Edema of the Spinal Cord in Experimental Allergic Encephalomyelitis.* (31536)

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Edema of the spinal cord has potentially serious consequences because of the anatomic relationships to its envelopes. Increased volume of cord parenchyma within a relatively inelastic pia and a relatively rigid vertebral canal might cause increased tissue pressure, with compression and collapse of blood vessels and subsequent ischemic necrosis. It has been suggested that this sequence of events is responsible for certain human cases of necrosis of the spinal cord (necrotic myelopathy) and certain sequelae of spinal cord trauma(1). The hyperacute form of experimental allergic encephalomyelitis (EAE) provides a useful model for the study of this problem because it is characterized histologically by striking accumulations of inflammatory edema fluid in the spinal cord(2). The present study concerns chemical confirmation and quantitation of spinal cord edema in hyperacute EAE.

Methods. Male Lewis rats 250-350 g in weight, had free access to Purina Laboratory Chow and tap water. Hyperacute EAE was induced by intradermal injection of 200 mg (wet weight) guinea pig spinal cord homogenized in 0.1 ml pertussis vaccine concentrate (20 billion organisms) into the right foot pads(2). The rats were sacrificed by exsanguination while under ether anesthesia. The brain was dissected after removal of the calvaria. "Forebrain" refers to the tissue isolated by passing a scalpel just in front of the frontal poles and just behind the occipital poles at right angles to the long axis of the brain; it included telencephalon, diencephalon, and part of midbrain; olfactory bulbs, optic nerves and chiasm were excluded. "Hindbrain" refers to cerebellum and remainder of brain stem down to the atlas. The spinal cord was blown out of the vertebral column with a blast of air from a syringe and needle placed in the lower end of the spinal canal. Tissues were weighed fresh and again after drying at 100°C until constant. Water content was the loss of weight, expressed as a percent of fresh weight. All estimations were done on groups of 3 rats, and the averages plus ranges are reported in the Tables.

Swelling refers to the augmentation in weight of edematous central nervous system (CNS) tissue compared to control CNS. It was calculated from the formula

$$\frac{P - P_1}{P_1},$$

where P = percent solids in control CNS and P_1 = percent solids in edematous CNS(3). This calculation required the assumption that the experimental procedure increased the wet weight of the CNS but had no effect on the dry weight. This assumption may be close to the truth for acute water intoxication if only water entered the CNS. In the case of EAE, a decrease in dry weight due to loss of solids from the CNS seems highly improbable, but it is reasonable to expect an increase in dry weight because of the influx of leukocytes into the CNS and the protein-rich character of inflammatory edema fluid. Values of swelling calculated on the basis of this false assumption of constant dry weight are too low (3) and must be considered as minimal estimates only.

Results. Water content was determined on successive days in groups of rats that had received encephalitogenic inoculum and in groups of untreated controls (Table I). Five and 6 days after inoculation, there were no clinical signs of EAE and the spinal cord water was normal. Inasmuch as microscopic lesions often antedate clinical signs, additional groups of rats were sacrificed at the same times for histological control. The sections revealed no lesions at 5 days and rare lesions of minimal severity at 6 days; this agreed with previous observations(2) and may be

* Supported by grant from USPHS, Nat. Inst. Neurol. Dis. and Blindness, NB 05727-01.

TABLE I. Water Content of Spinal Cord in EAE at Various Times.

EAE		Water (range)	Solids	Swelling*
		%		
None (normal controls)†		68.7 (1.2)	31.3	—
" (incubation period)	D + 5‡	68.5 (.1)	31.5	—
Histologic lesions only	D + 6	68.9 (.4)	31.1	—
Mild clinical signs	D + 7	69.7 (1.0)	30.3	2.9
Severe clinical signs	D + 7	73.2 (.2)	26.8	16.8
" " "	D + 8	73.5 (.2)	26.5	18.0

* $\frac{\%\text{ solids of control CNS} - \%\text{ solids of edematous CNS}}{\%\text{ solids of edematous CNS}}$.

† 9 rats. Other groups consist of 3 rats.

‡ Day of water determination, counted from day of encephalitogenic inoculation as D0.

TABLE II. Water Content of Forebrain and Cord.

Treatment	Avg wt of rats*	Forebrain		Spinal cord	
		Water (range)	Swelling	Water (range)	Swelling
	g	%		%	
EAE	256	78.4 (.1)	—	73.5 (.2)	16.9
None	343	78.5 (.3)	—	68.9 (.8)	—
Starved	289	78.7 (1.3)	—	69.0 (.5)	—
Starved + overhydrated	272	79.8 (.1)	5.5	70.8 (.0)	6.0
EAE + overhydrated	275	79.5 (.2)	3.6	74.4 (.5)	21.1

* Each value in Table represents average of 3 rats.

extrapolated to the rats whose CNS was used for water determination. Seven days after inoculation all the rats had clinical evidence of EAE and the spinal cord water was elevated. However, swelling was much greater in rats with severe disease (paralysis) than in rats with mild signs (limpness of tail). Some rats with mild signs were followed to the eighth day after inoculation, at which time their signs became severe and their spinal cord water content very high. The results indicated that swelling was proportional to the severity of the disease rather than its duration.

In another experiment, water content was determined in spinal cords and in forebrains of paralyzed rats, 8 days after inoculation (Table II). As before, there was marked swelling of cord, but forebrain water content was hardly different from that of normal animals. This finding was in agreement with the histologic evidence of striking predilection of hyperacute EAE for the spinal cord. In view of the loss of body weight in paralyzed rats, water contents were determined in normal rats in whom a similar loss of body weight was induced by starvation (with no restriction of water intake). Starvation had no effect on water content of CNS (Table II).

It was of interest to compare the water content in EAE with that in a non-inflammatory type of CNS swelling. For this purpose, normal rats were overhydrated by intravenous injection of distilled water, the method of Weed & McKibben and other workers(4-7). Thirty-eight ml water were instilled during 44 minutes through a catheter in the dorsal penile vein of starved rats under ether anesthesia. The injection was terminated as soon as respiratory distress in any one animal signalled the presence of severe water intoxication. This procedure caused 6% swelling in the spinal cord, much less than that observed in severe EAE. On the other hand, the swelling was not restricted to the cord; 5.5% swelling was found in the forebrain. Overhydration was also performed on rats with EAE; an additive effect on spinal cord water content was found (Table II). In another experiment of similar design, no attempt was made to keep body weight uniform and the dose of water was varied according to the body weight; very similar results were obtained. In that experiment hindbrains were analyzed also; like the forebrains, they exhibited swelling after overhydration but not after EAE.

Although brain swelling following IV overhydration has usually been attributed to hyposmolarity, the procedure also causes hemolysis and hemodilution. However, we and others have produced similar brain swelling after intragastric water administration, where hemolysis was not a factor(8). Contrariwise, we have been unable to produce brain swelling in 360 g rats after intravenous injection of enormous volumes (140 ml) of either saline or 5% glucose solutions, despite hemodilution. Therefore, hemolysis and hemodilution were not the major causes of brain swelling, but a contributory role cannot be excluded.

Discussion. The effect of EAE on water content has been studied before only in the ordinary variety of EAE, in guinea pigs. Fois *et al* found no abnormality in brain or cord water(9), but Wender and Hierowski reported marked swelling in both areas(10). Our finding of swelling in cord but not in brain of rats with hyperacute EAE is of particular interest because it paralleled the distribution of histologic lesions. According to electron microscopic observations, the increased water consists mostly of edema fluid in dilated extracellular spaces and excess cerebrospinal fluid(11). Part of the elevated water content may be due to infiltrating leukocytes.

The excess water in the brain following overhydration has been localized in glial cytoplasm by electron microscopy by some workers(5,6) but not by others(7). The present work demonstrates, apparently for the first time, that overhydration also causes spinal cord swelling, and the swelling is of similar magnitude.

The additive effect on water content of overhydration and hyperacute EAE is interesting in relation to the possibility that the excess water occupies the glial cytoplasmic compartment after overhydration(5,6) and the extracellular compartment in hyperacute EAE(11). Of particular importance is the severity of swelling obtained by this combination. Further experimentation is required to determine whether such a marked swelling can interfere with blood flow and cause ischemic necrosis, as has been hypothesized (1).

Summary. The hyperacute form of experimental allergic encephalomyelitis produced inflammatory edema of the CNS, with 3-18% swelling of the cord but not of the brain; the magnitude of change paralleled the severity of disease. Overhydration (water intoxication) caused 6-7% swelling of the spinal cord, and changes of similar degree occurred in the brain. The combination of overhydration and inflammatory edema had an additive effect on water content of the spinal cord.

1. Hoffman, H. L., Norman, R. M., J. Neurol. Neurosurg. Psychiat., 1964, v27, 116; Joyner, J., Freeman, L. W., Neurology, 1963, v13, 69.

2. Levine, S., Wenk, E. J., Am. J. Path., 1965, v47, 61.

3. Elliott, K. A. C., Jasper, H., Am. J. Physiol., 1949, v157, 122.

4. Weed, L. H., McKibben, P. S., ibid., 1919, v48, 531; Stern, W. E., J. Neurosurg., 1959, v16, 676.

5. Rosomoff, H. L., Zugibe, F. T., Arch. Neurol., 1963, v9, 26.

6. Luse, S. A., Harris, B., J. Neurosurg., 1960, v17, 439; Arch. Neurol., 1961, v4, 139.

7. Van Harreveld, A., Collewijn, H., Malhotra, S. K., Am. J. Physiol., 1966, v210, 251.

8. Rowntree, L. G., J. Pharm. Exp. Ther., 1926, v29, 135; Windle, W. F., Rambach, W. A., Arellano, M. I. R. R., Groat, R. A., Becker, R. F., J. Neurosurg., 1946, v3, 157.

9. Fois, A., Sereni, F., Sereni, L., Malandrini, F., Pieri, J., Neurology, 1961, v11, 681.

10. Wender, M., Hierowski, M., Acta Psychiat. Neurol. Scand., 1960, v35, 526.

11. Levine, S., Hirano, A., Zimmerman, H. M., Am. J. Path., 1965, v47, 209.

Received April 25, 1966. P.S.E.B.M., 1966, v123.