

6 AN INTERPRETATION OF CALCIUM PROTEIN FINDINGS
IN SERA OF LEPROSY REACTION CASES
BY MEANS OF THE McLEAN-HASTINGS EQUATION ¹

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In a recent paper (8) I showed that in the sera of leprosy patients the diffusible calcium level is well within the generally-accepted range of normal variation, regardless of the degree of advancement of the disease or the presence of lepra reaction. On the other hand, it was found that in the lepra reaction cases the values of the other constituents were abnormal. Out of 12 such cases examined the total calcium level was low in 7 (58%), the nondiffusible calcium and the albumin were low in 10 (83%), and the globulin was above normal in 9 (75%). The pertinent data are given later (Table 1 and Text-fig. 1).

It was concluded in that study that the reduction in concentration of total serum calcium is not due directly to any disturbance in calcium metabolism, as is believed by other leprosy workers, but to the lowering of concentration of the protein-bound (nondiffusible) calcium, and that probably that change is merely a sequela of the upset in the protein component system which results in the inversion of the albumin: globulin ratio, i.e., a shifting of the albumin level to below normal and of the globulin level to above normal.

In the present communication it is the purpose to show that in the McLean-Hastings equation we have the means of further explaining this situation, and also of revealing a factor which may be of importance to the problem of treating cases afflicted with lepra reaction.

THE McLEAN-HASTINGS EQUATION AND ITS APPLICATION

According to McLean and Hastings (6), in protein-containing fluids of human origin like the blood serum and the ascitic, pleural and subcutaneous edema fluids, the ionization of calcium is the function chiefly of an equilibrium between the calcium ions, the protein ions, and the undissociated calcium salts of protein, definable by the mass law equation:

¹ Published with the approval of the Director, Bureau of Hospitals.

$$\frac{[\text{Ca}^{++}] \times [\text{Protein}^{-}] }{[\text{Ca Proteinate}]} = K \quad (\text{I})$$

Their interpretation of this formula may probably best be stated in their own words:

From the standpoint of the ionization of calcium in protein-containing fluids, therefore, these fluids may be most simply thought of as solutions of calcium proteinate, which ionizes as a weak electrolyte into calcium and protein ions, with a residue of the unionized compound.

They also said, however, that this statement is an over-simplification of the conditions which actually exist in the fluids of the body.²

On applying this equation to the results obtained by me in lepro reaction cases it has seemed important, in view of the nature of the substances investigated, first to consider how the various substances under consideration may fit into the equation. This question may seem to be one that requires special investigation, but from the work of various investigators we have the following facts which are pertinent to the present discussion. (a) It is now generally accepted that the diffusible calcium is almost all ionized, if not completely so, and that the nondiffusible calcium is the fraction of that substance in physical or physico-chemical combination with protein (10). (b) These two forms of calcium are together sufficient to account for all or nearly all of the total calcium (6, 10). (c) The studies of Marrack and Thacker (5), Loeb and Nichols (4), Greenberg and Gunther (1), and Thomson and Collip (11) have yielded data which, according to them, suggest that the diffusible calcium is in equilibrium with the nondiffusible fraction. (d) Available evidence (2) shows that in the blood serum the protein-bound calcium is combined chiefly with the albumin, and that its quantity is in proportion to the quantity of that protein. In hyperproteinemia the increase in the protein-bound calcium is the calcium bound by albumin and not by the increased content of the euglobulin. In the sera of leprosy patients the findings of Ross (9) show that the increase in the total globulin content is chiefly in the euglobulin fraction.

² In this work of McLean and Hastings the calcium ions (Ca^{++}) were determined directly by the biologic method which they developed, using for indicator the isolated frog heart. Calcium proteinate and protein ions were calculated from values of total calcium and total protein, thus:

$$([\text{Total Ca}] - [\text{Ca}^{++}]) = [\text{Ca Proteinate}]$$

$$([\text{Total Protein}] - \text{Total Ca} + [\text{Ca}^{++}]) = [\text{Protein}^{-}]$$

It is plainly to be deduced from these facts that the equilibrium relationship can be significant only with respect to three of the five serum constituents under consideration, namely, the diffusible calcium, the nondiffusible calcium and the albumin, and that these three substances can be substituted for the reactants in the above given equation: the diffusible calcium for the $[Ca^{++}]$, the albumin for the $[protein^{- -}]$ and the nondiffusible calcium for the $[Ca\text{ proteinate}]$. The equation may therefore take the following form:

$$\frac{[Diffusible\ Ca^{++}] \times [Albumin^{- -}]}{[Nondissusible\ Ca] \quad (Ca\ albuminate)} = K \quad (II)$$

For reasons too obvious to require elaboration, this equation can at best be treated only qualitatively. That this is a sufficient basis for the present discussion will appear presently.

According to chemical equilibrium theory, when any change is made in a system where the reactants are in equilibrium concentrations, that change (of any one of the reactants involved) would by mass action law necessarily result in changes of concentrations of the other reactants by chemical action, in such a way as to make the value of the equilibrium constant K the same as it was originally. Take, for instance, the case of equation (II). By increasing the concentration of, say, the albumin, it is to be expected that more molecules of this protein fraction will react with the diffusible calcium to form more molecules of the undissociated calcium proteinate until constant K , temporarily disturbed as a result of the increased albumin concentration and consequently of the concentrations in the numerator, is restored to its original value. Stated otherwise, the concentration in the denominator (calcium proteinate) is also increased by the reaction involved in the restoration of the equilibrium. Conversely, should the concentration of albumin be decreased, the trend of adjustment would involve dissociation of calcium albuminate into calcium ions (diffusible) and albumin ions, and this process would continue until the rate of dissociation again equaled the rate of formation of this compound by the reaction between the diffusible calcium and the albumin. Here again it can be seen that a decrease in the product of concentrations in the numerator necessarily leads also to a decrease in the product of concentration in the denominator.

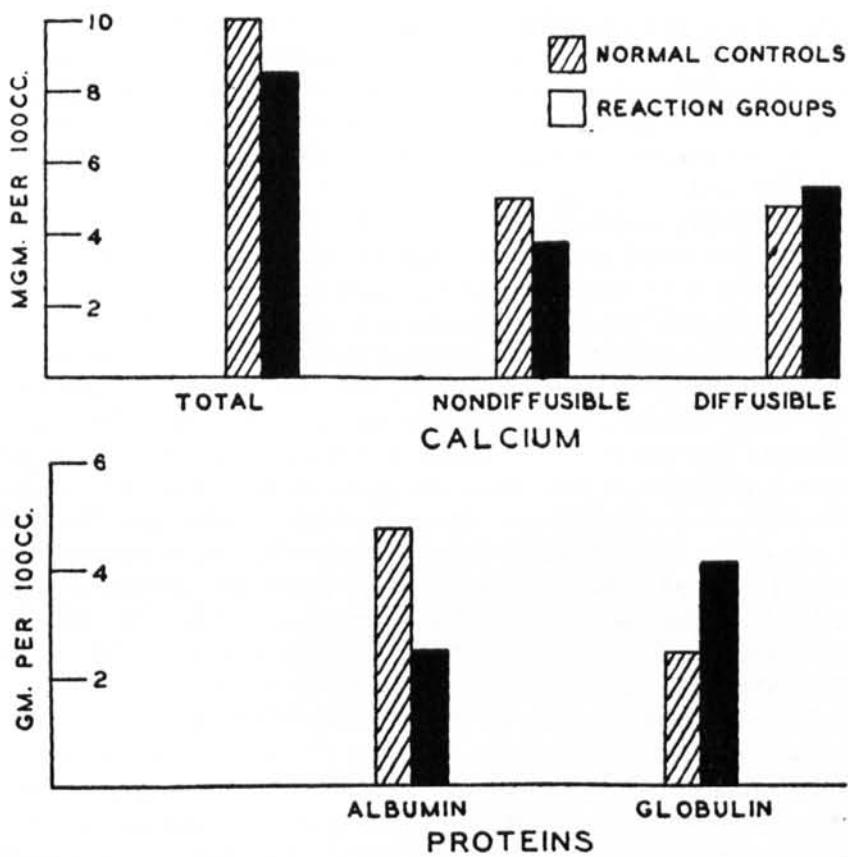
According to these principles, what can evidently be expected in conditions where the calcium metabolism is disturbed, as in

hypocalcemia due to the insufficiency of parathyroid hormone, is that both the diffusible and the nondiffusible fractions will be low; whereas in conditions caused by hyperfunction of this gland these fractions will be increased. In reported studies of such conditions this expectation was confirmed by the actual findings (7).

Referring again to the results obtained in the lepra reaction group in the previous study, Table 1 contains the essential data in comparison with accepted figures for normals, and they are illustrated in Text-fig. 1. Applying the mechanism just discussed to these data, it will be seen easily to refute the belief in the existence of a disturbance of calcium metabolism. Thus, while low total calcium and low nondiffusible calcium levels were actually observed in most of these cases, a result which could indeed be taken as indicative of such a metabolic disturbance, the diffusible calcium was normal in all, contrary to mass law. This constituent, according to this law, should also manifest lowering of its concentration if there were a metabolic disturbance. On the other hand, as equation (II) seems to reveal, the fall in the level of the albumin could very well account for the low levels of the nondiffusible calcium. This fact would show plainly that the observed low finding of total calcium is nothing but a consequence of the lowering of the albumin level. There can, therefore, be no sound basis for attributing these results to the existence of a disturbance in the metabolism of this mineral.

TABLE 1.—*Data from previous study, for controls and lepra reaction cases only; minima, maxima and averages.*

Constituents	Control cases			Reaction cases		
	Min.	Max.	Av.	Min.	Max.	Av.
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
Total calcium	9.0	11.0	10.0	6.82	9.92	8.65
Nondiffusible calcium	4.1	7.2	5.07	2.36	4.89	3.41
Diffusible calcium	4.2	6.8	4.96	4.27	6.1	5.33
	gm.	gm.	gm.	gm.	gm.	gm.
Albumin	3.77	5.24	4.4	2.54	5.73	3.34
Globulin	1.96	3.55	2.63	1.88	6.17	4.35



TEXT-FIG. 1.

TEXT-FIG. 1. Calcium and protein in blood of normal controls and leprosy patients in reaction.

The study of Wooley and Ross (12) should be mentioned at this juncture, not only because it is the only other work ever undertaken along this line, but also because of the apparent disagreement in our findings with regard to the diffusible and the nondiffusible calcium. They found the former fraction to be low and the latter fraction high in the active stages of the disease, and that the trend of both was to become normal with improvement of the case. Of the protein fractions, they found the albumin low and the globulin high; here we have no disagreement. As pointed out in my previous paper, the discordance in our findings may be only apparent, since by comparing their data for the diffusible fraction in leprosy cases with the normal figures used in my study, very good agreement in our findings is revealed. Furthermore, there can then also be seen good

accord for the nondiffusible fraction. My explanation of the disagreement was that these authors used for the low limit of normal a value which was relatively high as compared with the one which has found general acceptance among investigators.

If we should assume for the moment that the findings of Wooley and Ross are correct, then the determination of these constituents would provide a means of characterizing leprosy chemically, since in no other disease has it been demonstrated that those constituents are in such a state, i. e., one in which the levels of both the diffusible calcium and the albumin are low while at the same time the level of the nondiffusible calcium is high. It should be noted, however, that this increase of the nondiffusible calcium was explained by them as due to a concomittant increase of the globulin. From the point of view of the law of mass action this explanation seems plausible. Evidently, however, they shared the view, apparently advanced first by Loeb (8) in 1926, that it is the globulins rather than the albumin which have the major role in determining the diffusibility of calcium. That view, which at the time was purely hypothetical, has been invalidated by the more tangible evidence yielded by subsequent work of various investigators, and the current reviewers of literature no longer entertain that view.

Coming back to my data in the lepra reaction cases, it has been explained that, from what equation (II) appears to reveal, the deficiency in the albumin fraction may very well account for the abnormalities of concentration of the other constituents under discussion. It has also been explained that, to restore the equilibrium relationship in this equation, upset by the reduction of concentration of albumin, it is necessary to cause more dissociation of the calcium albuminate (nondiffusible calcium). Now, since the actual findings show there is already a drain below the physiological limit in the concentration of this compound, evidently as a result of this accelerated rate of dissociation, it follows that the only apparent way to help the body replenish the loss is to maintain an adequate supply of albumin in the blood stream. Assuming that the processes of body regulation function normally, we may then expect—according to the principles involved—that by increasing the albumin we would cause more association of the calcium and albumin ions to form more of the calcium albuminate. But while this process could be expected to continue as long as the needed protein material is maintained, it would do so only until the equilibrium relationship has been restored to its physiological requirement. The

consideration of this fact suggests that protein therapy in these cases may prove of value as a means by which to help the body correct the existing disturbance in the relationship. Supplementing this therapy with calcium administration may seem also advisable, not because there is a deficiency in this element in the active form but in view of the mechanisms involved in the equilibrium relationship between calcium and protein.

CONCLUSIONS

1. In leprosy, most markedly in lepra reaction cases, there occurs a lowering in the concentration of serum calcium, involving the biologically inert protein-bound (nondiffusible) fraction—and, in consequence, the total calcium—but not the diffusible fraction.

2. This condition is not due to a disturbance of the metabolism of calcium, but is a consequence of the reduction of the albumin concentration of the blood serum, a phenomenon of which an explanation is offered on the basis of the McLean-Hastings equation for mass law equilibrium relationship which they proved to exist between calcium and protein in body fluids.

3. It is suggested that protein therapy (parenterally administered protein), may prove of value in the amelioration of lepra reaction. Calcium administration may serve as a valuable complementary factor, not because of a deficiency in the physiologically active form of calcium but to provide ready material to facilitate the processes involved in the calcium protein equilibrium relationship.

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