THE SODIUM AND CALCIUM RATIO OF BLOOD AND NERVE TISSUE IN NORMAL DOGS AND IN DOGS SHOWING SIGNS OF TETANY

by

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INTRODUCTION

Although many experiments have been done on calcium and its relation to the cause of tetany, few have been attempted to show the relation of sodium and calcium to the disease. Consequently this experiment was designed to ascertain if there was any relation between the sodium and calcium ratio in nerve tissue and the onset of convulsions. Since no work has been done on the mineral balance of the whole brain of dogs, it became necessary first to establish what could be called a normal balance of sodium, potassium, and calcium. Tetany was produced in supposedly normal animals by the injection of large amounts of sodium bicarbonate.

REVIEW OF IMPORTANT LITERATURE

As early as 1699 Etmuller observed convulsions in infants, but it was not until 1852 that the name "tetany" was coined by Corvisart, the distinguished French clinician. Recent advances are due largely to the studies of Viennese investigators, to Frankl-Hochwart in the field of adult tetany, to Escherich and to Kassowitz in the elucidation of the aspects of infantile tetany, and to Erdheim and his assistants in the realm of experimental pathology. (1).

Stevens (2) defines tetany as a disease characterized by intermittent, tonic muscular spasms, especially in the hands and arms, and increased excitability of the peripheral nerves.

Tetany invariably occurs after complete parathyroidectomy and is often present in cases of parathyroidpriva. The condition often accompanies rickets and hence is extensively found in young children, although often in latent form. Indeed, the disease is so prevalent that Hess (1) states "The incidence of tetany, including its latent form, is comparable to that of infantile rickets and greater than that of any other constitutional disorder at this period of life." The condition also occurs in adults, but to a less extent.

There are various theories of the etiology of tetany. The most prevalent one is that the condition is due to a low calcium content of the blood and tissues. In trying to explain the smaller number of cases in which the blood calcium was normal other theories have been brought forth. Many investigators hold to the idea that only the diffusible or ionized calcium can affect the tissues. But the lack of reliable methods for measuring ionized and diffusible calcium has led to no widespread acceptance of this theory.

That guanidine and its various derivatives play a part in causing tetany has long been a matter of discussion and experimentation, but no conclusive results along this line have yet been established. Alkalinity appears to be a contributory factor in the onset of tetany, although results show that it is not the main one. Acidity of the intestinal tract doubtless influences absorption of calcium. This fact may account for the conflicting results regarding tetany and alkalinity.

A more recent theory of the cause of tetany was advanced by Tisdall (3) in 1922. He administered sodium phosphate to four dogs in doses of from 280 cc. to 480 cc. An analysis of the serum of these dogs showed that the sodium content before and after the injection was approximately the same, while in every case the calcium in the serum was lowered. All of these dogs became hyperexcitable and one showed signs of tetany. In three other dogs he injected phosphoric acid in amounts equivalent to the sodium phosphate injected in the other animals. These dogs showed no signs of hyperexcitability or tetany. Both the sodium and the calcium content of the blood dropped in equivalent amounts. Tisdall concludes from the experiment that "the sodium-calcium ratio is the important factor in the production of tetany."

DeGeus' (4) research on twenty dogs confirmed the hypothesis that tetany invariably develops when the calcium content of the serum falls below 8 mgs. per 100 cc. With a calcium content above this, tetany does not develop unless there is an excess of sodium present. Alkalosis alone does not induce tetany, and the phosphate ion does not seem to have any influence on its development. Greenwald (5) confirms the fact that tetany was not due to increased alkalinity in an experiment in which he produced convulsions in different dogs by the injection of sodium carbonate, sodium bicarbonate, sodium chloride, and sodium sulfate. He found that the amount of sodium in the plasma was the same in all the animals.

Mac Callum and Vogel (7) and Paton, Findlay, and Watson (8) studied the hyperexcitability of nerves in tetany. By anastomosing the blood vessels of an animal in tetany with those of a normal animal, they found that an excitability indentical with that found in tetany appeared very quickly in the nerves of the normal animal. They concluded that tetany must be due to a lack of calcium in the blood and tissues, to a circulatory poison which renders calcium inactive, or a substance like strychnnia directly causing hyperexcitability of the nerves.

In the twenty-one cases of infantile tetany studied by Usher and Scott (9), two were found which had a blood calcium of 9.0 and 9.9 mgs. If we consider that normal blood contains about 9.0 to 11.0 mgs. calcium per 100 cc., we immediately see that these two cases were within the normal limits. The authors remark that the calcium in these two cases was probably in an unavailable form, that is, in an unionized or non-diffusible state.

Greenwald (10) did not find any toxin in the blood of dogs subjected to parathyroidectomy. After showing also that the urine of such animals did not contain appreciable quantities of guanidine, methyl guanidine, or di-methyl guanidine, he concluded that the hypothesis that guanidine intoxication is the cause of tetany cannot be accepted. He found that tetany developed when the blood calcium fell to 7 mgs. per 100 cc. There was also an admínished excretion of phosphorus. The decreased calcium content of the serum was not due to increased excretions. He remarks that "it is quite probable that the proteolytic intestinal flora impedes calcium absorption, while an acid condition of the intestinal contents promotes it. The excess of calcium not used in combining with phosphorus as calcium phosphate might have been expected to maintain the calcium content of

plasma and serum, but it must have been deposited as some insoluble compound. Greenwald concludes that it is probable that the loss of calcium from certain of the tissues, particularly the neuromuscular junction, rather than any change in the blood itself is the cause of tetany.

We should naturally expect to find some valuable information as to the etiology of tetany by studying the various methods by which the condition may be relieved. The success of treatments of tetany seem to be due to increasing the amount of calcium in solution in the blood and other tissues (12).

Luckhardt and Goldberg (13), and Dragstedt and Sudan (14) found that the oral administration of calcium lactate (1.5 to 3 grams per kilogram of body weight) afforded a permanent relief from tetany and permitted the indefinite survival of thyro-parathyroidectomized dogs. A combination of a milk diet and the intravenous injection of small doses of calcium chloride (1 gram in 10% solution) has also been found effective (15). Calcium lactate is not more effective when given orally than is calcium carbonate, calcium nitrate, or calcium acetate, but monobasic calcium phosphate is entirely ineffective. (16). The oral

administration of ammonium chloride (17), magnesium chloride (18), and magnesium lactate (19) has preserved the lives of parathyroidectomized dogs, but not so efficiently as calcium salts.

Scott and Usher (9) reported positive results in the treatment of tetany in children by the oral administration of calcium chloride.

THE THEORY OF EMULSIONS AS RELATED TO THE NERVE CONDUCTIVITY

I have found in the partial list of references I have reviewed on this subject only two which mention that the sodium-calcium ratio may be the important factor in the production of tetany rather than the absolute amount of calcium in the blood and tissues.

The theory upon which I am working, as formulated by Dr. Hughes and Dr. King (32) is based on emulsions. Stable oil-water emulsions cannot be formed with a dispersed phase of over 2 per cent unless there is an emulsifying agent present. If calcium soaps are used to stabilize the emulsion, water droplets will be formed with a continuous phase of oil. However, if sodium soaps are used a continuous phase of water will be formed with small

droplets of oil suspended. Clowes (33) found that one type of emulsion could be changed into another by the addition of an excess of the proper antagonostic soap. Thus an oil-in-water emulsion stabilized by sodium oleate may be changed into a water-in-oil emulsion by the addition of an excess of calcium oleate. Bancroft explains this effect on the basis that such substances as calcium oleate which form water-in-oil emulsions are wetted more by oil than by water, while substances like sodium oleate, which form the opposite type of emulsion, are wetted more by water than by oil.

The conductivity of these two types of emulsions would differ greatly from one another. Where the continuous phase is water the conductivity is known to be greater. Since nerves contain a large percentage of lipins, that is substances not wetted by water, and also water, it seems plausible that a phenomena analogous to emulsions exists in nervous tissue. Essentially all the facts bear out this theory.

Although it is not known definitely just what a nerve impulse is, yet it is certain that an electric field is set up when a nerve impulse passes through a nerve fiber. Sometime ago Dr. Hall (35) conducted an experiment in which

he measured the conductivity of isolated nerves suspended in calcium oleate and sodium oleate. The calcium and sodium oleates were used because they showed no appreciable conductivity themselves. For the experiment sciatic nerves of frogs and chickens were used.

The sodium effected a persistant lowering in the conductivity of the nerves, while the Ca caused the resistance to increase at somewhat greater rate than the controls.

Other substances affect the nerves by changing the oil-water emulsions. Divalent atoms act similarly to calcium, while monovalent atoms act similarly to sodium. Anesthetics and drugs have a characteristic action upon the nerves.

METHOD FOR TREATING EXPERIMENTAL ANIMALS

The dogs used for this experiment were obtained from the police departments of Manhattan and Junction City, Kansas. These animals were kept on a known ration for only two days before they were killed. They were apparently normal, although no attempt was made to completely diagnose their condition. They were bled to death by a cannula inserted in the caratid artery. When the blood pressure had dropped to a point where respiration was very difficult the carotid was severed completely and the blood allowed to

drain from the brain as much as possible through the jugular vein.

Tetany was produced in similar dogs by the intravenous injection of large quantities of 5 per cent sodium bicarbonate. The solution was injected in the jugular vein in 40 to 80 cc. portions, allowing 1 to 3 minutes for each portion. When the dog showed definite signs of tetany, the carotid was cut and the animal allowed to bleed to death as in the case of the normal animals.

The brain was removed by the aid of bone forceps. Special care was taken to remove the brain without injury, in order to avoid as far as possible the chance of any small portions of bone remaining in the nerve tissue, Any portion of bone left in the tissue would obviously throw the calcium content high above normal. The brain was washed thoroughly with distilled water, weighed, dried in an oven at 110 degrees for 48 hours, and weighed again. The dried nerve tissue was heated in a muffle below redness, and leached with hot water. The leachings were filtered and the remaining ash and filter paper again heated in the muffle at a dull red heat. The residue was digested with one to one hydrochloric acid for several hours and filtered. The combined extractions were made up to 100 cc. with distilled water and 10 cc. portions were used to determine

sodium. One hundred cc. portions of plasma were ashed and extracted by a similar process and made up to 250 cc. Twenty-five cc. portions were used for the sodium determinations.

THE METHOD FOR THE DETERMINATION OF SODIUM

The sample contained in a 150 cc. beaker was made alkaline by adding strong ammonium hydroxide drop by drop. The solution was heated to boiling; 5 cc. of a solution of saturated barium hydroxide added and boiled. The precipitate settled on cooling and more barium hydroxide was added to determine if precipitation was complete. When no further precipitation was produced ammonia was added to give a distinct odor and then the sample was filtered and washed thoroughly with hot water. The filtrate was evaporated to about 20 cc.,and while hot 1 cc. of 1 plus 4 ammonia hydroxide and 15 cc. of 10 per cent ammonium carbonate solution was added to complete the precipitation of the barium, calcium, and other metals.

The solution was allowed to stand a short time on a water bath, filtered and washed thoroughly with hot water. The filtrate and washings were evaporated to dryness and ammonium salts were expelled by heating in a muffle below

redness. The dry residue was treated with a little hot water and a few drops of the dilute ammonium hydroxide, 2 cc. of the ammonium carbonate solution, and a few drops of saturated ammonium oxalate were added. The solution was filtered into a beaker, and 1 or 2 drops of 1 plus 4 hydrochloric acid, and 5 cc. of ammonium sulfate (75 grams per liter) added. The solution was digested several hours on a water bathed and filtered into a weighed silicon dish. The sulfate solution was evaporated to dryness and heated to a dull redness. Ten cc. of 10 per cent ammonium carbonate were added and the sulfates again evaporated to dryness and heated to full redness until a constant weight was obtained. The sodium and potassium sulfates were then taken up in hot water and filtered into small casseroles for the determination of potassium. The filter paper with any insoluble matter was returned into the original silicon dish which contained the sulfates and heated to full redness until constant weight was obtained. This weight was then subtracted from the weight of the combined sulfates.

THE METHOD FOR THE DETERMINATION OF POTASSIUM

The solution of the sodium and potassium sulfates from the preceding determination was evaporated to a thick paste after a few drops of strong hydrochloric acid and an

excess of chloroplatinic acid was added. The residue was treated with 80 per cent alcohol. The precipitate was washed thoroughly by decantation and on the filter until the washings were colorless. Then the contents of the crucible were washed five or six times with 10 cc. portions of ammonium chloride solution.(100 grams in 500 cc.). Finally the precipitate was washed with 80 per cent alcohol to remove any traces of ammonium chloride. The crucible was then dried at 110 degrees for one hour and weighed. The weight of potassium sulfate was calculated from the weight of potassium chloroplatinate. The amount of potassium sulfate was subtracted from the weight of the combined sulfates and the weight of sodium sulfate thus obtained. The final results were calculated from these weights of sulfates in terms of sodium and potassium. The gooch crucibles used for this determination were made with mats of paper pulp of the best obtainable filter paper.

THE METHOD FOR THE DETERMINATION OF CALCIUM IN PLASMA

The method for the determination of calcium in the plasma was a modification of the procedure of Kramer and Tisdall (36). Two cc. of plasma, 2 cc. of water, 2 cc. of

a saturated solution of ammonium oxalate, and 2 drops of concentrated ammonium hydroxide was added a a centrifuge tube and mixed by shaking. The solution was allowed to stand over night and again mixed and centrifuged at 2000 revolutions for 10 to 15 minutes. The tube was quickly inverted in such a matter that the liquid drained out without the precipitate in the bottom of the tube being disturbed. The tube was allowed to drain for at least 5 minutes with its mouth on a pad of filter paper. Three cc. of 2 per cent ammonium hydroxide was added to the tube and the contents thoroughly mixed by shaking vigorously. The precipitate was again centrifuged out and the tube drained as before. Two cc. of approximately normal sulfuric acid were added and the tube heated in boiling water for at least 2 minutes. The oxalic acid thus set free was titrated with 0.01 normal potassium permanganate until a definite pink color was obtained which persisted at least 1 minute. The number of cc. of 0.01 normal permanganate multiplied by 10 gave the number of milligrams of calcium per 100 cc. in the plasma. The 0.01 normal permanganate was standardized against a 2 cc. sample of 0.02 normal sodium oxalate. The standard sodium oxalate solution was made up fresh every time a determination was run. The permanganate was

standardized each time a set of titrations was made. Corrections were made both in the standardization and in the titration of the samples for the amount of permanganate necessary to produce the definite pink color.

THE METHOD FOR THE DETERMINATION OF CALCIUM IN BRAIN EXTRACTIONS

The method used for the determination of the calcium in the brains was an adaptation of the previously mentioned Kramer-Tisdall procedure. Three 10 cc. portions of the extractions of the brain were measured into a 50-cc. volumetric flask and 3 to 4 drops of methyl red indicator added. Concentrated ammonium hydroxide was added until the solution was just alkaline, and 1 plus 4 hydrochloric acid was added until the solution was barely acid. Then 5 cc. of one-half normal hydrochloric acid, and 5 cc. of 2.5 per cent oxalic acid was added and the volume made up to 50 cc. Ten cc. portions of this acid solution were measured into 15 cc. centrifuge tubes and placed in boiling water. Two cc. of ammonium oxalate were added and the tube allowed to remain in the boiling water for several minutes. The tubes were cooled to room temperature and 2 to 3 cc. of 20 per cent sodium acetate were added until the color of the indicator just began to change. The solutions were allowed

to stand over night and the calcium oxalate centrifuged out and washed with dilute ammonium as in the procedure for the determination of calcium in plasma. Titrations were carried out exactly as in the former procedure. The calcium was then calculated for the whole brain.

THE METHOD OF INJECTION OF SODIUM BICARBONATE

The 5 per cent sodium bicarbonate solution was injected into the jugular vein with a 40 cc. syringe. One to 3 minutes was allowed for the injection of each portion.

Dog No. 15. Killed April 17, 1930. Weight 15 pounds.

Time	Amount Injected
3:35-36	40 cc.
3:40-41	40 cc.
3:48-49	40 cc.
4:00-03	80 cc.
4:08-09	40 cc.
4:11-12	40 cc.
4:16-20	80 cc.
4:24-25	40 cc.
5:00-02	80 cc.
5:13-15	80 cc.
5:25	Convulsions
5:27	Bled to death

Dog No. 16. Killed May 1, 1930. Weight 21 pounds.

Time	Amount Injected
3:37-38	40 cc.
3:43-47	80 cc.
3:55-58	80 cc.
4:10-12	80 cc.
4:17-19	80 cc.
4:38-40	80 cc.
4:50-53	80 cc.
4:58	Tetany
5:20	Bled to death

Dog No. 17. Killed May 1, 1930. Weight 23 pounds.

Time	Amount Injected
5:58-59	80 cc.
5:06-08	80 cc.
5:18-19	80 cc.
5:28-30	80 cc.
6:43-45	80 cc.
6:55-56	80 cc.
7:05-06	80 cc.
7:15-16	80 cc.
7:25	Tetany
7:30	Bled to death

Dog No. 18. Killed May 2, 1930. Weight 34 pounds.

Time	Amount Injected
5:17-19	80 cc.
5:23-26	80 cc.
5:29-31	80 cc.
5:40-42	80 cc.
5:45	Vomiting
5:46-47	80 cc.
6:05-10	80 cc.
6:10-15	80 cc.
6:25	Tetany
6:35	Bled to death

Dog No. 19. Killed May 3, 1930. Weight 34 pounds.

Time	Amount Injected
3:28-30	80 cc.
3:34-36	80 cc.
3:39-41	80 cc.
3:46-48	80 cc.
3:55-57	80 cc.
4:04-06	80 cc.
4:10-12	80 cc.
4:18-20	80 cc.
4:30	Vomiting
4:36-39	80 cc.
4:45	Moderate tetany
4:50	Bled to death

CONCLUSION

The results show such a variation that it was impossible to draw any definite conclusions. In eleven out of the sixteen animals the calcium content of the whole brain varied between the limits of 3.07 and 3.76. This would seem that the calcium is least variable of all the mineral constituents determined. The sodium and potassium levels vary so greatly even in the pathological animals that no relation can be shown between the sodium-calcium ratio and tetany in this preliminary experiment. The difference is the dogs themselves would account for some of this variation. The variation in the calcium content may have been due to small particles of bone in the tissue.

BIBLIOGRAPHY

(1) Hess, A. F.
Lea and Febiger, Philadelphia, 1929.

(2) Stevens, A. A.
W. B. Saunders Co., Philadelphia, 1926.

(3) Tisdall, F. F.
Jour. Biol. Chem., 1922, liv. 35.

(4) d'Ceus, J. C. F.
Jour. Am. Med. Assoc., 1926, LXXXVI, 1955

(5) Greenwald, I.
Jour. Biol. Chem., 1924, lix. 1.

(7) MacCallam, W. C., and Voegtlin, C.
Jour. Exp. Med., 1913, XVIII, 618.

(8) Patton, Findlay, and Watson
Quart. Jour. Exp. Physiol., 1916, X, 243.

(9) Usher, S. J., and Scott, J. P.
Jour. Am. Med. Assoc., 1926, LXXXVIII, 1904.

(10) Greenwald, I.
Jour. Biol. Chem., 1924, LXI, 649.

(11) Greenwald, I.
Jour. Biol. Chem., 1922, liv.285.

(12) Greenwald, I.
Jour. Biol. Chem., 1926, LXVII, 1

(13) Luckhardt, A. B. and Goldberg, B.
Jour. Am. Med. Assoc., 1923, LXXX, 79.

(14) Dragstedt, L. R. and Sudan, A. C.
Am. Jour. Physiol., 1926, LXXVII, 296, 307

(15) Salvesen, H. A.
Jour. Biol. Chem., 1923, LVI, 443.

(16) Compere, E. L., and Luckhardt, A. B.
Proc. Soc. Exp. Biol. and Med., 1924, XXI, 526

(17) Boyd, T. E., Austin, W. C., and Ducey, E. F.
Amer. Jour. Physiol., 1927, LXXVII, 225

(18) Wenner, W. F.
Proc. Soc. Exp. Biol. and Med., 1926, XXIII, 432

(20) Dragstedt, L. R.,
Physiol. Rev., 1927, VII, 499.

(21) Stewart, C. P., and Percival, G. H.
Physiol. Rev., 1928, VIII, 283.

(22) Csapo, J.
Jour. Biol. Chem., 1927, LXXV, 509

(23) Koopmann,
ab. J. Am. Med. Assoc., 1926, LXXXVIII, 1780

(24) Denis, W., and Corley, R. C.
Jour. Biol. Chem., 1925, LXVI, 609

(25) Greenwald, I, and Gross, J.
Jour. Biol. Chem., 1926, LXVIII, 325

(26) Irving
Jour. Biol. Chem., 1926, LXVIII, 239

(27) Peterson, H. A., Pineus, J. B., and Kramer, B.
Jour. Biol. Chem., 1926, LXVIII, 601

(28) Moro, E.
Ab. Jour. Am. Med. Assoc., 1926, LXXXVII, 287

(29) Behrendt, H.
Ab. Jour. Am. Med. Assoc., 1926, LXXXVII, 984

(30) MacCallem, W. G., and Veegtlin, C.
Jour. Exp. Med., 1909, XI, 118.

(31) Dragstedt, L. R.
Physiol. Rev., 1927, VII, 499

(32) Hughes, J. S. and Ming, H. H.
Science, LVII, 590

(33) Clowes, G. H. A.,
Jour. Phys. Chem., 1916, XX, 407

(34) Bancroft, W. D.
Jour. Phys. Chem., 1913, XVII, 501

(35) Hall, J. L.
Unpublished data

(36) Kramer, B., and Tisdall, F. F.
1921, XLVII, 475

Table I

Mineral Content of Brain Tissue

	Brain Dry Weight	Brain Wet Weight	Mgs. Mineral per whole Brain			Mgs. Mineral per 100 gms. Wet B.			Mgs. Mineral per 100 gms Dried B.			
			Na	K	Ca	Na	K	Ca	Na	K	Ca	Na/Ca
Brain 9	17.052	83	100	156	3.37	122	189	4.06	589	921	19.8	297
Brain 10	16.060	77	94	228	5.45	122	296	7.08	585	1420	33.9	172
Brain 11	22.080	89	54	126	4.65	60.7	142	5.23	245	571	21.1	---
Brain 12	18.578	83	190	232	4.33	229	280	5.22	1023	1250	23.3	436
Brain 13	15.695	64	178	172	3.27	278	269	5.10	1135	1100	20.8	544
Brain 14	19.975	90	210	240	3.34	233	267	3.72	1052	1200	16.7	628
Pathological Dogs												
Brain 15	10.775	56	146	190	3227	261	349	5.34	1357	1763	30.4	446
Brain 16	16.897	66	165	179	3.42	250	271	5.18	976	1060	20.3	421
Brain 17	16.060	63	143	183	3.07	227	291	4.87	890	1140	19.1	468
Brain 18	15.935	67	154	214	3.76	230	320	5.61	970	1343	23.6	412
Brain 19	19.100	81	153	219	4.26	189	271	5.26	800	1145	22.3	359

Table II

Mineral Content of Blood

Dog. No.	Mgs. mineral per 100 cc. of plasma		
	Sodium	Potassium	Calcium
9	342	23	12.5
10	228	33	13.0
11	340	35	11.2
12	347	32	12.5
13	---	--	10.3
14	319	34	13.5
16	414	35	----
17	399	38	----