. Huffman, Conley, Lightfoot & Duncan, 1941; Duncan, Huffman & Robinson, 1935; Parr, 1957) have also demonstrated that milk-fed calves become hypomagnesaemic, usually before 3–4 months of age, and that vitamin D added to the diet does not prevent this. Herman (1936), however, kept eight calves for about 1 year or more on a milk diet with and without vitamin D supplements, and yet reported that no hypomagnesaemia occurred in any of his animals. The only manner in which his treatment appeared to differ appreciably from that of other workers was in the fact that his calves were kept in the sunlight as much as possible. This suggested that ultraviolet-light radiation might produce an effect not shown by the oral administration of vitamin D. A further part of the present work was undertaken in order to examine this possibility.

* Part 1: Smith (1957).

EXPERIMENTAL

Nine bull calves were used. One was an Angus × Ayrshire crossbred (7 A), another was an Angus × Shorthorn/Friesian crossbred (11 A) and the remainder were Friesians. Apart from the differences described below they received the same general treatment as did the calves used previously (Smith, 1957). They were given 4·40 l. of milk/day up to 10 weeks of age, 6·15 l. of milk/day from 10 to 20 weeks and 8·80 l. of milk/day from 20 weeks. The milk was sampled every day and magnesium and calcium were determined on 4-day batches. The mean magnesium and calcium contents of the batches were 12·5 and 121 mg./100 ml. respectively, measured over the whole experimental period. The standard deviations for individual 4-day batch determinations were ±0·6 mg./100 ml. for magnesium and ±5 mg./100 ml. for calcium. There were no marked changes in milk magnesium from one part of the experimental period to another such as occurred in 1954–55 (Smith, 1957), and it is not therefore considered necessary to give these milk analyses in detail. The calves were given a mineral supplement containing iron, copper and manganese as in the previous work (Smith, 1957). Details of the supplementary vitamin D added to the diet and the ages at which some of the calves were irradiated with u.v. light are shown in Table 1. In addition to the vitamin D supplements, the calves received whatever naturally occurring vitamin D activity was present in their milk diet. No vitamin D assay was carried out on the milk but it is probably valid to use the figures obtained by Henry & Kon (1942). These workers studied milk obtained from the same farm as that from which our milk was obtained. They found that winter milk, such as our calves received before supplementation with 70 000 i.u. of vitamin D_3/day or u.v.-light treatment, showed an antirachitic activity for rats equivalent to about 10 i.u. of vitamin D/l. For u.v.-light irradiation the calves were shaved on one side and the lamp (Alpine Sun Lamp, model 10, Hanovia Ltd.) was directed at this side. The shaved area was about 3000–4000 cm.^{2}, but part of this area (about 20–40 % depending on the calf) was black pigmented. After a milder introductory period of a few days the calves were irradiated for 15 min. daily with the lamp 60 cm. from the skin. This gave a daily dosage of about 10^7 ergs/cm.^{2} of u.v.-light radiation below 3132 Å. Erythema and granular exfoliation occurred but there was not much peeling or blistering, even though the treatment was continued for about 2 months. The treatments with 70 000 i.u. of vitamin D_3/day or with u.v. light were started when the plasma magnesium level was falling, usually when the level was between 1·5 and 1·8 mg./100 ml. This was done in an attempt to use any resultant change in plasma magnesium as an index of greater availability, if any, of dietary magnesium. Balance experiments over periods of 8 days were carried out as previously described (Smith, 1957), usually at approximately monthly intervals, during the whole experimental period. In each of the calves irradiated with u.v. light or given 70 000 i.u. of vitamin D_3/day the last two balance periods reported were carried out after such treatment had started. Four of the calves (nos. 2A, 4A, 7A and 11A) were given diets of magnesium-free synthetic milk (prepared from margarine, casein, glucose, inorganic salts and vitamins) and magnesium-supplemented milk for a period of about 1 month between these last two balance periods, but they continued to get 70 000 i.u. of vitamin D_3/day. Results obtained during this time, which gave information on the amount of endogenous faecal excretion of magnesium, will be reported later. The calves were returned to a normal milk diet at least 8 days before the final balance period.

All the calves remained in general good health, except for calf 14A which lost its appetite and had to be removed from the experiment at 18 weeks of age. There was no diarrhoea during any of the reported balance periods. None of the calves had convulsions or showed other signs of hypomagnesaemia during the reported experimental period, with the exception of calf 2A when it was receiving magnesium-free synthetic milk. The increase in weight of all the calves was regular (except when they were receiving synthetic milk) and generally similar to that found previously (Smith, 1957). The mean weights of the calves were 43 kg. (S.D. ± 2·5) at birth, 64·5 kg. (S.D. ± 2·5) at 10 weeks, 96·5 kg. (S.D. ± 1·5) at 20 weeks and, of the three calves that were kept that long, 142·5 kg. (S.D. ± 2·5) at 34 weeks of age.

The methods for determining calcium and magnesium in plasma, urine, faeces and milk were as described previously (Smith, 1957).

RESULTS

Plasma levels

Determinations of plasma magnesium and plasma calcium were made at weekly intervals throughout the experiment. All the calves eventually showed hypomagnesaemia before they were given 70 000 i.u. of vitamin D_3/day or were irradiated with u.v. light. As in previous experiments (see, for example, fig. 2, Smith, 1957) the age of onset of hypomagnesaemia was usually quite distinct. For comparative purposes, when weekly samples are taken, the age of onset of hypomagnesaemia can be defined as that age at which the plasma-magnesium level first shows a negative deviation of more than 15 % from the previous mean value, provided that this deviation tends to increase in subsequent determinations. A random deviation of this size was observed on only three occasions in 178 determinations during the initial period during which the plasma-magnesium level was normal. The mean age of onset of hypomagnesaemia, thus defined, was 17 weeks (S.D. ± 1·5). There was no significant difference in this value between calves given 300 or 400 i.u. of vitamin D_3/day and those given no extra supplementary vitamin D. Calves given 300 i.u. of vitamin D_3/day did not show hypocalcaemia when hypomagnesaemia began. Their plasma calcium remained near normal with this supplement for the subsequent 4–5 weeks before they were given a high level of vitamin D_3 or u.v.-light treatment. In accordance with previous

Table 1. *Ages in weeks at which the calves received different amounts of supplementary vitamin* D_3 *or were irradiated with ultraviolet light*

Supplementary vitamin D_3 was added to the milk as an aqueous suspension.

	Treatment				
Calf no.	No supplementary vitamin D	300 i.u. of vitamin D_3/day	400 i.u. of vitamin D_3/day	70 000 i.u. of vitamin D_3/day	u.v. irradiation
1A	0–5	5–15	15–26	—	26–35
2A	0–5	5–15	15–22	22–34	—
4A	0–5	5–15	15–23	23–35	—
7A	0–5	5–15	15–18	18–29	—
8A	0–5	5–15	15–22	—	22–31
11A	0–19	—	—	19–30	—
12A	0–14	—	—	—	14–24
13A	0–25	—	—	—	—
14A	0–18	—	—	—	—

findings (Smith, 1957), calves not given extra vitamin D showed a strong positive correlation between plasma-magnesium and plasma-calcium levels when hypomagnesaemia began. Correlation coefficients, with the numbers of determinations in parentheses, were 0·71 (20), 0·82 (14) and 0·87 (12) for calves 11A, 12A and 14A respectively. Calf 13A showed neither hypomagnesaemia nor hypocalcaemia up to 23 weeks of age and it was slaughtered immediately after the onset of hypomagnesaemia at 24 weeks. As was found previously (Smith, 1957), the addition of a high level of vitamin D to the diet of a hypocalcaemic calf (no. 11A) rapidly restored the calcium level to normal. Irradiation of the hypocalcaemic calf 12A with u.v. light had the same effect (plasma calcium restored from 7 to about 12 mg./100 ml. in 2 weeks).

Neither supplementation with 70 000 i.u. of vitamin D_3/day nor irradiation with u.v. light caused any appreciable improvement in plasma-magnesium levels, and these in fact continued to fall.

Balance experiments

Calcium. The percentages of dietary calcium excreted in the urine and faeces at different ages are shown in Figs. 1 and 2 respectively. The urine excretion was always very low and it was not appreciably affected by vitamin D supplementation or irradiation with u.v. light. In the calves given 300 i.u. of vitamin D_3/day from 5 weeks of age the rate of increase with age in the percentage of dietary calcium excreted in the faeces was less rapid than for the calves not given extra vitamin D. At 6–8 weeks of age, about 2 weeks after the vitamin D supplement was started, their mean calcium retention did not differ significantly from that of the group not given vitamin D, but at 11–13 weeks their mean calcium retention was significantly better ($P < 0{\cdot}02$). At 15–17 weeks the difference between the means was greater but because of the large variance introduced by the high retention shown by calf 13A (72 % compared with 27 and 39 % respectively for calves 11A and 14A) this difference was not statistically significant. In view of the exceptional resistance of calf 13A to hypomagnesaemia and hypocalcaemia this high retention was also probably exceptional. Determinations on calves 2 weeks or more after their treatment was changed, either by giving them 70 000 i.u. of vitamin D_3/day or by irradiating them with u.v. light, showed a marked improvement in calcium retention, even in calves already receiving 300 i.u. of vitamin D_3/day. It appeared that when either of these treatments was given calves retained calcium with about the same high efficiency at 5 months of age as at 3 weeks of age, but that between 5 and 8 months the retention efficiency decreased slowly.

Magnesium. The percentages of dietary magnesium excreted in the urine and faeces at different ages are represented in Figs. 1 and 3 respectively. The decrease in urine excretion and the increase in faecal excretion of magnesium with age in the present calves were similar to those shown previously (Smith, 1957). There were no significant differences in these changes between calves given 300–400 i.u. of vitamin D_3/day and those given no extra vitamin D. It was also clear that neither supplementation with 70 000 i.u. of vitamin D_3/day nor irradiation with u.v. light had any marked effect on magnesium utilization, although the results obtained did not lend themselves to statistical treatment. There was very considerable variation in the amounts of magnesium retained by the different calves and in general these differences were reflected in the times and rates of onset of hypomagnesaemia. Thus, to take extreme examples, calf 13A which was exceptionally resistant to hypomagnesaemia still retained about 40 % of the dietary magnesium and excreted about 8 % in the urine at 20–22 weeks of age. Calves 2A and 7A, both of which showed plasma-magnesium values of less than 1·6 mg./100 ml. before 20 weeks of age, retained only about 10 % of the dietary magnesium at 20–22 weeks and, in common with other hypomagnesaemic calves, excreted less than 2 % in the urine.

DISCUSSION

The general picture of falling efficiency in the utilization of dietary magnesium and calcium with increasing age, and eventual occurrence of correlated falls in plasma-magnesium and plasma-calcium levels, was similar in the present milk-fed calves not given supplementary vitamin D to that observed previously (Smith, 1957). The mean age of onset of hypomagnesaemia (17 weeks, S.D. $\pm 1{\cdot}5$) in the present nine calves differed significantly ($P < 0{\cdot}01$), however, from the mean age of onset of hypomagnesaemia (11 weeks, S.D. ± 1) in the eight calves given a milk diet without supplementary magnesium and examined in 1954–55 and 1955–56 (Smith, 1957). It is not clear why the present calves were relatively less susceptible to hypomagnesaemia. Treatment, apart from very small differences in the amounts of milk given, was similar and the magnesium content of the milk was not higher in the present experiments. The following facts might be relevant. (*a*) For practical reasons of supply most of the present calves were Friesians, whereas most of the earlier calves were Shorthorns. No difference in the susceptibility to hypomagnesaemia of calves of different breeds has been reported but the matter has not been adequately studied. (*b*) The calves were kept in unheated pens, except when they were undergoing balance experiments, and were thus subjected to different temperatures depending upon climatic conditions. Hegsted, Vitale & McGrath (1956) have reported that the dietary magnesium requirement of rats may be influenced by environmental temperature. Similar unexplained differences in susceptibility to hypomagnesaemia between groups of calves studied at different times are apparent from an examination of the literature. Thus, for example, despite closely similar treatment the calves of Knoop, Krauss & Hayden (1939) did not show hypomagnesaemia until about 4 months of age whereas those of Parr (1957) began to show hypomagnesaemia in 3–4 weeks.

The finding that magnesium utilization and plasma-magnesium levels, unlike calcium utilization and plasma-calcium levels, were apparently independent of vitamin D intake was also in accordance with previous findings (Smith, 1957). The additional finding that irradiation of some of the calves with u.v. light was also without marked effect on their magnesium utilization, means that no explanation is provided for the absence of

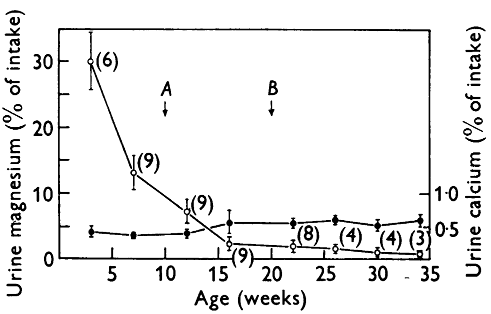

Fig. 1. Effect of age on the excretion of calcium (●) and magnesium (○) in the urine of milk-fed calves. There were no significant differences between the amounts excreted by calves given 300–400 i.u. of vitamin D_3/day and those given no supplementary vitamin D. Similarly, the addition of 70 000 i.u. of vitamin D_3/day or irradiation with u.v. light did not appear to affect the results. Consequently mean values, with standard deviations shown as vertical bars, are given for all the calves irrespective of treatment. The numbers of calves examined, which were the same for both the calcium and magnesium determinations, are shown in parentheses. Experimental points are situated with respect to age in the middle of the 2-weeks age ranges within which the determinations were made. Increases in the amounts of milk fed are indicated as follows: *A*, 4·40–6·15 l./day; *B*, 6·15–8·80 l./day.

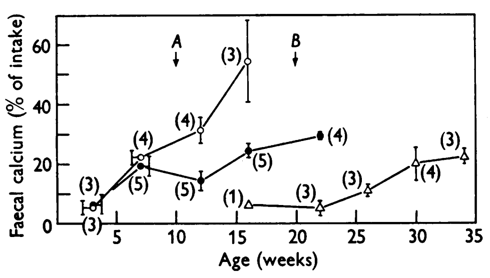

Fig. 2. Effect of age and of different vitamin D supplements on the excretion of calcium in the faeces of milk-fed calves. Treatments were as follows: ○, no supplementary vitamin D; ●, 300 i.u. of vitamin D_3/day from 5 weeks of age and 400 i.u. of vitamin D_3/day from 15 weeks of age; △, 70 000 i.u. of vitamin D_3/day or irradiation with u.v. light. Results for the last two treatments are recorded together since there was no apparent difference between them. Mean values are given with the numbers of calves examined in parentheses and with standard deviations shown as vertical bars. Experimental points are situated with respect to age in the middle of the 2-weeks age ranges within which the determinations were made. Increases in the amounts of milk fed are indicated as follows: *A*, 4·40–6·15 l./day; *B*, 6·15–8·80 l./day.

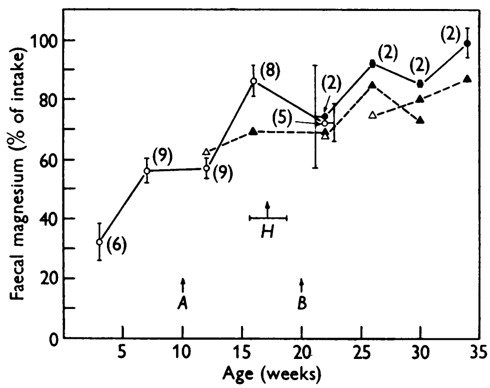

Fig. 3. Effect of age and of different vitamin D supplements on the excretion of magnesium in the faeces of milk-fed calves. Treatments were as follows: ●, 70 000 i.u. of vitamin D_3/day; ○, 300–400 i.u. of vitamin D_3/day or no supplementary vitamin D. Results for the last two treatments are recorded together since there was no significant difference between them. The above results are given as mean values, with the numbers of calves examined in parentheses and with standard deviations shown as vertical bars. The groups of three triangular symbols joined by a broken line each represent results for an individual calf. The first symbol in each group (△) represents a result obtained when the calf was receiving not more than 400 i.u. of vitamin D_3/day. The other symbols (▲) represent results obtained at least 2 weeks after daily u.v.-light irradiation of the calf had been instituted. Experimental points are situated with respect to age in the middle of the 2-weeks age ranges within which the determinations were made. Increases in the amounts of milk fed are indicated as follows: *A*, 4·40–6·15 l./day; *B*, 6·15–8·80 l./day. The arrow *H* indicates the mean age of onset of hypomagnesaemia, with the standard deviation shown as a horizontal bar.

hypomagnesaemia observed in the calves examined by Herman (1936). The results of this worker remain irreconcilable with those of other workers in the field.

According to Bechdel, Hilston, Guerrant & Dutcher (1938) the minimum requirement of normally fed calves for vitamin D is about 6·7 i.u./kg. body wt./day. This figure was, however, derived from experiments in which a diet rather low in phosphorus was given (Ca:P about 3:1). More recently Thomas & Moore (1951), using a better diet in this respect (Ca:P about 1·3–1·8:1), have reported that calves grew normally for 8 months with no blood abnormalities and no signs of rickets when their vitamin D intake was only about 4·8 i.u./kg. body wt./day (less during the first 3 months). Post-mortem examination showed normal bone formation and normal values for bone ash in these animals. Our calves (1A, 2A, 4A, 7A, 8A) which were given 300–400 i.u. of supplementary vitamin D/day from 5 weeks of age received a total intake of vitamin D (or its equivalent as naturally occurring vitamin D activity in the milk) of between about 4·5 and 7·0 i.u./kg. body wt./day. They therefore received dietary vitamin D in amounts which appear to be adequate for normally fed calves. Our results show, however, that this vitamin D intake was not sufficient for optimum calcium utilization in our calves. This regime was not continued for a sufficiently long time to provide evidence on whether any ill effects were potentially associated with the impaired calcium utilization, but hypocalcaemia has been shown in milk-fed calves on even higher vitamin D intakes (Smith, 1957; Parr, 1957). The available evidence favours the view that vitamin D requirements are greater in milk-fed calves than in normally fed calves.

Irradiation of some calves with u.v. light appeared to produce an effect on calcium metabolism similar to that produced by giving vitamin D in large amounts (70 000 i.u./day).

SUMMARY

1. The addition of a supplement of 300–400 i.u. of vitamin D_3/day to the diet of milk-fed calves reduced the rate of decrease in calcium utilization as the calves got older and prevented the plasma-calcium level from falling when the plasma-magnesium level fell.

2. The addition to the diet of 70 000 i.u. of vitamin D_3/day or irradiation of the calves with u.v. light greatly improved the utilization of calcium both in calves previously receiving no vitamin D and in those receiving 300–400 i.u. of vitamin D_3/day.

3. The mean faecal excretion of magnesium increased from 32 % of the dietary magnesium at about 3 weeks of age to 86 % at about 16 weeks of age, after which it did not change greatly. Neither the addition of vitamin D to the diet nor irradiation of the calves with u.v. light had any appreciable effect on this excretion.

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REFERENCES

Bechdel, S. I., Hilston, N. W., Guerrant, N. B. & Dutcher, R. A. (1938). *Bull. Pa agric. Exp. Sta.*, no. 364.

Duncan, C. W., Huffman, C. F. & Robinson, C. S. (1935). *J. biol. Chem.* **108**, 35.

Hegsted, D. M., Vitale, J. J. & McGrath, H. (1956). *J. Nutr.* **58**, 175.

Henry, K. M. & Kon, S. K. (1942). *Biochem. J.* **36**, 456.

Herman, H. A. (1936). *Res. Bull. Mo. agric. Exp. Sta.*, no. 245.

Huffman, C. F., Conley, C. L., Lightfoot, C. C. & Duncan, C. W. (1941). *J. Nutr.* **22**, 609.

Knoop, C. E., Krauss, W. E. & Hayden, C. C. (1939). *J. Dairy Sci.* **22**, 283.

Parr, W. H. (1957). *Vet. Rec.* **69**, 71.

Smith, R. H. (1957). *Biochem. J.* **67**, 472.

Thomas, J. W. & Moore, L. A. (1951). *J. Dairy Sci.* **34**, 916.

The Inhibition of Mitosis by the Reaction of Maleic Hydrazide with Sulphydryl Groups

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Maleic hydrazide, or 6-hydroxy-2*H*-pyridazin-3-one, inhibits the growth of plants (Leopold & Klein, 1951) and has been used to control the sprouting of stored potatoes and onions. McLeish (1953) demonstrated that the site of action of the chemical was on the chromosomes of the *Vicia* cell, so affecting the early stages of mitotic division. Studies on the effect of maleic hydrazide on the enzymes of intact cells suggest that maleic hydrazide affects the enzymes requiring sulphydryl (SH) groups for activity (Andrae & Andrae, 1953; Bertossi, 1955; Muir & Hansch, 1953; Isenberg, Odland, Prop & Jensen, 1951). No reaction, however, between maleic hydrazide and soluble SH