3. Emmelot, P., Bos, C. J., Benedetti, E. L., Runke, P. H., Biochem. et Biophys. Acta, 1964, v90, 126.

4. Sipski, V. P., Barclay, M., Archibald, F. M., Terebus-Kekish, O., Reichman, E. S., Good, J. J., Life Sci., 1965, v4, 1673.

5. Barclay, M., Skipski, V. P., Terebus-Kekish, O., Barclay, R. K., Fed. Proc., 1966, v25, 656.

6. Hargreaves, T., Lathe, G. H., Nature, 1963, v200, 4912.

7. Straus, W., Biochem. Biophys. Acta, 1956, v19, 58.

8. Beaufay, H., de Duve, C., Bull. Ste. Chim. Biol., 1954, v36, 1525.

9. Arias, I. M., J. Clin. Invest., 1962, v41, 2233.

10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., J. Biol. Chem., 1951, v193, 265.

11. Novikoff, A. B., Sixth Inter. Congress of Biochemistry, 1964, v6(8), 609.

12. Hoenig, V., Hoenigova, J., Vnitrni Leg., 1962, v8, 275.

13. Andrews, W. H.. H., del Rio Lozano, I., Quart. J. Exp. Physiol., 1961, v46, 238.

14. Arias, I. M., in Prog. in Liver Disease, H. Popper, F. Schaffner, eds., Grune & Stratton, Inc., New York, 1961.

15. Hanson, V., Acta Physiol. Scand., 1952, v28, Suppl. 101.

16. Cherrick, G. R., Stein, S. W., Leevy, C. M., Davidson, C. S., J. Clin. Invest., 1960, v39, 392.

17. Nosslin, B., J. Lab. Clin. Med., 1965, v65, 891.

18. Shotton, D., Carpenter, M., Rinehart, W. B., N. Engl. J. Med., 1961, v264, 550.

Received October 28, 1966. P.S.E.B.M., 1967, v124.

Suppressive Effect of Anti-Allergic Mediators on Experimental Encephalomyelitis.* (31820)

HELENE C. RAUCH AND OSCAR L. FRICK† (Introduced by S. Raffel)
Department of Medical Microbiology, Stanford University School of Medicine, Stanford, Calif.

Experimental allergic encephalomyelitis (EAE) is an autoimmune response induced in animals and man by the injection of central nerve tissue, or a basic protein extracted from central nerve myelin, most often in combination with Freund's adjuvant containing tubercle bacilli(1). After approximately 2 weeks, clinical symptoms including weight loss, paresis, incontinence and ascending paralysis follow the development of central nervous system lesions essentially marked by perivascular lymphocytic cuffing. The immune mechanism is a cellular response, passive transfers having been accomplished successfully only with viable lymphocytes in immunologically receptive animals(2-5).

However, the role of antibody, either produced or transported by infiltrating cells, has not been excluded from an inducing role or, at the least, as an enhancing factor(6). In the event that whole tissue is used to induce disease, antibodies to non-myelin brain tissue or vascular elements may possibly initiate a local inflammatory response. Increased vascular permeability, due to released mediators, may facilitate the infiltration of myelin-sensitized lymphocytes. Myelin antibody might also enhance tissue damage by infiltrating the parenchyma following the lymphocytic perivascular invasion. At any rate, if a local antigen-antibody reaction does influence the course of EAE, then pharmacologic mediators may also be involved. Histamine and serotonin(7,8), perhaps the best characterized mediators of hypersensitivity, have consequently been investigated for their effect on the occurrence and severity of EAE.

Although anti-histiminic agents, which compete with histamine for active cellular sites, have been found ineffective in EAE (9-11), it was of interest to study the effect of depletion of available histamine which has recently been shown to occur in guinea pigs treated with repeated doses of anaphylatoxin. This treatment renders these animals refractory to passive anaphylactic shock for about

* Supported in part by USPHS Research Grant NB 05513 from Nat. Inst. of Neurol. Dis. and Blindness, and USPHS Fellowship Grant EPD-12,239 from Nat. Inst. of Allergy and Infect. Dis.

† Present address: Dept. of Pediatrics, Univ. of California Med. Center, San Francisco.

3 weeks(12). Furthermore, recent studies by O'Brien *et al*(13) have elaborated on the role of lysergic acid diethylamide (LSD), primarily a serotonin antagonist, in reducing the clinical and histologic severity of EAE in guinea pigs.

The effect of LSD has been included in this report in order to compare its activity with that of a substituted derivative Sansert (methysergide maleate, Sandoz) and to record acceleration or suppression of disease after termination of drug administration. Both rats and guinea pigs have been included since anaphylaxis may be mediated primarily by serotonin or histamine respectively in these species(7,8,14).

Materials and methods. Preparation of anaphylatoxin. Pooled sera from rats of various strains were incubated with dextran (Pharmacia, mol wt 250,000) at 37°C for 2 hours (50 mg/ml). The insoluble dextran was then removed by centrifugation. One ml of the supernatant rat serum, when injected intravenously into a normal guinea pig, caused death by "anaphylactoid" shock within minutes in 6 test animals. Dextran-treated rat serum will be referred to as "anaphylatoxin."

Anaphylatoxin-treated guinea pigs. Two groups of 12 guinea pigs (450-650 g) were given increasing doses of anaphylatoxin intravenously 2 or 3 times daily for 7 or 10 days. The serum dose ranged from 0.3 ml to 1 ml per injection. Two groups of 6 control animals each received identical doses for 5 days of the same rat serum pool which had not been incubated with dextran. Longer treatment with normal serum was impractical because of the development of anaphylactic symptoms. Five days after initiating anaphylatoxin treatment, all animals received bovine spinal cord KCl extract‡ 0.5 mg and BCG 0.5 mg in Freund's (Difco) adjuvant in 0.1 ml volume intradermally. Between the sixth and ninth day after initiation of treatment, the anaphylatoxin-treated groups were passively sensitized intravenously with rabbit anti-egg albumin sera (50 μg antibody nitrogen) and challenged 24 hours later with 1 mg of egg albumin intravenously. Three animals died of anaphylaxis; the remaining animals failed to react and were consequently presumed to be depleted of endogenous histamine.

All animals were observed daily for signs of disease (weakness, paralysis or weight loss) until the 35th day, after which the survivors were sacrificed. The brains of all animals autopsied at the onset of disease or at the end of the experiment were fixed in formalin and stained with both hematoxylin-eosin, and luxol fast blue for observation of demyelination. Three slides of each brain were examined initially; if the specimen was negative, an additional 10 sections were prepared and examined for lesions of encephalomyelitis.

Drug-treated guinea pigs. Guinea pigs (400 to 600 g) were divided into 3 groups of 9 animals each. The first group was given daily injections of LSD. The second group received daily injections of Sansert, and the last group was untreated. Drug administration commenced 3 days prior to injection of the spinal cord preparation and continued for 30 days. Drugs were given in a dose of 0.1 mg/g body weight. The drug-treated animals were held for an additional 30 days with no further treatment. The control animals were sacrificed after the initial 30-day period. For the induction of encephalomyelitis, animals were injected subcutaneously on the back with 0.7 ml of 33% (weight/volume) normal guinea pig cord homogenized in 0.25% phenol/water mixed with an equal volume of oil-tubercle bacillus-aquaphor mixture (9 parts oil containing 3.7 mg BCG per ml, plus one part aquaphor). This mixture was held at 60°C. At the time of appearance of distinct clinical symptoms or at termination of the experiment, the brains of all animals were examined histologically.

Drug treatment of rats. Wistar rats (150 to 300 g) were divided into 3 groups containing 10 animals each. The 2 drug groups (LSD or Sansert) were injected daily with 0.1 mg/g of drug subcutaneously. The third group served as controls. Treatment with drugs began 4 days prior to injection of

‡ Prepared by Dr. Elizabeth Einstein, Dept. of Neurology, Univ. of California Medical Center, San Francisco.

TABLE I. Effect of Anaphylatoxin on Development of Experimental Allergic Encephalomyelitis in Guinea Pigs.

Group	Clinical disease	Histologic lesions
Control	10/12*	11/11†
Anaphylatoxin	16/19	17/17†

* Number reacting animals over number in group within 30-day period of experiment.
† Three animals were unavailable for histologic examination.

TABLE II. Effect of Drug Treatment on Development of Experimental Allergic Encephalomyelitis in Guinea Pigs.

Group	Clinical disease			Histologic lesions
	Drug period*	Post-drug period*	Total	
Control	12/12†	—	12/12	12/12
LSD	6/8	2/2	8/8	8/8
Sansert	7/9	1/2	8/9	9/9

* Drug and post-drug periods were each 30 days.
† Number reacting animals over number in group.

the encephalitogenic preparation and continued for 30 days afterward. Control animals were sacrificed 30 days after the inducing injection, but the drug-treated animals were observed for 30 days after discontinuance of drug treatment. Emulsified normal guinea pig cord as described above was used for inducing encephalomyelitis in these rats. Histological examination was carried out as above.

Results. Intensive treatment of guinea pigs with anaphylatoxin in no way affected their response to experimentally induced allergic encephalomyelitis. It may be seen in Table I that 10 of 12 untreated guinea pigs developed symptoms of EAE, as did 16 of 19 animals treated with anaphylatoxin. In both groups of guinea pigs, the time of onset of symptoms and the severity of histologic lesions were very similar. On day 18 animals in both the control and treated groups reacted to intracutaneously injected tuberculin.

Guinea pigs treated with either LSD or Sansert (Table II), on the other hand, responded somewhat differently from untreated controls to injection of the encephalitogen. Clinical disease was delayed until after withdrawal of drugs in 25% of treated animals, while all control pigs developed EAE within the test period. The final incidence of disease, however, was substantially the same in both groups.

The effect of drug treatment on development of allergic encephalomyelitis in rats is summarized in Table III. During drug treatment with LSD, clinical disease was reduced by about 33%. After discontinuance of the drug, disease actually became apparent in a higher percentage of animals than was seen in the control group. This may be an artifact since the control group was observed by experimental design for only 30 days in contrast to the 60-day observation period of the treated rats. Consequently, clinical disease could be delayed but not averted by LSD treatment in rats. However, when disease appeared, histologic lesions were milder in some animals that had undergone drug treatment.

These results demonstrate that during drug treatment EAE is suppressed in about 30% of the experimental animals. Anaphylatoxin-treated animals, depleted of histamine and insensitive to passive anaphylaxis are uniformly susceptible to experimental allergic encephalomyelitis.

TABLE III. Effect of Drug Treatment on Development of Experimental Allergic Encephalomyelitis in Rats.

Group	Clinical disease			Histologic lesions						
	Drug period*	Post-drug period*	Total	Drug period			Post-drug period			Total
				+	±	0	+	±	0	
Control	6/10†	—	6/10	9/10	1/10	0	—	—	—	10/10
LSD	4/10	4/6	8/10	4/4	0	0	0	2/5	3/5	6/9 ‡
Sansert	4/10	1/3	8/10	7/7	0	0	0	2/3	1/3	9/10

* Drug and post-drug periods were each 30 days.
† Number of reacting animals over number in group.
‡ Animals lost for histologic purposes.

Discussion. As apparent from both clinical and histologic data LSD, and to a lesser extent Sansert, can delay the development of experimental allergic encephalomyelitis, and perhaps reduce the severity of perivascular lesions. Animals treated with the serotonin antagonists in this study confirmed the report by O'Brien *et al*(13) that EAE is suppressed in guinea pigs during the course of drug administration. However, following discontinuance of these agents, the incidence of disease rose to that of the control groups. Taking into account the limited but random sectioning of brain for histologic observation, it appears that LSD-treated animals show a less severe cellular response than do untreated controls. Intensive treatment with anaphylatoxin, which results in the removal of endogenous histamine, is without effect on the incidence of EAE.

The anti-serotonin drugs may counteract increased vascular permeability produced by serotonin(15). This condition is well documented in EAE. Cerebral vessels have increased permeability as measured by infiltration of trypan blue(16) or of iodinated bovine serum albumin(17). If this permeability depends to some extent on serotonin, then the effect of these anti-serotonin drugs may be the diminution of perivascular infiltration of cellular or humoral components thus limiting to some extent the damage to the central nervous tissue.

These effects on EAE are similar to those observed by others(18,19) with corticosteroids, in that the disease was suppressed (clinically and histologically) during the course of intensive drug therapy, but subsequently became manifest upon discontinuance of treatment. The action of corticosteroids may be directly on the cellular and/or antibody components, while anti-serotonin agents probably act by decreasing the vascular permeability, thereby excluding cellular or humoral elements from the brain parenchyma. Although it must be recognized that LSD may have other actions as well, the end effect of these anti-allergic mediators is partial suppression of EAE.

Summary. Intensive treatment of guinea pigs with anaphylatoxin sufficient to prevent passively induced anaphylaxis did not alter the development of experimental allergic encephalomyelitis. In contrast, anti-serotonin drugs, lysergic acid diethylamide (LSD) and methysergide maleate (Sansert), did suppress the development of the auto-immune disease in about 30% of rats or guinea pigs during the period of drug treatment.

The authors wish to thank Mrs. Tamara Tsvetikov for excellent technical assistance in preparation of the histologic sections. We also thank Sandoz Pharmaceuticals, Division of Sandoz, Inc., San Francisco, Calif., for supplying LSD-25 and Sansert (UML-491).

1. Research in Demyelinating Diseases, Part III, Etiology of Experimental Allergic Encephalomyelitis, Ann. N. Y. Acad. Sci., 1965, v122, 161.
2. Koprowski, H., Paraf, A., Billingham, R., Jervis, G., Compt. rend., 1960, v250, 2956.
3. Paterson, P. Y., J. Exp. Med., 1960, v111, 119.
4. Stone, S. H., Science, 1961, v134, 619.
5. Aström, K-E., Waksman, B. H., J. Path. Bact., 1962, v83, 89.
6. Paterson, P. Y., Adv. Immunol., 1966, v5, 131.
7. Brocklehurst, W. C., Humphrey, J. H., Perry, W. L. M., J. Physiol., 1955, v129, 205.
8. Sanyal, R. K., West, G. B., Nature, 1957, v180, 1417.
9. Alvord, E. C., Jr., Correll, J. W., Ladd, A. T., Kellner, A., J. Immunol., 1948, v60, 345.
10. Halpern, B. N., Bertrand, I., Lhermitte, F., Presse med., 1950, v58, 684.
11. Lumsden, C. E., Brain, 1949, v72, 198.
12. Frick, O. L., Halpern, B. N., Liacopoulos, P., J. Physiol., 1962, v163, 191.
13. O'Brien, D. J., Hughes, F. W., Newberne, J., Proc. Soc. Exp. Biol. and Med., 1962, v111, 490.
14. Paton, W. D. M., Progr. Allergy, 1958, v5, 79.
15. Malcolm, J. L., in 5-Hydroxytryptamine, Pergamon Press, New York, 1958, p227.
16. Barlow, C. F., J. Neuropath. Exp. Neurol., 1956, v15, 196.
17. Vulpe, M., Hawkins, A., Rozdilsky, B., Neurology, 1960, v10, 171.
18. Kabat, E. A., Wolf, A., Bezer, A. E., J. Immunol., 1952, v68, 265.
19. Kibler, R. F., Ann. N. Y. Acad. Sci., 1965, v122, 469.

Received October 21, 1966. P.S.E.B.M., 1967, v124.