CCLXIX. THE TOTAL AND DIFFUSIBLE CALCIUM OF SERUM AND THE CALCIUM OF CEREBROSPINAL FLUID IN HUMAN CASES OF HYPOCALCAEMIA AND HYPERCALCAEMIA.

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CASES of hypocalcaemia may be divided into three groups. Firstly, there are the various types of low-calcium tetany. Secondly, there are cases of hypocalcaemia associated with low serum-protein, in which no symptoms of tetany occur; this condition may be present in nephrosis, chronic nephritis with oedema, kala-azar and some other conditions. Thirdly, there are cases of chronic nephritis with uraemia, in which the inorganic phosphorus of the serum is increased, and in which the serum-proteins may be normal or only moderately reduced; in some instances neuromuscular irritability and carpopedal spasm occur in addition to uraemic manifestations.

One would expect that in tetany a low $[Ca^{++}]$ would be the significant change. The ionic calcium may or may not account for the whole of the diffusible calcium of serum, but it must be included in the diffusible fraction. It would also be expected that when the serum-calcium falls in response to a rise in the inorganic phosphate, the diffusible fraction would be primarily affected, though the change in the diffusible fraction would lead to a secondary change in the protein-bound fraction as a result of the disturbance of equilibrium. On the other hand, where the serum-calcium falls as a result of a fall in the serum-protein, one would expect the protein-bound fraction to be reduced and the diffusible fraction normal, and the absence of tetany would thus be explained.

Now those authors who have used the concentration of calcium in the cerebrospinal fluid as a measure of the diffusible calcium of the serum have arrived at results which are inconsistent with the above views, for it has been shown that when the serum-calcium falls in tetany, the calcium of the cerebrospinal fluid is relatively little changed. Authors who have determined the diffusible calcium of the serum by ultrafiltration or dialysis have arrived at variable results, but, on the whole, work done on ultrafiltration of human sera has shown that the diffusible calcium is decreased in tetany and normal in cases of hypocalcaemia with low serum-protein. The view that the calcium of cerebrospinal fluid is equal to the diffusible calcium of serum has been attacked, but as yet only a few comparisons between the cerebrospinal fluid and the serum ultrafiltrate have been made in cases where the total serum-calcium is abnormal.

At the outset of the present work the intention was to make comparisons between the calcium of serum and of cerebrospinal fluid in conditions where the

serum-calcium was markedly raised or lowered, and this has been done in cases of tetany, uraemia, osteitis fibrosa and some other diseases. Later the scope of the work was extended, and the serum was analysed for total calcium, diffusible calcium, protein and phosphorus. It is unfortunate that some cases of tetany were examined before the experiments on ultrafiltration were begun.

METHODS.

Calcium was estimated by the method of Kramer and Tisdall as described by Harrison [1930], but the following technical points require comment. Firstly, the time allowed for precipitation of the calcium oxalate was in most instances more than 2 hours and often overnight. This was done as a precaution, though half an hour is probably adequate for the majority of sera. Secondly, the conditions of washing the precipitate of calcium oxalate must be considered. The precipitates obtained from cerebrospinal fluids or from ultrafiltrates do not pack closely enough to be left undisturbed by decantation, and removal of the supernatant with a pipette is therefore preferred. In analyses of pure calcium chloride solutions it was found that the soluble oxalate was completely removed by the second washing, and that a third washing caused no loss of calcium oxalate (ordinarily the residue was twice washed). In these and all other determinations the "blank" value was obtained by taking a little of the last supernatant fluid (comparable with that left with the final residue) and treating it in the same way as the residue. In this way correction is made for any soluble oxalate remaining with the calcium oxalate. The "blank" value was almost constantly 0.03 cc. N/100 permanganate.

Wherever possible, 4 cc. of cerebrospinal fluid or ultrafiltrate were used, but in some cases it was not possible to obtain so much ultrafiltrate, and some of the figures for diffusible calcium are based on single determinations on 2 cc. fluid. The errors likely to occur under these conditions are not great enough to affect the main significance of the results.

Phosphate. Inorganic phosphate was estimated in serum or oxalate-plasma by the method of Briggs [1922]. The time elapsing between collection of the blood and removal of the plasma from the corpuscles was not more than half an hour and was usually about 10 to 15 minutes.

Serum-proteins. Only total protein estimations have been made in the present series of cases. Some of these were made by the micro-Kjeldahl method using Pregl's apparatus [Harrison, 1930] but the majority by the colorimetric method in the form used by Greenberg [1929, 1].

In a preliminary comparison of these two methods, using sera and exudate fluids in which the ratio of albumin to globulin varied through a wide range, it was found that the results agreed well for total protein, but that considerable discrepancies sometimes occurred in the determination of the ratio of albumin to globulin. Tuchman and Sobotka [1932] have obtained similar results in comparing the Kjeldahl method with the colorimetric method as described by Wu.

Albumin and globulin have different tyrosine equivalents in the colorimetric method, but in spite of this there was a very constant relation between the total protein-nitrogen as determined by the Kjeldahl method and the tyrosine equivalent of total protein in the colorimetric method. Greenberg's factor, 1 mg. tyrosine $\equiv$ 16.0 mg. total protein, was found to hold good in spite of variations in the ratio of albumin to globulin, and it was therefore considered justifiable to make total protein estimations by the colorimetric method without separating the albumin and globulin fractions.

In the two cases where Bence-Jones protein was present in the serum, the total protein was estimated by the Kjeldahl method.

Urea was determined by the method of Archer and Robb [1925].

Ultrafiltration. Collodion sacs were cast inside tubes 10 cm. in length and 1.6 cm. in diameter. The solution used was 3 % pyroxylin in alcohol and ether (40 parts alcohol to 60 parts ether) and 3 cc. ethylene glycol were added to 100 cc. of the pyroxylin solution. The sacs retained protein completely, as was shown by testing the ultrafiltrates with salicylsulphonic acid. The permeability of the sacs to calcium was checked by filtering the same sample of serum through the different sacs and showing that all the ultrafiltrates contained the same concentration of calcium. The permeability of the sacs was very constant and regular, and sacs which had been in use some time gave the same results as new ones.

Table I.

Case	Age	Sex	Diagnosis	Date	Cerebro-spinal fluid-calcium mg. per 100 cc.	Serum-calcium mg. per 100 cc.			Serum-protein %	Plasma-phosphate (inorganic) mg. P per 100 cc.	Blood-urea mg. per 100 cc.	
						Dif. fusible	Total	Non-dif. fusible				
												Dif. fusible Ca % of total
C. F.	18	F	Osteitis fibrosa with parathyroid hyperplasia	14. ix. 32	—	19.2	—	—	—	1.70	26	
				19. ix. 32	6.4	17.8	—	—	—	—		
				11. x. 32	—	17.8	8.6	52	—	—		
				17. x. 32	—	15.6	6.2	60	—	—		
S. H.	52	F	Osteitis fibrosa with parathyroid hyperplasia	28. x. 32	5.9	16.1	6.8	58	—	—	—	
				2. vi. 33	—	13.0	6.1	53	1.68	—		
				19. vii. 33	6.3	8.4	14.7	6.3	57	—	—	
				25. vii. 33	6.9	7.2	16.9	9.7	43	12.6	4.2	120
A. Ho.	54	F	Multiple myelomatosis	6. v. 31	—	15.6	—	—	—	2.8	38	
												10. vii. 31
M. L.	11 to 13	F	Late rickets	8. v. 33	—	6.3	10.8	4.5	58	2.35	—	—
J. R.	28	M	Acute nephritis	—	—	—	—	—	—	—	—	—
R. F.	22	F	Carpopedal spasm and dwarfism. Hysteria	31. v. 32	5.6	—	10.5	—	—	4.72	—	—
				5. v. 33	—	5.3	10.8	5.5	49	6.4	3.58	30
M. S.	—	F	? Tetany. Radium menopause	21. iii. 33	—	6.0	10.7	4.7	56	6.1	2.72	48
H. H.	55	M	Multiple myelomatosis	13. ii. 33	—	5.9	10.6	4.7	56	6.5	4.67	102
K. L.	42	F	Congenital cystic kidney	21. iv. 33	—	5.8	10.4	4.6	56	6.2	2.86	30
D. O.	28	F	Convulsions during pregnancy	21. iii. 33	—	6.0	10.3	4.3	58	6.4	3.22	—
W. McG.	16	M	? Osteitis fibrosa	8. vi. 33	—	5.1	9.8	4.7	52	6.2	3.07	—
				2. v. 33	—	5.4	9.6	4.2	56	5.9	3.26	25
C. T.	13	F	Osteitis fibrosa	27. ix. 33	—	4.6	9.5	4.9	48	6.2	1.91	—
R. B.	22	F	Hysteria and carpal spasm	29. vii. 33	4.7	—	9.2	—	—	—	—	—
M. R.	—	M	Progressive muscular atrophy	16. i. 33	5.7	5.7	9.1	3.4	63	—	—	90
R. H.	11	M	Subacute nephritis	8. ii. 33	—	4.7	9.1	4.4	52	5.8	4.60	203
A. T.	30	F	Chronic nephritis	3. iv. 33	—	5.2	8.5	3.3	61	5.5	7.05	282
T. H.	33	M	Congenital cystic kidney	11. xi. 32	6.2	6.0	8.4	2.4	71	6.1	12.70	400
G. G.	58	M	Chronic nephritis	—	—	—	—	—	—	—	—	—

P. M.	56	M	Chronic nephritis	14. iii. 33	—	5.9	8.1	2.2	73	4.5	3.04	30
J. McP.	56	F	Myxoedema, pyelonephritis	22. x. 32	—	4.9	8.1	3.2	61	5.1	3.92	70
J. T.	33	M	Nephritis	26. v. 33	—	5.3	7.7	2.4	69	5.3	6.60	475
J. St.	18 to 19	M	Acute to subacute nephritis	22. x. 32 23. ix. 33 25. ix. 33 4. x. 33	— 5.2 4.9	4.5 2.8 3.2	8.0 7.3 6.0	3.5 — 2.8	56 38 53	4.9 4.6 —	7.3 — 12.6 12.2	112 — 198
M. H.	17	F	Acute to subacute nephritis	30. i. 33 7. ii. 33 20. ii. 33 27. ii. 33 28. iv. 33 22. ii. 32 4. iv. 32	— — — — — 4.5	5.7 4.4 4.0 4.0 3.8	7.8 7.3 6.8 7.1	2.1 2.9 2.8 3.3	73 60 59 54	5.7 5.3 5.2 5.4 5.3	8.15 14.2 13.8 13.1 5.98	215 — 368 320 337
F. W.	35	F	Chronic nephritis	28. iv. 33	—	3.8	7.1	3.3	54	5.3	5.98	337
H. D.	22	F	Tetany. (In attack)	22. ii. 32 4. iv. 32	4.5 —	—	6.7 7.0	— —	— —	5.6 —	2.32 3.22	21
E. T.	15	F	Late rickets and tetany	20. v. 32	4.9	—	6.4	—	—	—	—	—
T. Y.	43	M	Chronic nephritis. Tetany	19. iv. 33	5.7	4.0	6.4	2.4	63	5.4	28.1	690
A. Hi.	34	F	Chronic diarrhoea and tetany { Tetany present Tetany improving No symptoms Tetany present	25. iv. 33 9. v. 33 16. v. 33 2. vi. 33	4.3 — 4.9 4.6	3.0 3.9 4.5 4.1	5.4 6.3 6.9 5.4	2.4 2.4 2.4 1.3	56 62 65 76	5.0 — — —	3.20 — — —	— — — —
J. Sh.	36	M	Chronic nephritis	21. x. 32	5.3	2.8	6.2	3.4	45	7.0	26.2	410
G. P.	7	M	Chronic nephritis, uraemia and tetany	1. v. 31 6. v. 31 14. v. 31	— 5.3 4.8	— — —	5.6 — 6.1	— — —	— — —	— — —	10.4 — 6.6	156 — —
R. A.	39	M	Chronic nephritis	2. ii. 32	4.7	—	5.1	—	—	—	—	480
E. F.	39	F	Exophthalmic goitre. Postoperative tetany	19. vii. 32	4.4	—	4.4	—	—	—	6.3	—

NOTES

C. F. and S. H. In each case hyperplastic parathyroid glands were found at operation, and the blood chemistry returned to normal after their removal.
A. Ho. Bence Jones protein was present in serum and urine. *Post mortem* examination showed multiple bone tumours (plasma-cell myeloma), gross renal disease, and normal parathyroid glands.

M. L. An unusual case of late rickets of unknown origin and resistant to treatment.

R. F. Carpopedal spasm, believed to be due to hysteria and probably not alkalosis tetany.

R. B. Carpal spasm certainly due to hysteria. The electrical excitability of the motor nerves was normal.

H. D. Idiopathic tetany, probably not of parathyroid origin.

E. F. Tetany after thyroidectomy for exophthalmic goitre. The observations were made two months after the operation.

In the course of ultrafiltration, the sacs tended to dry above the level of the serum contained in them. In time this made it necessary to discard old sacs. The drying caused them to become less permeable, in the sense that filtration became unduly slow, though it did not appear to alter the composition of the ultrafiltrate. The sacs were stored in saline solution in the refrigerator. Before use they were dried as completely as possible with filter-paper and rinsed with a little of the serum to be filtered. The first drops which formed when ultrafiltration began were blotted off the sac with filter-paper. Ultrafiltration was carried out under a diminished pressure of about 150 mm. mercury in the apparatus described by Greenberg and Gunther [1929]. The rate of filtration was such that with new sacs containing 5 to 6 cc. of serum about 1 cc. ultrafiltrate was collected per hour.

The figures obtained at the moderate pressure used in this method are comparable with those of the majority of other authors who have worked on human material. High pressure ultrafiltration may give higher figures for diffusible calcium [Nicholas, 1932].

Ultrafiltration is preferred to compensation dialysis as a method of estimating the diffusible calcium, because dialysis against any external fluid which does not contain the same concentration of free calcium as the serum is likely to cause some disturbance of the equilibrium between protein-bound calcium and diffusible calcium, with a consequent change in the distribution ratio. The concentration of the fluid inside the sac during ultrafiltration does not lead to any such disturbance of the distribution ratio, provided that the filtration is not too long continued [Stewart and Percival, 1928, 1]. In the course of the present work it has been noted that the serum inside the sac can be concentrated to at least half the original volume without causing any change in the composition of the ultrafiltrate.

RESULTS.

The results are given in Table I.

Normally the total serum-calcium ranges between 9 and 11 mg. per 100 cc.; the cerebrospinal fluid-calcium varies from 4.5 to 5.5 mg. per 100 cc. and the diffusible serum-calcium is approximately equal to the cerebrospinal fluid-calcium.

The present series of observations includes nineteen strictly simultaneous estimations of total serum-calcium and cerebrospinal fluid-calcium, in seventeen of which the serum-calcium is abnormal. The serum-calcium values range from 4.4 mg. per 100 cc. to 17.8 mg. per 100 cc., but the whole range of variation in the cerebrospinal fluid-calcium is from 4.4 to 6.9 mg. per 100 cc. The calcium of cerebrospinal fluid never definitely falls below normal in hypocalcaemia and only rises slightly above normal in hypercalcaemia.

There are three comparisons between the diffusible serum-calcium and cerebrospinal fluid-calcium in cases of hypercalcaemia. In the two cases of hyperparathyroidism, the diffusible calcium is markedly raised and forms a normal proportion of the total calcium, and there is a striking difference between the calcium of the ultrafiltrate and that of the cerebrospinal fluid. The case of C. F. shows that the discrepancy cannot be due to a delay in attaining equilibrium between serum and cerebrospinal fluid. When the observation was made on Oct. 28th, it was known that the total serum-calcium had ranged between 15.6 and 19.2 mg. per 100 cc. for more than six weeks, and in fact it had been rising for months. The diffusible calcium had been observed to remain in the region of 9.3 mg. per 100 cc. for seventeen days before the final observation, yet the cerebrospinal fluid-calcium was 5.9 mg. per 100 cc., only slightly above normal.

In the case of A. Ho., the hypercalcaemia was mainly due to a rise in the non-diffusible fraction of the serum-calcium, and there was also a rise in the serum-protein as a result of the presence of Bence-Jones protein in the serum. There was a moderate rise in the diffusible serum-calcium, and very little difference between the ultrafiltrable calcium and cerebrospinal fluid-calcium.

When the total serum-calcium is markedly reduced in tetany or in uraemia, the diffusible calcium of the serum is also low and falls definitely below the cerebrospinal fluid-calcium (6 observations).

In uraemia, a moderate fall of serum-calcium (9.0 to 7.5 mg. per 100 cc.) is usually associated with normal figures for diffusible calcium. This may occur in spite of moderate rises in the inorganic phosphate of the plasma (6.6 to 12.7 mg. P per 100 cc.). Higher phosphate figures are associated with more marked hypocalcaemia and a fall in the diffusible calcium of the serum. In most cases the serum-proteins are low, but the distribution of calcium between the diffusible and non-diffusible fractions does not bear a regular relationship to protein and phosphate figures.

There are only two cases of hypocalcaemia with low serum-protein and normal plasma-phosphate (P. M. and J. McP.); in both of these the diffusible calcium is normal.

Observations on diffusible calcium have been made in one case of tetany with low serum-calcium. The diffusible calcium was 4.1 mg. per 100 cc. or lower at all times when symptoms were present; on one occasion when symptoms were absent the diffusible calcium was 4.5 mg. per 100 cc.

The series includes two cases of carpopedal spasm of unknown causation, with normal figures for both total and diffusible calcium in the serum (R. F. and R. B.). These are not regarded as cases of tetany. Greenberg and Gunther [1931] mention three similar cases of "hysteria and carpopedal spasm."

DISCUSSION.

The relationship between the total calcium of serum and cerebrospinal fluid.

Parallel determinations of calcium in serum and cerebrospinal fluid in experimental animals have been made by Cameron and Moorhouse [1925], Merritt and Bauer [1931, 2], Morgulis and Perley [1930] and Hertz [1930]. By the work of these authors it has been established that in parathyroidectomised animals the calcium of the cerebrospinal fluid is only slightly lowered in spite of the hypocalcaemia, and that when the total serum-calcium falls to the level of 4 to 5 mg. per 100 cc. the figures for calcium in serum and cerebrospinal fluid become approximately equal. On the other hand, when hypercalcaemia is induced by administration of parathormone, the cerebrospinal fluid-calcium is only slightly raised, and figures of 12 to 17.2 mg. per 100 cc. in serum have been recorded in association with figures of 5.2 to 6.8 mg. per 100 cc. in cerebrospinal fluid. These results are shown in Fig. 1. Similar, though rather more variable results were found when hypercalcaemia was induced by continuous intravenous injections of calcium salts [Morgulis and Perley, 1930]. The findings in the present series of human cases agree well with those recorded in experimental hypo- and hyper-calcaemia.

Parallel observations of calcium in serum and cerebrospinal fluid in various human diseases have been made by Cantarow [1929, 1, 2], Leicher [1922], Pincus and Kramer [1923], Berenczy [1929], Merritt and Bauer [1931, 1, 2], Critchley and O'Flynn [1924], Lennox and Allen [1930], Kral *et al.* [1929], Weston and Howard [1922], Parhon and Ornstein [1930], Hamilton [1925], Halverson and Bergeim [1917], McCance and Watchorn [1931; 1932, 1], and Nourse *et al.* [1925]. The vast majority of the observations of these authors were made in cases where the total serum-calcium was normal or only slightly raised or lowered. The data obtained in meningitis must be considered separately, for, as Merritt and Bauer [1931, 1] have pointed out, the cerebrospinal fluid-calcium

may rise above normal in such cases. The rise in protein in the cerebrospinal fluid in inflammatory conditions will cause some alteration in the distribution of calcium between plasma and cerebrospinal fluid, but there may be other factors involved. Observations in such cases are not comparable with those in which the meninges are normal and ought not to be used as a basis for the study of the normal equilibrium between plasma and cerebrospinal fluid. The data of McCance and Watchorn [1932, 1] for cases of meningitis include some definitely high figures for cerebrospinal fluid-calcium (6.5 to 7.9 mg. per 100 cc.), but the majority of the results fall between 5.2 and 6.5 mg. per 100 cc. McCance and Watchorn [1931] also record a number of high figures for cerebrospinal fluid-calcium in cases of general paralysis, cerebral haemorrhage and cerebral arterial sclerosis.

A very large number of analyses have been made in other human diseases—including non-inflammatory diseases of the nervous system, mental diseases and a wide variety of diseases not affecting the nervous system. In all these conditions the cerebrospinal fluid-calcium ranges between 4.5 and 6.5 mg. per 100 cc. with only occasional exceptions.

Parhon and Ornstein [1930] reported low figures for the calcium of cerebrospinal fluid in epilepsy, but this is not confirmed by other authors.

Data for hypocalcaemia in human subjects are given by Cantarow [1929, 2] in three cases of bronchial asthma; by Merritt and Bauer [1931, 2] in three cases of tetany; by Leicher [1922] in one case of tetany; by Lennox and Allen [1930] in three cases of tetany; and by Nourse *et al.* [1925] in seventeen cases of tetany. The results show that it is extremely unusual for the calcium of the cerebrospinal fluid to fall below 4 mg. per 100 cc.

In Fig. 2 are assembled the data for tetany and for various other human diseases, collected from the literature, and including the observations of the present paper. The data for normal subjects and for cases of mental and nervous disease are excluded as being too numerous to plot. Also Cantarow's results [1929, 1] cannot be shown owing to his method of recording them.

Observations on the calcium of cerebrospinal fluid after parathormone administration in human subjects have been made by Merritt and Bauer [1931, 2] in cases of epilepsy, by Cantarow [1929, 2] in cases of epilepsy and bronchial asthma, and by Berency [1929] in various diseases. In the cases of Merritt and Bauer the calcium of cerebrospinal fluid remained normal in spite of hypercalcaemia, and in many of Cantarow's cases the same result was obtained, though some showed definite rises in the cerebrospinal fluid-calcium. All Berency's cases showed rises in the cerebrospinal fluid-calcium, but in all it was abnormally high before parathormone was given.

The relation between the calcium of cerebrospinal fluid and the diffusible serum-calcium.

Normally the figures for calcium are approximately the same in cerebrospinal fluid and in ultrafiltrate. Very few comparisons have been made in cases where the serum-calcium is abnormal. McCance and Watchorn [1931], and Greenberg [1929, 2] have made comparisons, and found some discrepancies greater than can be accounted for by experimental error. In some cases the ultrafiltrate contained more calcium than the cerebrospinal fluid, in others the reverse discrepancy occurred, but very few of the figures were definitely abnormal. Greenberg considers that "when the blood-calcium is at a normal stable level, there is an approximate approach to an equilibrium between blood and spinal fluid, and the spinal fluid-calcium is then a close measure of the diffusible

calcium; but on the other hand, when the blood constituents are undergoing marked fluctuations the spinal fluid changes do not keep pace.' On this basis the relatively constant figures for cerebrospinal fluid-calcium in animals subjected to parathyroidectomy or treated with parathyroid hormone are explained. The present work shows that there must be some other factor involved beside the time factor—in the case of C. F. the cerebrospinal fluid failed to come into equilibrium with the blood after prolonged hypercalcaemia. Hertz [1930] has shown that in parathyroid tetany in animals, the diffusible calcium may be low while the cerebrospinal fluid-calcium is normal. The results of the present paper show similar discrepancies.

The observation that in tetany the cerebrospinal fluid-calcium is not significantly lowered, whilst the diffusible serum-calcium is lowered, may explain the fact that the nervous symptoms of tetany are peripheral and not central. A reduction in the $[Ca^{++}]$ of the fluid in contact with the central nervous system has been shown to cause convulsions of central origin [Huggins and Hastings, 1933.]

The distribution of calcium between the diffusible and non-diffusible fractions in protein solutions and in serum.

For the purpose of the present discussion it will be assumed that the non-diffusible calcium is bound to protein. A number of observations show that the diffusible and non-diffusible fractions of the serum-calcium are not to be regarded as independent entities but are in equilibrium with each other. The removal of calcium ions by precipitation with oxalate leads to a dissociation of the protein-bound fraction, so that the whole of the serum-calcium can be precipitated as oxalate. Similarly, the addition of citrate to the serum causes the whole of the serum-calcium to become diffusible [Stewart and Percival, 1928, 2], presumably owing to the removal of calcium ions by the formation of a non-ionised complex. The serum-calcium can be completely removed by dialysis if very large volumes of external saline solution are used; this shows that removal of the diffusible calcium disturbs the equilibrium and causes dissociation of the protein-bound fraction [Loeb and Nichols, 1923]. The balance between the two fractions may also be disturbed by the addition of more calcium in diffusible form; the added calcium becomes distributed between both fractions so that at the new equilibrium the non-diffusible fraction forms approximately the same proportion of the total as before. This occurs both *in vivo* and *in vitro* [Smith and Sternberger, 1932].

The conditions of this equilibrium have been studied in dialysis systems by Marrack and Thacker [1926] and by Loeb and Nichols [1923; 1925; 1927, 1, 2]. This work has shown that the proportion of calcium which becomes bound to protein, in serum or other protein-containing mixtures, depends upon the following factors: the protein concentration; the nature of the protein; the calcium concentration in the external solution; the hydrogen ion concentration; the chloride concentration; and the temperature. Some of these factors will vary in different pathological conditions, and there may be other significant factors as yet unrecognised.

If the protein concentration varies, other factors being constant, the ratio of the protein-bound calcium to the diffusible calcium varies directly as the protein concentration.

If the protein, p_H and chloride concentrations are constant, and the calcium varies, there is a regular relationship between the protein-bound calcium and the calcium of the external solution, as is shown by the experiments of Loeb and

Nichols [1927, 1]. The regularity of these results is shown in Fig. 3, where the results of Loeb and Nichols are plotted in a manner comparable with the other charts in the present paper—*viz.* the total calcium values in the protein solution are plotted as abscissae, and the calcium values of the aqueous solutions as

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 1. Relation between serum-calcium and cerebrospinal fluid-calcium in animals at different levels of parathyroid function (parathyroidectomy; normal; and parathyroid-treated). Data collected from the literature.

Fig. 2. Relation between serum-calcium and cerebrospinal fluid-calcium in human tetany and other human diseases. Data collected from the literature together with those of the present paper.

Fig. 3. The distribution of calcium between serum and external fluid in a dialysis system. Data of Loeb and Nichols. Each straight line represents the results obtained on one serum sample when dialysed against external fluids of varying calcium content.

Fig. 4. Relation between total and diffusible serum-calcium in various human diseases. Data collected from the literature including the present paper.

- Cases of tetany.
- Cases of uraemia.
- ▲ Serum-proteins below 6 % in cases other than uraemia or tetany.
- Hyperparathyroidism.

ordinates. For each serum the result is a straight line, but the straight lines do not pass through the origin—with low calcium concentrations a greater proportion of calcium is protein-bound. Greenberg and Gunther [1929] have pointed out that these results are in accordance with Langmuir's adsorption isotherm.

A change in the hydrogen ion concentration towards the acid side will cause a decrease in the amount of protein-bound calcium, whereas a change to the alkaline side will cause an increase. If the reaction is at the isoelectric point of the proteins the whole of the calcium becomes diffusible [Loeb and Nichols, 1925; Pincus *et al.*, 1926]. The changes likely to occur in pathological conditions will probably cause only slight alterations in the proportions of diffusible and non-diffusible calcium. Marrack and Thacker [1926] state that a change from p_H 7.4 to 7.8 will cause the diffusible calcium to fall from 5.4 to 4.5 mg. per 100 cc. They make the suggestion that alkalosis tetany may be due to this effect, but Greenberg and Gunther [1931] have found that in alkalosis tetany the diffusible calcium is normal. Hertz [1929] found a slight, but scarcely significant, fall in the diffusible calcium in this condition.

A fall in chloride concentration causes an increase in the proportion of calcium bound to protein, and the effect is large enough to be of importance in pathological conditions. The figures of Loeb and Nichols [1927, 1] indicate that if the total calcium remained steady at 13.0 mg. per 100 cc. one would expect that a fall in chloride concentration from approximately 130 to approximately 30 *mM* would cause a fall of diffusible calcium from 8 mg. per 100 cc. to approximately 5 mg. per 100 cc.

In this discussion the diffusible fraction has been treated as a single entity. If it in fact includes two components, an ionised portion and a non-ionised diffusible complex, then there will be two balanced reactions to consider—between ionic calcium and protein-bound calcium, and between the ionised and non-ionised fractions of the diffusible calcium. Such subdivisions are still a matter of controversy. Benjamin and Hess [1933] subdivide both diffusible and non-diffusible fractions according to the amount removed by adsorbing agents.

Total and diffusible calcium in pathological conditions.

The distribution of calcium in human sera in various diseases has been studied by McCance and Watchorn [1931; 1932, 2], Benjamin and Hess [1933], Greenberg and Gunther [1930, 1, 2, 3; 1931; 1932], Greenberg [1929, 1, 2], Liu [1927; 1928], Kirk and King [1926], Pincus *et al.* [1926], Snell [1930], Weill [1932, 1, 2] and Hertz [1929].

For the purposes of the present discussion the results of these authors, together with those of the present paper, have been grouped as follows: (1) tetany, excluding alkalosis tetany, 28 cases; (2) uraemia, 33 cases; (3) serum-proteins below 6 % in cases other than uraemia and tetany, 20 cases; (4) pregnancy, 16 cases; (5) hyperparathyroidism, 5 cases, and (6) all conditions not included in the preceding categories, 180 cases. Some observations have been omitted owing to difficulty in classifying them. The last group serves as a control group. The "normal" diffusible calcium as judged from these data may be taken as 4.5 to 6.5 mg. per 100 cc. In 90 % of the control cases the diffusible calcium forms 45 to 65 % of the total and is on the average 52 %.

Data in cases of pregnancy vary. McCance and Watchorn [1932, 2] found in most instances an unusually high proportion of diffusible calcium; Kirk and King [1926] recorded the reverse effect.

The data from the other groups of human cases collected from the literature, together with those reported in the present paper, are assembled in Fig. 4. Here the total serum-calcium values are plotted as abscissae and the diffusible calcium values as ordinates. The line *OA* is drawn through the origin and the point corresponding to total calcium 10.0, diffusible calcium 5.2; any observations where the diffusible calcium forms 52 % of the total will fall on this line,

which marks the average percentage of diffusible calcium in the control cases. Similarly *OC* and *OB* represent 45 % and 65 % respectively, and mark the range within which 90 % of the "control" data fall. The actual "control" data are not plotted, but the lines *OA*, *OB*, and *OC* are derived from them and serve as guides to show whether, in the other cases, the diffusible calcium keeps the normal proportion or not. Points falling above *OB* indicate a disproportionately low non-diffusible fraction, and points falling below *OC* indicate a disproportionately low diffusible fraction.

In the group of cases where the serum-protein is below 6 % (plotted in Fig. 4 as solid triangles), the diffusible calcium is almost always normal, and when the total calcium is low, the protein-bound fraction is reduced. This is most clearly shown in those cases where the serum-protein is very low [Liu, 1927].

McCance and Watchorn [1932, 2] state that the protein concentration of the serum has no effect on the proportion of non-diffusible calcium. Their data, however, were obtained on sera in the majority of which the proteins were normal. It appears that so long as the variations in the serum-protein fall within the normal range, the relationship between protein and protein-bound calcium is obscured by other factors affecting the equilibrium, but when the protein is markedly reduced, the protein concentration becomes the predominant factor.

The cases of uraemia (plotted as solid circles in Fig. 4) are collected from the data of McCance and Watchorn [1932, 2], Pincus *et al.* [1926], Hertz [1929] and the present paper. The proportions of diffusible and non-diffusible calcium vary widely, and although in general low serum-protein is accompanied by a high proportion of diffusible calcium, and marked phosphate retention causes a fall in both total and diffusible calcium, it is not possible to explain the figures entirely in terms of variations in protein and phosphate. The effects of acidosis and of chloride retention probably play a part also. The phosphate retention alone should not cause any disturbance of the normal distribution ratio [Marrack and Thacker, 1926].

It is of interest that a normal diffusible calcium concentration may exist in spite of phosphate retention—examples of this occur in the data of Pincus *et al.* and in the present paper. In the more severe degrees of hypocalcaemia the absolute value of the diffusible calcium falls, and in such cases tetany may occur. No definite "tetany level" of diffusible calcium seems to exist in uraemic cases.

In the 15 cases of tetany (plotted in Fig. 4 as open circles) the diffusible calcium ranges from 2.0 to 4.2. There are four observations where the points fall above *OA*; three of these are the data from the case A. Hi. of the present paper, where the serum-protein was below normal. The majority of the other observations show a disproportionate reduction in the diffusible calcium as compared with the total calcium. This would be expected in hypocalcaemia with normal serum-protein (see Fig. 3). Some of the cases of tetany described by Greenberg and Gunther [1931] show a definite reduction in the diffusible calcium associated with a normal total calcium figure. The variation in the proportion of diffusible to total calcium may possibly be connected with variations in chloride metabolism. According to Morris *et al.* [1931] there are abnormalities of chloride metabolism in tetany. Although the proportion of diffusible calcium to total calcium is so variable in tetany, these observations on human cases show very definitely that the absolute value of the diffusible calcium is always subnormal; all cases of tetany show diffusible calcium values of 4.2 mg. per 100 cc. or lower. It is striking that a horizontal line drawn across Fig. 4 at the level of 4.2 mg. diffusible calcium per 100 cc. precisely separates the tetany cases from the cases

in the "low protein" group. (The data on human tetany are derived from: Greenberg and Gunther [1931]; Liu [1927; 1928]; Pincus *et al.* [1926]; Snell [1930], Hertz [1929] and the present paper.)

The data for diffusible calcium in experimental parathyroid tetany are much less regular than those just discussed. Such observations have been made by Von Meysenbug and McCann [1921], Cruikshank [1923], Pincus *et al.* [1926], Reed [1928], Hertz [1930], Benjamin and Hess [1933], Snell [1930] and Moritz [1925].

The results of Von Meysenbug and McCann, and Cruikshank were obtained using compensation dialysis as a method of determining the diffusible calcium and Lyman's nephelometric method for the calcium analyses. (Lyman's method has been criticised as giving irregular results.) The majority of the data of the remaining authors, who used ultrafiltration, show that the diffusible calcium falls in parathyroid tetany though the proportion of the diffusible to the total calcium is variable.

The data obtained for dogs at different levels of parathyroid function (normals, parathyroidectomised dogs and dogs treated with parathyroid extract) are collected in Fig. 5, which is compiled from the data of Pincus *et al.*, Reed, Hertz, Benjamin and Hess, and Snell. The results of Moritz for rabbits are similar.

Fig. 5. Relation between total and diffusible calcium in dogs at different levels of parathyroid function. (Parathyroidectomy; normal; parathyroid-treated).
Data collected from the literature.

There are observations in five cases of human hyperparathyroidism—one recorded by Benjamin and Hess [1933], two by Snell [1930] and two in the present paper. These show that the total and diffusible calcium both rise, maintaining their normal proportions (Fig. 4). The data in animal experiments are very variable (Fig. 5). Probably in the human subjects, suffering from chronic disease, the metabolic conditions are more constant and regular than in animals subjected to experimental procedures. The observations in hypo- and hyper-parathyroidism do not suggest that the action of the parathyroid hormone in any way affects the distribution of calcium between the diffusible and non-diffusible fractions. It seems probable that whatever may be the concentration of the diffusible calcium, and whatever the factors that determine it, the concentration of the protein-bound calcium will adjust itself to come into equilibrium with the diffusible calcium, the particular distribution ratio being determined by the nature and quantity of the protein, the hydrogen ion concentration, and the chloride concentration. No description of the variations in the distribution ratio can be complete without a knowledge of all these factors.

SUMMARY.

1. In human cases of hypocalcaemia and hypercalcaemia, the calcium of the cerebrospinal fluid remains relatively constant in spite of very wide variations in serum-calcium.

2. The cerebrospinal fluid-calcium cannot be taken as a measure of the diffusible calcium of serum. In hyperparathyroidism the diffusible calcium of the serum is greater than the cerebrospinal fluid-calcium; in tetany and in some cases of uraemia the diffusible calcium falls below the cerebrospinal fluid-calcium.

3. In hypocalcaemia associated with low serum-protein and normal inorganic phosphate, the diffusible calcium is normal.

4. In the hypocalcaemia of uraemia, the diffusible calcium of serum may be normal if the reduction in total calcium is only moderate. With the more severe degrees of hypocalcaemia, the diffusible calcium falls below normal, and in such cases tetany may occur.

5. In tetany, the diffusible calcium falls below normal; the relation of diffusible to total calcium is variable.

6. In hyperparathyroidism, the diffusible calcium rises, and the normal ratio of diffusible to total calcium is maintained.

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REFERENCES.

- Archer and Robb (1925). *Quart. J. Med.* **18**, 274.
 Benjamin and Hess (1933). *J. Biol. Chem.* **100**, 27.
 Berenczy (1929). *Klin. Woch.* **8**, 2428.
 Briggs (1922). *J. Biol. Chem.* **53**, 13.
 Cameron and Moorhouse (1925). *J. Biol. Chem.* **63**, 687.
 Cantarow (1929, 1). *Arch. Int. Med.* **44**, 670.
 — (1929, 2). *Arch. Int. Med.* **44**, 834.
 Critchley and O'Flynn (1924). *Brain*, **47**, 337.
 Cruikshank (1923). *Biochem. J.* **17**, 13.
 Greenberg (1929, 1). *J. Biol. Chem.* **82**, 543.
 — (1929, 2). *Proc. Soc. Exp. Biol. Med.* **27**, 514.
 — and Gunther (1929). *J. Biol. Chem.* **85**, 491.
 — (1930, 1). *Arch. Int. Med.* **45**, 983.
 — (1930, 2). *Arch. Int. Med.* **46**, 67.
 — (1930, 3). *Arch. Int. Med.* **46**, 72.
 — (1931). *Arch. Int. Med.* **47**, 660.
 — (1932). *Arch. Int. Med.* **50**, 855.
 Halverson and Bergeim (1917). *J. Biol. Chem.* **29**, 337.
 Hamilton (1925). *J. Biol. Chem.* **65**, 101.
 Harrison (1930). Chemical methods in clinical medicine. (Churchill, London.)
 Hertz (1929). *Z. Kinderheilk.* **47**, 215.
 — (1930). *Biochem. Z.* **217**, 337.
 Huggins and Hastings (1933). *Proc. Soc. Exp. Biol. Med.* **30**, 459.
 Kirk and King (1926). *J. Lab. Clin. Med.* **11**, 928.
 Kral, Stary and Winternitz (1929). *Z. ges. Neurol. Psych.* **122**, 308.
 Lennox and Allen (1930). *Arch. Neurol. Psych.* **24**, 1199.
 Leicher (1922). *Deutsch. Arch. klin. Med.* **141**, 196.

- Liu (1927). *Chinese J. Physiol.* **1**, 331.
— (1928). *J. Clin. Invest.* **5**, 259.
Loeb and Nichols (1923). *J. Gen. Physiol.* **6**, 453.
— (1925). *J. Gen. Physiol.* **8**, 451.
— (1927, 1). *J. Biol. Chem.* **72**, 687.
— (1927, 2). *J. Biol. Chem.* **74**, 645.
Marrack and Thacker (1926). *Biochem. J.* **20**, 580.
McCance and Watchorn (1931). *Quart. J. Med.* **24**, 371.
— (1932, 1). *Brain*, **55**, 91.
— (1932, 2). *Biochem. J.* **26**, 54.
Merritt and Bauer (1931, 1). *J. Biol. Chem.* **90**, 215.
— (1931, 2). *J. Biol. Chem.* **90**, 233.
Morgulis and Perley (1930). *J. Biol. Chem.* **88**, 169.
Moritz (1925). *J. Biol. Chem.* **66**, 343.
Morris, Watson and Morris (1931). *Biochem. J.* **25**, 786.
Nicholas (1932). *J. Biol. Chem.* **97**, 457.
Nourse, Smith and Hartman (1925). *Amer. J. Dis. Child.* **30**, 210.
Parhon and Ornstein (1930). *Bull. Mem. Soc. Med. Hôp. Paris*, **54**, 742.
Pincus and Kramer (1923). *J. Biol. Chem.* **57**, 463.
— Petersen and Kramer (1926). *J. Biol. Chem.* **68**, 601.
Reed (1928). *J. Biol. Chem.* **77**, 547.
Smith and Sternberger (1932). *J. Biol. Chem.* **96**, 245.
Snell (1930). *Proc. Staff Meetings Mayo Clinic*, **5**, 17.
Stewart and Percival (1928, 1). *Biochem. J.* **22**, 548.
— (1928, 2). *Biochem. J.* **22**, 559.
Tuchman and Sobotka (1932). *J. Biol. Chem.* **98**, 35.
Von Meysenbug and McCann (1921). *J. Biol. Chem.* **47**, 541.
Weill (1932, 1). *Compt. Rend. Soc. Biol.* **109**, 925.
— (1932, 2). *Compt. Rend. Soc. Biol.* **109**, 927.
Weston and Howard (1922). *Arch. Neurol. Psych.* **8**, 179.