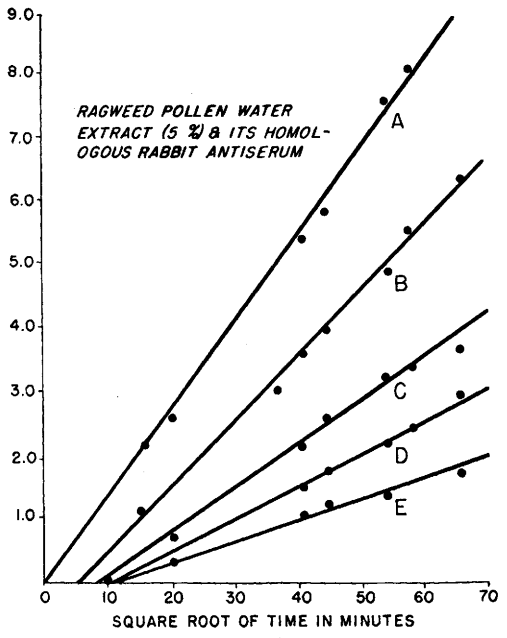

FIG. 1.

Movement of 5 bands of precipitate obtained with ragweed pollen extract and its homologous antiserum plotted against the square root of the time.

least 5 distinct substances in ragweed pollen extracts which are antigenic for rabbits. That the antigenic complexity may be even greater than this is indicated by the observation that complete absorption of the ragweed extract with rabbit antiserum markedly reduces the direct skin reactivity in specifically sensitive human beings, but does not abolish it.[13] The relationship of these 5 antigens to the various chemical[1] and electrophoretic[2] fractions shown to exist in ragweed pollen extracts is not known at present, and more work is needed to elucidate this problem. The technic of Oudin shows great promise in the fractionation of antigenic mixtures. It has been applied by Oudin to the fractionation of horse serum[6] and by us to demonstrate the presence of at least 3 antigens in cytochrome c of 0.34% iron content.[14] Moreover, work from Oudin's laboratories[9] and from our own (unpublished experiments) indicates that it may be possible to apply his method as a quantitative tool for the determination of either antigen or antibody concentrations.

Conclusion. Application of the technic of Oudin to ragweed pollen extracts and their antisera demonstrated the presence of at least 5 different antigens in such extracts.

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[14] Becker, E., and Munoz, J., *J. Immunol.*, 1949, **63**, 173.

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Allergic Encephalitis Following Injection of Dialysate of Brain Tissue. (17410)

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The encephalitic process which follows the injection into monkeys[1,2] and guinea pigs[3,4] of homologous or heterologous brain tissue has been variously termed meningo-encephalomyelitis,[7] acute disseminated encephalomyelitis,[1] isoallergic encephalomyelitis[3] or allergic encephalitis,[5] and has been compared with the post-vaccinal (rabies) paralysis of human beings.[10] The nature of the factor in brain

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[1] Kabat, E. A., Wolf, A., and Bezer, A. E., *J. Exp. Med.*, 1947, **85**, 117.

[2] Morgan, I. M., *J. Exp. Med.*, 1947, **85**, 131.

[3] Freund, J., Stern, E. R., and Pisani, T. M., *J. Immunol.*, 1947, **57**, 179.

[4] Kopeloff, L. M., and Kopeloff, N., *J. Immunol.*, 1947, **57**, 229.

[5] Bell, J. F., Wright, J. T., and Habel, K., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 457.

which is responsible for this process is not known, although reports have appeared showing that the factor is present in the phosphatide fraction of brain[7,11] and in the protein fraction remaining after extraction of all brain lipoids.[11] Work on the purification of rabies vaccine[5] has shown that the factor which causes allergic encephalitis is soluble in a solution of calcium acetate. This factor was separated from rabies vaccine by washing the infected brain tissue carefully with calcium acetate solution after extraction with benzene and ether. When incorporated into the mineral oil adjuvant described by Freund and McDermott,[6] the separated factor produced allergic encephalitis in guinea pigs. This report describes some observations made during a study of the properties of the factor causing allergic encephalitis.

The procedure by which dialysates of brain tissue were prepared is an extension of the method[5] for freeing rabies vaccine from the factor causing allergic encephalitis. Brain tissues from rabbits and cattle were minced in a Waring Blendor, suspended in distilled water, and the suspensions dried from the frozen state. The dried brain was extracted with benzene followed by two extractions with ether. After removal of the ether, the brain tissue was suspended in distilled water. When the suspension had become thoroughly hydrated, it was further diluted with an equal volume of M/5 calcium acetate solution, or in some experiments with distilled water. The mixture was placed in cellophane tubing and suspended in 4 volumes of distilled water. All cellophane tubes were tested by filling them with water and observing for leaks while moderate pressure was applied to the top of the tubes. Care was taken to wash the end of the tubing through which brain suspension was introduced so that the dialysate would not be contaminated. After storage at 6° C for 3 to 4 days with frequent agitation, the clear dialysate, which now had a slightly amber tint, was removed and dried from the frozen state. This dialysate usually contained 2 to 4 mg of solids per milliliter at a concentration equivalent to an original 10% brain emulsion.

The presence of the allergic factor in the brain dialysate was determined by mixing a 5 ml sample with 5 ml of the adjuvant (4.5 ml of mineral oil containing 9 mg of dried acid fast bacilli [B.C.G.], plus 0.5 ml Arlacel A) and injecting 1 ml of the mixture subcutaneously into each 200-250 g guinea pig. The animals were observed for at least a month following injection. Those which developed only slight weakness in the hind quarters are recorded as having doubtful symptoms, those with definite weakness or paralysis as positive. Forty-six of the 74 animals used were killed for histological study in various stages of the disease, after clinical recovery, or after 30 to 60 days had elapsed without clinical evidence of allergic encephalitis. Therefore, final mortality figures could not be tabulated. Brains and spinal cords were fixed in formalin and sections through the thalamus, pons, and several levels of the cord were examined. The lesions found were similar in all respects to those previously produced by the injection of unmodified brain tissue in adjuvant. They consisted of scattered, perivascular cuffs of mononuclear and microglial cells about venules in the cerebral white matter, hippocampus, thalamus, pons, cerebellar white matter, and cord. The size and number of the lesions varied greatly in individual animals.

Brain preparations which produced either positive clinical signs or characteristic microscopic brain lesions were listed as containing the factor. In most cases a positive histopathological diagnosis was related to positive clinical signs. In a few cases characteristic lesions were found in animals which showed no clinical signs of the disease. The results of 6 experiments on dialysis are summarized in the accompanying table.

It will be noted that animals injected with the brain preparations either before or after dialysis showed evidence of allergic encepha-

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TABLE I.
Allergic Encephalitis Produced in Guinea Pigs by Various Fractions of Brain Tissue.

Exp. No.	Material*	Amt inj.,† mg	Diagnosis‡		
			Clinical		Histopathological
			Positive	Doubtful	Positive
216-A	Cal acetate extr. of normal rabbit brain before dialysis	50	2/6	2/6	1/6
B	Dialysate of normal rabbit brain	50	9/20	8/20	12/18
254	Dialysate of normal rabbit brain	40	3/5	0/5	3/3
241	(Same)	25	2/10	2/10	2/2
233-A	Water extr. of normal beef brain before dialysis	100	6/9	2/9	1/3
251-B	Dialysate of normal beef brain	250	2/10	2/10	5/10
233-B	(Same)	100	2/9	2/9	1/3
D	(Same)	50	1/5	0/5	1/1

* All tissues were previously dried and extracted with benzene and ether.
† Expressed as equivalent weight of wet whole brain.
‡ Denominator of fraction indicates number of animals in each experiment.

litis. In no instance, however, did all of the animals injected with any one preparation develop the characteristic signs or lesions of the disease. Since the incidence of this disease following one dose of whole brain in adjuvant has been variable[4] no attempt was made to determine the amount of factor present by the incidence of positive reactions in animals. All observations which showed a positive picture of allergic encephalitis are regarded as significant.

On the basis of the above it is evident that the substance which causes allergic encephalitis is capable of passing through a cellophane membrane and, therefore, has a molecular weight of approximately 10,000 or less. It will be recalled that Alvord[7] found the factor to be a heat-stable, phosphatide-like substance which might possibly resemble the alcohol-soluble brain-hapten of Lewis[8] and Rudy.[9] Very little is known regarding the properties of the materials recovered in the dialysate, except that the mixture contains salts, amino acids and other substances and is Biuret negative at a concentration equivalent to 200% brain tissue, *i.e.*, a solution with 4 to 8% total solids.

Summary. The dialysate of benzene-ether extracted brain tissue is capable of causing signs and lesions of allergic encephalitis in guinea pigs. The material recovered in the dialysate is free of protein and presumably contains only substances of low molecular weight.

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