SOME EFFECTS OF INJECTIONS OF HYALURONIDASE INTO THE SUBARACHNOID SPACE OF EXPERIMENTAL ANIMALS*

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STUDIES of the influence of enzyme injections on fluid absorption, hematoma dissolution and prevention of excessive fibrosis† have been carried on in most areas of the body with the exception of the central nervous system. To extend such studies to this area an investigation of the effects of injecting hyaluronidase† in the subarachnoid space was undertaken.

METHOD AND MATERIALS

To test initially the influence of cerebrospinal fluid on the spreading effect of hyaluronidase in tissues, cerebrospinal fluid in 1 cc. amounts obtained from the cisterna magna of Macacus rhesus monkeys and cats was used to dissolve 150 turbidity reducing units of hyaluronidase. At 30-min. intervals up to 3 hours these solutions mixed with 2 cc. of 0.5 per cent methylene blue or with 100 cc. of saline were injected subcutaneously to provide a visual method of recognizing the spreading effect of the enzyme. Control injections were made simultaneously in symmetrical regions using equal quantities of the same solutions without hyaluronidase. The spreading effect of the hyaluronidase appeared to be unaltered by the cerebrospinal fluid.

A second group of animals anesthetized by intravenous pentobarbital (30 mg./kg.) were studied. Each animal was placed on its right side during the first stages of the experiment. Increments of 1500 units of hyaluronidase were dissolved in 1 cc. amounts of cerebrospinal fluid obtained from the animal being used and injected into the subarachnoid space. Pressure changes were followed for 15 minutes after returning the solution to the cisterna. This procedure was repeated until the total enzyme injected ranged from 6000–9000 units.

Small quantities of cerebrospinal fluid were withdrawn and studied for cellular reactions at the end of 2, 4, 24 and 72 hours by smears stained by Wright's technique and in some instances with supravital preparations using neutral red and Janus green. Total protein determinations were made at the end of 4, 24 and 72 hours. Alteration in the permeability of the blood-brain barrier was tested in 3 instances by injecting into the peritoneal cavity 1 gm. of trypan blue dissolved in 10 cc. of saline, and the time of appearance of the dye in the cerebrospinal fluid was noted. The 3 Macacus rhesus monkeys used were kept for observation for 36 hours, 1 week and 6 months. The 6 cats were sacrificed at 4 hours, 2, 5, 7, 10 and 12 days. Gross and microscopic studies were made of the brains of all the animals.

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In a third group 4 cats received daily instillation of 1500 units of hyaluronidase for 4 days into the cisterna magna and 4 other cats for 8 days. Daily cerebrospinal fluid cell counts and neurological observations were made. In 2 of these animals trypan blue was injected intraperitoneally as previously described. Fourteen days following the last hyaluronidase instillation all animals were sacrificed. The vessels to the head were perfused with normal saline followed by 10 per cent formalin, and gross and microscopic studies of the brains were made.

RESULTS

All animals in the second group, which received intrathecal hyaluronidase over a short period of time, demonstrated similar responses with only slight variations in degree. Representative changes of cerebrospinal fluid pressure and cells, and alterations in the blood-brain barrier in these animals are illustrated in Fig. 1. A marked cerebrospinal fluid pressure elevation occurred although there was drainage of a total of from 4 to 6 cc. from each animal over a 4-hour period. Red blood cells were found in varying quantities depending probably on the degree of trauma produced by the needle insertions and subsequent movement. A minimal increase in polymorphonuclear cells was seen in all instances. More impressive were the more numerous large epithelial cells with round densely basophilic nuclei which were found in the cerebrospinal fluid of each animal (Fig. 2). Supravitral preparations initially revealed centrally located nuclei but in subsequent smears nuclei lying peripherally were seen. At around 48 hours loss of nuclei of some cells was noted giving to the cell remnant with its open end a basket-like

Fig. 1. Representative cerebrospinal fluid changes in cat #4 following cisternal instillation of 9000 units of hyaluronidase.