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Calcium and Magnesium Metabolism in Calves

4. BONE COMPOSITION IN MAGNESIUM DEFICIENCY AND THE CONTROL OF PLASMA MAGNESIUM*

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Orent, Kruse & McCollum (1934) first showed that bone magnesium is depleted in rats kept on magnesium-deficient diets. This was subsequently also shown to be true for calves (Knoop, Krauss & Hayden, 1939; Blaxter, Rook & MacDonald, 1954; Blaxter & Sharman, 1955; Parr, 1957). It has also been shown that in neither of these animals are the soft tissues usually appreciably depleted even under the most severe conditions of magnesium deficiency (Cunningham, 1936; Blaxter *et al.* 1954; Parr, 1957; and our own unpublished observations) although MacIntyre & Davidsson (1958) have recently reported a small fall in skeletal-muscle magnesium in magnesium-deficient rats.

It appears therefore that bone magnesium represents a store of this element which can be called upon under conditions of deficiency to supply the needs of the soft tissues. It has been shown by killing rats at different times after introducing a magnesium-deficient diet that their bone magnesium can rapidly be mobilized (Duckworth, Godden & Warnock, 1940; Duckworth & Godden, 1941). Similar experiments with calves have not been carried out but Blaxter (1956) has related bone magnesium to plasma magnesium in calves slaughtered with differing degrees of hypomagnesaemia developed as a result of protracted milk feeding.

Our experiments on the development of hypomagnesaemia in milk-fed calves (Smith, 1957, 1958, 1959) made it desirable to follow associated changes in bone composition in such animals and other magnesium-deficient calves. The use of vertebrae taken from the tail of the living animal for this purpose will be described.

* Part 3: Smith (1959).

EXPERIMENTAL

Sample preparation and analysis

Bones examined were the caudal vertebrae, rib shaft, femur head and shaft and the first phalanges. They were removed either after slaughter or, with some of the caudal vertebrae, from sections of the tail taken from the living animal, a local anaesthetic (Nupercaine) being used. In this way at least ten successive samples could be obtained from one calf if sections containing only one vertebra were removed at a time. The bones were thoroughly cleaned of adhering soft tissue, cartilage and fat. Samples of the vertebrae and phalanges consisted of whole bones which were split longitudinally. Samples taken from the femur and rib shafts (which were cut transversely from about the middle of the bone) and from the femur head each weighed about 1–2 g. The samples were extracted in a Soxhlet extractor first for 12 hr. with ethanol and then for 6 hr. with ether. They were dried in an oven at 100° to constant weight and then ashed at 600° for about 18 hr. The ash was dissolved in 2N-HCl, the solution made up to a suitable volume and a 5 ml. sample containing about 0·05 mg. of magnesium added to a 15 ml. conical centrifuge tube. With methyl orange as an indicator saturated sodium acetate was added dropwise to give a pH of about 4–5. Calcium was then precipitated as the oxalate and titrated with permanganate; magnesium was precipitated as magnesium ammonium phosphate and determined colorimetrically. The method was that of Green & Allcroft as described by Godden (1937). The accurate determination, by chemical means, of magnesium in the presence of a large excess of calcium is difficult. We found that with the above method for a solution containing magnesium and calcium in the ratio of 1:55 (about the ratio in normal bone) magnesium was recovered to an extent of 98·4 S.D. ±0·2% (six determinations). For a solution in which this ratio was 1:140 (about that in bone grossly depleted of magnesium) the magnesium recovery was 94·5 S.D. ±0·2% (six determinations). In view of the simplicity and good reproducibility of the method and since we were principally concerned with comparative measurements it was considered that this error could be tolerated. It should be remembered, however, that our figures for the percentage of magnesium in bone ash are probably too low by about 0·01–0·02.

Plasma magnesium and calcium were determined as described previously (Smith, 1957).

Treatment of animals

The bone composition in several calves fed normally and in two groups of calves developing hypomagnesaemia was examined. The first group developing hypomagnesaemia consisted of Shorthorn calves (nos. 1, 3, 4, 6, 7) which were fed almost exclusively on cow's milk supplemented when they got older with vitamin D, iron, copper and manganese. Some were also given magnesium supplements for part of the time. Caudal vertebrae were removed at approximately monthly intervals. These animals were also used for balance experiments etc., and further details of their treatment have been described previously (Smith, 1957). The second group consisted of four Friesian bull calves which, at about 2–4 weeks of age, were transferred from a magnesium-adequate diet (magnesium-supplemented synthetic milk or cow's milk, 4·40 l./day) to one of low-magnesium synthetic milk (4·40 l./day). Caudal vertebrae were removed before this change when the magnesium status of the calves was normal, and at intervals afterwards. The composition and method of preparation of the synthetic milk has been described previously (Smith, 1959). It contained about 0·6–0·8 mg. of magnesium/100 ml.

RESULTS

Caudal vertebrae as an index of bone composition

In order to examine the variability between different caudal vertebrae from any one calf the whole tails from a number of slaughtered calves showing different degrees of hypomagnesaemia were obtained. The individual vertebrae were analysed separately. The results given in Table 1 show that the composition of most of these vertebrae in an individual animal was closely similar and that any one could be used to assess, with reasonable accuracy, the composition of the remainder. This appeared to be true whatever degree of magnesium depletion had occurred. Indeed the variability seemed to be somewhat less in the hypomagnesaemic animals than in the normal animals but this was probably due to the fact that the former were older (about 8 months) than the latter (about 1 week). The results given in Table 1 do not include those for the very small bones at the tip of the tail. These tended to vary slightly in composition from the other caudal vertebrae (maximum observed variation in ash content about 12%). We avoided them in subsequent experiments by using only vertebrae with a dry weight greater than 0·1 g.

Table 2 shows the relation between the composition of the caudal vertebrae and the composition of certain other bones in normal and magnesium-depleted calves. Although the ash contents of the different bones were somewhat variable the only marked consistent difference was for the femur

Table 1. *Variability in the composition of different caudal vertebrae from individual calves*

Each column gives the results for an individual calf. Mean values for each calf are given with the standard deviations for determinations on individual vertebrae in parentheses.

	Normal		Magnesium-depleted		
No. of vertebrae	9	11	12	12	11
Ash in dry bone (%)	—	52·2 (1·6)	57·1 (0·9)	59·3 (0·5)	59·4 (0·7)
Magnesium in ash (%)	0·751 (0·026)	0·825 (0·022)	0·401 (0·006)	0·386 (0·009)	0·236 (0·009)
Calcium in ash (%)	38·5 (0·4)	37·9 (0·5)	39·4 (0·3)	39·2 (0·3)	39·2 (0·2)

Table 2. *Relationship between the composition of the caudal vertebrae and the composition of certain other bones in normal and magnesium-depleted calves*

Composition of each of the different bones is expressed as a percentage of the composition of the caudal vertebrae from the same animal. Mean values (±S.D.) are given for two normal calves (mean caudal-vertebrae ash in dry bone 53·3%, calcium in ash 38·6% and magnesium in ash 0·73%) and for three magnesium-depleted calves (mean caudal-vertebrae ash in dry bone 58·5%, calcium in ash 39·1% and magnesium in ash 0·37%).

		Phalanges	Femur head	Rib shaft	Femur shaft
Ash in dry bone	Normal	106±3	98±1	111±8	130±7
	Depleted	102±2	104±3	99±5	123±2
Calcium in ash	Normal	101±0	99±1	101±1	101±0
	Depleted	100±2	100±1	100±1	99±1
Magnesium in ash	Normal	99±1	101±3	101±3	101±5
	Depleted	101±5	109±2	115±7	137±13

Table 3. *Composition of the caudal vertebrae in calves of different ages and with different degrees of magnesium depletion*

Mean results are given (±S.D.).

	Normal		Magnesium moderately depleted	Magnesium grossly depleted
No. of calves	5	3	3	3
Age (weeks)	1–3	30–40	30–40	30–40
Ash in dry bone (%)	55·4±1·1	58·3±1·0	58·6±0·7	58·5±0·6
Calcium in ash (%)	38·9±0·5	39·1±0·3	39·0±0·3	39·3±0·2
Magnesium in ash (%)	0·76±0·02	0·71±0·03	0·43±0·04	0·29±0·04

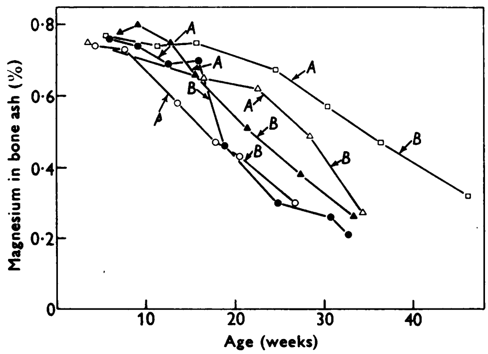

Fig. 1. Relation between bone (caudal vertebrae) magnesium and age in calves fed on cow's milk and developing hypomagnesaemia at different rates. ○, Calf 1; ●, calf 3; □, calf 4; △, calf 6; ▲, calf 7. Ages at which the different calves showed concentrations of plasma magnesium of 1·6 and 0·7 mg./100 ml. are indicated by arrows labelled *A* and *B* respectively.

shaft. This contained about 20–30 % more ash than did the caudal vertebrae in both the normal and magnesium-depleted calves. The calcium in the ash was closely similar for the different bones in all the calves, as was the magnesium in the ash for the normal calves. In the magnesium-depleted calves, however, there were marked differences in the magnesium contents of the ashes of the different bones. Thus although magnesium depletion in these calves occurred in all the bones examined it did not occur to the same extent in all of them. Of those studied the femur shafts were depleted to the least extent and the vertebrae and first phalanges to the greatest extent. It appears therefore that the caudal vertebrae offer a sensitive guide to the magnesium status of the skeleton but that changes observed in these vertebrae during magnesium depletion do not exactly reflect the changes occurring in the skeleton as a whole.

Bone composition in magnesium deficiency

The results given in Table 3 show some small differences in composition between caudal vertebrae from calves of different ages. These differences were not, however, statistically significant and the difference for magnesium in the ash was in any case so small as to be of little importance in relation to changes occurring in magnesium deficiency. The changes in bone-ash magnesium for calves receiving cow's milk, shown in Fig. 1, can be regarded therefore as reflecting changes in the magnesium status of the animals and were not to any appreciable extent due intrinsically to the increasing age of the calves.

There appeared usually to be a broad direct relationship between the rate of onset of hypomagnesaemia in the different calves (itself probably controlled by the rate of fall of efficiency of magnesium absorption, Smith, 1957, 1958, 1959) and the rate of depletion of their bone magnesium. Thus with one exception (calf no. 3, which will be discussed below) falls to concentrations of plasma magnesium of 1·6 and 0·7 mg./100 ml. were associated with bone-ash magnesium figures of 0·60–0·67 and 0·40–0·48 % respectively, whatever the rate at which the concentration of plasma magnesium fell. The plasma magnesium decreased only slowly after it reached about 0·7 mg./100 ml. (see for example Fig. 2 of Smith, 1957) and further depletion of bone magnesium occurred with only a small decrease in this concentration. When the last bone samples were taken, at or within 2 weeks of death (the calves either died, presumably from magnesium deficiency, or were slaughtered with severe symptoms of magnesium deficiency), the mean plasma magnesium level was 0·47 S.D. ± 0·05 mg./100 ml., and the mean magnesium in the bone ash was 0·27 S.D. ± 0·02 % (about 38 % normal). The relationship between plasma magnesium and bone magnesium in these calves is illustrated in Fig. 2.

Fig. 3 shows the changes occurring in bone magnesium and plasma magnesium after the abrupt onset of an acute condition of magnesium deficiency brought about in a group of calves by replacing a magnesium-adequate diet with one of low-magnesium synthetic milk. These results are expressed as percentages of the starting (normal) values since the different calves varied somewhat in their normal levels. After the introduction of the magnesium-deficient diet, the depletion of bone magnesium appeared to occur at an approximately constant rate, presumably at a rate necessary to provide adequate magnesium for the growing soft tissue and for endogenous faecal excretion [urine excretion falls nearly to zero as soon as calves are transferred to a magnesium-deficient diet (Smith, 1959)]. Plasma magnesium fell, rapidly at first, and then more slowly. The growth rate of the calves, and their consequent need for magnesium, was depressed when they received synthetic milk (average weight increase about 1 kg./week compared with about 2 kg./week on a similar amount of cow's milk) and the effects of the magnesium deficiency were consequently less dramatic than might have been expected. The relation between plasma magnesium and bone magnesium for these calves is also illustrated in Fig. 2.

When the bone-ash magnesium values of two of the calves fed on low-magnesium synthetic milk had fallen to 84 and 68% respectively of the normal value they were given daily subcutaneous injections of 1 g. of magnesium (as the sulphate). After 5 days of injections the plasma magnesium of both calves ranged between about 3·7 and 2·2 mg./100 ml. (about 160–95% of normal) after each injection. After 9 days of injections in one case and 16 days of injections in the other the bone-ash magnesium for both calves was found to be normal (each was 102% of the value obtained before the magnesium-deficient diet was fed).

The bone samples taken periodically from each of the calves used in the above experiments did not show any marked changes in ash content or in the percentage of calcium in the ash concomitant with magnesium depletion. Some of the calves showed evidence of faulty calcium utilization for part of the time, due apparently to vitamin D deficiency (Smith, 1957), but bone samples were not usually taken during these periods. Calf 7 showed some indication of slightly reduced bone ash (reduced by about 5–10%) in two samples taken when the calf was hypocalcaemic, but no other apparent abnormalities were observed. The results in Table 3 all refer to calves receiving adequate vitamin D. They show no statistically significant differences in bone-ash content or in the percentage of calcium in the ash between calves of similar age but widely differing magnesium status.

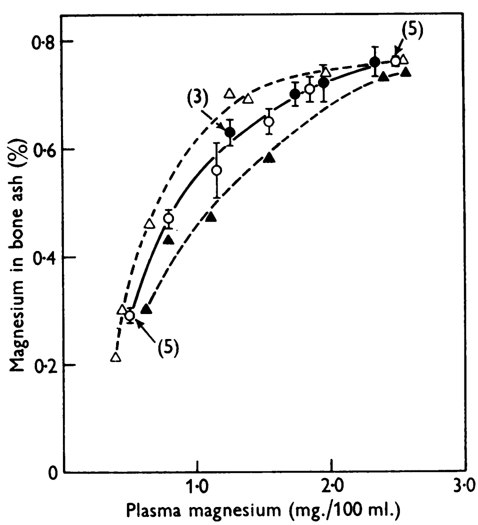

Fig. 2. Relation between bone (caudal vertebrae) magnesium and plasma magnesium in calves developing hypomagnesaemia. Mean results (●, ○) were for groups of calves in each of which the plasma-magnesium values did not differ by more than ±0·15 mg./100 ml. from the mean. Standard deviations for the corresponding bone-ash magnesium values are shown as vertical bars. There were four animals in each group except for those in which the number of animals has been shown by a figure in parentheses. Calves fed on cow's milk and low-magnesium synthetic milk are indicated by ○ and ● respectively. Results for two individual calves are also given and are indicated by the symbols ▲ (calf no. 1) and △ (calf no. 3) with the appropriate curves shown as broken lines.

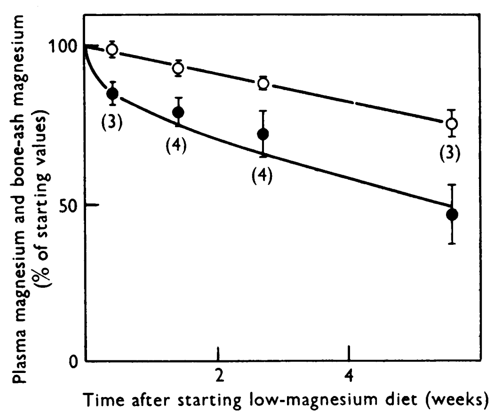

Fig. 3. Changes in bone (caudal vertebrae)-ash magnesium (○) and plasma magnesium (●) after an abrupt change from a magnesium-adequate diet to one of low-magnesium synthetic milk. The calves were 2–4 weeks old at the time of the change in diet. The results given are mean values (with standard deviations shown as vertical bars and the numbers of calves in parentheses) expressed as a percentage of the initial values obtaining on the magnesium-adequate diet. These initial values (mean results for four calves) were 2·34 S.D. ±0·06 mg./100 ml. for plasma magnesium and 0·76 S.D. ±0·03% for bone-ash magnesium.

DISCUSSION

The removal of caudal vertebrae appears to provide a satisfactory means of assessing bone composition in the living calf showing magnesium deficiency (and could probably also be used in studying bone composition in other conditions). The method offers obvious advantages over rib biopsy, which has been used by Thomas & Okamoto (1958) to assess bone magnesium. It should be borne in mind, however, that although in the normal animal we found magnesium to be uniformly distributed in the ash of the different bones which we examined, in magnesium deficiency this distribution was not uniform. This finding is not in agreement with the conclusions of Blaxter (1956). However, these conclusions were based on comparisons between bones (rib shaft, rib end and metatarsal shaft) different from those which we examined. Moreover, it is possible that in the final stages of depletion the bones which lose magnesium more rapidly reach a minimum value, while the bones losing magnesium more slowly catch up. Thus although in moderate depletion such as we examined differences exist, in gross depletion they may tend to disappear.

Our results suggest that magnesium behaves similarly to calcium in the lack of uniformity in depletion of different parts of the skeleton during deficiency. Thus calcium has been shown to be removed more readily from cancellous bone (e.g. vertebrae) than from compact bone (e.g. femur shaft) in calcium-deficient sheep (Benzie, Boyne, Dalgarno, Duckworth, Hill & Walker, 1955).

The relationship between bone and plasma magnesium shown in Fig. 2 is similar in general form to that reported by Blaxter (1956) for data obtained from calves slaughtered with varying degrees of hypomagnesaemia developed as a result of milk feeding. Such absolute differences as exist may be partially accounted for by the fact that Blaxter (1956) examined bone from the metatarsal shaft whereas we used caudal vertebrae.

The relationship suggests that in hypomagnesaemia an equilibrium exists between bone and plasma magnesium. It also suggests that normal bone, from a physiological standpoint, is virtually saturated with magnesium. This was further indicated by the fact that daily injection of magnesium into two calves, so that the plasma magnesium did not fall appreciably below normal and for part of the time was considerably above normal, did not lead to any appreciable increase in bone magnesium above normal amounts. Thus it appears that under normal conditions, when excess of magnesium is entering the blood, the bone plays little or no part in regulating concentration of plasma magnesium. This control appears to be largely effected by changes in urine excretion. This excretion is high when the diet contains more than adequate magnesium (Smith, 1957, 1958), increases enormously when magnesium is injected so that plasma magnesium rises above normal (Smith, 1959), and decreases, eventually nearly to zero, when the magnesium available from the diet decreases (Smith, 1957, 1958). When the available magnesium is so low as to be inadequate even with almost no loss in the urine then bone magnesium is apparently liberated. Under these conditions plasma magnesium is not maintained at a normal concentration, the liberated magnesium is utilized elsewhere and as the bone becomes more and more depleted plasma magnesium drops to maintain the relationship shown in Fig. 2. After depletion has started injection of a small amount of magnesium causes an increase in plasma magnesium, which is followed by a drop nearly to the previous depleted amount (unpublished results). Urine excretion does not increase appreciably if the plasma magnesium remains below normal and the drop is presumably due to magnesium being taken up by the bone to restore the equilibrium. Our present work shows that repeated injection eventually restores both bone magnesium and plasma magnesium to normal.

All the animals studied showed a relationship between bone and plasma magnesium similar in general form to the mean curve shown in Fig. 2. Different animals, however, showed marked differences of detail; extreme differences were shown by calves nos. 1 and 3 respectively and the individual curves for these calves are also shown in Fig. 2. Such differences must affect the rate of fall of plasma magnesium during a period of magnesium deficiency. For example, the depletion of say 13 % of the bone magnesium in calves nos. 1 and 3 would lead to the animals showing plasma-magnesium levels of about 1·8 and 1·1 mg./100 ml. respectively. Plasma magnesium appears therefore to be only an approximate guide to the magnesium status of any individual animal. This helps to explain apparent anomalies in the different rates at which different milk-fed calves develop hypomagnesaemia, for although such different rates can usually be broadly related to corresponding differences in the efficiency with which the calves utilize dietary magnesium (Smith, 1957, 1958) this does not represent the whole picture. Thus, for example, calves nos. 1 and 3 on a milk diet showed almost identical rates of fall of plasma magnesium (that for calf no. 1 is given in Fig. 2 of Smith, 1957) and yet calf no. 1 showed a considerably more rapid decrease in utilization of dietary magnesium (Smith, 1957). These facts can be reconciled by the above considerations.

Shortly before death or the appearance of serious clinical symptoms of magnesium deficiency our calves all showed values of about 0·2–0·3 % of magnesium in the bone ash (about 60–70% depletion). Similar values at death from magnesium deficiency have also been reported by Blaxter & Sharman (1955). These values appear to correspond approximately with the first achievement of a lower limiting level for plasma magnesium of about 0·3–0·5 mg./100 ml. (Fig. 2). Dallemagne & Fabry (1956) have suggested that about two-thirds of bone magnesium is adsorbed on the surface of the bone-salt crystals, with the remaining one-third replacing calcium in the phosphate molecule. It seems possible that the adsorbed fraction is, in fact, that part which is available in magnesium depletion. If this were so the plasma magnesium, about 0·3–0·5 mg./100 ml., would correspond to the virtually complete removal of this adsorbed magnesium. This concentration may therefore be that at which there is magnesium equilibrium between some other tissue or tissues and the extracellular fluid.

Although our results showed no marked change in bone calcium associated with magnesium depletion they were too few to give any reliable information on the small change which would result from the replacement of magnesium by calcium in the bone salt as suggested by Blaxter (1956).

SUMMARY

1. Individual caudal vertebrae have been shown to provide a satisfactory guide to the composition of the skeleton in magnesium-deficient calves. The depletion of bone magnesium occurring in this condition was uniform in the different caudal vertebrae although it varied somewhat in other parts of the skeleton.

2. Periodic removal and examination of these vertebrae from the living calf have been used to follow changes in the magnesium status of the skeleton during conditions of magnesium deficiency resulting from feeding with cow's milk or low-magnesium synthetic milk. Bone-ash magnesium fell progressively from a normal figure of about 0·75% eventually to about one-third of this value when serious clinical symptoms of magnesium deficiency became manifest. Injection of sufficient magnesium readily restored normal values.

3. A relationship between bone-ash magnesium and plasma magnesium has been shown. The evidence suggests that the bone plays little or no part in the control of plasma magnesium under conditions of magnesium excess but that in magnesium deficiency in calves the concentration of plasma magnesium is determined by the concentration of bone magnesium.

4. No significant changes in total bone ash or in the proportion of calcium in the ash were observed concomitant with the depletion of bone magnesium.

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