by temperature over a range of 20°C to 30°C. When the pH of the culture medium reaches 6.0 or lower, morphological changes in the flagellates are produced. Vacuolation and rounding up occur in both control and antibiotic-containing cultures and do not seem to be due to the influence of tyrocidine, but rather to a change in the hydrogen-ion concentration of the culture medium, with possibly the actions of metabolites in an ageing culture.

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Water, Sodium and Potassium Content of Normal and Encephalomyelitic Mouse Brain.* (18617)

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Changes in the water and electrolyte content of brain tissue have been described in a variety of conditions. By using the method of extracellular electrolyte depletion described by Yannet and Darrow(1), Swinyard, Toman and Goodman(2) were able to show that a marked reduction occurred in electroshock and chemoshock threshold in the first 4 hours and that the threshold returned to normal in 24 hours. In a later study from the same laboratory(3) this phenomenon was further investigated and it could be demonstrated that a small but not significant increase in total brain water occurred concomitantly with a decrease in electroshock seizure threshold and that an increase in extracellular sodium and chloride concentration of cerebral cortical tissue was more closely correlated with the electroshock seizure threshold than with other factors examined. In micro-incinerated preparations and by employing a microcrystallographic method(4), changes of local distribution of potassium and sodium in the cerebral cortex in relation to experimental convulsions could be detected. It was further shown that these neuron changes involved a loss of potassium and a gain in sodium. These changes were found to be consistent in animals given metrazol or electroshock convulsions, but were absent in asphyxia, exercise, ether anesthesia and did not depend on cortical topography. In experimental cerebral concussion in the cat an increase in the volume of the brain up to 5.5% could be found(5). In dogs, however, there was no indication of swelling and there was no shifting of water from the extracellular to the intracellular phase or vice versa as determined by the water, chloride, sodium, potassium, calcium, magnesium and total nitrogen content of cerebral tissue(6). Furthermore, the ionic environment has a pronounced influence on brain tissue respiration and it could be shown(7) that potassium caused an increase in aerobic glycolysis and inhibited anaerobic glycolysis, whereas sodium lacked these effects(8).

In view of clinical data suggesting that salt depletion and hemoconcentration may be predisposing factors to the invasion of the poliomyelitis virus(9) and the low serum potassium levels found in patients with severe infantile paralysis(10), studies on the water and electrolyte content of brain tissue of mice infected by the intraperitoneal route with a

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1. Yannet, H. and Darrow, D. C., *J. Biol. Chem.*, 1940, v134, 721.

2. Swinyard, E. A., Toman, J. E. P., and Goodman, L. S., *J. Neurophysiol.*, 1946, v9, 47.

3. Swinyard, E. A., *Am. J. Physiol.*, 1949, v156, 163.

4. Colfer, H. F. and Essex, H., *Am. J. Physiol.*, 1947, v150, 27.

5. White, J. C., Brooks, J. R., Goldthwaite, J. C., and Adams, R. D., *Ann. Surg.*, 1943, v118, 619.

6. Eichelberger, L., Kollros, J. J., and Walker, E., *Am. J. Physiol.*, 1949, v156, 129.

7. Ashford, C. A. and Dixon, K. C., *Biochem. J.*, 1935, v29, 157.

8. Dixon, K. C., *J. Physiol.*, 1949, v110, 87.

9. Rinehart, J. F., *J. Ment. Nerv. Dis.*, 1944, v99, 822.

TABLE I. Composition of Mouse Brain. (Mean value, standard deviation, and probability).

	No. of animals	Wet wt, mg	Dry wt, mg	Dry wt, mg/g wet wt	Water, mg/g of wet wt	Na, mg/g of wet wt	K, mg/g of wet wt
Normal mouse brain	23	419.3 ±24.3	91.5 ±4.7	218.6 ±11.7	781.4 ±11.7	1.0 ±0.1	3.7 ±0.4
Infected mouse brain	28	430.8 ±31.4	89.9 ±6.4	208.9 ± 8.4	791.1 ± 8.4	1.3 ±0.2	3.6 ±0.5
P values for difference					.02	.01	.40

neurotropic virus seemed to be desirable.

Experimental. Four- to 6-weeks-old mice of the ZBC strain‡ were inoculated intraperitoneally with a 10^{-5} dilution of the MM encephalomyelitis virus.§ When the first signs and symptoms of the disease developed the mice were decapitated and the wet and dry weight of the whole brain were determined in a platinum crucible. The dry tissue was then moistened with 4 N sulfuric acid and ashed at 500°C for 15 hours. The ash was dissolved in 5 cc of distilled water and analyzed for sodium and potassium by means of a Perkin-Elmer flame photometer. The brains of normal mice of the same strain and age served as controls. Table I shows the results obtained in 23 normal and in 28 infected brains.

Discussion. The data show that the invasion of brain tissue with the MM encephalomyelitis virus is accompanied by significant changes in the water and electrolyte content. Of all the factors studied the most marked changes are the increase in the content of water and of sodium. These results might be interpreted in either of two ways. There might be either an accumulation of extracellular fluid containing sodium, or an increase in intracellular sodium and water. There is evidence that some of the sodium in brain tissue is intracellular(11). The proportions might change in disease, however the more simple interpretation of our findings would be the first mentioned, namely that extracellular edema occurs. Encephalomyelitic viruses affect selectively certain centers of the brain stem and do not produce uniform damage. Therefore shifts of water or electrolytes in the diseased portions may have been masked by analysing the whole brain. Such an assumption can be supported by the finding of mutual replacability of neuronal potassium and sodium in the axoplasm of the giant squid(12). It has previously been postulated that edema of the cord may contribute to the pathogenesis of the paralysis in poliomyelitis (13) and the presence of edema in the central nervous system in poliomyelitis has been described(14). Recently therapeutic measures for severe cases of poliomyelitis, like repeated blood plasma transfusions, have been based on this assumption(15,16).

Summary. 1. The infection of mice with the MM encephalomyelitis virus causes a significant increase in the water and sodium content of the brain, as compared with normal controls. 2. The potassium content of the infected brain shows a slight decrease which is not statistically significant. 3. A plausible but not conclusively demonstrated interpretation of the results is that an extracellular edema of the brain occurs with MM encephalomyelitis virus in mice.

‡ From the colony of Dr. John J. Bittner, University of Minn.

§ Obtained from Dr. Raymond Bieter, University of Minn.

10. Earle, A. M., *J. Ped.*, 1950, v36, 715.

11. Eichelberger, L. and Richter, R. B., *J. Biol. Chem.*, 1944, v154, 21.

12. Steinbach, H. B., and Spiegelman, S., *J. Cell. and Comp. Physiol.*, 1943, v22, 187.

13. Wickman, I., *Nerv. and Mental Dis. Monograph Series* 1913 No. 16.

14. Courville, C. B., *Pathology of the Central Nervous System,* p. 234, 2d ed., Pacific Press, Cal., 1945.

15. Eaton, R. M., and Bower, A. G., *Science,* 1949, v110, 428.

16. Bower, A. G., Eaton, R. M., Chudnoff, J. S., Affeldt, J. E., and Chaney, A. L., *Am. J. Med. Sc.,* 1950, v220, 46.

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