CCLXXXIX. BLOOD CALCIUM AND MAGNESIUM OF THE COW IN HEALTH AND DISEASE.

BY WILLIAM MILLER ALLCROFT AND HENRY HAMILTON GREEN.

From the Veterinary Laboratory, Ministry of Agriculture, Weybridge.

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THE present paper continues investigations into the levels of blood-minerals in the domestic animals in health and disease and deals specifically with serum-magnesium and calcium under normal and definable pathological conditions in the bovine¹.

In general the serum-Ca of the normal bovine ranges around 10 mg. and the serum-Mg rather over 2 mg. per 100 ml. For calcium a figure such as 8 mg. is considered low and 13 mg. high but, in using the terms hypocalcaemia and hypercalcaemia, figures such as the 3-7 mg. of "milk fever" of cows and the 15-20 mg. of bovine equisetosis [Meetz, 1932] come most naturally to mind. In the case of magnesium the most characteristic disorder associated with change of serum level is "lactation tetany" or "grass tetany" of cows, a disease which is most prevalent during the period of luxuriant growth of spring pasture, although occasionally reported even in stalled animals in mid-winter, and in which the serum-Mg may vanish almost entirely.

The normal value of serum-Mg however is not so clearly established as that of serum-Ca and to some extent this complicates the biochemical diagnosis of lactation tetany, acute and chronic. In the present paper the data are plotted so as to show frequency distribution and so render possible an assessment of the probability of any given value falling within the normal range.

For normal cattle Sjollemma [1930] gave an average value of 1.66 mg. Mg per 100 ml. serum in one set of observations and 2.0-2.24 mg. in another [1932]. In a group of cows with a previous history of lactation tetany he observed considerably lower values at the end of the winter stall period than after summer at pasture. Dryerre [1932] gave an average of 1.9 mg. with a range 1.8-3.1 mg. for random samples. Blakemore and Stewart [1932-33] record an average value of 2.13 mg. with a range 1.04-3.17 mg. for clinically healthy cows on farms with a history of lactation tetany. They also state that of 70 cattle at the Cambridge Institute of Animal Pathology, showing an average of 2.97 mg., not one showed a value below 2.4 mg.; of a dozen cows from a district in Cheshire not one showed less than 2.8 mg. per 100 ml.

The difference between a Dutch group averaging 1.66 mg. and an English group averaging 2.97 mg. represents a difference of 80 % and is obviously perturbing from the point of view of diagnosis of lactation tetany. In the case of Sjollemma's figures it is not always certain however how many of the values are "random normals" and how many are "clinically normal animals" from grass tetany farms at the season of high incidence of the disease, so that the divergence may be more apparent than real. Nevertheless the differences in different sets

¹ Work of W. M. Allcroft, commenced at the Rowett Institute, Aberdeen, continued on a Carnegie Research Scholarship at Weybridge.

of records at least serve to emphasise the need for further work on the physiological range in the bovine under different conditions of life and upon the factors affecting the individual level of blood-Mg.

Factors known to be, or suspected of being, able to effect an alteration in the level of serum-Mg are few in number, and certain experimental data are rendered more difficult to interpret by the fact that normal values vary more widely in small laboratory animals than in the larger mammals. Thus Watchorn [1933] found a normal range of 3.30–6.89 mg. per 100 ml. serum in young rats and 3.63–5.69 mg. in adult rats, and in periodic observations on the rabbit an erratic jump of 100 % in the same individual has occasionally been recorded.

The influence of dietary Mg on blood Mg is still debatable but changes in the Mg content of a ration over fairly wide limits do not seem to effect consistent alterations in serum-Mg although extreme changes may do so. Magnesium starvation, induced in rats and dogs by Kruse *et al.* [1932; 1933] on a synthetic diet containing only 1.8 parts Mg per million, was associated with an early and progressive fall in serum-Mg, the level falling to half the normal within 3 weeks. At the other extreme Cunningham [1933] reports marked rise of serum-Mg in rats following ingestion of large amounts of magnesium compounds and less convincing elevation in sheep and cows [1934]. Brookfield [1933] associated changes in level of serum-Ca and Mg with varying mineral intake in the diet of rabbits. On the other hand Watchorn [1932] was not prepared to attach significance to the differences in serum-Mg of rats on diets ranging so widely as 0.2 and 1.6 % in Mg content, and limited Weybridge data (unpublished) show no significant change following oral administration to cattle of considerable doses of magnesium salts over short periods.

On the experimental side Jacoby and Freidel [1932] report lowered serum-Ca and raised Mg following intravenous injection of sodium oxalate (0.1–0.15 g. per kg.) into rabbits. Weaver and Reed [1930] found that complete thyroparathyroidectomy had no effect on the level of serum-Mg whilst depressing Ca and elevating inorganic P. Scholtz [1931] reported that injections of parathyroid extract produced a small transitory rise of serum-Mg followed by a fall before maximum elevation of serum-Ca. Greenberg and Mackey [1932] observed a transient rise in serum-Mg of the order 0.4–1.0 mg. per 100 ml., reaching its peak 2–6 hours after subcutaneous injection of 100 units of parathyroid extract into dogs. Cannavò [1932] reported marked and progressive elevation of serum-Mg following subcutaneous injections of large amounts of prolan (anterior pituitary extract) in rabbits and dogs. Cannavò and Indovina [1932] later reported similar effects in man.

Under pathological conditions in the human subject there is no such dramatic change in serum-Mg as in bovine lactation tetany, but lesser variations have been frequently reported. Peters and Van Slyke [1931] give the wide normal range of 1–3 mg./100 ml. for serum-Mg in man and state that the level is little affected by pathological conditions, but it now seems doubtful if 1 mg. can really be regarded as normal and whether many of the minor variations are not more significant than they appear at first sight. Watchorn [1924–25] found that chronic infections such as eczema, catarrh, iritis, diarrhoea and infections of the nares, sinuses and tonsils were attended by distinct hypermagnesaemia, values up to 3.5 mg. occurring in some of these conditions as compared with normal values of 2.08–2.50 mg. Weil *et al.* [1923] reported a higher level in cases of atheroma of the aorta and chronic deforming arthritis—up to 3.7 mg. as compared with their normal of about 1.8 mg. A consistent, small reduction in serum-Mg is reported in hyperthyroidism (Watchorn, personal communica-

tion). Krüger [1932] has reported low serum values in children with active and healing rickets, and Becher and Hauman [1932] record high values in nephritis—up to 3.49 mg. in severe cases with nitrogen retention. Watchorn and McCance [1932] record values even up to 5.77 and 10.74 mg. in uraemia. Hirschfelder [1933] on the other hand has reported lowered serum-Mg (0.9–1.37 mg.) in patients with nephritis, epilepsy and parathyroid tetany associated with twitchings or convulsions. Sjollem and Seekles [1932] observed normal values in two cases of tetany, one manifest after removal of a goitre, the other latent in a case of pyloric stenosis, but reduced serum-Mg as well as reduced serum-Ca in three patients after parathyroidectomy.

In view of some of the high values of serum-Mg earlier recorded for normal human patients by Marriott and Howland [1917] and Denis [1920] and the low average accepted as normal by Weil *et al.* [1923] it is difficult to assess the significance of observed changes in human diseases. In sharp distinction to this is the bovine “grass tetany” of the Continent, or “lactation tetany” as it is termed in Britain and in New Zealand [Hopkirk *et al.*, 1933]. In this disease, characterised by extreme nervous hyperirritability in acute cases, values for serum-Mg as low as 0.1 mg. per 100 ml., or only one-twentieth of a common normal value, have been found, and values well below half the normal are the rule. The fall may be quite sudden, and return to normal may occur within 24 hours of spectacular recovery following a single injection of a magnesium salt, although quite commonly the normal value may only be regained after several weeks [Blakemore and Stewart, 1932–33]. In milk fever of cows the typical hypocalcaemia, with serum-Ca commonly below half the fairly consistent normal value, may be associated with distinct hypermagnesaemia, as reported by Pribyl [1933], although the association is not invariable and may be a pathological exaggeration of the physiological changes reported by Allcroft and Godden [1934] for clinically healthy dairy cows at parturition.

As will be shown presently, pronounced hypomagnesaemia in the bovine can be associated with any range of blood-Ca, and pronounced hypocalcaemia may be associated with either low or high serum-Mg. For equines there is also provisional evidence (unpublished) that the hypocalcaemia observed by Montgomerie *et al.* [1929] in “transit tetany” of Welsh mountain ponies after a railway journey to market, may also be associated with pronounced hypomagnesaemia.

The present data concern blood levels of Ca and Mg in normal cows and heifers in cases of lactation tetany, milk fever, advanced tuberculosis and contagious abortion.

Technique.

Serum-Ca was determined by the Clark and Collip [1925] modification of the technique of Kramer and Tisdall [1921]. Serum-Mg was determined by a modification of the procedure of Denis [1922], the final colorimetric determination utilising the technique of Fiske and Subbarow [1925]. Checks on a standard solution containing 10 mg. Ca and 2 mg. Mg per 100 ml. were regularly inserted at random amongst sera to be analysed in order to ensure consistent manipulation.

Results.

Normal animals. Two lots of data were secured. Samples of blood from 108 cows and heifers in the dairy herd of the National Institute for Dairy Research at Reading constituted the first lot, procured with the co-operation of the Director, Dr H. D. Kay, and Mr N. Barron, the Ministry veterinary

Distribution charts for values of serum-Ca and serum-Mg on a percentage basis for comparison.

- Fig. 1. 108 normal cows and heifers (Reading).
 Fig. 2. 31 normal cows (Wiltshire and Isle of Wight).
 Fig. 3. Combined Fig. 1 and Fig. 2.
 Fig. 4. 53 cows suspected of contagious abortion.
 Fig. 5. 35 cows with advanced tuberculosis.

adviser attached to the University. The second lot is represented by 31 random samples procured through veterinarians in Wiltshire and the Isle of Wight.

Figs. 1 and 2 show the distribution of values for serum-Ca and serum-Mg in both groups, which for graphical purposes are treated on the basis of 100 animals so as to permit direct comparison. It is apparent that distribution of values is much the same in both groups and, since the numbers in each lot are different and the selection of graphing intervals is arbitrary, no significance is attached to the fact that in the random lot the calcium tends to run a little higher and the magnesium a little lower than in the selected dairy herd. Figs. 1 and 2 may therefore be combined, presented as a group of 139 animals in Fig. 3, and treated as percentage distribution of random samples of normal blood.

The range of serum-Ca is 8.65–11.65 mg. per 100 ml., or $\pm 15\%$ around the mean, but the proportion of extreme values is small. 85% of the values lie between 9 and 11 mg. and 57% lie between 9.5 and 10.5 mg. Only 3% fall below 9 mg. and only 5% exceed 11.5 mg.

For serum-Mg the range is 1.85–3.17 mg., a wider variation of about $\pm 26\%$, but again the great majority of values fall between much narrower limits. Nearly 90% fall between 1.9 and 2.7 mg. and 59% lie between 2.1 and 2.5 mg., *i.e.* within $\pm 9\%$. Only 4% are down to 1.7–1.9 mg. and only 2% reach 3.1–3.3 mg. The general shape of the charts is what would be expected from random sampling.

Contagious abortion. Samples sent to the Weybridge laboratory for routine diagnosis were also available and 53 of these, from widely scattered districts of Great Britain, were taken for analysis. 32 showed positive agglutination tests and 21 were negative. Fig. 4 shows distribution charts for serum-Ca and Mg for the group as a whole, and positive and negative reactors are marked individually on Fig. 8. Taken as a whole group of "cows suspected of contagious abortion" the charts of Fig. 4 do not differ materially from the charts of Figs. 1, 2 and 3. In further comparison of reactors with non-reactors no significant difference could be discerned in serum-Ca but the serum-Mg showed a somewhat greater tendency to diverge from the normal. In the positives the range was 1.7–3.3 mg. with 81% of the values between 2.1 and 2.7 mg. In the negatives the range was 1.9–2.7 mg. with 80% of the values between 2.1 and 2.5 mg. If these differences can be regarded as significant they would support the observations of Watchorn and others, already discussed, which indicate greater fluctuations in individual values with a general upward tendency for localised bacterial infections.

Advanced tuberculosis. Advantage was taken of the presence of advanced cases of bovine tuberculosis under study at Weybridge, and blood samples were secured from 35 animals. The Ca and Mg values for citrated plasma are charted in Fig. 5 and show a decided general shift to the left. The serum-Ca is slightly lowered, 42% of the values lying between 8.5 and 9.0 mg. in distinction to 57% of values between 9.5 and 10.5 mg. in Fig. 4. The Mg values are of still greater interest. They are definitely lower in all except three cases, so much so that 79% of the values are below the lower normal limit of Fig. 3, 37% lying between 1.5 and 1.7 mg. and 42% falling below 1.5 mg. 9% of the cases were down to 0.9–1.1 mg., approximately half the average normal value.

Lactation tetany. These samples were obtained from a few veterinary practitioners co-operating in the investigation but, since the incidence of the disease seems to have been low in 1934, the number of samples is not so large as desired. The 18 cases recorded, including a few 1933 samples, are shown in Figs. 7 and 8.

All display the pronounced hypomagnesaemia characteristic of the disease, with values 0.3–1.16 mg. per 100 ml. of serum, but it is of interest to mention that in the 1933 cases serum-Ca was either normal or only moderately low while in 1934 marked hypocalcaemia was also shown in all cases, values ranging from

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 6. Spot chart for lactation tetany cases.

• Sjollema. □ at Weybridge.

Fig. 7. Distribution chart for values of serum-Ca and serum-Mg in 55 cases of lactation tetany (Sjollema).

Fig. 8. Spot chart for values of serum-Ca and serum-Mg of cows in health and disease.

- Normal cows and heifers.
- + Positive reactors to *Brucella abortus*.
- × Negative reactors to *Brucella abortus*.
- Advanced cases of tuberculosis.
- Lactation tetany.
- Milk fever.
- ▣ 6 cows sampled 0 to 2 days *post partum*.
- ▤ The same cows sampled again 4 to 5 days *post partum*.

7.6 mg. down to 4.8–5.8 mg., *i.e.* of the order commonly encountered in milk fever. Judging from the clinical notes of the practitioners who supplied these bloods it would appear that symptoms in the corresponding cows were not always typical and that clinical diagnosis was occasionally in doubt. In one case, which terminated fatally, the cow was treated for lactation tetany at the

first visit and for milk fever the following day. It showed considerable improvement after inflation of the udder and injections of calcium but relapsed into hypomagnesaemic tetany a day later.

A clinical note by Wilkinson [1933] raised the question whether milk fever and lactation tetany can co-exist. The present evidence at least shows that the biochemically diagnostic features of the two diseases can co-exist, irrespective of the specific clinical symptoms of the particular case. Fig. 8 shows a cluster of five cases circumscribed between the limits 0.4–1.1 mg. serum-Mg and 4.7–5.7 mg. serum-Ca.

In his 1930 paper Sjollem gave the data of 55 grass tetany cases showing an average of 6.65 mg. Ca, but without drawing special attention to the distribution of values. The individual Dutch figures are therefore charted in Fig. 6 along with the Weybridge data. It is immediately obvious that the two sets of spots correspond, that hypomagnesaemia is characteristic of the disease, but that blood-Ca varies independently; marked hypocalcaemia is as common as slightly subnormal Ca.

Milk fever. This disease was not specifically sought in 1934 but the few cases encountered are spotted in Fig. 8 and suggest an interesting tendency towards high Mg although the figures are actually covered by the variations shown higher up for normal cows. Pribyl, already quoted, definitely associated the hypocalcaemia of typical milk fever with distinct hypermagnesaemia and indeed suggested that his results confirmed the view of Kloubok [1932] that the coma of milk fever could be interpreted as a magnesium narcosis. Recent information from Godden and Duckworth (unpublished) emphasises the association from another point of view. From data procured at calving, Godden and Allcroft [1932] considered that the extreme hypocalcaemia of milk fever might well be a pathological manifestation of the physiological depression of serum-Ca noted in clinically normal dairy cows at the time of parturition. Some of the supplementary observations of Allcroft and Godden [1934], in which determinations of serum-Mg were included, are also inserted in Fig. 8. From these it will be noted that for the first 2 days after parturition the relevant spots dip downwards to the right, *i.e.* towards hypocalcaemia and hypermagnesaemia. The same cows, sampled 4–5 days after calving, show a slight tendency towards over-compensation, so that the spotting slants upwards and to the left and suggests slight physiological hypomagnesaemia and perhaps slight hypercalcaemia.

DISCUSSION.

Little further discussion is needed but the main features of the observations may be recapitulated as follows:

- (1) Although the extreme range of serum-Mg in clinically healthy cows is comparatively wide the common physiological range is considerably narrower. In some cases extreme values may possibly be due to disorders not clinically manifest at the time blood samples are taken for average values.

- (2) Comparatively localised infection with *Brucella abortus* appears to have no marked effect upon serum-Mg although the values tend to fluctuate more widely.

- (3) When most of the organs are involved as in advanced generalised tuberculosis there is a distinct lowering of serum-Mg associated with slight diminution of serum-Ca. In how far the changes are specific for the disease and in how far they are due to secondary involvement of the obscure physiological mechanism controlling the level of blood-Mg, or to malnutrition, is not yet known.

(4) The pronounced hypomagnesaemia and nervous hyperirritability of lactation tetany of cows may be associated with normal serum-Ca or with marked hypocalcaemia. There is no necessary correlation between the blood levels of these two minerals in this disease, and the Ca/Mg ratio is not the determining factor in precipitating the nervous symptoms.

(5) The specific hypocalcaemia of milk fever may be associated with normal, high or low serum-Mg. When hypomagnesaemia and hypocalcaemia are both pronounced the case may be diagnosed as atypical milk fever or atypical tetany, and the train of symptoms may even change in the same animal within 24 hours.

(6) The period of parturition in the modern high-yielding dairy cow is commonly characterised by sudden physiological hypocalcaemia and fluctuating serum-Mg, apparently in the direction of hypermagnesaemia closely succeeding the hypocalcaemia. These changes may be followed by over-compensation in the opposite direction. Blood samples taken shortly after calving should therefore be excluded in considering normal values. Sufficient cases of "milk fever" at periods atypically remote from parturition, and of "grass tetany" incidentally associated with recent calving, have not yet been obtained to correlate the changes in serum-Ca and -Mg more specifically with the sexual life of the cow.

(7) Since, as is particularly apparent in the data of Blakemore and Stewart [1932-33], a reduced level of serum-Mg may persist in some cases for several weeks after apparent recovery from lactation tetany, and chronic hypomagnesaemia may be found in the bovine without specific symptoms, unusually low "apparent normal values" should be viewed with suspicion of metabolic disorder.

(8) Hypomagnesaemia in the bovine cannot be correlated with low dietary intake of magnesium. It may appear and disappear on the same diet within 48 hours. Its seasonal incidence however is commonly associated with change in environmental and dietary (as distinct from nutritional) conditions.

SUMMARY.

The normal range in values of serum-Ca and serum-Mg in 139 clinically healthy cows is recorded as 8.65-11.65 mg. and 1.85-3.17 mg. per 100 ml. respectively. 85 % of the values for Ca lay between 9 and 11 mg. and 57 % between 9.5 and 10.5 mg. Only 3 % fell below 9 mg. and only 5 % exceeded 11 mg. Nearly 90 % of the Mg values lay between 1.9 and 2.7 mg., and 59 % between 2.1 and 2.5 mg. Only 4 % were below 1.9 mg. and only 2 % reached 3.1-3.3 mg.

No definite change in distribution of values could be associated with *Brucella abortus* infection, but in advanced generalised tuberculosis definite hypomagnesaemia was shown in 32 out of 35 cases.

Eighteen cases of lactation tetany showed serum-Mg between 0.3 and 1.1 mg. and serum-Ca ranging from 10.8 mg. down to 4.7 mg. No correlation existed between these two minerals in clinically diagnosed cases of tetany. In five cases of milk fever serum-Ca ranged from 3.8 to 6.8 mg. and serum-Mg from 1.8 to 3.1 mg. with, perhaps, a tendency to high values for the latter. Certain cases showing the typical hypomagnesaemia of lactation tetany combined with the typical hypocalcaemia of milk fever showed atypical symptoms of both diseases.

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