Influence of Lysergic Acid Diethylamide on Experimental Allergic Encephalomyelitis.* (27832)

DANIEL J. O'BRIEN, FRANCIS W. HUGHES, AND JAMES NEWBERNE
Department of Pharmacology, Indiana University School of Medicine and Department of Pathology, Pitman-Moore Company, Indianapolis

Many classes of pharmacologic agents have been investigated to determine their value in preventing or altering the severity of experimental allergic encephalomyelitis (EAE). Antihistaminics(1,2,3) as well as anti-coagulants(4) have had little effect on the course of the disease. In one study the prophylactic use of salicylates alone or combined with p-aminobenzoic acid reduced the incidence of paralysis and mortality(5). However, salicylates did not alter the severity of the disease when administered after injection of the brain-adjuvant emulsion. Kabat *et al.*(6) were able to prevent the disease in monkeys by starting large doses of cortisone prior to the brain-adjuvant and continuing the drug for the duration of the experiment. Kolb *et al.*(7), demonstrated the prophylactic effectiveness of cortisone in guinea pigs. Ferraro and Roizin(8) have confirmed the prophylactic effectiveness of cortisone in guinea pigs but they have also indicated that it has no permanent inhibitory effect on the disease processes. Gammon and Dilworth(9) reversed the disease process in 69% of guinea pigs treated with corticotropin (ACTH) starting the drug within 24 hours after onset of paralysis and continuing for 21 days. Kolb *et al.*(7) were able to demonstrate some reduction in the severity of the disease by administration of nitrogen mustard one week prior to giving brain-adjuvant and continuing for 60 days. Hoyer, Condie and Good(10) prevented the occurrence of EAE with large daily doses of 6-mercaptopurine. However, when this drug was discontinued, EAE developed.

Although delayed hypersensitivity is generally believed to be involved in the pathogenesis of EAE, no specific pharmacologic antagonism has been developed. The failure of the antihistamines to be effective in such situations suggests the formation or liberation of substances other than histamine.

A substance which is receiving increasing attention as a possible important factor in diseases of hypersensitivity is 5-hydroxytryptamine (serotonin). Serotonin is known to be involved in the anaphylactoid reaction elicited by dextran and egg white in rats(11,12). These observations suggest that serotonin may play an important role in allergic phenomena. In view of this possibility an anti-serotonin compound, such as lysergic acid diethylamide, was considered for study in experimental allergic encephalomyelitis in guinea pigs.

Materials and methods. Animals. Male guinea pigs initially weighing 250 to 300 g were used. The *brain-adjuvant emulsion* was prepared from fresh guinea pig brain according to the method of Freund(13). Each animal received 3 ml of the emulsion injected subcutaneously along the lumbar region of the back. The animals were grouped according to the following schedule: Groups HO-1 and HO-2 received brain-adjuvant plus saline (0.85%) in the same volume and on the same administration schedule as the animals receiving injections of drug. Groups HLSD-1 and HLSD-2 received brain-adjuvant and lysergic acid diethylamide–dosage schedule below. Groups LSDO-1 and LSDO-2 received lysergic acid diethylamide only–dosage schedule below. *Lysergic acid diethylamide (LSD)* was injected subcutaneously 3 times weekly to groups HLSD-1 and HLSD-2 and LSDO-1 and LSDO-2 starting on the day of injection of brain homogenate and continued over the 60-day period of experiment. Lysergic acid diethylamide was prepared in distilled water as a 20 μg/ml solution for administration to the HLSD-1 and LSDO-1 groups and in a 50 μg/ml concentration for administration to the HLSD-2 and LSDO-2 groups. Groups HLSD-1 and LSDO-1 re-

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TABLE I. Incidence of Paralysis and Death (Guinea Pigs).

Series	No.	A Paralysis, survived	B Paralysis, death	C Death, no symptoms	Total paralysis	Total death
					%	%
I Brain homog. only	20	7	5	3	12 (60)	8 (40)
Brain homog. + LSD 20 γ/kg 3 × weekly	20	1	1	4	2 (10)	5 (25)
II Brain homog. only	10	1	5	0	6 (60)	5 (50)
Brain homog. + LSD 50 γ/kg 3 × weekly	10	1	1	0	2 (20)	1 (10)

ceived LSD, 20 μg/kg, subcutaneously, 3 times weekly for the 60-day duration of experiment. Groups HLSD-2 and LSDO-2 received 50 μg/kg on the same administration schedule. Groups HO-1 and HO-2 received injections of 0.85% saline in the same volume and on the same schedule as the drug was administered. *Histopathology.* For histopathologic study the brains were fixed in 10% formalin and sectioned at various levels; basal ganglia, thalamus, mid-brain, pons, medulla, cerebellum, and several areas of cerebrum. For routine study the tissues were imbedded in paraffin, cut at 7 μ and stained with hematoxylin-eosin. For study of detail selected specimens were prepared with the prussian blue reaction for iron, Gomori's tichrome stain, gallocyanin stain for nissl substance and nuclear detail, and the luxol blue-periodic acid Schiff reaction for myelin and neutral mucopolysaccharides(14).

Results. Table I summarizes the results of the 2 experiments. When LSD was administered in either dosage, 20 μg/kg or 50 μg/kg tri-weekly, a decrease in numbers of animals with paralysis as well as total mortality resulted. Deductions from these gross findings suggest that the protective action of LSD is not dose-related. However, in Table II, the histopathologic evaluation shows that in the series receiving the higher dosage level, LSD shifted the severity of brain lesions from moderate-severe to moderate-slight.

Significant histologic alterations were demonstrated in all guinea pigs of both groups in which clinical paralysis had occurred. In addition, slight to moderate alterations were also found in several animals which had not developed untoward clinical signs. The latter were usually much less severe and occurred only in a few sites.

The histologic changes were generally similar to those described by other investigators (15). Briefly, there were areas of damage to blood vessels characterized by cellular reactions in various sites, especially the white matter. In the more severely affected there were widely disseminated lesions, some of which were confluent and rather extensive. In those with mild or slight alterations the

TABLE II. Histopathologic Observations on Guinea Pig Brains.

	Animals		Brain lesions: Distribution		Brain lesions: Reaction		
Series	No.	No. with lesions	Focal*	Dissem-inated	Severe	Moderate	Slight
I Brain homog. only	20	9	6	3	1	1	7
Brain homog. + LSD 20 γ/kg 3 × weekly	20	7	6	1	—	—	7
II Brain homog. only	10	9	1	8	3	6	—
Brain homog. + LSD 50 γ/kg 3 × weekly	10	9	5	4	—	1	8

* 1 to 3 sites.

lesions tended to be focal and limited in distribution. Wherever tissue reactions occurred, they consisted of foci of lymphoid cells, plasma cells, and neutrophils located in the adventitia of blood vessels with varying degrees of proliferation of the adventitia. Frequently, many cells appeared epithelioid in character and there was some proliferation of glial elements without any apparent damage to axis cylinders or neurones in the vicinity of the lesions.

Although rather severe brain lesions were found in all guinea pigs in which clinical paralysis had occurred, there was very little correlation between the presence of mild lesions and clinical paralysis. In the first series that received brain homogenate alone, 9 of 20 animals developed lesions during the experimental period (Table II). Of these, 3 were disseminated and 6 were limited in distribution. The tissue reaction was severe in one, moderate in another, and mild in the remaining 6. Of those that received brain homogenate plus LSD (20 μg/kg 3 times weekly), 7 of 20 developed lesions. Of these, 6 were focal and one was disseminated in distribution, and in all 7 the tissue reaction was rather mild.

In the second series, 9 of 10 receiving brain homogenate only developed lesions. Eight of these were disseminated and one was focal. The tissue reaction was severe in 3 and moderate in the remaining 6. Of those receiving brain homogenate plus LSD (50 μg/kg 3 times weekly), 9 developed lesions but only 4 of these were disseminated and 5 were focal in distribution. The tissue reaction was moderate in one and slight in the remaining 8. The data indicate that in the groups which received brain homogenate alone greater numbers were affected with more severe, disseminated lesions than in those which also received LSD. The apparent sparing effect of LSD was more noticeable in those on the higher level.

Discussion. As seen from the histologic studies the groups which received LSD along with the brain homogenate had fewer numbers of animals affected and in those affected the lesions were limited in distribution and the reaction was rather mild. Thus, LSD produced an apparent sparing effect which was more noticeable in those on the higher dosage level.

There was little evidence of demyelination or damage to neurones or axons in any of these guinea pigs and rather poor correlation between clinical signs and histologic lesions. These findings are in general agreement with the observations of others(15).

Several investigators have demonstrated that a change in the permeability of the cerebral blood vessels does occur in EAE. Barlow, using the trypan blue technic, found no increase in permeability where the lesions were restricted to the vessel walls, but in regions where the lesions also involved the brain parenchyma an abnormal passage of dye occurred(16). Vulpe, Hawkins, and Rozdilsky used iodinated radioactive bovine albumin and the autoradiographic method to assess the changes of permeability of cerebral blood vessels in EAE(17). In the initial stage of the disease an increased permeability to albumin was noted in the inflamed vessels. Later in the disease the blood vessels lacked the increased state of permeability although signs of inflammatory changes were still present. In some acute animals, increased permeability with no inflammatory changes was present and in many cases increased permeability was observed around blood vessels without any inflammatory signs. They have suggested that these latter findings indicate either that the primary vascular lesions may be an early phenomenon of EAE or that radioactive cerebrospinal fluid alone can penetrate the normal blood vessels into the surrounding brain tissue.

In the rat paw, 5-hydroxytryptamine has been shown by Rowley and Benditt to cause a marked increase in capillary permeability when given in low (1-10 μg) concentrations (12). That serotonin will produce vasodilatation of blood vessels of the cortex with subsequent edema has been shown by Malcolm after an intracarotid injection of 100-200 μg of 5-hydroxytryptamine(18). Spector and Willoughby have demonstrated the presence of serotonin in early inflammatory exudates (19).

The limiting effect on the lesions of EAE

attained with LSD in this study may be due to a direct anti-serotonin effect on the cerebral vessels or within the parenchymal elements. The possibility of a specific action of LSD other than its anti-serotonin property can not be excluded.

Summary. Lysergic acid diethylamide (LSD) was administered to guinea pigs that had received subcutaneous injections of brain homogenate. Dosages of 20 or 50 μg/kg triweekly reduced the incidence of paralysis as well as mortality rate. In animals that had demonstrable histopathologic lesions after the drug, the severity of the lesions was reduced.

1. Alvord, E. C., Jr., Correll, J. W., Ladd, A. T., Kellner, A., *J. Immunol.,* 1948, v60, 345.

2. Halpern, B. N., Bertrand, I., Lhermitte, F., *Presse med.,* 1950, v58, 684.

3. Lumsden, C. E., *Brain.,* 1949, v72, 198.

4. Waksman, R. H., Morrison, L. R., *J. Immunol.,* 1951, v66, 421.

5. Good, R. A., Campbell, B., Good, T. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 341.

6. Kabat, E. A., Wolf, A., Bezer, A. E., *J. Immunol.,* 1952, v68, 265.

7. Kolb, L. C., Karlson, A. G., Gayre, G. P., *Trans. Am. Neurol. A.,* 1952, v77, 117.

8. Ferraro, A., Roizin, L., *J. Neuropath. and Exp. Neurol.,* 1953, v12, 373.

9. Gammon, G. A., Dilworth, M. J., *Arch. Neurol. and Psychiat.,* 1953, v69, 649.

10. Hoyer, L. W., Condie, R. M., Good, R. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 205.

11. Parratt, J., West, G., *J. Physiol.,* 1957, v139, 27.

12. Rowley, D. A., Benditt, E. P., *J. Exp. Med.,* 1956, v103, 399.

13. Freund, J., Stern, E. R., Pisani, T. M., *J. Immunol.,* 1947, v57, 179.

14. Armed Forces Inst. of Pathology, *Manual of Histologic and Special Staining Technics,* 1957.

15. Waksman, B. H., *Internat. Arch. Allergy and Applied Immunol.,* 1959, v14, suppl.

16. Barlow, C. F., *J. Neuropath. and Exp. Neurol.,* 1956, v15, 196.

17. Vulpe, M., Hawkins, A., Rozdilsky, B., *Neurology,* 1960, v10, 171.

18. Malcolm, J. L., In *5-Hydroxytryptamine,* Pergamon Press, New York, 1958, p227.

19. Spector, W., Willoughby, D., *Nature,* 1957, v179, 318.

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Accentuation of Plaques of Myxoma and Fibroma Viruses by Immune Serum.* (27833)

MERRY S. MOORE AND DUARD L. WALKER

Department of Medical Microbiology, University of Wisconsin Medical School, Madison

Production of plaques on cell monolayers by myxoma and fibroma viruses has been described(1). This report deals with the influence of specific rabbit antiserum on the appearance of these plaques. It was found that when antiserum was incorporated in the agar overlay, the plaques produced by both viruses were not reduced in size or number, were made much more distinct and, without preliminary cell staining, could be counted earlier.

Materials and methods. The Moses strain of myxoma virus (MV) and the Patuxent strain of fibroma virus (FV) were used in all experiments. The origin of the viruses, the details for cultivation of the viruses, preparation of monolayers of secondary rabbit kidney (RK) cells, and composition of the routine nutrient agar overlay (RO) were the same as previously described(1) with the exception that the virus was centrifuged onto the cell monolayers according to the methods of Padgett and Walker (to be published). In brief, monolayers of secondary RK cells grown in 1-oz prescription bottles were placed cell side out in a basket rotor of an International centrifuge (size 1, model CM) and centrifuged at 3500 rpm for 30 minutes at room temperature. The inoculum was removed and the cell sheet covered with 5 ml of the

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