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A STUDY OF CALCIUM AND MAGNESIUM IN THE CEREBROSPINAL FLUID†

In the course of studies conducted in this laboratory on the distribution and concentration of metal ions in the central nervous system (CNS),[15] similar studies were made on the cerebrospinal fluid (CSF). Of the fifteen ions found in the CNS tissue, only four (Na, K, Mg, and Ca) were found consistently, and Cu occasionally in CSF. The rôle of magnesium in biological systems has been well studied.[10, 12, 19] The rôle of calcium in the same systems is not so well understood. In general, it would appear that calcium and magnesium are antagonistic to each other in their action on enzyme systems. The mode and site of action of these antagonists is not clear but may involve the shifting of equilibria through activation or inhibition of different mutually dependent components of the same system.[1, 6, 11, 13, 16, 17, 20, 24, 25] There is also evidence that these ions may be competitive. Magnesium, the concentration of which is very important in cells where high metabolic activity or high energy production-requirement exists, has been shown to be required for the activation of most of the enzymes concerned with the transfer and removal of phosphate in biological systems.[14] The CNS has been shown to have such an energy requirement.[18, 22] It has further been shown that the CSF is not a filtrate but is more in the nature of a dialysate of the plasma, and, while the origin of this fluid and the location of the barriers interposed between it and the blood are still a matter of controversy, nevertheless, it is generally accepted that this fluid is more nearly in equilibrium with the cells of the CNS than is the plasma.[21, 26] It seemed worthwhile, therefore, to make a study of magnesium and calcium in the CSF which might elicit some information of the metabolic components of the CNS cells since the interstitial fluid in which they are bathed closely resembles the CSF.

A search of the literature revealed that the Clark-Collip method for the determination of calcium in the CSF was most widely used.[4] There was also

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revealed a number of methods for magnesium. However, in spite of the fact that normal values had been established for both ions, in no case were the levels of both of these ions reported in the same fluid. It was felt that the levels of magnesium and calcium and their ratios in the CSF could be of significance. It was decided to adapt the ethylenediaminetetraacetate (EDTA) methods for calcium and magnesium determinations in body fluids to CSF.[2,8,7] While this work was in progress, Stutzman and Amatusio reported calcium and magnesium levels and their ratio in the CSF.[27]

EXPERIMENTAL

The method finally decided upon for the determination of calcium was a modification of the method of Buckley and Bartolotti.[2] The total magnesium and calcium was determined by using eriochromeschwarz-T indicator and titrating with standard EDTA. Thus, by subtracting calcium values from calcium-magnesium values, calcium and magnesium levels were easily and rapidly determined in a single specimen of the CSF. These methods were checked by the Clark-Collip method for calcium in body fluids and the method of Fales for magnesium.[8] In standard addition and recovery experiments of calcium and magnesium done on pooled samples of the CSF, the percentage deviation was less than 2%. In studies of the reproducibility of calcium and magnesium determinations, the percentage deviation was also less than 2%.

PROCEDURE

CALCIUM: One ml. aliquot of CSF was placed in a 50-ml. white porcelain evaporating dish; 5 ml. of distilled water, 0.15 ml. of 5-N sodium hydroxide, and 1 ml. of ammonium purpurate (murexide) were added. The solution was titrated with standardized EDTA from a salmon-pink to an orchid purple at the end-point.* Milligrams per cent calcium were calculated.

CALCIUM-MAGNESIUM: One ml. aliquot from the same CSF was placed in a clean Erlenmeyer flask; 10 ml. of buffer solution and one drop of erichrome-schwarz-T indicator were added. The mixture was titrated with standardized EDTA from a starting red color to a clear blue end-point. Results were calculated as magnesium. Since the total calcium and magnesium were determined by this titration, by subtracting the calcium determined previously from the total calcium-magnesium, calcium, and magnesium concentrations in the particular CSF were obtained.

* It was found that the delicate color change could not be read accurately in glassware. Contrary to other investigations, no difficulty was found in reading the end-point if a clean, white, porcelain dish was used and a bit of care was taken to notice the color contrasts produced by EDTA.The end-point is reached when no more color contrast exists. Reproducibility was obtained with a little practice.

REAGENTS

EDTA: Approximately four grams of EDTA was dissolved in a small amount of distilled water and diluted to one liter. One ml. of this is equivalent to approximately one mg. of calcium carbonate. The exact equivalence for calcium was determined by titrating with standard calcium carbonate. The exact equivalence for magnesium was determined by titrating with standard magnesium chloride. For determining calcium and magnesium in CSF, this solution is diluted one in forty with distilled water. One ml. of this reagent is then equivalent to one mg.% of calcium and 0.600 mg.% of magnesium.

AMMONIUM PURPURATE (murexide): 0.1 grams of the powdered indicator are dissolved in 50 ml. of distilled water and stored in a dark place. This solution should be made fresh every three days.

ERIOCHROMESCHWARTZ-T: 0.5 grams of the indicator are mixed with 50 ml. of triethanolamine and placed in a glass bottle. If this solution is kept slightly basic, it is quite stable and easily handled.

BUFFER SOLUTION: 67.5 grams of ammonium chloride are mixed with 570 ml. concentrated ammonium hydroxide and diluted to one liter.

SODIUM HYDROXIDE: A 5-N solution of sodium hydroxide is prepared.

RESULTS

Calcium and magnesium levels were determined on the CSF obtained from forty patients who met the following conditions:

a. No sign of neurological condition.

(b) No psychosis or neurosis.

The mean level for calcium was found to be 4.95 mg.% $\pm$ 0.11. The mean level for magnesium was 3.01 mg.% $\pm$ 0.06. The calculated Ca^{++}/ $Mg.^{++}$ ratio was 1.64 $\pm$ 0.03 (Table 1).

In addition, calcium and magnesium were determined on the CSF and serum obtained at the same time from ten patients selected as above. As shown in Table 2 the CSF calcium averaged 4.97 mg.% $\pm$ 0.11 and magnesium 3.14 mg.% $\pm$ 0.25, with a ratio of 1.61 $\pm$ 0.07. Serum calcium averaged 9.97 mg.% $\pm$ 0.15 and magnesium 2.49 mg.% $\pm$ 0.18 with a ratio of 4.1 $\pm$ 0.7. The value of 4.26 mg.% CSF magnesium in patient No. 6 was the reason for the deviation of the CSF magnesium and Ca^{++}/ $Mg.^{++}$ ratio in this series from that of the values obtained in the original series of forty patients.

DISCUSSION

The EDTA methods for the determination of magnesium and calcium in the CSF was found to be rapid, simple, and reliable. It has the added advantage of requiring only one ml. of fluid for the analysis of each ion. Previous investigations of the concentrations of calcium and magnesium

TABLE 1. CALCIUM AND MAGNESIUM IN THE CEREBROSPINAL FLUID OF PATIENTS SELECTED AS NORMALS

Patient No.	Ca mg%	Mg mg%	Ca++/ Mg++	Patient No.	Ca mg%	Mg mg%	Ca++/ Mg++
1	4.79	2.94	1.63	21	4.73	2.90	1.63
2	4.79	2.97	1.62	22	5.16	3.12	1.66
3	4.73	2.97	1.60	23	4.79	2.90	1.65
4	4.81	2.90	1.66	24	5.21	3.21	1.67
5	4.84	2.93	1.65	25	5.11	3.03	1.68
6	4.94	3.06	1.62	26	4.78	2.99	1.62
7	4.79	2.90	1.65	27	4.87	3.03	1.62
8	5.16	3.09	1.67	28	4.94	2.96	1.67
9	4.84	2.96	1.64	29	5.00	3.03	1.65
10	4.94	3.12	1.60	30	4.89	2.99	1.64
11	4.79	2.93	1.64	31	5.11	3.09	1.65
12	4.94	2.99	1.65	32	5.00	3.12	1.60
13	4.94	2.99	1.65	33	5.32	3.22	1.65
14	5.00	3.06	1.63	34	5.05	3.06	1.65
15	5.01	3.00	1.66	35	5.11	3.09	1.65
16	5.06	3.16	1.60	36	4.79	2.93	1.64
17	4.85	3.02	1.61	37	5.06	3.06	1.65
18	4.89	3.03	1.61	38	4.79	2.93	1.64
19	4.79	2.99	1.60	39	5.00	3.06	1.63
20	4.84	2.90	1.67	40	5.01	3.00	1.66
				Mean	4.95	3.01	1.64
				Av. Dev.	0.11	0.06	0.03

in the CSF have shown wide normal ranges. Thus, Merritt and Fremont-Smith[23] have reported a range of 4.5-5.5 mg.% for calcium with an average of 3.05 mg.%. Flexner[9] has reported a range of 4.0 to 7.0 mg.% for calcium and 3.04-3.65 mg.% for magnesium. Cohen[5] has reported 1.0-3.5 mg.% for magnesium. More recently Stutzman and Amatusio[27] have determined calcium and magnesium in the CSF using the Clark-Collip method for calcium and the Simonson hydroxyquinoline method for magnesium. These investigators reported 4.86 ± 0.13 mg.% for calcium and 2.88 ± 0.17 mg.% for magnesium. This compares very well with the results of the present investigation where CSF calcium is found to be 4.95 ± 0.11 mg.% and magnesium 3.01 ± 0.06 mg.% (see Table 1). It seems fairly certain that the normal range of calcium and magnesium in the CSF is maintained with very narrow limits.

Another interesting finding is apparent from this work for there is a remarkable constancy in the Ca++/Mg.++ ratio as shown in our forty cases selected as normals. The ratio of 1.64 ± 0.03 (see Table 1) agrees very well with the value of 1.66 ± 0.06 found by Stutzman and Amatusio.

TABLE 2. SERUM AND CEREBROSPINAL FLUID CALCIUM AND MAGNESIUM FROM A SELECTED GROUP OF RELATIVELY NORMAL PATIENTS

		CSF			*Serum*	
No. Patient	*mg% Ca*	*mg% Mg*	*Mg++ Ca++/*	*mg% Ca*	*mg% Mg*	*Mg++ Ca++/*
1	4.89	2.96	1.65	9.89	2.45	4.1
2	4.89	2.93	1.67	9.80	2.39	4.1
3	5.00	3.03	1.65	10.09	2.23	4.9
4	4.84	2.96	1.63	9.83	2.26	4.3
5	5.05	3.00	1.68	9.95	2.32	4.3
6	5.16	4.26	1.21	9.95	2.87	3.5
7	4.94	2.96	1.66	10.00	2.19	4.6
8	5.26	3.26	1.62	9.80	2.53	3.8
9	5.06	3.06	1.65	10.15	2.51	4.0
10	4.79	2.93	1.64	10.20	2.33	4.3
Mean	4.97	3.14	1.61	9.97	2.49	4.1
Av. Dev.	0.11	0.25	0.07	0.15	0.18	0.7

This value holds even though the absolute amounts of calcium and magnesium may vary. In view of the fact that calcium and magnesium are mutually antagonistic, and that magnesium is associated with high metabolic activity and high energy production systems, it is suggested that the CNS requirement for these ions is different from other body systems and that the ratio of these ions is rigidly controlled by some highly selective mechanism under normal conditions. The ten cases where the CSF and the serum Ca++/Mg++ ratio were determined supports this assumption since the serum ratio was at 4.1 as against 1.65 for the CSF. Investigations are now under way to determine serum and CSF calcium and magnesium and their ratios in patients with varying degrees of mental disturbance. Deviations such as occurred in patient No. 6 (Table 2) may be significant since preliminary work suggests that there is a distinct change in the Ca++/Mg.++ ratio of disturbed patients.

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REFERENCES

1 Braverman, I. and Margulis, S.: The inhibition of the adenosine triphosphate activity of actomysin. J. Gen. Physiol., 1948, *31,* 411.

2 Buckley, E. S., Jr. and Bartolotti, T. R.: Determination of serum calcium. J. Biol. Chem., 1953, *204,* 577.

3 Buckley, E. S., Jr., Gibson, J. B., and Bartolotti, T. R.: Simplified titrimetric techniques for assay of calcium and magnesium in plasma. J. Laborat. Clin. M., 1951, *38,* 751.

4 Clark, E. P. and Collip, J. B.: A study of the Tisdall method for the determination of blood serum calcium with suggested modification. J. Biol. Chem., 1925, *63,* 461.

5 Cohen, H.: The magnesium content of the cerebrospinal fluid and other body fluids. J. Med. Oxf., 1924, *20,* 289.

6 DuBois, K. F., Albaum, H. G., and Potter, V. R.: Adenosine triphosphate in magnesium anesthesia. J. Biol. Chem., 1943, *147,* 699.

7 Elliot, W. E.: Voumetric determination of calcium in blood serum. J. Biol. Chem., 1952, *197,* 641.

8 Fales, F. W.: A micromethod for the determination of serum calcium. J. Biol. Chem., 1953, *204,* 577.

9 Flexner, L. B.: The chemistry and nature of the cerebrospinal fluid. Physiol. Rev., 1954, *14,* 1934.

10 Gordon, J. J.: Observations on brain phosphatase. Biochem. J., Lond., 1953, *55,* 821.

11 Gordon, J. J.: Properties of brain phosphatase. Biochem. J., Lond., 1950, *46,* 96.

12 Gore, M. B. R.: Adenosine triphosphate activity of brain. Biochem. J., Lond., 1951, *50,* 18.

13 Green, D. E.: Carboxylase. J. Biol. Chem., 1941, *138,* 327.

14 Green, D. E.: *Mechanisms of biological oxidations.* Cambridge University Press, 1940.

15 Harris, W. H., Beauchemin, J. A., Herschenson, H. M., Roberts, S. H., and Matsuyama, G.: A study of metal ions in the central nervous system. I. Preliminary considerations. J. Neuropath., 1954, *13,* 427.

16 Hoff, H. E., Smith, P. K., and Winkler, A. W.: Effects of magnesium on the nervous system in relation to its concentration in serum. Am. J. Physiol., 1940, *130,* 292.

17 Katchman, A.: Enzymatic synthesis of triphosphopyridine nucleotide. J. Biol. Chem., 1950, *182,* 805.

18 Kety, S. S., Schmidt, C. F.: The nitrous oxide method for the quantitative determination of cerebral blood flow in man: Theory and procedure, and normal values. J. Clin. Invest., 1948, *27,* 476.

19 Lehninger, A. L.: Rôle of metal ions in enzyme systems. Physiol. Rev., 1950, *30,* 393.

20 Lowry, O. H., Roberts, N. R., Wu, Mei-Ling, Hixon, W. S., and Crawford, E. J.: The quantitative histochemistry of brain. II. Enzyme measurements. J. Biol. Chem., 1954, *207,* 1954.

21 Manery, J. F.:*The biology of mental health and disease.* The twenty-seventh annual conference of the Milbank fund. New York, Paul Hoeber, Inc. 1952. p. 126.

22 McIlwain, H.: Brain metabolism and activity. Brit. Bull., 1950, *6,* 301.

23 Merritt, H. and Fremont-Smith, F.: *The cerebrospinal fluid.* Phila., W. B. Saunders Co., 1937. p. 12.

24 Mommaerts, W. F. H. H.: The reaction between actomysin and adenosine triphosphate. J. Gen. Physiol., 1948, *31,* 361.

25 Pauling, Linus: *The nature of the chemical bond.* Ithaca, N. Y. Cornell University Press, 1948. Chap. II.

26 Spiegal, Adolf M.: Cerebrospinal fluid. Progr. Neur. Psychiat. N. Y., 1952, *7,* 275.

27 Stutzman, F. L. and Amatusio, D. S.: A study of serum and cerebrospinal fluid calcium and magnesium in normal humans. Arch. Biochem. Biophys., 1952, *39,* 1952.