

SUPPRESSION OF EXPERIMENTAL "ALLERGIC" ENCEPHALOMYELITIS IN GUINEA PIGS BY ENCEPHALITOGENIC PROTEINS EXTRACTED FROM HOMOLOGOUS BRAIN\*· ‡

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Experimental "allergic" encephalomyelitis (EAE) can be produced in many mammals and birds by the injection of a vaccine containing mammalian or avian brain and Freund's adjuvants, killed mycobacteria in water-in-oil emulsion (1). The pathogenesis of this disorder is thought to be due to delayed sensitivity to the brain in association with the adjuvants (2). The evidence for this hypothesis is not completely consistent, the rabbit (2-4) but not the guinea pig (5) developing delayed cutaneous hypersensitivity to homologous nervous tissue following injection of homologous nervous tissue with adjuvants.

Since the concentration of antigen in whole brain might be too low to permit a visible skin reaction to develop in the guinea pig, experiments were performed using partially purified encephalitogenic proteins. These water-soluble proteins are approximately 30 times as active as the whole homologous brain from which they were extracted by dilute acid after removal of lipids (6). In these experiments sensitivity to tuberculin was simultaneously determined 11, 15, 21, and 28 days after the injection of whole guinea pig brain with adjuvants. The severity of EAE was made to vary by using encephalitogenic vaccines containing widely differing amounts of killed tubercle bacilli with a constant amount of homologous brain. The results were quite surprising (Fig. 1): not only was there no convincing evidence of sensitivity to the homologous encephalitogenic extract, but also those animals repeatedly skin-tested failed to develop significant clinical or histological evidence of the disease.

Other investigators have reported that EAE can be *prevented* by pre-treatment of newborn (7-9) or adult (10, 11) animals with incomplete vaccines

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containing whole brain, encephalitogenic brain extracts (12), or tubercle bacilli (12, 13). *Suppression* of the disease subsequent to the injection of the complete encephalitogenic vaccine has not been reported following skin tests with whole brain (2-5, 14, 15), tuberculin (15), or tubercle bacilli (3) or even following desensitization with whole brain or tuberculin (16). Condie *et al.* (17) have recently reported suppression by the intravenous administration of whole brain emulsion to heparinized rabbits 7 days after challenge, but they have not yet reported histological confirmation of their results.

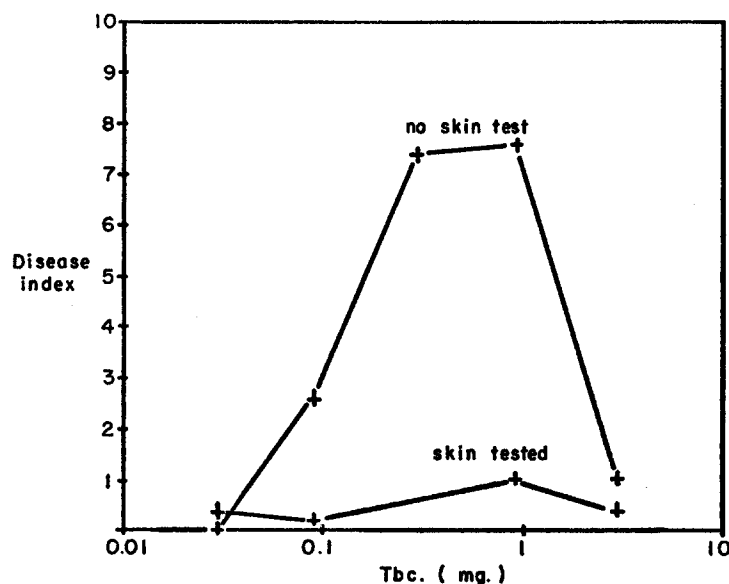


FIG. 1. Suppression of EAE by skin tests at 11, 15, 21, and 28 days with acid extract of guinea pig brain and PPD. (Experiment 1, Table II; original encephalitogenic vaccines contained 0.3 mg. lyophilized guinea pig brain and various amounts of *Mycobacterium tuberculosis*. Note also range of concentrations of tubercle bacilli for optimal production of EAE in guinea pigs not skin-tested.)

Since this was the first demonstration of suppression of EAE so long after the injection of encephalitogenic vaccine, further experiments were performed to determine whether the suppression was real or some statistical artifact, whether it was due to the tuberculin, to the brain extract, or to some non-specific stress reaction, and whether there was any quantitative relation between the amount of antigen and the degree of suppression.

#### Method

In the first 4 experiments encephalitogenic vaccines were made containing 0.5 mg. (dry weight) heat-killed *Mycobacterium tuberculosis* and 0.5 mg. (dry weight) lyophilized whole

guinea pig brain in each 0.1 ml. of emulsion (2 parts Bayol F or heavy mineral oil, 1 part aquaphor, 1 part 0.85 per cent saline). Intradermal injection of 0.1 ml. was made over the sternum.

Skin test solutions of tuberculin (generously supplied as Corper's PPD by Parke, Davis and Company) were made in saline, and of acid extract of guinea pig brain (6) in Hanks buffered salt solution. Dialysis of the brain extract against cold Hanks solution was carried out for 24 to 48 hours followed by centrifugation to clarify the solutions which were of approximately neutral pH as indicated the phenol red indicator. Single or multiple intradermal injections of 0.1 ml. each were given in the previously shaved skin of the back. The amounts of antigens contained in each 0.1 ml. varied as follows:

PPD: 0.002 to 0.03 mg.

Acid extract: 0.4 to 0.6 mg. (representing about 50 per cent of the original extract, the remainder being insoluble in Hanks solution after dialysis).

Skin damage of approximately the same size (1 cm. in diameter) as the above skin tests was produced by the intradermal injection of 0.2 ml. of 20 per cent ethanol.

TABLE I  
*Calculation of Disease Index (19)*

Severity of histologic disease	Severity of clinical disease				Presence of lipemia
	0, ?	Definite paralysis	Killed when moribund	Died	
0, ?	0	1	2	3	0
	1	2	3	—*	+
Slight	1	4	6	7	0
	3	5	7	—*	+
Moderate to marked	4	8	9	10	0
	5	9	10	—*	+

\* Blood not collected from animals found dead.

The general design of the first 4 experiments was as follows: white male guinea pigs of mixed breed, each weighing about 500 gm., were obtained from May Rabbitry, San Antonio. Groups of 50 to 80 animals were injected with a particular encephalitogenic vaccine. Each animal was kept in an individual cage randomly arranged in the animal room with respect to each other. Subgroups of 5 to 10 animals previously identified by code numbers were injected with the skin test solutions on predetermined days (8 to 11, 13 to 15, and sometimes 18 to 21 and 28) following the injection of the encephalitogenic vaccine. Uninjected controls were shaved and handled the same way but were not skin-tested. Each animal was observed once or twice daily for evidence of weakness, paralysis, difficulty in righting, fecal impaction, weight loss, or ruffled fur. Unless definite weakness or paralysis was noted, the animal was considered only questionably involved; but if definite signs followed the development of questionable signs, the day of onset was considered to be that of the questionable signs. If the animal appeared moribund, it was killed; in any event all animals were killed on the 30th day, serum collected, and perfusion with agar-formalin (18) accomplished. The brain and spinal cord were examined in longitudinal sections stained with hematoxylin and eosin after embedding in paraffin. In the graphs of the incidence of disease by day of onset, those animals having significant histologic evidence but not having demonstrated clinical evidence of disease were arbitrarily added

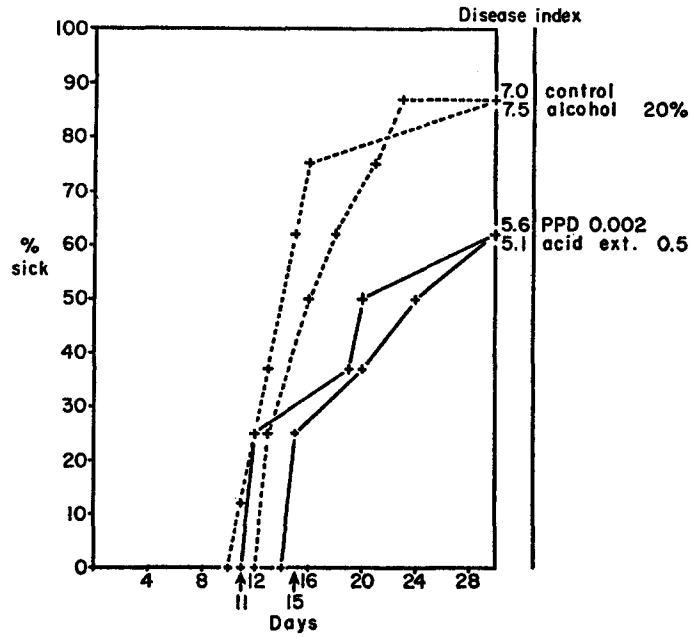


FIG. 2. Effect of skin tests at 11 and 15 days with acid extract of guinea pig brain, PPD, or alcohol (Experiment 2, Table II).

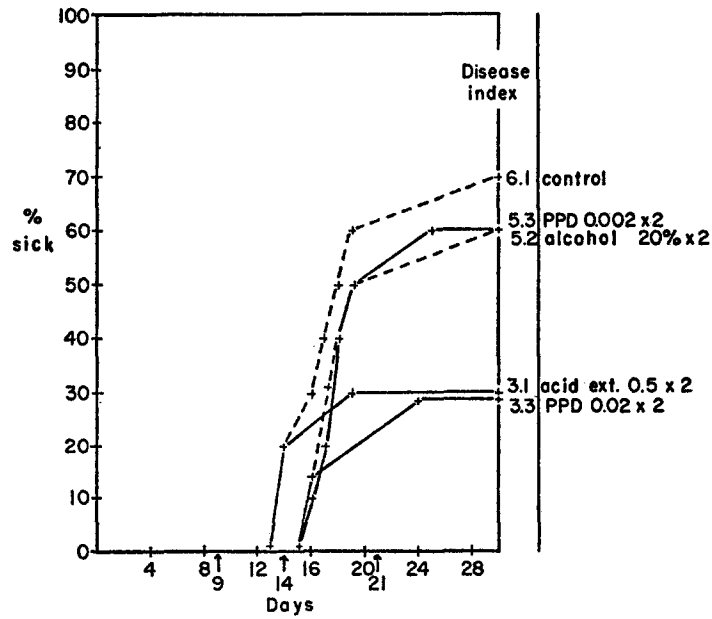


FIG. 3. Effect of skin tests at 9, 14, and 21 days with larger amounts of acid extract of guinea pig brain, PPD, or alcohol (Experiment 3, Table II).

to the graph on the 30th day. In addition, each animal was scored according to a graded response "disease index" (19), based on the severity of clinical, hematological, and histological abnormalities. Table I indicates the numerical score given each animal. An average disease index was obtained for each group of animals for statistical analysis and comparison.

Experiments 5 and 6 differed only in the following ways: the encephalitogenic vaccines contained 0.1 mg. *Mycobacterium butyricum* and 0.3 mg. lyophilized guinea pig brain. "Borate-soluble protein" (20) extracted from the acid extract (6) of guinea pig brain was dissolved in physiological saline at concentrations of 1 to 5 mg./ml., 2 intradermal injections of 0.1 ml. being given on the 8th, 13th, and 18th day after the challenge vaccine. Groups of 14 to 30

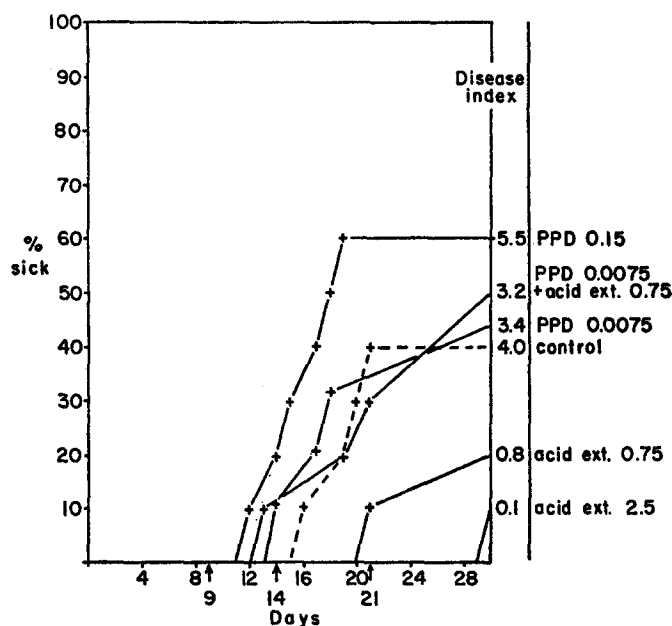


FIG. 4. Effect of skin tests at 9, 14, and 21 days with still larger amounts of acid extract of guinea pig brain or PPD (Experiment 4, Table II).

guinea pigs were obtained from a closed colony maintained at the National Institutes of Health, Bethesda.

#### RESULTS

The results can best be presented graphically (Figs. 2 to 4) and demonstrate that suppression of EAE induced in guinea pigs with whole homologous brain and adjuvants occurred regularly following intradermal injections of the acid extract of guinea pig brain. Furthermore, the more brain extract that was given, the more complete was the suppression: no statistically significant suppression occurred when 0.5 mg. was given on the 11th and 15th day (Fig. 2), but practically complete suppression occurred when 5 such injections were given on each of the 9th, 14th, and 21st days (Fig. 4).

A non-specific stress, such as provided by the intradermal injection of 20

per cent alcohol in amounts which produced approximately the same size skin reaction but with central necrosis, did not produce suppression of EAE

TABLE II  
Suppression of EAE by Acid Extract of Guinea Pig Brain, Alcohol or PPD after Challenge with Whole Guinea Pig Brain and Adjuvants\*

Experiment No.	Amount and No. of intradermal injections of			On days after challenge*	Clinical EAE‡	Disease index ± standard error of mean
	Acid extract	PPD	Alcohol			
1	mg 0	μg 0	0	0	4/5	7.6 ± 1.5
	0.5, 0.05	2, 0.2	0	11, 15, 21, 28	0/5	1.0 ± 0.8
2	0	0	0	0	6/8	7.0 ± 1.1
	0	0	×1	11, 15	6/8	7.5 ± 1.3
	0	2	0	11, 15	4/8	5.6 ± 1.6
	0.5	0	0	11, 15	4/8	5.1 ± 1.6
	0.5	2	0	11, 15	4/8	5.4 ± 1.6
3	0	0	0	0	6/10	6.1 ± 1.4
	0	0	×2	9, 14, 21	5/10	5.2 ± 1.4
	0	2, 2	0	9, 14, 21	6/10	5.3 ± 1.4
	0	20, 20, 2, 0.2	0	9, 14, 21	2/7	3.3 ± 1.3
	0.5, 0.5, 0.1, 0.02	0	0	9, 14, 21	3/10	3.1 ± 1.3
4	0	0	0	0	6/10	4.0 ± 1.0
	0	5, 2.5	0	9, 14, 21	3/9	3.4 ± 1.3
	0	30 × 5	0	9, 14, 21	6/10	5.5 ± 1.5
	0.5, 0.25	5, 2.5	0	9, 14, 21	3/10	3.2 ± 1.0
	0.5, 0.25	0	0	9, 14, 21	0/10	0.8 ± 0.4
	0.5 × 5	0	0	9, 14, 21	0/10	0.1 ± 0.1

\* Encephalitogenic vaccine in experiment 1 contained 0.3 mg. brain and 0.9 mg. *Mycobacterium tuberculosis*; all other groups were injected with vaccines containing 0.5 mg. of each in 0.1 ml. injected intradermally over the sternum.

‡ Numerator is number of guinea pigs with clinical EAE (definite paralysis), and denominator is number of animals injected.

(Figs. 2 and 3), and only inconstant effects were obtained with tuberculin (PPD).

The results of these and other similar experiments are summarized in Table II.

A similar series of experiments was performed using as skin test antigen that portion of the acid extract of guinea pig brain which is soluble in a pH 9 borate buffer. Recent evidence indicates that this soluble portion ("borate-soluble protein") has an even greater encephalitogenic activity than the acid

extract (20). Experiment 5 (Table III) with whole brain as the inciting material and borate-soluble protein ("BSP") as the skin test material showed complete suppression of clinical signs of disease. However, the histologic and clinical results were not well correlated in this series; *i.e.*, 6 out of 7 of the treated animals showed mild but definite inflammatory lesions. In spite of this, the disease index of the treated series was significantly less than that of the controls (3.4 as compared to 8.0). There is at the moment no explanation for the lack of correlation between clinical and histological results in this series. Experiment 6 repeated and extended these results by testing simultaneously a smaller dose of "BSP" and by omitting 1 or 2 of the 3 skin tests. It is evident that 1 mg. of

TABLE III  
Suppression of EAE by Skin Tests with "Borate-Soluble Protein" (BSP) after Challenge with Whole Guinea Pig Brain and Adjuvants\*

Experiment No.	BSP	On days after challenge*	Clinical EAE†	Disease index ± standard error of mean
5	mg. 0	0	7/7	8.0 ± 1.0
	0.5 × 2	8, 13, 18	0/7	3.4 ± 0.6
6	0	0	4/6	6.0 ± 1.7
	0.1 × 2	8, 13, 18	3/6	4.8 ± 1.6
	0.5 × 2	8	4/6	4.5 ± 1.4
	0.5 × 2	8, 13	3/6	5.0 ± 1.4
	0.5 × 2	8, 13, 18	0/6	0.7 ± 0.2

\* Encephalitogenic vaccine contained 0.3 mg. of brain and 0.1 mg. *Mycobacterium butyricum* in 0.1 ml. injected intradermally over sternum.

† Numerator is number of guinea pigs with clinical EAE (definite paralysis), and denominator is number of animals injected.

"BSP" injected intradermally on each of 3 days following the initial whole brain injection suppressed the disease clinically and decreased the severity of histological lesions. One or 2 such injections were not sufficient to alter the disease significantly, nor was 0.2 mg. injected 3 different times adequate for suppression.

It may be added that there was no systemic reaction to the intradermal injection of even large amounts of either PPD or neural extracts. Furthermore, the cutaneous reactions to the neural extracts did not differ in appearance from those in normal controls (21).

#### DISCUSSION

The results of the present experiments indicate that suppression of EAE induced by previous injection of whole homologous brain in Freund's adjuvants can be produced by skin-tests with relatively large amounts of aqueous solutions

of the same chemical component of homologous brain which most effectively produces the disease when mixed with Freund's adjuvants. The inconstant suppressive effects of tuberculin are most easily attributed to statistical variations which occur commonly in biological systems.

Although the vaccines were prepared on the basis of previous experiments (19) to produce maximal disease with an average disease index of about 8, the present results in the controls were generally not quite so great (disease indices of 6.0 to 8.0); and in the particular experiment (Fig. 4) in which the most complete suppression occurred the encephalitogenic vaccine seemed the least effective (disease index of only 4.0). The reasons for this variation from the expected results in the controls are certainly not clear. It is quite likely, however, regardless of whether EAE is due to hypersensitivity or not, that the immunologic events developing in the treated animal are quite complicated: not only must there be some competition of the many antigens in the original encephalitogenic vaccine (tubercle bacilli and whole brain) with the production of many circulating antibodies and tissue hypersensitivities, but in addition there must be some competition between the encephalitogenic power of the vaccine containing whole brain and the suppressive ability of the brain extract which contains relatively few neural proteins. In such a complicated biological system one must await further experiments before any definitive conclusions can be drawn, but one may anticipate that more complete suppression will be obtained if the original encephalitogenic vaccine and the skin test solution contain the same neural extract, that better routes and timing of the treatments will be found, and that the interrelationships between the many chemical components of whole brain and their ability to produce EAE may be demonstrated by the suppressive ability of each individually or in combination.

A distinction probably should be made between *prevention*, *suppression*, and *therapy* of EAE: *prevention* by treatments given before the injection and *suppression* by treatments given after the injection of the encephalitogenic vaccine, and *therapy* by treatments given after the onset of clinical evidence of disease. These distinctions are admittedly arbitrary, and the mechanisms underlying these 3 phenomena may or may not be identical or related. There are probably several different immunologic mechanisms underlying *prevention* (22): immunologic tolerance (7), circulating protective or blocking anti-brain antibodies (reference 23, but not reference 24), and a Koch reaction (13, 25). Several other non-immunological methods have been used to prevent and/or to suppress EAE: x-irradiation, cortisone (26, 27), ACTH (28), salicylate and *para*-aminobenzoic acid (29), not always successfully (30, 31).

According to these arbitrary definitions, the present experiments are considered to represent *suppression*, rather than prevention or therapy. The lack of suppression by intradermal injections of dilute alcohol producing comparable cutaneous lesions indicates that the suppression by the encephalitogenic neural proteins is not due to a non-specific stress reaction (9), but



unfortunately no further conclusions are warranted at this time as to the specificity of suppression for encephalitogenic as compared to non-encephalitogenic extracts of brain or as to the pathogenesis of the suppression. Some clue concerning the pathogenesis may be afforded by the quantitative data, which indicates that the greater the amount of active extract given, the more suppression is produced, and which suggest some specific desensitization, deflection, or neutralization of antibodies, or paralysis of antibody-forming mechanisms. If suppression is on an immunologic basis, however, it seems strange that the skin reaction was no greater than that which occurred in controls (21).

#### SUMMARY

The intradermal injection of aqueous solutions of certain homologous neural proteins will suppress the encephalomyelitis which is induced in guinea pigs by the previous injection of whole homologous brain with Freund's adjuvants. These neural proteins extracted by dilute acid from defatted guinea pig brain are themselves highly encephalitogenic when injected with adjuvants, but the specificity of this suppression for encephalitogenic as compared to non-encephalitogenic extracts remains to be proven.

Suppression is probably not due to a non-specific stress reaction, as indicated by the absence of suppression by intradermal injections of alcohol and by statistically insignificant and inconstant effects of similar injections of tuberculin.

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