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AN ESSAY

on

SOME ASPECTS OF CALCIUM METABOLISM.

by

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I.

Introduction.

The object of the present paper is to present the results which have been obtained by the author in an investigation into the variations of the calcium content of human serum in certain pathological conditions, to give a resumé of the literature dealing with the source, functions, and variations of the blood calcium, and to point out some of the directions to be taken by future work.

At present, our knowledge of calcium metabolism is fragmentary; not only are there many points on which no work seems to have been done, but the results of different investigators are often contradictory. Doubtless, the relationships between calcium and the other inorganic substances present in living tissues are very complex, and the complexity is not diminished by the presence of organic ampholytes. Moreover, apart even from these obstacles to a clear understanding of the metabolism of calcium, there appear to be other less obvious factors to which at present we possess no clue. In this connection one may mention the apparently well-established fact that calcium chloride, fed by the mouth, is absorbed to a much greater extent than is calcium lactate.(20) This assertion rests on no isolated observation; it has been made time and again by reliable workers who have adduced what appears to be ample evidence. Yet in either case, one would imagine, the effect of the gastric juice would be the production of free calcium ions in the presence of a large excess of chlorions. In the intestine, where, presumably, absorption takes place to the greatest extent (if not exclusively), it is true that the reaction is alkaline, but the anion present in greatest concentration is still the chlorion. Why, then, should calcium chloride be absorbed so much more easily than the lactate?

Certain lines of enquiry in biological chemistry have already yielded results such that a coherent and plausible account can be given of the phenomena involved and their relationships, though doubtless in no case can the complete and final story be told. It is evident, however, that in the case of calcium we are still far from being able to correlate the various experimental results and employ them in the weaving of a single harmonious hypothesis; the gaps are too great, and much of what passes for knowledge is uncertain.

II.

The Absorption of Calcium.

Calcium salts, whether soluble or insoluble, appear to be absorbed with considerable difficulty. Even under the most favourable conditions, the greater part of the ingested calcium is lost in the faeces, having apparently escaped absorption. Nevertheless a certain amount is assimilated. Various estimates of this amount have been made, some depending on the difference between the intake and the faecal output, others on the amount excreted by the kidney. The consensus of opinion seems to be that the adult man requires approximately 0.4 g. of calcium per day (I, 56). In the growing child the amount per kilo. body weight is naturally greater, and Herbst (32) states that the normal child stores about 0.3 g. of calcium per day. Even in a well-balanced diet, of course. calcium must be present in amounts much greater than these to allow for the low efficiency of absorption - I.0 to I.5 g. of calcium seems to be the average daily dietary requirement (I).

The assimilation of calcium is affected by many dietary factors. Very important is the balance of the mineral constituents of the diet. While a large excess of sodium chloride, or, in general of chlorion, appears to be a distinct aid to calcium absorption (68), an excess of potassium (76, 95), magnesium (75), or phosphate (2I, 37, 38, 60) is definitely detrimental. So great is the influence of these substances,

especially in the growing animal, that a diet in which the phosphate is greatly in excess of the calcium, though both are far above the actual requirements, leads, almost invariably, to deficient bone formation and rickets (26).

This influence of a large excess of phosphate is perhaps no more than would be expected when one considers the insolubility of calcium phosphate. That potassium should have a similar effect is not so obvious, but has been explained by Seemann (76) who states that the ingestion and absorption of excess potassium results in the excretion of the excess along with chlorine ions, that the resulting depletion of chlorine results in a deficient secretion of hydrochloric acid in the gastric juice, and that with this abnormally low concentration of chlorides in the digestive fluids, calcium cannot be absorbed. Zander (95), supporting this theory, remarks that whereas in the case of healthy infants the mother's milk contains sodium and potassium in the ratio of 2:I. in the case of rachitic children the ratio may be I:2. Excess of magnesium in the diet, besides interfering with the absorption of calcium, appears to prevent its proper utilisation in the same way as potassium interferes with sodium, causing an increased urinary excretion (30).

Many workers have shown that the absorption of calcium is greatly aided by the addition of fat to the diet (e.g. 37). It is difficult to picture the mechanism of the fat action. One would imagine that the presence of fatty acids in amounts greater than usual would aid the formation of insoluble calcium soaps and so depress the absorption of calcium in much the same way as excess of phosphate is known to do. Moreover, as has already been mentioned, such a relatively simple organic salt of calcium as the lactate is known to be absorbed with great difficulty. These considerations, supported by the observation that only some fats, at any rate, were efficacious in improving calcium absorption (64, 8I), naturally led to attention being turned to the vitamin content of fat as a

possible explanation. McCollum and his co-workers (6I) found, however, that cod-liver oil was equally effective whether administered fresh or after oxidation for twelve hours whereby its content of vitamin-A had been entirely destroyed. It was also found by the Aberdeen workers that, except in extreme cases, olive oil and linseed oil aided calcium absorption to the same extent as did cod-liver oil(37). Butter fat, though rich in vitamin-A, was one of the fats which did not assist the absorption of calcium (64, 8I). These facts were disturbing, since they indicated that after all, the explanation of the phenomenon must be sought in some action of the fat itself. More recent work, however, developing from the effect of irradiation with ultra-violet light, first of the animal itself, later of the whole diet, and then of otherwise inactive fats, (34, 9I) has led to the view that fat contains a special anti-rachitic factor - vitamin-D - which is more resistant to oxidation than is vitamin-A, (and so was not destroyed in McCollum's experiments with cod-liver oil), is present in butter in very low concentration, and can be produced in such otherwise inactive fats as olive oil by exposure to light (against which, probably, no precautions were taken by Husband Godden and Richards (37)). This question of the influence of vitamin-D is a very important and intricate one, and will be dealt with more fully at a later stage.
Here it is sufficient to point out that many claims have been advanced in favour of an increased absorption - as distinct from utilisation - of calcium under the influence of the anti-rachitic vitamin or of ultra-violet radiation.

III.

The Excretion of Calcium.

It is usually supposed that calcium is excreted both by the kidney and through the epithelium of the large intestine, the greater part going by the latter route (I5). The relative quantities, however, vary under the influence of a number of factors. Thus an excess of chlorine ions in the body fluids leads to a greatly increased excretion of calcium in the urine (29), while with an excess of phosphate more calcium appears in the faeces and less in the urine (an effect which, as experiment shows, is only partly due to diminished calcium absorption)(I5). Generally speaking, an ion which forms a soluble salt with calcium causes an increased excretion in the urine, an insoluble combination tends to appear in the faeces.

Telfer (83) has recently suggested that the kidney provides the sole, or at any rate the main excretory route for calcium, and that when insoluble calcium salts are ingested, the decreased urinary and increased faecal calcium is due, not to increased excretion via the large intestine, but simply to decreased absorption.. While the decreased absorption undoubtedly does account for some of the alteration in the relative amounts of calcium appearing in the urine and faeces, it is difficult to avoid the conclusion that intestinal excretion plays more than an inconsiderable part in the removal of calcium. Thus von Noorden (88) has shown that intravenous injection of calcium salts, whereby difficulties of absorption were avoided, was followed by appearance of calcium in the bowels. At present, therefore, we must believe that both mechanisms are employed in the excretion of calcium, to a relative extent depending on the total amount of calcium and on the nature and concentrations of the other ions.

IV.

The Functions of Calcium.

The most obvious function of calcium is, of course, the formation of bone, and, indeed, it is from studies of bone formation, and especially of conditions leading to imperfect ossification, that much of our information concerning the absorption and metabolism of calcium has been derived. Yet we are far from possessing a full knowledge of the mechanisms involved in the transference of calcium from the blood to the growing bone. Freudenberg and György (24) have suggested that the cartilage protein forms a compound with calcium by virtue of its acidic groups, that the resulting compound, having now a preponderance of basic groups, combines with phosphate ions, and that the calcium-cartilage protein-phosphate complex so formed breaks down with formation of the original cartilage protein and deposition of insoluble calcium phosphate. The same authors (23) have postulated a similar mechanism for the transference of calcium to tissues other than bone, and though their work has been adversely criticised - e.g. by Liesegang (48) - it is supported by the experiments of Budde (10) who, using gelatin in vitro, claimed to have demonstrated the consecutive uptake by protein of calcium and phosphate. More light has been thrown on the matter by the researches of Robison and his co-workers (e.g.70, 71). They hold that the bone and ossifying cartilage of young animals contain an enzyme (which they designate "phosphoric esterase") capable of rapidly hydrolysing hexose monophosphoric ester, which is present in normal blood - particularly in the erythrocytes. Hexose monophosphate, they claim, combines with calcium and the salt so formed is hydrolysed by phosphoric esterase with deposition of calcium phosphate, They find that if strips of young bone, whether from a normal or a rachitic animal, are placed in a solution of calcium hexose phosphate at the correct pH, the bone grows through deposition of calcium phosphate.

The importance of calcium to the animal, however, is by no means confined to the building up and maintaining of the bony framework. Calcium is present in every normal cell. and though it appears to be possible for a cell to live for some time in complete absence of calcium (insofar as any element can be said to be completely absent), the element is essential for normal functioning. The demand of the soft tissues for calcium is shown by the fact that even in adults, continued absence of calcium from the food may result in the withdrawal of calcium from the bones and their consequent softening (I6). Many investigations have been undertaken with the object of demonstrating or explaining the necessity of calcium ions, and many curious and interesting facts have come to light.

It was early found that more than the maintenance of a definite osmotic pressure was necessary for the continuance of normal cell life. Besides isotonicity, there was necessary in the fluid bathing the living cells, or with which isolated organs were perfused, the presence of definite ions, and more, the presence of these ions in very definite proportions - the solution of salts must be physiologically balanced.

The isolated frog's heart, for example, survived much longer, and worked much more efficiently, when the perfusing fluid contained calcium than when sodium chloride alone was present, and the contractions became much more nearly normal when potassium chloride was also added. On the other hand, the effect of potassium salts on the isolated heart or on skeletal muscle was neutralised by the addition of traces of calcium salts. Here we have an example of ion antagonism.

Meltzer (63) gives another of the many examples of this phenomenon when he observes that the toxic effects of large doses of magnesium salts (narcosis and anaesthesia) can be completely removed by injections of calcium salts. Loeb's suggestion (49) is that calcium is not directly essential for life, but that the various ions neutralise one another's toxic effects. Loeb found that the fertilised eggs of

fundulus develop equally well in sea-water or in distilled water but that they will not develop in pure solutions of sodium chloride, potassium chloride, or calcium chloride isotonic with sea-water. When, however, the tonicity remaining constant, calcium chloride is added to the sodium chloride solution, the eggs live for a longer time, and when potassium chloride is also added, a nearly non-toxic solution is obtained in which the eggs develop at a rate approaching the normal. The solution is near the physiological balance, when the ion antagonisms overcome the individual ion toxicities. This explanation marks a certain advance in our understanding of the need for the various ions, but we still need to know how the ions antagonise one another, and in what their toxic action consists. To the latter question the experiments of Loeb and of Osterhout (96) appear to reply that the toxicity is in some way connected with an increased permeability of the cell walls. The antagonism of the ions has been explained by Loeb - since it is found among ~~cations~~ anions as well as among ~~anions~~ cations - as due to a maintenance of the equilibrium among the various protein salts. A calcium chloride solution is toxic because, with increased permeability of the cell walls, more calcium ions enter the cell and an excess of calcium proteinate is formed, the properties of protoplasm being thereby modified. However this may be, it is certain that for maximal furtherance of the vital activities a correct balance of the various inorganic ions is no less necessary than a correct osmotic pressure, and that calcium is an important factor in maintaining this balance.

Mention may be made here - the point will be referred to again - of the sugggstion which has more than once been advanced that calcium plays some part in regulating the pH of the blood - and presumably of the tissues also. Conclusive experiments appear to be lacking, but it is perhaps significant that Stewart and Haldane (82) found an immediate rise in the serum calcium in response to ammonium chloride

ingestion or to carbon dioxide inhalation - both of which tend to lower the blood pH - , while an alkalosis produced by ingestion of sodium bicarbonate was accompanied by a lowered serum calcium.

One important function of calcium ions is to aid in the coagulation of shed blood. Several explanations have been offered of the mechanism of blood coagulation, and there are differences (inter alia) in the rôle assigned to calcium. According to the theory proposed independently by Morawitz (65) and by Fuld and Spiro (25) and later strongly supported by the work of J.Mellanby (62), fibrinogen is converted to fibrin by the action of thrombin, and the insoluble fibrin, entangling the corpuscles, forms the clot. Thrombin does not exist in the blood as such, but is formed, when blood is shed, from prothrombin (which is closely associated with fibrinogen) by the combined action of thrombokinase and calcium salts. The enzyme thrombokinase is supposed to be present in the formed elements of blood and in the tissues - particularly those rich in nucleo-protein. Mellanby showed that the action of calcium is specific, since, although the ions of the same group (Mg, Sr, and Ba) will, in presence of thrombokinase, convert prothrombin to thrombin, none of them compare in potency with calcium. Incidentally, he found that increase of the concentration of calcium beyond a certain point lengthened the coagulation time of serum just as did the lowering, the effect being more particularly clear when the minimum amount of thrombokinase was present. Howell (35) differs from these workers in believing that prothrombin is converted to thrombin under the influence of calcium alone, and that this action is prevented from taking place in the blood vessels by the presence of an inhibitory substance ("anti-thrombin") which, however, is neutralised when the blood is shed by a "cephalin-protein" liberated from the blood and tissue cells.

The precise influence of calcium on the individual organs of the body is somewhat difficult to determine since,

unless it is supplied to the tissue under investigation along with sodium, changes due to the absence of the latter render the results difficult to interpret. On the heart muscle it appears to exert a tonic influence, the contractions becoming more forcible and relaxation less complete when the perfusing fluid contains an excess af calcium, while toxic amounts cause the heart to stop in systole (69). If calcium is excluded from the perfusing fluid the heart comes to a standstill in diastole, but continues to consume glucose (5I), and the electrical charges remain strong (50). It would thus appear that calcium is in some way concerned in the utilisation of chemical energy for the production of contraction. Lack of calcium has been suggested as an explanation of the greatly increased excitability of striped muscle which occurs in tetany. Calcium figures prominently in the physiology of the conduction of nerve impulses, and is said to be necessary for the transference of impulses at the neuromuscular junctions (5I) and through synapses (66). In excess, it exerts a paralysing action similar to that of curare, and its removal is said to increase the irritability of the terminations of the autonomic nerves in mammals (I5). There is, however, at least one exception to this in the vagus, which loses its inhibitory action when the heart is perfused with calcium free fluid (36). Intravenous infusion of soluble calcium salts, besides acting on the cardiac musculature, causes a constriction of the blood vessels, and a marked contraction of the pupil which may be completely developed when ne other signs of calcium intoxication are yet evident (4). The myosis is apparently due essentially to a stimulation of the muscle of the constrictor of the pupil, since response to light and other mydriatic influences is lost. It has been stated (II) that intravenous or subcutaneous injection of calcium chloride in animals partially or completely inhibits the pleural effusion produced by iodide administration, and that it prevents the development of oedema and inflammation of the conjunctiva after the instillation of brucinine. For

these reasons its use has been recommended in man to hasten reabsorption of pleural effusions and exudates, but in the few cases in which it was used by the author there was no apparent benefit.

V.

The Calcium Content of Blood.

Very wide variations are found in the figures given in the literature for the calcium content of whole blood, serum, plasma, and corpuscles. To no small extent this is due to the different methods which have been used for its estimation. Few of the older figures are trustworthy, and many even of the more recent ones must be regarded with some suspicion.

The methods available are of all types, colorimetric, nephelometric, gravimetric, volumetric, and even, in one case, the simple counting of particles of precipitated calcium oxalate. In the author's experience, and in that of his collaborator, the method of Kramer and Tisdall (46) has proved the most reliable, and to be capable of yielding results with a maximum error of 5% and an average error of only 2%. As applied to blood serum, the method consists in precipitating the calcium directly from I - 2 cc. of serum by means of ammonium oxalate, collecting and washing the precipitate by centrifuging, and finally titrating, in presence of sulphuric acid, with potassium permanganate.

Jones and Nye (40) give, for children varying in age from four weeks to fourteen years, the values 9.4 mg. calcium per I00 cc, for whole blood, I0.0 mg. for serum. and 8.7 mg. for corpuscles. Mazzocco (58) states that the calcium content of the plasma is practically identical with that of the serum. Jones and Nye's values for serum are thus roughly confirmed by Koechig (45) who finds values from 9.5 to II.0 mg. per I00 cc. of plasma, and by Matz (57) who gives the range 9.0 to I2.0 for normal serum. Their results for

whole blood, however, seem to be incorrect. Howland and Marriott (97) had already found that the calcium content of whole blood was only half that of serum, and Alport (2) gives 5.8 mg. as the mean calcium content of I00 cc. of whole blood. Jones (98) gives 8.8 for whole blood, 5.0 for corpuscles, amd I2.3 for plasma.

These examples are sufficient to show the widely diverging figures found in the literature. Some of the variations, as has been said, are doubtless due to the use of different methods of estimation, some of them far from reliable. Examination of the detailed results of each author, however, shows considerable variations on each side of the mean, and in view of the comparatively small range obtained by the author and by other workers who have used the same or an equally reliable method, it is difficult to avoid the conclusion that some at least of the published figures are the result of faulty technique.

Turning now to results obtained by the use of the Kramer and Tisdall method, we find that those authors found ten normal sera to contain I0.5 to 9.5 mg. calcium per I00 cc. (six lay between 9.5 and I0.0)(46); Kramer and Howland (47) recorded seven normals within a range of 9.3 to 9.9; while the author, from eight normal cases, obtained results ranging from 9.4 to 9.9. Watchorn (90) gives a higher figure for the normal, but here too, the range, I0.0 to I0.8, is small.

In view of these variations in the normal which, though reduced by the use of the Kramer and Tisdall method, are not entirely eliminated, the author, in a paper shortly to be published, has urged the inclusion by every author of a series of determinations on normal sera. Without such a guide it becomes, when dealing with physiological or pathological variation from the normal, very difficult to compare the results of one author with those of another and to estimate the value of the conclusions drawn from them.

Calcium is supposed to be present in the serum in two

forms, part in the ionised state, part in combination with some colloid complex. By filtering ox-serum through a collodion membrane, Cushny (18) found that the filtrate contained the whole of the sodium and potassium, but only about 60% of the calcium present in the original serum. The remainder of the calcium, he concluded, was present in some non-diffusible form. It has been suggested that the amount of ionised calcium must be constant in order to conserve the osmotic relationships between the body fluids and the tissues, but according to Vines (87) this is by no means the case, and he quotes examples - certainly of diseased conditions - where the total calcium content of the serum is normal while the ionic portion is reduced by half. The author, while investigating the effect of parathyroid feeding on the serum calcium in a series of cases of psoriasis found that during the preliminary period the ionised calcium varied considerably from day to day.

Certainly the methods available for the determination of the ionised calcium are very unsatisfactory, and conclusions drawn from them are only valid when the variations are of a gross character.

Vines' method consists in adding to the serum an amount of ammonium oxalate equivalent to the calcium present. In these circumstances, he considers, the ionic or "active" calcium is precipitated, whereas the remainder comes down only on addition of a considerable excess of oxalate. The impossibility of adding an exact equivalent of ammonium oxalate, the disturbance of the equilibrium between the ionised and unionised calcium, and the time factor, all conspire to make this method unreliable.

The method of dialysing the serum against a solution of known calcium concentration is slow, and for various reasons cannot be expected to yield results of a very high degree of accuracy.

The author has used the method of collodion filtration, but has not succeeded in obtaining very consistent results,

and even when, by suitable precautions, a moderate degree of consistency is obtained, it is impossible to be certain that one is estimating the ionic, the whole ionic, and nothing but the ionic calcium. The collodion membrane, even when subjected to a preliminary drying period of constant length and under constant pressure, still contains moisture (in variable amount) which dilutes the first portion of the filtrate. With the comparatively small amounts of serum available in work on human subjects, this constitutes a factor of some importance. Further, the time during which filtration is allowed to take place affects the concentration of calcium in the filtrate. The increase, in the later stages, of the "organic" calcium of the mother-liquor causes a shift in the equilibrium with an increase in the ionic calcium. The effect of these two factors is shown in the following table.

Table I.

Total calcium content of sample : 9.I mg./I00 cc.

	A. Membrane dried one hour under pressure.	B. Membrane surface dried.
	Mg. Ca/I00 cc. filtrate	Mg. Ca/I00 cc. filtrate.
-3 hours	7.45	4.40
-7 "	7.60	7.40
24 "	8.70	8.50

Moreover, no membrane which the author has as yet obtained has proved capable of preventing entirely the passage of the serum proteins; in every case the filtrate has given a positive biuret test. The author is informed by Dr. Stedman that in his experiments collodion filtration has similarly failed to give a filtrate entirely free from protein.

It seems, then, that though calcium does exist in the blood in two distinct forms of combination, there is no method at present available of measuring accurately their relative amounts.

VI.

Physiological Variations in the Serum Calcium.

Lyman, quoted by Jones and Nye (40) reports a sex variation in the calcium content of whole blood, his figures being 6.I mg. calcium per I00 cc. blood for males and 7.I for females. These reults, however, do not seem to have been confirmed, and they receive no support from the author's determinations of the serum calcium in a series of dermatological cases.

Age is said to influence the serum calcium which, according to Jansen (39), increases during the suckling period and thereafter undergoes a gradual diminution which continues throughout adult life. The serum calcium is thus highest during the period of calcium storage. Jones (98) supports this to some extent, finding that the plasma calcium is higher during the first few days of life than later. On the other hand she states that the calcium content of thecorpuscles and of whole blood is lower in very young children than in older ones.

In I908, Bell (7) observed a rise in the serum calcium just before menstruation, this rise being followed by a marked fall. Later work, however, fails to support this (79, 93) and Mr.J.B.S.Haldane informs the author that in an investigation shortly to be published from Cambridge by Miss Watchorn, no regular variations were observed preceding or during menstruation; variations there often were, but they were quite irregular.

The most marked alteration in the serum calcium occurs during pregnancy, and is doubtless due to the drain on the maternal tissues to meet the demands of the growing foetus, demands which increase from 0.006 g. per day during the first four months of gestation to over 0.6 g. per day at term. Corresponding to this rapidly increasing foetal requirement the calcium in the maternal serum tends to fall, as Mazzocco and Moron (59) have shown in the following series of cases.

Table II.

I0 healthy, non-pregnant women.....	9.I9 mg. Ca/I00 cc. serum
I7 healthy, puerperal women.....	8.79
29 pregnant women.....	8.77

Similarly, Bogart and Plass (9) found that the average calcium content of the serum was 9.I mg./I00 cc. for 23 women at the time of labour, and I0.2 for twelve normal and non-pregnant women. Widdows (92) states that the serum calcium falls during the last month of pregnancy and shows a tendency to rise after confinement and during the early stages of lactation. There have, it is true, been one or two denials of a decrease in the serum calcium in pregnancy (e.g.33), but on the whole it seems to be well attested.

VII.

Experimental Alterations in the Serum Calcium.

In man, it appears to be by no means difficult to alter the calcium content of the serum. Apart from the administration of large doses of calcium chloride which György (28), Stewart and Haldane (82) and others have shown to raise the serum calcium, Stewart and Haldane (82) have brought about increases by ingestion of ammonium chloride, by inhalation of carbon dioxide, and by over-breathing. They found, too, that the serum calcium fell below normal when sodium bicarbonate was ingested. It has been suggested that the calcium concentration of blood is intimately connected with the pH, and Paasen (67) states that the concentration of calcium ions is given by the expression $(Ca^{\cdot\cdot}) = K.(H^{\cdot})/(HCO_3^{!})$, where K = 350. Now both the ingestion of ammonium chloride (or, indeed, of calcium chloride) and the breathing of excess carbon dioxide cause an increase in the hydrogen ion concentration and consequently, according to Paasen, of the calcium ions. Conversely, ingestion of sodium bicarbonate increases the denominator in Paasen's formula and gives a decrease in the calcium ion concentration. So far, then, the results of Stewart and Haldane support the idea of a relationship

between calcium ions and hydrogen ions. Their exception, overbreathing, which should have caused a decrease in the calcium, is perhaps susceptible of explanation in the following way. During the long continued overbreathing, tetany was developed (private communication) and, as will be shown later, in tetany there is an excessive excretion of calcium which, since absorption remains normal, can only mean that calcium is poured into the blood-stream from the tissues. Hence it may well be that in Stewart and Haldane's experiments the fall in the serum calcium due to the alkalosis was masked by the influx from the tissues.

Very few investigations appear to have been carried out on the effect of oral or intravenous administration of calcium salts on the calcium content of the blood in animals, and the recorded experiments have yielded very indefinite results. Clarke (12) found that intravenous or subcutaneous injection of calcium salts in rabbits caused a transient rise in the serum calcium, though the feeding of a calcium-rich diet had no such effect. Denis and Minot (20), working with cats, were able to bring a sub-normal plasma calcium to normal by administration (orally) of large doses of calcium lactate, but were unable to affect an already normal serum. In Clarke's experiments the effect on the serum calcium of the loss of blood entailed in the frequent withdrawal of samples for analysis was ascertained in a control animal. It was found that there was an initial fall of 10-12% but a return to normal within seven hours.

The author has carried out a few experiments on the effect of intravenous injection of calcium lactate on the calcium content of the serum in rabbits and cats, and on the effect of extensive haemorrhage. The results are shown in the accompanying table.

Table III.

	Animal	Wt. in g.	Mg. Ca/I00 cc. Serum.	Procedure	Mg. Ca/I00 cc. serum after (mins) 30	60	90	I20
I	Rabbit	I500	I2.5	600 mg Ca lactate	I2.5	I3.3	I4.7	
II	Cat	2730	7.7	"	24.4	20.2		
III	Rabbit	I450	I0.3	I00 mg. Ca lactate	I3.0	II.8	I0.7	
IV	Cat	2500	9.0	I5 cc. blood drawn every 30 minutes	9.0	9.0	9.0	
V	Cat	2600	9.5	"	9.5	9.5	9.5	9.5

In animals I to III a marked rise in the serum calcium has followed the injection of calcium lactate, but when it is considered that, from approximate calculations as to the total amount of serum present, enough calcium has been added for at least a twelve-fold increase in experiment I, six-fold in II, and two-fold in III, it is evident that excess calcium is removed with very great rapidity, either by transference to the tissues or by excretion.

Contrary to Clarke's experience with rabbits, it is evident from experiments IV and V that in cats the withdrawal of considerable quantities of blood - up to 25-30% of the total calculated amount - is without effect on the serum calcium. It is possible (though hardly probable) that one is dealing with a real species difference which would help to account for the much greater rise of the serum calcium in experiment II than in I and III. It is intended, shortly, to amplify these results (and, incidentally, to clear up this particular point) and to study in animals the effect on the serum calcium of alterations in the blood pH.

Experimental alterations in the serum calcium of dogs by means of parathyroid feeding have been reported by Collip (I3) and the author has obtained similar results in human subjects. This matter it is proposed to discuss in a later section of the paper.

VIII.

Pathological Variations in the Serum Calcium.

From the large number of estimations of the serum calcium which have been made in pathological conditions, it appears that there are wide variations from the normal. In some diseases the variation is always in the same direction - e.g. in diabetes the serum calcium, provided it shows any abnormality at all, is always low - but in other diseases individual cases may differ both in the degree and the direction of the abnormality. Generally speaking, variation is as common in one direction as the other. The subjoined table, giving the author's results, shows this very clearly, and is in no way atypical.

Table IV.

Sex	Disease and Comments	Mg. Ca/I00 cc. serum
F	Rheumatoid arthritis	9.3
F	"	9.7
F	"	9.0
F	"	9.5
M	Diabetes mellitus Ketonuria present	9.2
F	" "	9.3
F	" "	9.3
F	" "	9.I6
F	Chronic interstitial nephritis	8.6
M	"	8.7.
F	"	8.2
F	"	8.0
M	Acute nephritis	8.6
M	"	8.9
F	Chronic parenchymatous nephritis	7.5
M	Pituitary deficiency - Infantilism	9.5
F	" -)besity	9.2
M	Addison's disease	II.0
M	Cretinism	9.9
F	Tetania parathyreopriva	6.9
F	Idiopathic epilepsy	I0.2
F	"	I0.0
M	"	9.45
F	"	I0.0

Table IV. - continued.

Sex	Disease and Comments	Mg. Ca/100 cc. Serum.
M	Psoriasis	10.0
M	"	10.0
F	"	10.4
F	"	11.2
M	"	10.7
M	"	10.2
F	"	10.5
F	"	10.0
M	Pityriasis rubra	12.0
M	"	11.2
M	"	9.8
M	Varicose dermatitis	13.0
M	"	12.7
M	Chronic leg ulcer	12.0
M	"	11.5
M	"	10.2
F	"	9.6
F	Erythema induratum scrofulosorum	9.5
F	"	9.5
F	Papulonecrotic tuberculide	8.5
F	Lupus erythematosus	8.0
F	"	8.6
M	"	9.0
M	"	10.2
M	"	10.5
F	Erythema multiforme	10.4
F	"	10.0
F	Erythema pernio	9.4
F	Raynaud's disease	8.5
F	Syphilis	10.9
M	"	10.0
M	"	11.0
F	Purpura	6,9
M	Epistaxis	10,2
F	Neurasthenia	9.7
F	"	9.0
M	Severe dyspepsia , achlorhydric type.	9.1
M	T.B. meingitis	7.15
M	Sciatica	9.2
M	Haemophilia	10.0
F	Precocious puberty - menstruating.	10.8

To what factor the high serum calcium so often seen is to be attributed is as yet unknown, but it seems possible that many of the subnormal values are due, not to the disease itself, but to a concomitant acidosis. Stewart and Haldane (82) found that ana acidosis produced, e.g. by ingestion of ammonium chloride, was accompanied by a high serum calcium, but in their experiment, which was of short duration, there was a return to normal during the acidosis period, and even a suggestion of a fall below normal. Vines (87) states that calcium loss (with a low serum calcium) always accompanies acidosis, and other workers have reported similar results. It may well be that acidosis is followed by a mobilisation of calcium (and therefore a high serum content) but that the increased excretion (29, 82) brings about a depletion of the calcium stores until, in an acidosis of some days standing, the serum calcium has fallen below normal, to return only when the acidosis disappears. An experiment by the author bears out this idea to some extent. Epileptics were - for another reason - placed upon a ketosis producing diet. One case (Fig.I) showed a rapid rise in the serum calcium immediately upon the appearance of acetone bodies in the urine, and the rise was followed by a fall. A second showed an increased serum calcium almost coincident with ketonuria, but observations were perforce discontinued at this point. A third, in whose case no early observations were possible, showed a steadily decreasing serum calcium from the twelfth day onwards.

The regular accompaniment of acidosis by a low serum calcium, independently of the actual disease present, is further illustrated by the cases of diabetes and nephritis. Kahn and Kahn (41) have found the serum calcium to be low in cases of diabetes with acidosis, and the same observation has been made by Loeper and Béchamp (52). The author has confirmed these results (Fig.II), and has found further that after disappearance of ketonuria the serum calcium returns to normal. In these cases, it is right to mention, the

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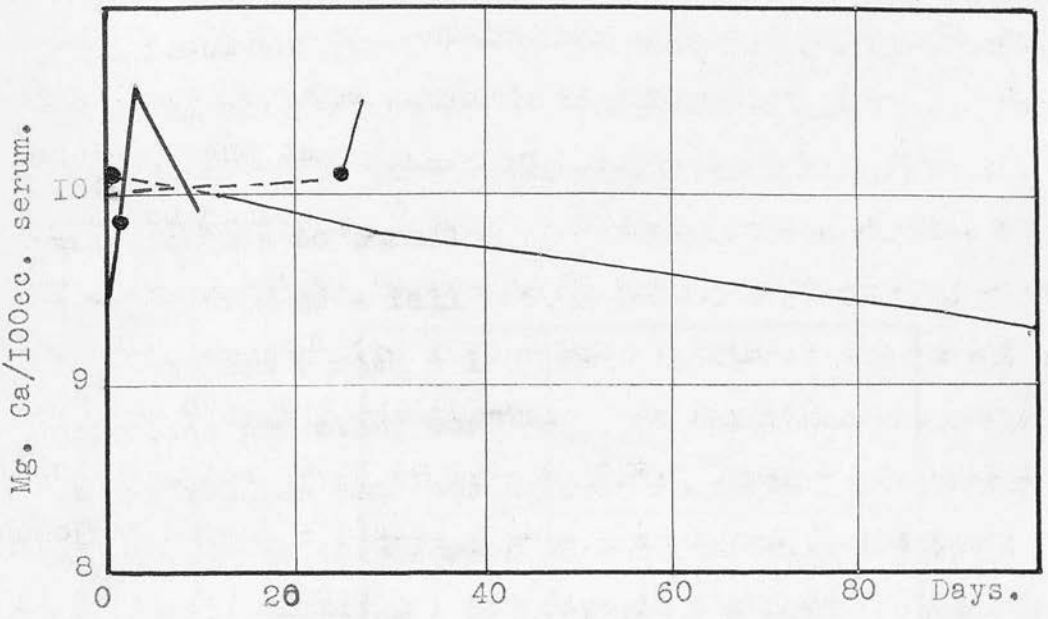

Fig.I.

Effect of acidosis on the serum calcium in epileptics.

Acetonuria commenced.....●

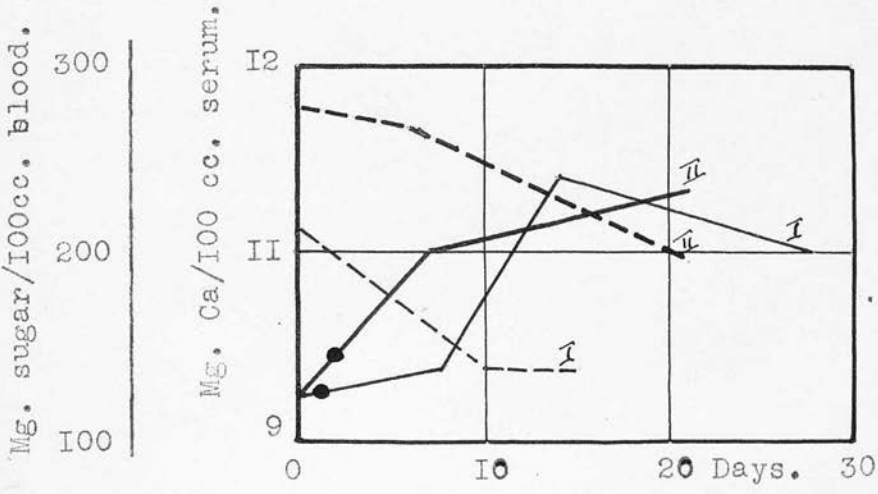

Fig.II.

The serum calcium and blood sugar in diabetes.

Continuous lines.....Ca
Broken lines.....Sugar.
Disappearance of ketonuria.....●

blood sugar fell as the calcium rose, but it is difficult to imagine any direct relationship between the two substances capable of causing such a state of affairs, and the exactly similar calcium variations in other diseases with a normal blood sugar throughout indicate that the essential factor was the removal of acidosis.

Marriott and Howland (55) have reported low values for the serum calcium in cases of nephritis accompanied by acidosis, while Denis and Hobson (I9) found low values only in about one fifth of the cases examined. They, however, do not appear to have looked for an acidosis, so that their results cannot be held to contradict those of Marriott and Howland. All cases examined by the author (Fig.III) showed a low serum calcium long enough after admission to hospital for stabilisation on the new diet to have taken place. Unfortunately, at the time these observations were made, the importance of acidosis was not realised, and no data bearing on this point were collected.

As opportunity arises, it is proposed to extend these observations of the serum calcium, and by making, simultaneously, measurements of the blood pH, bicarbonate reserve, etc., to test more fully the hypothesis that many such low values for the serum calcium are due rather to an acidosis than to the actual disease present.

Nor must it be forgotten that an alkalosis appears to be capable of producing a lowering of the serum calcium, so that the lowering in disease may be due to a variation (or tendency to vary) of the blood pH in either direction. Besides the subnormal serum calcium obtained by Stewart and Haldane (82) in alkalosis induced by ingestion of sodium bicarbonate, it has repeatedly been shown (despite a few statements to the contrary (e.g. 54,89)) that in tetania parathyreopriva, where the serum calcium is abnormally low, there is an accompanying alkalosis (8,I4,27,94). It is not, of course, suggested that in <u>all</u> disorders

Fig.III.

Serum Calcium in Nephritis.

		Mg. Ca/100 cc. Serum. Initial Value.	After $CaCl_2$ feeding.
Acute nephritis	I	8.6	10.5; 9.7
	II	8.9	
Chronic interstitial nephritis	I	8.6	
	II	8.7	
	III	8.2	
	IV	8.0	
Chronic parenchymatous nephritis	I	7.5	9.3; 9.7; 9.5

resulting in a lowering of the serum calcium the essential is an alteration in the blood pH - one can readily imagine other mechanisms capable of bringing about such a state of affairs.

Vines (87) distinguishes three types of disordered calcium metabolosm. A failure to absorb calcium from the alimentary canal constitutes his first type, and he cites rickets as the sole didease in which such a cause has been suggested. In his second type there is a failure by the tissues to utilise the calcium absorbed; calcium is present in the blood stream in, perhaps, normal amounts, but is in such a form that the tissues cannot use it. In the various acute or chronic infective processes which make up this type it is the "ionic" or "active" calcium which is increased, while the "inactive", "colloidal", or "organic" calcium remains normal or even above normal. Tetany and spasmophilia illustrate Vines' third type in which there is an error in the regulation of calcium excretion. In all cases, he says, decalcification of the tissues tends to occur, though it is only when the rate of excretion exceeds the rate of absorption from the alimentary canal and from the tissues that a lowering of the blood calcium is found. Nothing in this, of course, is contradictory to the author's hypothesis that the blood pH is often the determining factor in the serum calcium level, and indeed it is noteworthy that both in acidosis and in alkalosis there is increased - often grossly increased - excretion of calcium.

IX.

Rickets.

From studies in the morbid anatomy of rickets it has long been thought that this condition might be attributable to a defect in the intake or utilisation of calcium. Recent work has gone far to support this view, and it is now established beyond reasonable doubt that the disease is due

to imperfect calcium metabolism. It can be produced experimentally in animals by feeding them on a diet which is deficient either in calcium (2I) or phosphorus (80) or in which the ratio of these elements differs markedly from the normal of I:I (3I). The addition of the missing element, or the adjustment of the P:Ca ratio in such diets will avert the disease. On the other hand, a deficiency of both calcium and phosphorus in the diet is followed by the development, not of true rickets, but of a condition of osteoporosis (78). To ensure normal growth and health, then, there must be both an ample supply and a correct balance of the inorganic elements in the diet.

According to Tisdall, Kramer, and Howland (84), the serum calcium is low in rickets (8.5 mg./I00 cc.) and still lower (5.8) when spasmophilia is manifested in addition. Hess, Calvin, Wang, and Fletcher (I0I) confirm this statement so far as rickets is concerned.

With the controversy of the last few years as to whether rickets are due to lack of vitamin-A (E.Mellanby) or to lack of sunlight (Paton), it is not proposed to deal here. Both schools of thought appear to be satisfied by the referring of anti-rachitic power to a fat-soluble substance, vitamin-D, which is produced in otherwise inactive fats under the influence of sunlight and more especially of ultra-violet radiation. The addition to a rickets producing diet of fat containing vitamin-D either preformed or produced by exposure to ultra-violet rays, effectively prevents the development of morbid processes in the bones, while the serum content and urinary excretion of calcium become (or remain) normal, and the body stores calcium (9I). In the already quoted experiments of Husband, Godden, and Richards (37), where cod-liver oil, olive oil, and linseed oil were found equally efficacious in preventing the onset of rickets and in aiding the absorption of calcium (in spite of the absence of vitamin-A from the latter two oils), no mention, of course, is made of

the fact that exposure to light may have brought about a product of vitamin-D. They conclude that the effect was due to the fats themselves, and contradict the earlier results of Kochmann and Petzsch (44), Steinitz (81), Rothberg (72), Meyer (64), and others who, usually using butter, found a reduced absorption and decreased retention of calcium to follow the addition of extra fat to the diet.

Although vitamin-D occurs in close association with vitamin-A, both being fat-soluble, they do not necessarily occur in the same relative amounts in different fats, and butter-fat, for example, though rich in the anti-xerophthalmic and growth promoting factor, is poor in the anti-rachitic vitamin (22). Moreover, vitamin-A is more readily destroyed by heat and oxidation than is vitamin-D, so that an oil, originally rich in both, may be so treated as to become incapable of promoting growth or of counteracting xerophthalmia, while remaining an effective anti-rachitic agent(60). Since exposure to light or ultra-violet rays activates otherwise inactive oils and fats, their unsaponifiable fractions, or even cholesterol, rendering them capable of preventing the onset of rickets and of increasing calcium absorption, it is now thought that vitamin-D is produced, through the agency of radiant energy by an actual chemical change in cholesterol or one of the related alcohols.

To sum up, the modern view seems to be that rickets is due to inefficient calcium absorption, due either to an actual deficiency of calcium in the diet (or to a badly balanced diet) or to lack of vitamin-D which is a chemical entity closely related to cholesterol.

X.

Some Factors Controlling the Calcium Content of the Serum.

At one time or another almost all the endocrine organs have been stated to possess a controlling influence over calcium metabolism. The loss of calcium in hyperthyroidism and in

diabetes has been held to show a direct control by the thyroid and the pancreas. The excessive bone formation in acromegaly and in eunuchism is considered evidence of the influence of the pituitary and the gonads. Battaglia (40), on the strength of some good results in the union of fractures with adrenalin and calcium chloride administered orally, claims the adrenals as controlling the mobilisation of salts. It has has already been suggested that the disorder of calcium metabolism found in diabetes is due, not directly to the pancreatic failure, but to the accompanying ketosis, so that the pancreas need not be invoked as the presiding deity over calcium metabolism. Further, it is evident that any factor causing increased growth will also cause an increased demand for calcium, so that the thyroid, pituitary and gonads in all probability act merely indirectly. In Battaglia's experiments, one may be permitted to doubt whether the adrenalin ever reached the blood stream.

The case of the parathyroids is on a different footing. Mainly on account of the very low serum calcium found after parathyroidectomy, it used to be stated categorically that calcium metabolism is controlled by the parathyroids. Much evidence can be brought forward in support of this view. In tetania parathyreopriva there is no doubt that the serum calcium is abnormally low. MacCallum(99) showed that administration of calcium salts decreased the symptoms of post-operative tetany, and Salvesen (73) claims that the beneficial effect of milk in such cases is due to its calcium content. Kishi(42) finds that after removal of the parathyroids there is increased urinary excretion of calcium, though the intestinal excretion remains constant. Trendelenberg and Meyer (85) state that in tetania parathyreo--priva it is the ionic rather than the "inactive" calcium that is decreased, and this conclusion is supported by Salvesen and Linder (74).

Since Koch (43) isolated methyl-guanidine from the

urine of parathyroidectomised dogs, much work has been done, largely by Noël Paton and his co-workers (I00), to show that tetania parathyreopriva is due, not to a calcium deficiency, but to an excess of guanidine derivatives in the blood. Into this question we cannot enter here; we are concerned, not with calcium deficiency as a cause of tetany, but with the parathyroids as a controlling factor in calcium metabolism, and however tetany may be caused, there is no doubt that it is accompanied by a low serum calcium and that this condition regularly follows removal of the parathyroids.

From this point of view the important question in tetany is: can the serum calcium be restored to normal by administration of parathyroid? Unfortunately, conflicting replies have been given to this question. Collip (I3) says that it can, but in a single case studied by the author, the serum calcium remained constant for six weeks at 6.9 mg. per I00 cc. despite parathyroid administration both orally and intravenously. In this case, too, calcium chloride by the mouth was equally inefficacious; it was only when parathyroid and calcium chloride were given simultaneously that the serum calcium rose. Evidence derived from the study of tetany, though suggestive of parathyroid control of calcium metabolism, is thus not conclusive. It may be that the lower--ing of the serum calcium is merely a secondary effect of the increase in the guanidines.

Other evidence, however, is not lacking to show that the parathyroids do play some part in calcium metabolism. Collip (I3) has found that in normal dogs massive doses of parathyroid are able to bring about a temporary raising of the calcium level. The author has obtained similar results in human subjects, though cases were encountered in which the serum calcium was unaffected by parathyroid administration. (Figs. IV-VII)

At present an attempt is being made to find an explanation of this varying result. While it is as yet too

early to make a definite pronouncement, it does seem significant that in all cases in which parathyroid feeding raised the serum calcium, the initial value was either xxxnormal (Figs. IV and VII) or had been subnormal for only a short time (Fig.V). On the other hand, cases in which no rise was produced were definitely subnormal when observations began (Fig.VI).
If it proves to be true that parathyroid is capable only of raising a serum calcium which is already normal or has not been subnormal long enough to cause serious calcium depletion of the tissues, it becomes of interest that administration of calcium salts - except in massive doses - seems to be incapable of causing more than a transient rise in a normal serum calcium, though it can usually raise a subnormal one to the normal level (the failure in the quoted case of tetany is probably due to the excessive calcium excretion in that condition).

If it be granted that the parathyroids do exert a control over the calcium content of the blood - and the evidence given seems to justify such a conclusion - it is pertinent to enquire in what way the control is exercised. There are three ways in which parathyroid may act. It may control the rate of calcium absorption from the intestine; it may control the rate of excretion; and it may control the exchange of calcium between the blood and the tissues. That it controls the rate of absorption is unlikely, since Underhill (86) found that in tetany absorption is normal. It is true that in the case of tetany studied by the author, feeding of calcium chloride did not raise the serum calcium, but this may well have been due to the excessive excretion of calcium which is known to accompany tetany (42,86). There is, in view of the increased elimination of calcium in tetany, a possibility that parathyroid acts by influencing the rate of excretion. Further information on this point is, however, lacking, and the author is at present engaged in estimating the urinary excretion of calcium in a series of cases in which parathyroid administration has raised the serum calcium to a level above normal.

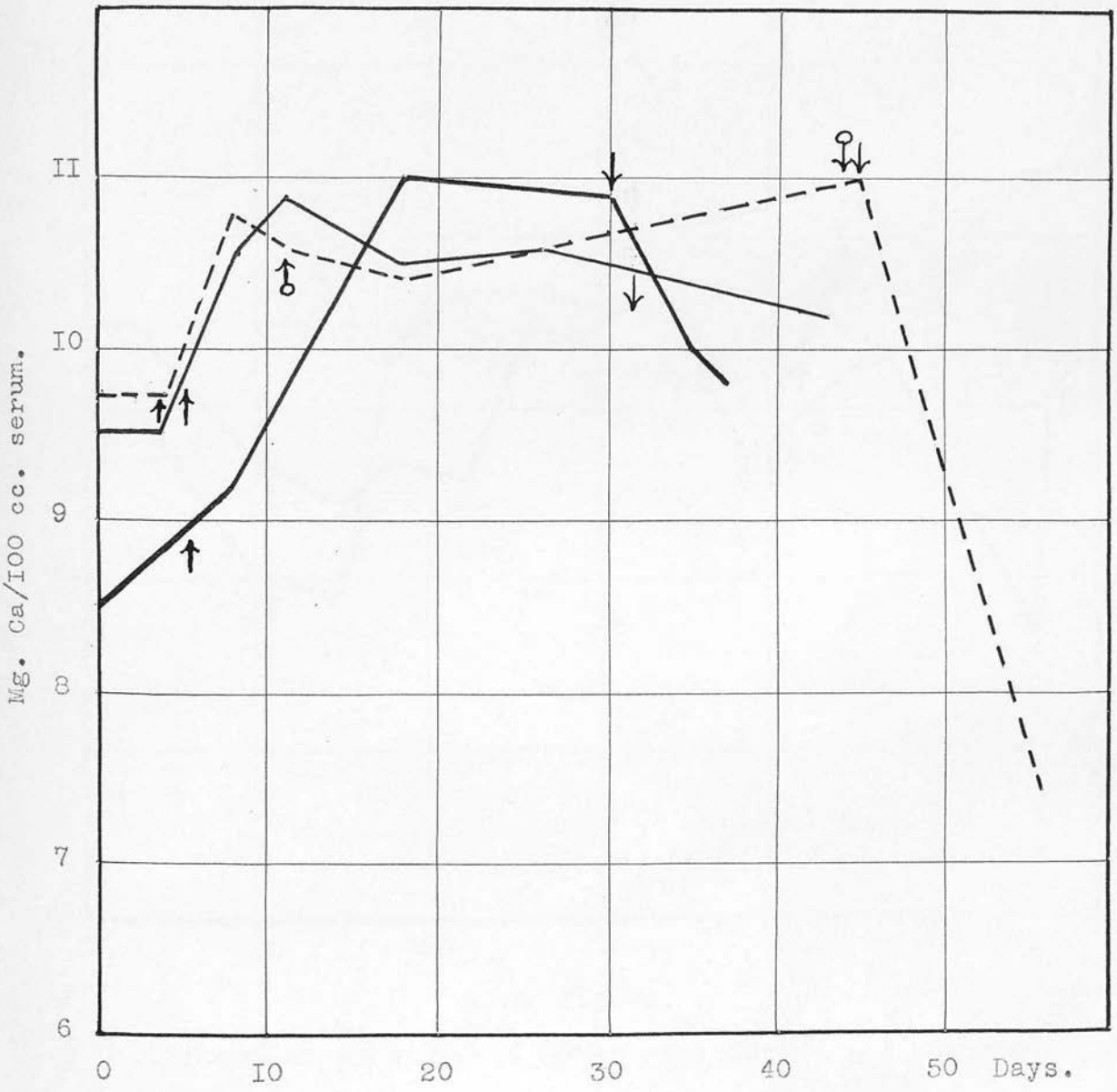

Fig.IIII.

Effect of parathyroid administration on the serum calcium.

↑ Start ↕
Parathyroid per os ↓ Finish ↕ Calcium chloride.

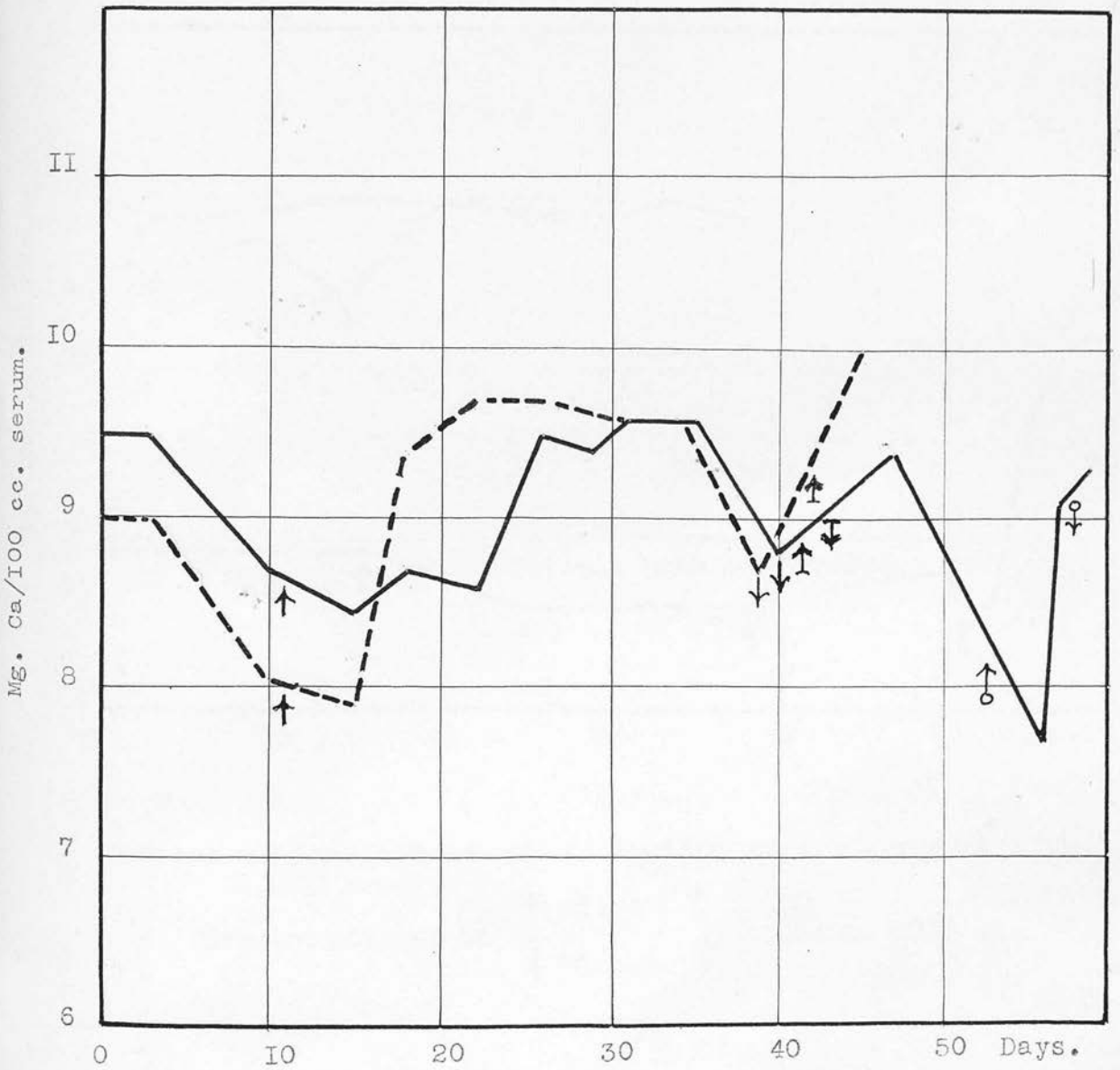

Fig.V.

Effect of parathyroid administration on the serum calcium.

Parathyroid per os ↑ Start ↓ Finish — Calcium salts per os. ↑ Start ↓ Finish

Parathyroid hypodermically ↑ Start ↓ Finish

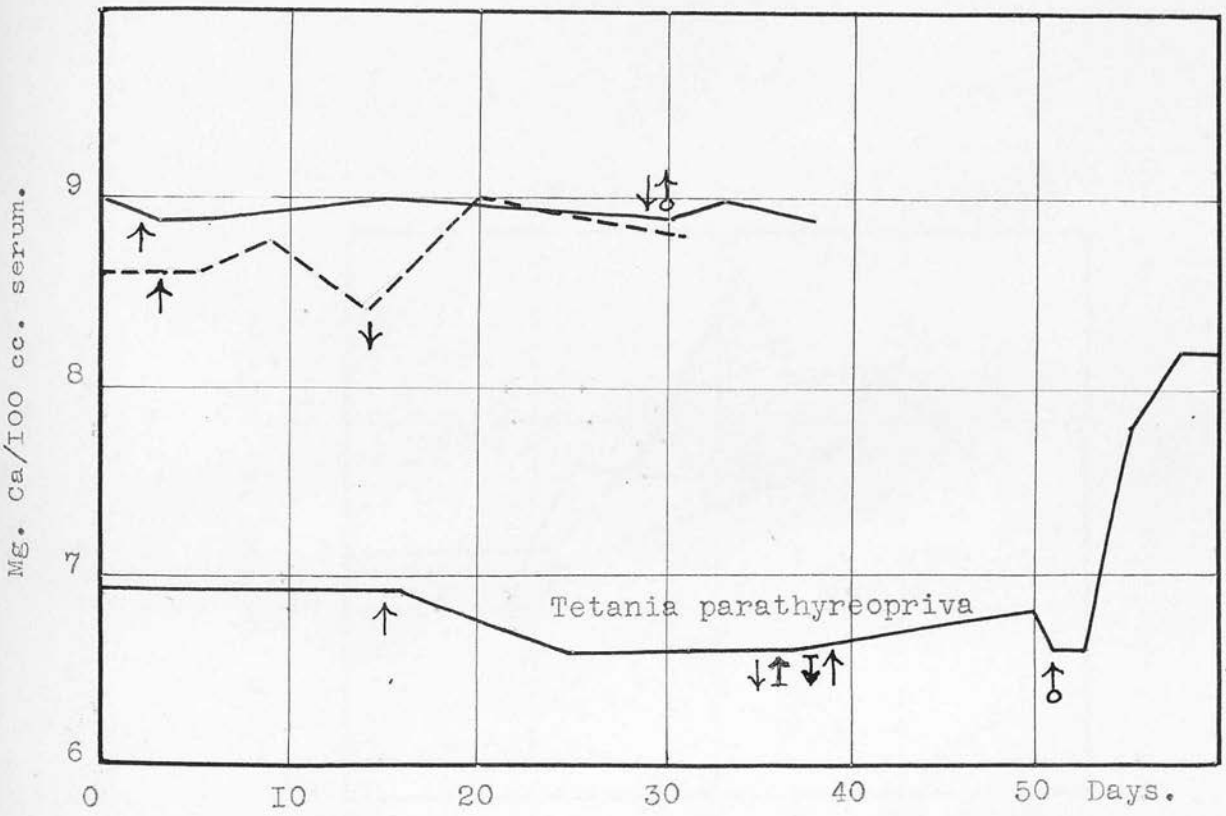

Fig.VI.

Effect of parathyroid administration on the serum calcium.

Parathyroid per os ↑ Start ↓ Finish — Calcium salts per os.

Parathyroid hypodermically ↥ Start ↧ Finish

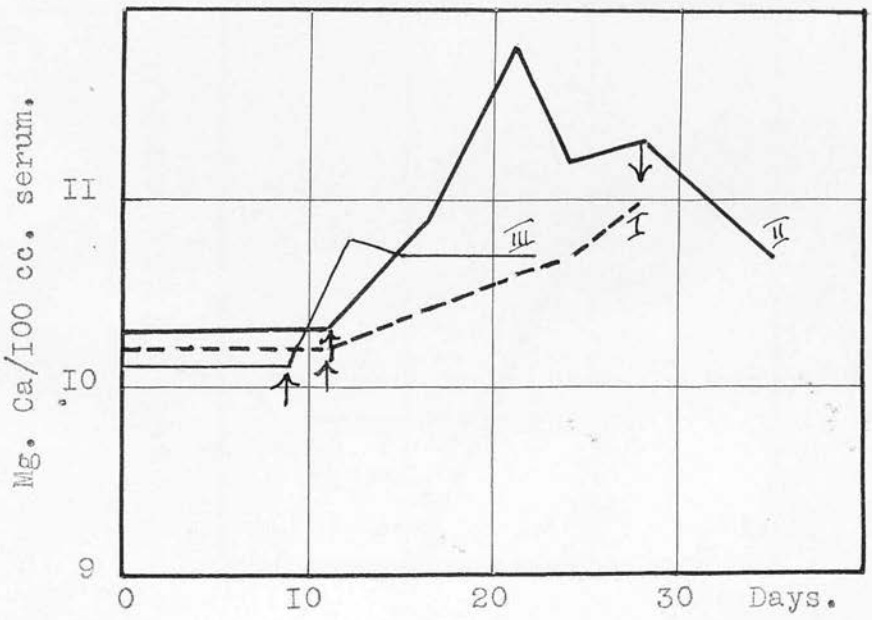

Fig.VIIa.

Effect of parathyroid feeding on the total serum calcium.

Parathyroid per os ↑ Start ↓ Finish

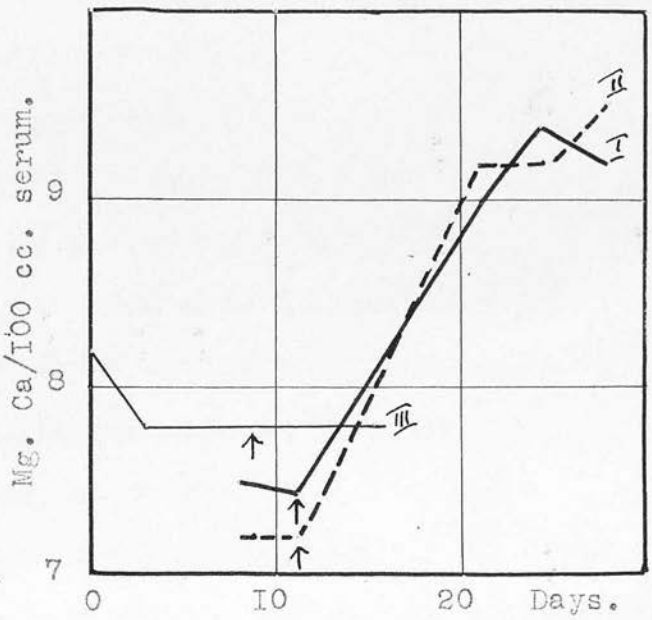

Fig.VIIb.

Effect of parathyroid feeding on the "ionic" serum calcium.

Parathyroid per os ↑ Start ↓ Finish

On the evidence at present available, it seems justifiable to advance, tentatively, the suggestion that parathyroid acts by controlling the transfer of calcium from the blood to the tissues (and vice versa). On this hypothesis, one can readily see why excess parathyroid should be able to raise a normal serum calcium - when there is an abundant supply of readily available calcium in the tissues - but not one that has for some time been subnormal - with consequent depletion of the tissue stores. Moreover, it explains the author's observation (Fig.V) that in certain cases, maintenance of the serum calcium at a high level by means of administration of parathyroid has resulted after some time in a fall of the serum calcium to a level below normal. The increased serum calcium, with the consequent increased excretion (29) may cause a depletion of the tissue stores, when the parathyroid is , naturally, no longer able to maintain a high level in the serum. An interesting consequence of this hypothesis, and one that can be tested experimentally, is that a long continued excess of parathyroid should lead to the same result as parathyroidectomy - a very low serum calcium with, if calcium is the controlling factor in that disease, production of tetany. It is admitted, of course, that the facts at present known are sufficient merely to exclude the possiblity of the action of the parathyroids being exerted on the rate of absorption of calcium; they cannot decide definitely between the excretory mechanism and the tissue-blood equilibrium as the site of parathyroid action. The latter of the two possibilities is merely the author's choice of a working hypothesis and will be modified or rejected - or accepted definitely - as future results may demand. Further, there is, of course, no suggestion that the parathyroids constitute the sole controlling factor in calcium metabolism. The effect of variations or tendencies to vary in the blood pH has already been considered, and it is obvious that many of these variations are quite independent of any action of the parathyroid.

XI.

Conclusion.

The disjointed state of our knowledge of the place of calcium in the bodily economy is evident from the foregoing account. In spite of much painstaking research, we are still shrouded in the mists of uncertainty but, as has truly been said,

> "the principles of science have been worked out by slow degrees and much blundering"

and

> "by the mistakes of others and by their misconceptions we are guided into more fruitful paths".

Nevertheless, progress has been made, and though doubtless there will be further failures and disappointments, the relentless march of all-conquering science will not be stayed, and ultimately the truth will be made plain to all men.

The original work described in this paper has been carried out in collaboration with Dr. C. P. Stewart in the Departments of Medical Chemistry, Therapeutics, and Pharmacology.

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