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Magnesium and Calcium in the Cerebrospinal Fluid of the Rat (33064)

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Atomic absorption spectrophotometry is a specific, sensitive, accurate method for measuring numerous cations. This technique was used to obtain previously unavailable data on the content of magnesium and calcium in the cisternal cerebrospinal fluid (CSF) of the rat. The results are given in the present report.

Methods. Three groups of male albino rats (Sprague-Dawley strain) fed a stock laboratory diet and tap water *ad libitum* were studied. The concentrations of Mg and Ca were measured in plasma water (P), ultrafiltrate of plasma (UF), and CSF from animals weighing 120–130 gm and 320–380 gm. The P-Mg and CSF-Mg were also determined in a third group of animals weighing 150–220 gm.

Collections of samples. All glassware was cleaned with a 1:2 dilution of concentrated HNO_3 and rinsed thoroughly with distilled–deionized water prior to use.

CSF. Short-beveled no. 25 hypodermic needles (Becton, Dickinson and Co.) were removed from their hubs and soldered into the empty hubs from no. 22 needles so that the blunt end of the needle projected posteriorly approximately 0.5 cm into the hub. A removable 6-cm length of polyethylene tubing (B-D PX022) was inserted over the blunt end of the needle. Under ether anesthesia, hair was clipped from the posterior cervical and occipital areas of the rat, and the animal was pinned to a stand designed to

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facilitate cisternal puncture (1). Following insertion of the needle into the cisterna magna, CSF was collected directly into a tared 5-ml volumetric flask through the polyethylene tubing. Amounts (20–80 mg) of clear CSF may be obtained from 90 to 100% of the animals within 1 minute when one person controls the needle, a second holds the volumetric flask and a third applies gentle pressure to the rat's abdomen. After completion of the collection, the flask was immediately stoppered and reweighed. Chemical determinations were made either within 6 hours, or the CSF was frozen at −20°C and assayed the next day. Specimens visibly contaminated with blood or weighing less than 10 mg were discarded.

Plasma. After collection of the CSF, the animal was rapidly exsanguinated via aortic puncture. The blood was collected in syringes containing approximately 0.04 ml of ammonium heparin (1000 units/ml). By analysis, no detectable Mg or Ca was found in the heparin. After centrifugation, aliquots of fresh plasma were taken for determination of specific gravity and ultrafiltration, and the remainder was frozen in polyethylene tubes until analyzed for Mg and Ca. The plasma was ultrafiltered at room temperature (24°C) by the method of Toribara *et al.* (2) as modified by Walser (3). No contamination was detected from the dialysis tubing after ultrafiltration of distilled–deionized water. Three ml of plasma centrifuged at 2500 rpm for 3 hours produced 500–800 μl of ultrafiltrate. A 400–μl sample of UF was transferred to a 10-ml volumetric flask and frozen until analyzed the next day.

Analytic methods. The specific gravity of the plasma was determined by the $CuSO_4$ method (4) and the concentrations of Mg and Ca in plasma were corrected to meq/kg plasma water, using the factors reported by Sunderman (5). The water content of several samples of plasma in which the specific gravity had been measured was in excellent agreement with Sunderman's values for serum.

The concentrations of Mg and Ca in the various samples were measured on a Perkin-Elmer model 303 atomic absorption spectrophotometer using the operating conditions recommended by the manufacturer. The standards for magnesium were prepared freshly each day. The standards for calcium contained 330 ppm Na (6) and were stored, frozen, in polyethylene bottles between determinations. When treated this way, the Ca standards were stable for at least 1 month. Initially, a solution of strontium (10,000 ppm) and trichloroacetic acid (20% w/v) was used to prevent anion interference and to precipitate proteins. Later, lanthanum was substituted for strontium at the same concentration to decrease the background noise in the determination of Ca. Neither the precision of the method nor the results of the recovery studies noted below were affected by this change.

Plasma and UF. All plasma samples were run in duplicate. A 0.4-ml sample of plasma was washed into 7.6 ml of distilled–deionized water and 2.0 ml of the La-TCA solution was added. Following centrifugation, the protein-free supernatant was transferred to another test tube and analyzed for Mg and Ca. Standards, appropriate blanks, and UF (in the 10-ml volumetric flasks) were prepared in an identical manner. Any UF containing a precipitate was discarded. The precision of the method (7) based on 20 pairs of samples was ± 0.02 meq/liter for Mg, and ± 0.07 meq/liter for Ca; 99.3 ± 0.6% (mean ± SE) of a known amount of Mg, and 99.8 ± 0.8% of a known quantity of Ca were recovered when added to the plasma.

CSF. Two-tenths ml of a 12 ppm Mg standard and 0.2 ml of a 70 ppm Ca standard were washed into the 5-ml volumetric flask containing the CSF. After the addition of 1.0 ml of La-TCA solution, the sample was diluted to the mark with deionized–distilled water. Three separate control samples containing everything but CSF were also prepared in 5-volumetric flasks. The standards and blanks were made up as described above. The concentrations of Mg and Ca, corrected to 1000 gm of CSF, were calculated from the difference between the unknown samples and the mean value for Mg and Ca in the three control solutions. Recovery

TABLE I. The Concentration of Mg in Plasma Water, Ultrafilterable Plasma, and Cisternal Cerebrospinal Fluid in the Rat.[a]

Wt. range of rats (gm)	Plasma sp gr (mean)	(A) Plasma Mg (meq/kg of H_2O)	(B) Plasma ultrafilterable Mg (meq/liter)	B/A × 100 (%)	CSF Mg (meq/kg)
120–130	1.023	1.59 ± 0.04 (10)	1.18 ± 0.03 (8)	74.5 ± 2.1 (8)	1.56 ± 0.03 (10)
150–220	1.025	1.50 ± 0.05 (6)	—[b]		1.71 ± 0.05 (5)
320–380	1.025	1.58 ± 0.07 (8)	1.14 ± 0.09 (7)	71.5 ± 0.33 (7)	1.66 ± 0.04 (6)

[a] Values expressed as mean ± SE; (no. of animals given in parentheses).
[b] Not measured.

studies for the CSF were almost identical with those in plasma. All statistical methods were taken from Snedecor (8).

Results. The data are summarized in Tables I and II. For all practical purposes, the concentration of the cations in a liter of UF is equal to that in 1000 gm of UF water (the difference being 0.003 meq for Mg and 0.007 meq for Ca).

The age of the animals had no statistically significant effect on any of the results. The CSF-Mg/UF-Mg ratio was greater than unity in all cases; the mean value was 1.3 for young and 1.5 for mature rats. With the exception of one young rat with a low UF-Ca, the CSF-Ca/UF-Ca ratio in the young and older rats was less than one. The mean combined figure for both groups was 0.82.

Discussion. I have been unable to find published data on the normal rat with which to compare the results for UF and CSF Mg and Ca reported in this study. The mean percentage UF-Mg and Ca are somewhat greater than have been found in man (2,3, 9); however, the validity of the method used is indicated by the fact that when applied to human plasma, a mean of 64% of Mg and 49% of Ca were ultrafilterable. These figures correspond well with those of others.

The concentration of Mg in the CSF of the rat is about the same as in the horse (10) and dog (11), although in the latter case the reported values are somewhat conflicting (12). When compared to other animals and humans, the CSF-Mg in the rat is low (10, 13–16). The general rule that the CSF-Mg exceeds the measured or assumed UF-Mg at normal concentrations of magnesium in the plasma is true in the rat. Apparently the rabbit is the only mammal studied so far in which the UF-Mg and CSF-Mg are equal (13). The results for the CSF-Ca and the ratio of CSF-Ca to UF-Ca are in agreement with observations in several other species including man (10,11,13,14,16,17). In all probability, the concentration of Mg and Ca in the cisternal cerebrospinal fluid is the same as that in the remainder of the unobstructed ventriculosubarachnoid system (14,15,17), although a difference in the amount of Ca and Mg between newly formed fluid from the choroid plexus and cisternal CSF has been noted in cats (16).

Thus, in the normal rat and in many other mammals, a concentration gradient exists between the ultrafilterable plasma and the cerebrospinal fluid for these two neurophysiologically important cations. The mechanisms which maintain these gradients have not been adequately characterized, but they appear to operate at the sites of both formation and reabsorption of the CSF (12,16). Acute and chronic hypermagnesemia and hypercalcemia have little effect on the concentration of Mg and Ca in the CSF, indicating the homeostatic function of the blood–CSF barrier (11–13). In the case of magnesium, however, chronic, severe hypomagnesemia is associated with a delayed but marked decline in the concentration of Mg in the CSF in rats (18) and humans (18,19).

Summary. The concentration of Mg and Ca were measured in the plasma, ultrafilterable plasma, and cisternal cerebrospinal fluid of young and mature rats using atomic absorption spectrophotometry. Aging had no

TABLE II. The Concentration of Ca in Plasma Water, Ultrafilterable Plasma, and Cisternal Cerebrospinal Fluid in the Rat.[a]

Wt. range of rats (gm)	Plasma sp gr (mean)	(A) Plasma Ca (meq/kg of H_2O)	(B) Plasma ultrafilterable Ca (meq/liter)	B/A × 100 (%)	CSF Ca (meq/kg)
120–130	1.023	5.64 ± 0.05 (10)	3.45 ± 0.25 (8)	61.8 ± 4.77 (8)	2.89 ± 0.14 (10)
320–380	1.025	5.65 ± 0.05 (8)	3.43 ± 0.06 (7)	60.8 ± 0.39 (7)	2.77 ± 0.04 (6)

[a] Values expressed as mean ± SE; (no. of animals given in parentheses).

effect on any of the results. The CSF-Mg exceeded UF-Mg, and CSF-Ca was less than UF-Ca, indicating the existence of a blood–CSF concentration gradient for these cations in the rat.

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Study by Peptide Mapping of Antihapten Antibody of Varying Affinity from Individual Rabbits* (33065)

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It is well known that antihapten antibody is heterogeneous with regard to its affinity for the homologus haptenic determinant (1–4). It is further known that the average affinity of the antihapten antibody present in the serum of an immunized animal increases progessive-

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