radiated mice. With intravenous injections of 125 mg/kg on pre-irradiation days 14 and 3, a significant reduction in the ST_{50} day was observed. Similar results were obtained with both doses when injections were given after irradiation. The beneficial effects of zymosan in the treatment of radiation injury in mice reported by Ross and Pillemer could not be confirmed.

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Effect of Hyaluronidase on the Cat's Brain. (22287)

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The existence of interstitial spaces between elements constituting the nervous system has been assumed. Investigations of Bairati and Mattioli(1,2) confirmed and proved the existence of these interstitial spaces which probably were designed for the flow of cerebrospinal fluid. The experiments of Bairati(3), Bairati and Tripoli(4), Bairati and Patterno (5), Bartoli and Bertaccini(6), and Massari and Marsico(7) demonstrated by histochemical tests on different groups of animals the existence of a ground substance, the main element of which is a mucopolysaccharide or a mucoprotein contained in the interstitial spaces of the nervous tissue. The results of Hotchkiss' reaction were clearly positive in nervous tissue of fishes, amphibia, and lower mammals, whereas the results were doubtful when the test was performed on the brain of birds and higher mammals. These authors studied the behaviour of the nervous tissue under the influence of hyaluronidase and proved that the ground substance is affected by hyaluronidase, which allows a more rapid and extensive diffusion of the injected liquids and an easier mechanical dissociation of fragments of nervous tissue. Thus mucopolysaccharide may be present in interstitial spaces of the nervous substance. Freedman(8) also proved by means of histochemical reactions the existence of a substrate hydrolyzed by hyaluronidase, probably hyaluronic acid, in the nervous system, *i.e.* in the cerebral cortex, nucleus caudatus, and thalamus. Recently, Hess(9) stated too that the ground substance of the nervous tissue is a mucopolysaccharide, rather than a mucoprotein; since it is not hydrolyzed by pepsin, trypsin, or pancreatin. Hess found that hyaluronidase had no effect, and this indicates that this substance is not hyaluronic acid. In the present work, the behaviour of the nervous tissue under action of the hyaluronidase enzyme was examined.

Materials and methods. 13 young adult cats of both sexes were used, with a weight of 1.5-2 kg. All animals were operated on under

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aseptic conditions and ether anesthesia. Symmetrical points of the skull were opened, and after incising the duramater, the following procedures were carried out. In the *first test,* on 2 animals, a superficial wound of the cerebral cortex was made by application of the trephine on both sides of the chosen area for 10 seconds. After stopping the hemorrhage, a square piece of paper 7 mm side length, soaked in physiological saline solution was applied to the control side. On the other side, another piece of paper of the same form and size was applied, which immediately before had been soaked in a recently prepared hyaluronidase solution (30 units of Schering's "Kinaden" in 0.1 ml of physiological serum). Both wounds were covered with a piece of cellophane paper applied to the bone between it and the muscles. After closing the wound, 10 ml of a solution of Merck's trypan blue of 1% in physiological serum was injected intravenously and repeated every 3 hours. The animals were sacrificed 10 hours later by bleeding under anesthesia. In the *second test,* 2 cats were treated in the same way, but the quantity of hyaluronidase used was 10 units. Trypan blue was 20 ml divided into 2 injections separated by 40 minutes. The animals of this group were sacrificed 10 hours later. In the *third test,* cerebral lesions were made by application to the cortex of a square piece of paper, 5 mm side length, soaked with carbol-xylol. This was kept in contact with the cortex for 40 seconds. Thereafter, the symmetrical points of the cortex treated in this way were irrigated with serum at 37° and then the animals were treated exactly as in the first test. One of the animals was sacrificed 2 hours after, and the remaining 2 after a period of 10 hours. In the *fourth test,* 4 cats were treated with hyaluronidase inactivated by heat, the enzyme having been dissolved in serum at 70° and the solution incubated one hour at 70°. The experimental procedure was the same as in the third test. Two animals of this group were sacrificed and the remaining ones 10 hours after. In the *fifth test,* in 2 cats a deep injection of 0.2 ml of trypan blue solution at 1% in physiological serum containing 20 units of Kinaden was injected deeply into the cortex on one side. In a symmetrical point of the cortex of the other side, a 0.2 ml of trypan blue dissolved in physiological serum at 1% was injected, to the same depth and the animals sacrificed after a period of 24 hours. The brains were fixed in an aqueous formol solution at 10% and afterwards examined microscopically with a magnifying glass.

Results. In cats of the first test, a larger diffusion of the dye was seen on the side which had been treated with hyaluronidase (Fig. 1-A). The stain is broader on the treated side than on the control side to which physiological serum had been applied.

In the second test the results were analogous. On the side treated with hyaluronidase the dye extended over a broader area than that which was reached on the control side. The diffusion area was smaller in this test than in the first one.

In the third test, the results were exactly the same as in the previous ones.

In the fourth test, in which the hyaluronidase had been inactivated by heat, the diffusion area of the dye was essentially the same on both sides. (Fig. 1-B).

In the fifth test, the dye formed on the control side a small sediment of quite precise limits while on the side in which hyaluronidase had been injected, it had spread into the adjoining area, in the immediate nervous tissue following the needle's course and forming a halo of pale blue color.

Discussion. The behavior of the nervous tissue in the presence of the hyaluronidase enzyme was also studied by Bairati(10) using the method of injection—at low pressure—of coloring substances (India ink) diluted in solutions of the enzyme with physiological serum. Injections were performed in nervous tissue obtained immediately after death and in living animals. In the technic applied by Bairati, the quantity injected is not exactly determined and the pressure at which the injection is performed cannot be regulated with accuracy, a fact which possibly may influence the degree of diffusion of the coloring substance thus rendering doubtful the interpretation of the results.

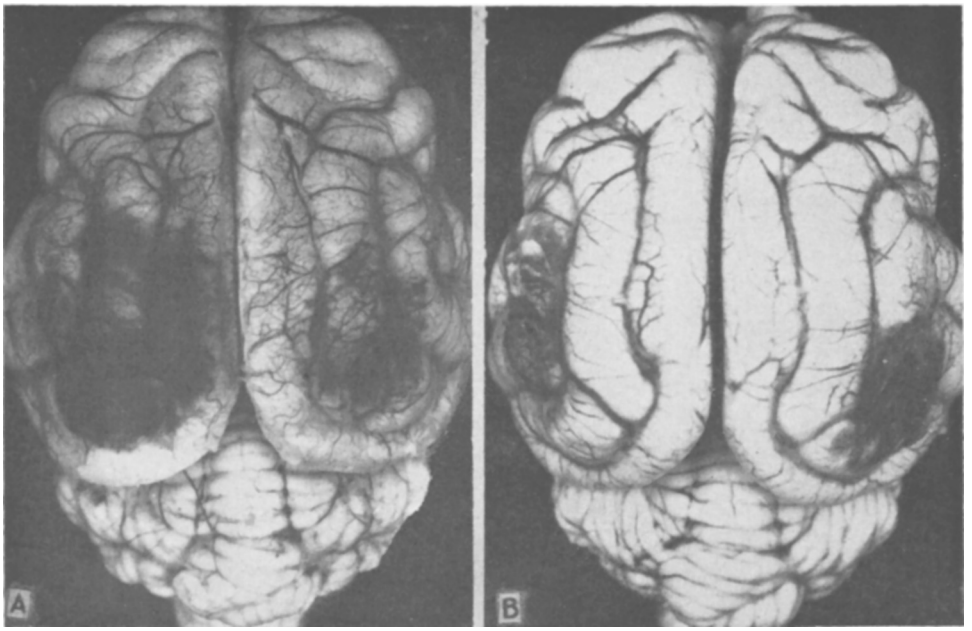

FIG. 1. A. First test. Left side treated with hyaluronidase. Right side (control) treated with physiological serum. Notice broader diffusion of coloring substance on left side. B. Fourth test. Left side treated with hyaluronidase inactivated by heat. Diffusion area of coloring element is approximately the same as on the right (control) side.

The technic used in our experiments avoids any such errors. Passage of the dye takes place through the blood vessels at the same pressure and at the same concentration on both sides, and the animal serves at the same time for test and control.

It can be deduced from our results that in the area of the cortex to which the enzyme was applied, the diffusion of the dyestuff is greater than on the control side. The degree of diffusion was influenced by concentration of the enzyme in the tested solution, and by the time over which it was allowed to act. This effect of the enzyme disappears when it is inactivated.

These results indicate the existence of a substance—probably hyaluronic acid—in the cerebral cortex of the cat which is susceptible to the action of the hyaluronidase.

Summary. 1) The existence of a substance susceptible to the action of hyaluronidase in the cat's brain is demonstrated. 2) The action of hyaluronidase on the cerebral substance is influenced by the concentration of the enzyme and by the duration of its action. 3) When the enzyme is activated by heat, the effects of the diffusion factor on the nervous system disappear.

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