II. STUDIES IN EXPERIMENTAL TETANY.

I. ON THE DISTRIBUTION OF CALCIUM IN THE PLASMA AND CELLS.

II. ON THE VARIATIONS IN COLLOIDAL AND IONIC CALCIUM.

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I.

THEORIES PUT FORWARD WITH REGARD TO THE CAUSATION OF TETANY.

ALTHOUGH much research work has been done and a great deal written on this subject, it may not be out of place here briefly to recapitulate the main theories which have been put forward in an attempt to explain the chemical changes occurring in the blood and their relationship to the symptoms following a loss of function of the parathyroid glands.

The first hypothesis formulated was that the condition was due to a disturbance of the metabolism of calcium salts whereby the tissues and body fluids were deprived of soluble calcium, hence the irritability of the nervous system. This was based upon work of MacCallum [1902-1904] and Loeb [1902, 1, 2; see also 1915] in 1902 and upon researches by Sabbatani who showed that salts of bivalent metals have a depressing effect on the central nervous system, and that such salts counteract the effect of calcium-precipitating substances on the cerebral cortex.

Further confirmation of this theory was forthcoming in the work by Quest [1906] upon the calcium content of cerebral tissue in tetany and by the demonstration of the curative effect of calcium salts upon tetany by MacCallum and Voegtlin [1909]. These workers had previously clearly shown that tetany was associated with a deficiency of calcium salts. That the injection of calcium salts is efficacious in the treatment of tetany in children has been more recently confirmed by Howland and Marriott [1916].

In 1915 Wilson, Stearns and Thurlow [1915] brought forward evidence to the effect that after parathyroidectomy a condition of alkalosis may develop, which condition is neutralised by acid products formed by the muscular activity associated with the muscle tremors incident to tetany.

That the condition preceding an acute attack of tetany is one of alkalosis, they deduced from a study of the variations in the values of the dissociation constant of oxyhaemoglobin, the alveolar carbon dioxide pressure, and the

hydrogen-ion concentration of the blood of dogs subjected to parathyroidectomy. They further showed that the condition of acidosis following several attacks of tetany and the injection of a mineral acid are associated with relief from tetany. In this condition of acidosis the dissociation constant of oxyhaemoglobin is lowered as is also the alveolar carbon dioxide pressure.

Wilson, Stearns and Janney [1915] also indicate that there is a sudden diminution in the excretion of acids and ammonia and a decrease in the ammonia ratio and the hydrogen-ion concentration of the urine immediately following parathyroidectomy. With the onset of tetany there is an increased elimination of acids and ammonia with an increase in the hydrogen-ion concentration value of the urine.

The third hypothesis which has been brought forward is that the condition of tetany is due to the circulation of some toxin or toxins produced by the breakdown of body protein and to be found in the blood and urine of the patient. This theory was the outcome of work by Noël Paton, Findlay and Burns [1915, 1916] and was first published in 1915.

The idea that some product of muscle protein decomposition was the responsible factor was not then entirely new, it having been suggested by Pekelharing and Van Hoogenhuyze [1910] some five years previous to the publication of the researches of Paton and his co-workers.

It is well known that the injection of guanidine sulphate or methylguanidine [Paton, Findlay and Burns, 1915] gives rise to symptoms of hyper-irritability of nerves and it is to Koch [1912] that the credit is due for first discovering the presence of methylguanidine in the urine of dogs suffering from parathyroid tetany.

THE DISTRIBUTION OF CALCIUM IN THE PLASMA AND CELLS.

Numerous estimations upon the total calcium of blood, of plasma and of serum have been carried out, and while a certain divergence of results exists, the general average of results arrived at by various workers is practically the same. In their earlier work MacCallum and Voegtlin stated that the per cent. of calcium in whole normal blood was about 13.3 mg. while in tetany blood it was about 5.4 mg. Several years later these workers by means of a different analytical method arrived at the conclusion that their former results were too high and that more correct figures for the percentage of calcium in normal and tetany blood, would be 6.1 mg. and 2.7 mg. respectively.

Variations in results are undoubtedly due to difference in the methods used for the estimation of blood calcium. Of the two methods more generally used in recent years, namely those of Lyman [1917] and Halverson and Bergheim [1917], it seems that the experimental error is much more under control with the former than with the latter. A nephelometric method which depends upon the accurate matching of shades or depth of colour, always introduces a personal error, and such a method cannot safely be adopted until by practice he who uses it is assured by stringent tests that his readings

are within 0.5 to 1 mm. of the correct figure. The latter method is one of titration with potassium permanganate, a titration in which the end point is rather difficult of determination and one in which one drop of the permanganate solution too many or too few may result in an error of $\pm 5\%$.

In the present investigation Lyman's method has been used and has proved very satisfactory for estimating small amounts of calcium. The nephelometer used was made by taking a Duboscq colorimeter, and replacing the cups and plungers with hard rubber cylinders into which short prepared test-tubes fitted accurately. The test-tubes were supported by means of their flanged edges resting in two lateral slits cut into a hard rubber plate which was screwed on to the colorimeter beneath the prism. The cylinders, supported by movable rings resting on the cup supports, were so adjusted that when the tops were in contact with the hard rubber plate, thereby excluding all light, the indicators on the millimeter scale stood at the zero mark.

The nephelometer-colorimeter attachment or illumination box was made of wood painted black on the outside and white within, the partition placed between the lamps: the colorimeter contained a plate of frosted glass, and the illumination consisted of one set of two 100 c.p. 110 volt lamps and one set of two 100 c.p. 220 volt lamps, so arranged that any number could be used for illumination.

In determining the distribution of calcium in plasma and cells, it was necessary to obtain accurate values for the proportion of cells to plasma in whole blood. For this purpose it was found that the ordinary haematocrit was not satisfactory and the following method was adopted. A piece of pressure glass tubing 10 cm. long having an internal diameter of 1 mm. and a wall thickness of 3 mm. was taken and the ends made smooth with a file.

To a 50 cc. metal centrifuge holder a small right-angled piece of iron was attached so that it formed an inverted L, the arm of the letter being placed over the mouth of the cup and having a tapped hole at the distal end, placed accurately over the centre of the cup. The glass tube filled with blood was placed in a stout rubber band, a small brass cup was placed over the upper end of the tube the lower end of which was then pressed firmly into the rubber cushion in the bottom of the centrifuge cup, by means of a nut which passing through the tapped hole fitted into a narrow and comparatively deep hole in the brass cup.

This device (Fig. 1) was used in duplicate and after centrifuging the specimen of blood for 15 minutes very accurate measurements could be made of the proportion of cells to plasma in a length of 10 cm. of blood.

Fig. 1. A 50 cc. metal centrifuge tube adapted for obtaining haematocrit readings with a 10 cm. length of blood.

Table I. *The distribution of calcium in the cells and plasma of normal dogs.*

Dog	p _H	Total Ca mg. %	Haematocrit reading mm.	Plasma Ca per 100 cc. blood	Cell Ca per 100 cc. blood	Cellular Ca % of total
I	7.5	9.60	3.7	8.36	1.24	12.19
II	7.5	8.1	3.6	7.20	0.90	11.1
III	7.5	8.00	8.4	7.26	0.74	9.3
IV	7.5	8.00	2.8	7.28	0.72	9.0
V	7.55	7.75	3.4	6.79	0.96	12.2
VI	7.55	8.10	3.1	7.24	0.86	11.6
VII	7.55	10.00	3.1	8.83	1.17	11.7
VIII	7.50	8.20	3.2	7.8	0.40	4.9
IX	7.4	7.50	3.2	7.20	0.30	4.0
X	7.5	7.85	3.5	6.95	0.90	10.2
XI	7.45	9.12	3.5	8.25	1.87	9.6
XII	7.45	9.60	3.0	8.40	1.20	12.5
XIII	7.45	10.80	2.7	9.56	1.24	11.5
XIV	7.5	11.00	3.4	9.90	1.10	10.0
XV	7.5	9.70	2.8	8.85	0.85	8.8
XVI	7.5	10.00	2.5	8.70	1.30	13.0

Table II. *The distribution of calcium in the cells and plasma of tetany dogs.*

Dog	p _H	Total Ca mg. %	Haematocrit reading mm.	Plasma Ca per 100 cc. blood	Cell Ca per 100 cc. blood	Cellular Ca % of total
I	7.6	5.82	3.0	5.32	0.50	8.5
II	7.70	5.37	1.9	4.86	0.51	9.5
III	7.65	5.62	2.8	5.19	0.43	7.6
IV	7.75	5.75	3.2	5.16	0.51	10.26
V	7.6	6.5	8.5	6.04	0.46	7.1
VI	7.7	6.25	3.3	5.83	0.42	6.7
VII	7.6	6.50	3.6	6.1	0.40	6.2
X	7.65	4.93	3.5	4.45	0.48	9.7
XI	7.65	4.75	2.7	4.41	0.34	7.1

From Table I it will be seen that the average result of the total calcium of the blood of 14 dogs is 9.12 mg. %. In computing this average the figures for dogs Nos. VIII and IX are not included as these dogs were suffering severely from mange.

The average figure for the amount of calcium in plasma from 100 cc. of blood is 8.11 mg. while for the corpuscles from the same amount of blood it is 1.01 mg. that is to say in a hundred parts by volume of blood we find a distribution of calcium between the plasma and the cells in the proportion approximately of 8 to 1.

In a series of some twelve rabbits in which similar estimations were made it was found that the figures were slightly higher, being for whole blood 10.0 mg. %, for plasma from 100 cc. of blood, 8.6 mg. and for the cells 1.4 mg.

Estimations were performed upon the parathyroidectomised dogs usually on the third day after operation. If the animals survived for two or three days longer, a second determination was made. On several of these animals a partial parathyroidectomy was performed but no conclusive results were obtained immediately following such an operation and the results quoted are those in totally parathyroidectomised animals. The p_H was estimated on the

day following operation on blood drawn from the left ventricle, the method used being that of Levy, Rowntree and Marriott [1915]. The alkaline reserve was determined by the colorimetric method of Marriott [1916]. The relation of the p_H to alkaline reserve will form the subject of a further communication in reference to the carbon dioxide combining power of the plasma of tetany dogs.

Average figures on seven completely parathyroidectomised dogs quoted in Table II show a whole blood calcium content amounting to 5.72 mg. %, with a plasma and cell content per 100 cc. of whole blood of 5.26 mg. and 0.46 mg. respectively. It is thus seen that in experimental tetany the ratio of plasma calcium to cell calcium is approximately 11.5 to 1.0.

Table III. *A comparison of the average figures for calcium distribution in normal and tetany dogs.*

Ca % in mg. whole blood	Plasma Ca mg. per 100 cc. of blood	Plasma Ca % of total Ca	Cell Ca mg. per 100 cc. blood	Cell Ca % of total Ca	Remarks
9.12	8.11	88.9	1.01	11.1	Normal dogs
5.72	5.26	91.9	0.46	8.1	Tetany dogs
37.4	35.2	—	54.4	—	Percentage fall in calcium

The alteration in the distribution may be more clearly seen from Table III, in which the relative percentage of calcium in the plasma and cells to the total blood calcium is shown and in which the comparative alterations are indicated as percentages of the normal contents. It is here shown that in the condition of tetany there is a considerable loss of cell calcium amounting to 54.4 %, as compared with a plasma loss of 35.2 %.

II.

OBSERVATIONS ON DIFFUSIBLE AND COLLOIDAL CALCIUM IN NORMAL AND PARATHYROIDECTOMISED DOGS.

In 1921 a method for the estimation of diffusible calcium was introduced by von Mysenbug and his colleagues [1921]. This method is one of compensation dialysis, in which the diffusible salt in the dialysee is balanced by the addition of an equal amount of the salt to the dialysing fluid.

The separation by filtration of the diffusible salts of blood serum can be effected by subjecting the filter to a pressure of 150 mm. of Hg. This method was employed by Cushny [1920], but to determine accurately the amounts of a salt in ionic and colloidal form, it is essential not to alter the balance between them by removal from the solvent of the diffusible salt. According to the law of mass action the removal of the ionic calcium would result in a further breakdown of calcium in combination in order to restore the normal balance. For this one reason therefore apart from others the compensation method of dialysis is the better and has been made use of in this work.

There are however difficulties associated with the method, the chief of which is the phenomenon of negative osmosis, a phenomenon in which water

may pass from the more concentrated to the more dilute side, that is against osmotic pressure. It may be mentioned here that in dialysing blood serum it is not always sufficient that the dialysate be a solution isotonic with blood serum.

Alterations in permeability of the membrane may be brought about by alterations in the membrane itself, by changes in the solutions within or without the membrane, and by changes in the electrical double layer on the surface of the membrane. If a solute of opposite electrical sign come within the sphere of electrical attraction of the membrane it will be adsorbed. If the adsorbed material be an amphoteric colloid the electrical charge on the membrane will be modified. It is this adsorption by membranes of amphoteric colloids which is responsible for the phenomenon of negative osmosis; water therefore will pass through the membrane at a rate depending upon the density, not the sign, of the electrical charge on the membrane.

The diffusion may be caused either by pure osmosis or by electrical osmosis, the latter being dependent upon the presence of electrolytes and also upon the fact that collodion membranes adsorb amphoteric colloids, thus producing electrical charges on the membrane.

Such salts as oxalates, citrates and phosphates attract water markedly but the effect of the anion may be neutralised by the cation, and this explains the weak action of calcium chloride where the electrical charge on the cation practically neutralises the two charges on the anions.

The difficulties associated with semi-permeable membranes are well known [see Burns, 1921 and Loeb, 1917].

It has been found that very thin sacs made of parlodion are too readily permeable to protein while sacs which are too thick are useless because of negative osmosis whereby the volume of the serum is increased. In such sacs the degree of permeability to electrolytes is also considerably reduced. In a very interesting paper on the degrees of permeability of collodion sacs Eggerth [1921] has shown that the speed of diffusion of electrolytes, "non-dialysable" colloids, like congo red, and proteins of serum and of blood cells depends upon the permeability of the membranes, and the relative permeability of the membrane depends upon the proportions in which alcohol and ether are present in the alcohol ether solvent used for dissolving the pyroxylin, or parlodion to give it the name under which it is manufactured. Sacs made by dissolving collodion in ether and alcohol in the proportion of 60 to 40 parts, were found to be impermeable to protein after three hours' dialysis. They still retained their maximal permeability to crystalloids, all of which were found to pass through in 15 minutes. To obtain sacs which will withstand dialysis of 24 hours at 15–20°, it is necessary to employ a "30 to 40 alcohol" membrane. With such a membrane the diffusion of electrolytes is complete in 10 to 15 minutes and it has been demonstrated that in the presence of protein the diffusion of calcium salts is complete well within 24 hours.

While a considerable drop in the total calcium of the blood and of its cellular elements has been noted in parathyroid tetany, the question as to

whether or not this was due to a loss in free or combined calcium has not been definitely settled. By dialysis we can determine the amounts of free and combined calcium in blood plasma or serum but no method has been perfected whereby a similar determination can be carried out with regard to the cellular elements of the blood.

That alterations may occur in the diffusible calcium in rickets and in tetany was suggested by von Maysenbug and his co-workers, but they in a series of four experiments found no distinct variations from the normal.

METHODS.

Preparation of parlodion sacs. The shreds of parlodion are dried in the air and dissolved in ether and 95 % alcohol in the required proportions. After solution of the parlodion shreds is complete, the bottle, firmly stoppered, is allowed to stand for two weeks.

Two small test-tubes 15 mm. internal diameter and 60 mm. long are filled with the mixture, centrifuged to bring all air bubbles to the surface, the hardened surface then peeled off and the contents emptied into the stock bottle, the tube being rotated meanwhile, in order to secure uniform thickness of the wall of the sac. The tube is clamped in the inverted position for 15 minutes, then placed in cold distilled water for half-an-hour. The upper rim is loosened by means of a knife and a thin glass rod with a bulbous tip gently pushed between the sac wall and the glass to the bottom of the test-tube, which is kept half filled with water. The sac and the glass rod are withdrawn together and the sac immersed in cold distilled water.

Compensation dialysis. In view of the fact that in parathyroid tetany variations in diffusible calcium may be great or small, it was necessary to make up a series of dialysing calcium solutions in order to balance as nearly as possible, the amount of free calcium found in the serum. For this purpose therefore ten dialysing solutions were made up giving a percentage of normal serum calcium varying from 40 to 80. These solutions were made from a stock solution of calcium chloride of about 1 % strength, the amount of calcium in the stock solution having been determined by the nephelometric method. The following is the series of solutions employed:

COMPENSATION DIALYSING SOLUTIONS.

Solution	Amounts of Ca in mg. in 2.5 cc.		Amounts of Ca in mg. %		% of normal Ca
	Estimated	Calculated	Estimated	Calculated	
A	0.206	0.193	8.25	7.71	39.28
B	0.213	0.210	8.54	8.40	40.65
C	0.243	0.241	9.75	9.65	46.42
D	0.257	0.262	10.30	10.50	49.10
E	0.290	0.289	11.62	11.58	55.33
F	0.312	0.315	12.50	12.60	59.52
G	0.343	0.338	13.75	13.50	65.47
H	0.367	0.363	14.68	14.70	69.90
I	0.390	0.355	15.62	14.19	74.38
J	0.406	0.420	16.25	16.70	77.38

To 2.5 cc. of the solution employed, 2.5 cc. of a double strength dialysing fluid were added the composition of which is as follows:

Dialysing fluid (Greenwald).

Salt	% amounts single strength g.	Molar strength	Amounts in 2000 cc. double strength
NaHCO ₃	0.2520	0.003	10.08 g.
KH ₂ PO ₄	0.0088	0.000065	12.9 cc. <i>M/5</i>
MgCl ₂	0.01059	0.00011	0.2118 g.
KCl	0.02960	0.000397	1.184 g.
NaCl	0.6250	0.01069	25.0 g.

Fig. 2. Diagram of the method of setting up a series of six sacs for compensation dialysis of the blood serum.

Since the balance between diffusible and colloidal calcium is in some measure dependent upon the hydrogen ion concentration of the serum, it is essential to bring the CO₂ tension of the serum into equilibrium with an atmosphere of CO₂ of a tension approximating that of alveolar air. To do this a large spirometer was filled with CO₂ and air, these were thoroughly mixed, and the percentage of CO₂ estimated daily by the Henderson and Morriss [1917] method.

The gas was passed through a bottle containing moist glass beads then through two separatory funnels placed in series, the first containing about 20 cc. of serum the second about 10 cc. of the double strength dialysing fluid. This equilibration was carried out at room temperature which during the course of these experiments varied from 30 to 33°.

The dialysis. The sacs to be employed were thoroughly washed in distilled water, shaken free of water and allowed to drain, but not allowed to dry in the air. The test-tubes used for dialysis were $\frac{3}{4}$ " wide by 4" long and into these were placed 2.5 cc. of the double strength dialysing fluid, and CO₂ passed into the test-tubes for a few minutes.

The sacs supported on small hard rubber cylinders, were filled with 5 cc. of serum, CO₂ was passed into the sacs, a small cork firmly inserted into the top of each cylinder, and the whole supported in the test-tube as shown in Fig. 2. Dialysis was carried out for 24 hours at a temperature of 12 to 15°.

DISCUSSION OF RESULTS.

In the following series of Tables Nos. IV to VII, the results obtained in normal, partially and totally parathyroidectomised dogs are given.

Table IV. *Total calcium in normal, partially and completely parathyroidectomised dogs (mg. per 100 cc.).*

Normal	Partial	Complete
11.24	6.25	5.0
10.50	6.13	5.10
11.20	8.20	5.00
11.30	Average 7.04	4.70
12.70		5.20
12.60		4.60
9.00		5.40
Average 11.30		Average 4.95
100 %	→ drop 37.72 %	→ drop 56.20 %

Table IV shows the variations in percentage of the calcium content of serum under the above three conditions. From a normal average of 11.30 mg. %, the calcium fell in partially parathyroidectomised animals to an average of 7.04 mg. %, to be followed by a further fall upon complete parathyroidectomy to an average figure of 4.95 mg. %. It is thus seen that the loss of calcium from the serum amounts to 56.20 % of the whole.

With regard to diffusible calcium, the figures quoted in Table V, which comprises results obtained from a series of seven normal dogs, are comparable to those of Cushny and von Mynsbug, namely that in normal animals the diffusible calcium amounts to between 60 and 70 % of the whole calcium content of the blood taking 10.5 mg. as the percentage of calcium in normal blood serum.

In comparing the results recorded in Tables VI and VII, results from partially and completely parathyroidectomised dogs respectively, it is seen that there is no essential difference in the amounts of calcium recorded.

It is somewhat difficult to say to what extent a single parathyroid gland may or may not be functioning. The upper left parathyroid was left with a small piece of thyroid tissue. In all of these cases the gland retained was distinctly identified, but we must admit that this method of leaving a gland

RESULTS OF COMPENSATION DIALYSIS OF SERUM CALCIUM.

NORMAL DOGS.

Table V.

Dog I. p_H 7.5; Total Ca = 10.5 mg. %; Ca in 5 cc. serum = 0.525 mg.; CO_2 tension = 49.64 mm. Hg.

Ca in mg. in 5 cc. serum		Ca in mg. in 5 cc. dialysate		Diffusible calcium %	Compensation dialysing solution
Begin	End	Begin	End		
.525	.584	.347	.317	55.5	G
.525	.534	.343	.316	55.4	G
.525	.493	.312	.332	67.1	F

 p_H 7.5; Total Ca = 11.24 mg. %; Ca in 5 cc. serum = 0.562 mg.; CO_2 tension = 50.16 mm. Hg.

.562	.503	.312	.337	64.4	F
.562	.516	.343	.413	61.4	G
.562	.462	.462	.383	70.6	H

Dog II. p_H 7.5; Total Ca = 10.5 mg. %; Ca in 5 cc. serum = 0.525 mg.; CO_2 tension = 50.92 mm. Hg.

.525	.530	.344	.344	65.5	G
.525	.515	.367	.354	64.9	H

Dog III. p_H 7.45; Total Ca = 11.7 mg. %; Ca in 5 cc. serum = 0.585 mg.; CO_2 tension = 50.92 mm. Hg.

.585	.505	.312	.342	63.5	C
.585	.558	.343	.362	65.1	G
.585	.550	.367	.382	67.9	H
.585	.557	.367	.377	66.1	H

Dog IV. p_H 7.45; Total Ca = 11.30 mg. %; Ca in 5 cc. serum = 0.565 mg.; CO_2 tension = 44.14 mm. Hg.

.565	.480	.312	.343	66.1	F
.565	.491	.343	.323	59.1	G
.565	.511	.367	.362	63.1	H
.565	.537	.367	.372	66.7	H

Dog V. p_H 7.4; Total Ca = 12.7 mg. %; Ca in 5 cc. serum = 0.635 mg.; CO_2 tension = 42.56 mm. Hg.

.635	.580	.212	.362	64.8	F
.635	.571	.343	.363	60.1	G
.635	.550	.367	.380	62.0	H
.635	.550	.367	.387	64.0	H

Dog VI. p_H 7.4; Total Ca = 12.6 mg. %; Ca in 5 cc. serum = 0.630 mg.; CO_2 tension = 50.0 mm. Hg.

.630	.590	.312	.332	55.9	F
.630	.589	.343	.352	56.8	G
.630	.621	.367	.372	59.3	H
.630	.599	.367	.367	57.8	H

Dog VII. p_H 7.4; Total Ca = 9.0 mg. %; Ca in 5 cc. serum = 0.450 mg.; CO_2 tension = 49.4 mm. Hg.

.450	.470	.312	.312	69.3	F
.450	.481	.367	.342	70.4	H

PARTIALLY PARATHYROIDECTOMISED DOGS.

Table VI.

Dog I. Total Ca = 6.75 mg. %; Ca in 5 cc. serum = 0.337 mg.; CO₂ tension = 50.0 mm. Hg.

Ca in mg. in 5 cc. serum		Ca in mg. in 5 cc. dialysate		Diffusible calcium %	Compensation dialysing solution
Begin	End	Begin	End		
.337	.340	.210	.250	90.0	B
.337	.368	.257	.291	96.4	D
.337	.363	.312	.296	83.1	F
.337	.368	.367	.352	100.0	H

Dog II. Total Ca = 6.13 mg. %; Ca in 5 cc. serum = 0.306 mg.; CO₂ tension = 47.83 mm. Hg.

.306	.335	.257	.302	103.5	D
.306	.302	.257	.272	93.2	D
.306	.352	.312	.293	90.0	F
.306	.291	.406	.372	109.0	J

Dog III. Total Ca = 8.2 mg. %; Ca in 5 cc. serum = 0.410 mg.; CO₂ tension = 48.64 mm. Hg.

.410	.410	.257	.281	84.5	D
.410	.391	.367	.392	101.6	H
.410	.391	.406	.412	101.9	J

COMPLETELY PARATHYROIDECTOMISED DOGS.

Table VII.

Dog I. July 21, operation; July 22, hyperpnoea of Cheyne-Stokes type with salivation, fine tremor of muscles, tetany not severe; 70 cc. of blood drawn from femoral artery.

Total Ca = 5.0 mg. %; Ca in 5 cc. serum = 0.250 mg.; CO₂ tension = 49.4 mm. Hg.

.250	.314	.312	.280	99.2	F
.250	.246	.257	.221	90.0	D
.250	.310	.367	.312	102.8	H
.250	.291	.406	.322	95.2	J

Dog II. July 20, complete operation at noon; July 21, 2 p.m., tremors of neck and shoulder muscles, increased respiration with slight salivation; 8 p.m., marked tremors of muscles of the neck and of the hind limbs. Respirations very rapid and of Cheyne-Stokes type; 9 p.m., 75 cc. of blood drawn aseptically from the femoral artery under ether anaesthesia; 10 p.m., animal much improved, able to stand and able to drink on account of reduced respiratory rate; animal mentally alert.

Total Ca = 6.09 mg. %; Ca in 5 cc. serum = 0.305 mg.; CO₂ tension = 41.8 mm. Hg.

.305	.279	.257	.272	69.0	D
.305	.273	.312	.291	90.0	F
.305	.273	.367	.322	90.3	H

July 22, dog in marked tremor, capable of standing but legs stiff; respiration very irregular and rapid with a rate of over 200 per minute; 70 cc. of blood taken aseptically under ether; respiration rate normal under ether. Definite improvement for 36 hours after withdrawal of blood, the dog becoming quite lively and responsive. Tremors commenced within 36 hours, being felt over the shoulder-girdle; respiration rate again rapidly rising from 60 per minute to 180 per minute.

Total Ca = 5.1 mg. %; Ca in 5 cc. serum = 0.255 mg.; CO₂ tension = 44.84 mm. Hg.

.255	.287	.312	.261	82.0	F
.255	.299	.406	.322	93.4	J

Dog III. Total parathyroidectomy performed July 21; dog died in the night about 20 hours after operation, having developed hyperpnoea and slight tremor during the evening of the 21st; unable to obtain blood from veins because of clotting.

Dog IV. July 20, total parathyroidectomy; July 26, this dog has shown no signs of tetany.

Total Ca = 8.3 mg. %; Ca in 5 cc. serum = 0.415 mg.; CO₂ tension = 51.68 mm. Hg.

.415	.358	.367	.332	72.4	H
.415	.425	.257	.262	64.4	D

August 5, no signs of tetany have developed. Total calcium = 7.5 mg. %.

Dog V. July 22, total parathyroidectomy performed; dog in excellent condition. July 23, slight hyperpnoea, muscular twitching, and salivation developing. July 24, all the muscles of the abdomen, shoulders, head and neck and legs in constant and rapid twitching; dog is very thirsty but unable to drink because of the very rapid respiration rate amounting to 240 per minute; the dog is able to stand, is constantly on the move and constantly attempting to drink. July 24, 10 a.m., because of the severity of the condition 70 cc. of blood were drawn from the femoral artery; at 2 p.m. the condition had greatly improved; all tremors had gone and respirations were almost normal. July 26, noon, tetany attack developing with rapid respirations, salivation, muscle tremors and spasticity of the limbs. July 27, 9 a.m., severe tetany has developed with strong spasmodic contractions of muscles of the head and jaw, the latter causing sharp snapping of the teeth together, causing the tongue to be bitten. Respiration rate 250 per minute; salivation and thirst marked; animal lying soaked in perspiration. 11 a.m., because of the distressing condition animal bled to death.

July 24, Total Ca = 5.0 mg. %; Ca in 5 cc. serum = 0.250 mg.; CO₂ tension = 47.12 mm. Hg.

Ca in mg. in 5 cc. serum		Ca in mg. in 5 cc. dialysate		Diffusible calcium %	Compensation dialysing solution
Begin	End	Begin	End		
.250	.313	.257	.254	100.4	D
.250	.313	.312	.282	100.8	F

July 26, Total Ca = 4.7 mg. %; Ca in 5 cc. serum = 0.235 mg.; CO₂ tension = 46.36 mm. Hg.

.235	.256	.257	.246	100.0	B
.235	.268	.312	.271	97.8	C

July 27, Total Ca = 4.7 mg. %; Ca in 5 cc. serum = 0.235 mg.; CO₂ tension = 42.56 mm. Hg.

.235	.305	.315	.227	96.6	F
.235	.332	.367	.300	99.1	H

Dog VI. July 22, total parathyroidectomy performed. July 30, usual signs of tetany; 70 cc. of blood taken from femoral artery for estimations and animal bled to death.

Total Ca = 4.6 mg. %; Ca in 5 cc. serum = 0.230 mg.; CO₂ tension = 50.16 mm. Hg.

.230	—	.312	.263	93.1	F
.230	.322	.367	.284	87.4	H

Dog VII. July 29, total parathyroidectomy performed; very definite signs of tetany developed within 48 hours.

Total Ca = 5.2 mg. %; Ca in 5 cc. serum = 0.260 mg.; CO₂ tension = 50.16 mm. Hg.

.260	.269	.257	.251	94.3	D
.260	.307	.367	.312	98.8	H

Dog VIII. Aug. 2, noon, total parathyroidectomy performed. Aug. 3, 9 a.m., marked tremor in muscles of the head and neck, shoulders, abdomen; respiration rate 220 per minute; 70 cc. of blood taken from femoral artery for estimations. Aug. 4, condition relieved temporarily by withdrawal of 70 cc. of blood. Aug. 5, 2 p.m., tremors of muscles very marked; perspiration and salivation marked; respiration rate about 200 per minute. 7 p.m., tremors of muscles as at 2 p.m.; haemorrhage from the tongue due to injury from spasmodic contraction of jaw muscles; perspiration profuse; spasticity of limbs marked; animal unable to stand; condition very severe.

Aug. 3, Total Ca = 4.6 mg. %; Ca in 5 cc. serum = 0.230 mg.; CO₂ tension = 50.16 mm. Hg.

.230	.280	.312	.241	73.5	F
.230	.290	.312	.240	73.0	F

Aug. 4, Total Ca = 6.0 mg. %; Ca in 5 cc. serum = 0.304 mg.; CO₂ tension = 51.68 mm. Hg.

.304	.310	.312	.302	97.6	F
.304	.309	.367	.283	98.5	H

Aug. 5, Total Ca = 5.4 mg. %; Ca in 5 cc. serum = 0.270 mg.; CO₂ tension = 49.56 mm. Hg.

.270	.179	—	.120	88.8	No compensa-
.270	.138	—	.126	93.3	tion fluid used

NOTE. The percentage of calcium which is diffusible calcium is calculated as follows:

$$\text{Diffusible calcium} = \frac{\text{Ca in dialysate after dialysis} \times 2 - \text{Ca added}}{\text{Original calcium in serum}}$$

in situ while all other similar tissue is removed is not par excellence the method that should be followed in the investigation of the internal secretion of any gland. Just as in partial depancreatization so in partial parathyroidectomy, the gland or piece of gland should be transplanted and the activity of the graft definitely proved before it is totally removed. In the former method one can not with assurance state whether or not there has been any alteration in the normal blood and lymph supply due to operative interference. In all of these operative animals subcutaneous suture was employed, no dressings were applied and without exception excellent healing of the neck wounds was obtained.

It seemed that as far as the purpose of the investigation was concerned no practical end would be served by the method of partial parathyroidectomy: it was therefore resolved to perform the complete operation on all other animals in one stage.

From the average percentage of diffusible calcium recorded for these tetany animals namely 93.70, a percentage which is computed from the total amount of calcium in each sample of serum, it is evident that the loss of colloidal calcium is indeed a marked one.

It is true that the compensation in dialysing was not complete, but in view of the high percentages recorded and also in view of the results obtained on the final serum from dog VIII, in which no compensation was attempted, one is forced to the conclusion that in severe tetany the major part, if not all, of the colloidal calcium is freed from its protein combination. It would also seem from certain of the results that it would be possible to determine the rate of the breakdown of this calcium proteinate linkage. It appears however that the rapid loss of diffusible calcium from the blood demands a correspondingly rapid liberation of calcium from its colloidal state.

The great demand of the organism for calcium one can understand upon witnessing the severity of the condition. It seems feasible therefore to postulate that there is an attempt on the part of the organism to supply the bivalent salts necessary for the maintenance of a normal condition of irritability of the nervous system and the method adopted is that of a rapid liberation in ionic form of all the colloidal calcium in the blood. But unfortunately in virtue of its free state in the circulation the calcium is very readily lost by way of the kidneys, which organs being devoid of judgment and exercising no discrimination are not to be expected to respond to the abnormal condition by setting up any mechanism of curative adaption. Under these conditions it is evident that once the store of colloidal calcium in the blood is utilised, there must be a call upon the calcium of the tissues. That there is a muscle protein breakdown in tetany has been amply proved by several workers, more notably Paton, Findlay and Burns. It is possible therefore that there is a continual breakdown of muscle protein, which is responsible for the production of a toxic substance, and it is this steady decomposition of protein which is the mechanism whereby an attempt is made to meet the call of the nervous and other tissues for calcium.

In view of the urgent needs of the organism and probably as a result of the protein decomposition, the calcium is thrown out in that form in which it is most easily utilised.

In considering the reason for the presence of diffusible calcium in the blood in tetany the somewhat contradictory facts that both hydrochloric acid and calcium salts are effectual in the treatment of tetany open up a suggestion that the increase in the hydrogen ion concentration of the blood during acute tetany may be a means whereby more calcium can be liberated from protein combination. The lessened alkalinity of the blood would favour the dissolving of the calcium, and would thereby make immediately available for the body calcium as soon as it was broken out from its protein combination.

It must not be forgotten that the alteration in calcium metabolism is a sign of tetany, and that the causative factor is probably a body associated with the creatine of muscle protein.

Watanabe [1918, 1] has shown that the administration of guanidine induces severe acidosis, with a retention of phosphates and a decrease of calcium in the blood, and he expresses the possibility that the fundamental cause of tetany is the increased formation of guanidine nitrogen brought about by the disturbance of the function of the parathyroid glands. But he also points out elsewhere [1918, 2] that the tetany due to guanidine injection is not abolished by calcium administration. This last observation is contradictory to that of Fühner [1915] in which he states that the administration of calcium removes the effects of guanidine, an observation which tends still further to correlate these two conditions namely parathyroid and guanidine tetany. A factor in favour of the toxic theory which has been fully proved during this investigation is that the withdrawal of blood amounting to about 100 cc. and the injection of about 200-300 cc. of calcium-free saline, immediately effects an amelioration of the symptoms of tetany.

THE SIGNS OF PARATHYROID TETANY.

In all of these dogs with the single exception of No. IV definite symptoms of tetany developed. It is evident from the figures for dog IV that there was no derangement of the free and combined calcium balance, but it will be noted that there is a slight though definite loss in total calcium.

Dogs II, V, VI, VII and VIII developed signs of tetany within 36 hours and it will be seen from the notes in the tables that the attacks were with only two exceptions of a severe type. None of these animals suffering from severe tetany survived for more than five or six days. The signs noted as definitely indicating parathyroid tetany were the increased respiratory rate of from 200 to 250 per minute, with noisy expiration, often of a Cheyne-Stokes type, the profuse salivation, and nasal secretion, all of which developed within 48 hours. The characteristic muscle tremors were invariably in evidence within 24 hours as fine muscle twitchings which could be felt most clearly over the shoulder girdle. These tremors became more and more marked until

in some cases by the end of the second day after operation they had become coarse spasmodic twitchings. These were markedly in evidence in dogs V, VII and VIII, in which cases the spasmodic contractions of the temporal and masseter muscles were undoubtedly distressing, the latter causing injury to the tongue by the sudden snapping together of the teeth.

The spasticity of the limbs, on account of which the animal is forced to lie on its side, the inability to stand, the cardiac irregularities, vomiting, and at times profuse perspiration and loss of appetite were all in evidence two and at the latest three days after operation.

The mild or more chronic types of tetany described by others in which the animals lived for two or three weeks after operation have not been seen throughout this investigation; all were definite, and most were severe cases of tetany terminating fatally within one week. The severity of these attacks was always greatly ameliorated by the withdrawal of the 70 cc. of blood for the necessary examinations.

This severe condition occasioned by complete removal of the thyro-parathyroid apparatus may well account for the results recorded.

THE HYDROGEN ION CONCENTRATION AND CO_2 -COMBINING POWER OF THE BLOOD IN TETANY.

The p_{H} and the CO_2 -combining power of the blood were determined in these dogs. The normal p_{H} was found to vary between 7.45 to 7.50 while within 24 hours after parathyroidectomy it was usually somewhat higher varying from 7.55 to 7.75 but with the rapid development of the tetany it invariably fell to about or below the normal and after the third day it varied from 7.2 to 7.35.

In accordance with the findings of others the signs of initial alkalosis were undoubtedly present. Following this slight increase in the p_{H} , there was an indication that with the falling p_{H} the alkaline reserve was encroached upon by non-volatile acids, and as a condition of acidosis is produced the alkaline reserve becomes more and more depleted, until subsequently the compensatory loss of carbon dioxide by way of the alveolar air, whereby the p_{H} is maintained normal, is of no avail and the p_{H} falls, and continues to fall until the death of the animal.

That such a process may take place is suggested by the figures for the CO_2 -combining power of the blood which have been obtained. As far as the CO_2 -combining power of the plasma is concerned the high figures of McCann [1918] have not been confirmed.

The graph (Fig. 3) shows the average findings for CO_2 -combining power and the p_{H} of the blood arrived at in this laboratory. This aspect of the problem still requires further investigation, and it is hoped that the results of further research on this part at present being undertaken here will shortly be published.

SUMMARY.

(1) The calcium content of 100 cc. of normal blood amounts to the following average figures (in mg.), total 9.12, plasma 8.11, cells 1.01; in tetany these drop to the average figures of whole blood, 5.7, plasma, 5.26 and cells, 0.46. These figures show a loss of calcium amounting to 37.2 % for whole blood, to 54.4 % for the cells and 35.2 % for the plasma.

(2) Diffusible calcium in normal serum averages from 60 to 70 %, while in severe parathyroid tetany it amounts to 94 % of the total calcium.

(3) While there is an immediate state of alkalosis following parathyroidectomy, this condition is not necessarily marked and with the development

of the signs of severe tetany it passes rapidly into a condition of acidosis as shown by a steadily falling p_H of the blood.

(4) The immediate relief of the condition consequent upon the withdrawal of 70–100 cc. of blood is indicative of a toxic causative factor.

(5) The calcium deficiency and the great loss of colloidal calcium is merely indicative of a rapid protein disintegration.

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