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The Partition of Serum Calcium into Diffusible and Non-Diffusible Portions.

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Various methods of determining the diffusible and non-diffusible calcium have given discordant results. The question whether the non-diffusible calcium is protein-bound is unsatisfactorily answered. The present communication gives, first, the relative importance of the two fractions of blood calcium in maintaining the normal irritability of neuro-muscular system, second, the correlation between non-diffusible calcium and the protein content of blood or other body fluids, and third, the influence of cod liver oil and parathyroid hormone on the calcium partition in blood.

For the determination of diffusible and non-diffusible calcium, the method of ultra-filtration and dialysis as described by Moritz[1] and improved by Updegraff, Greenberg and Clark[2] was employed with a few modifications. Total calcium, diffused calcium and non-diffused calcium were separately analyzed, using the method of Clark and Collip.[3] Wu and Ling's method[4] was used for albumin and globulin content of blood or other body fluids.

The calcium distribution in blood serum was determined in 9 adults with conditions not known to be associated with disturbances in calcium metabolism, with a total serum calcium ranging from 9.2 to 10.6 mg. per 100 cc. The non-diffusible calcium varied from 6.1 to 4.1 mg. or 57.6 to 42.3 per cent, while the diffusible calcium from 5.6 to 4.5 mg. or 57.7 to 42.4 per cent. The average values of 5.0 mg. or 51 per cent for diffusible calcium, and of 4.8 mg. or 49 per cent for non-diffusible calcium obtained in this series agree very well with the results of Moritz and Updegraff and her co-workers.

Two cases of tetany were studied. In one case with a total serum calcium of 8.0 mg., the non-diffusible fraction was 4.3 mg. or 58.6 per cent, while the diffusible fraction 3.7 mg. or 46.4 per cent. In the second case with a total serum calcium of 6.5 mg., the results were 3.8 mg. or 56.4 per cent for the non-diffusible portion and 2.6 mg. or 43.6 per cent for the diffusible portion. In these two cases of tetany, both the diffusible and the non-diffusible calcium was decreased with a greater decrease in the diffusible fraction.

In the three cases of nephrosis examined, all showed a reduction of total serum calcium, the values being 8.6 mg., 7.9 mg. and 7.7 mg. respectively. The non-diffusible fraction was greatly reduced, namely, 3.3 mg., 2.3 mg. and 2.0 mg., while the diffusible fraction remained normal or slightly above normal, namely, 5.3 mg., 5.6 mg. and 5.7 mg. Similar results were obtained in 4 cases of kala-azar studied. The total serum calcium ranged from 6.6 mg. to 7.6 mg. In spite of the low total serum calcium in these cases of kala-azar and nephrosis, none of them showed any increased irritability of nerves to either mechanical or electrical stimulation. The greater reduction of diffusible than of non-diffusible calcium in tetany, and the reduction of the non-diffusible portion without any reduction in diffusible calcium in kala-azar and nephrosis without tetany, suggest strongly that it is the diffusible, rather than the non-diffusible calcium that is physiologically active in the control of irritability of neuro-muscular system.

The reduction of non-diffusible calcium in nephrosis and kala-azar seems to be associated with a reduction in plasma protein, especially albumin. In these cases, plasma albumin was invariably low, ranging from 1.2 to 2.3 per cent with an average of 1.9 per cent, in contrast to 3.7 per cent in the 9 control cases. Plasma globulin was distinctly low in four out of seven cases. Studies on 8 pleural and ascitic fluids seem to substantiate further the close parallelism between non-diffusible calcium and protein content. In these fluids, with a total calcium ranging from 5.3 to 7.2 mg., the non-diffusible portion varied from 1.7 to 0.2 mg., while the diffusible ranged from 5.7 to 4.4 mg., showing that almost all the calcium is in the diffusible fraction. The protein content of these fluids was extremely low; albumin 0.4 to 0.06 per cent, and globulin 0.7 to 0.04 per cent.

The influence of cod liver oil on serum calcium partition and plasma protein was studied in two cases. One case of tetany with a total serum calcium of 8.0 mg., consisting of 4.3 mg. of non-diffusible and 3.7 mg. of diffusible calcium was given 30 cc. of cod liver oil daily for 12 days. At the end of the period, the total serum calcium rose to 9.8 mg., 4.7 mg. non-diffusible and 6.1 mg. diffusible, showing a more marked rise in the diffusible calcium. Similar results were obtained in a case of nephrosis. In these cases the plasma proteins were not markedly changed, although the albumin showed a tendency to increase toward the end of the period.

Next, the effect of parathyroid hormone was studied in two cases of tetany. In one case, which was already convalesceent, 730 units of Collip's parathyroid extract were given in 8 days. The serum calcium rose from 9.2 mg. to 10.4 mg. In regard to the calcium par-

tition, the non-diffusible portion increased from 4.2 mg. to 4.6 mg., while the diffusible portion from 5.2 mg. to 5.8 mg. The second case, while showing active tetany, received 530 units of the extract in 8 days. The blood calcium increased from 6.5 to 11.1 mg. The non-diffusible fraction changed from 3.1 to 4.6 mg., while the diffusible fraction rose from 3.6 to 6.6 mg. In both cases, under the influence of parathyroid hormone the rise in the diffusible calcium was greater than in the non-diffusible calcium. Plasma proteins again were not significantly altered, though the tendency for the albumin to diminish was appreciable.

To summarize, in the study of serum calcium, emphasis should be laid on its partition, for there are conditions unassociated with tetany but showing a low total serum calcium. In such cases, unlike those of tetany, the reduction is entirely in the non-diffusible calcium, coinciding with a reduction of plasma albumin. The importance of protein in relation to the level of non-diffusible calcium is again brought out by the analysis of pleural and ascitic fluids which with a very low protein content contain mostly diffusible calcium, the non-diffusible being sometimes negligible in amount. Under the influence of either cod liver oil or parathyroid hormone, the elevation of blood calcium seems to be more in the diffusible than in the non-diffusible portion, apparently independently of any change in the plasma proteins. The increase of the diffusible calcium in the cases of tetany is always accompanied by relief of symptoms.

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[1] Moritz, A. R., *J. Biol. Chem.*, 1925, lxiv, 81.

[2] Updegraff, H., Greenberg, D. M., and Clark, G. W., *J. Biol. Chem.*, 1926, lxxi, 87.

[3] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, lxiii, 461.

[4] Wu, H., and Ling, S. M., *Chinese J. Physiol.*, 1927, i, 161.

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The Alleged Ephedrine Action of Two California Species of Ephedra.

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In a recent paper by Clark and Groff[1] it was inferred that ephedrine or closely related substances are present in two California