much controversy have been reported not to contain hyaluronidase(9). Experiments are now in progress to determine the role of estrogen and progesterone upon the elaboration of the spreading factor by the mammary gland.

Summary. 1. Spreading type assays made on extracts of mammary glands taken from female albino rats pregnant from 3 to 18 days indicated the presence of a spreading factor. The concentration of the factor increased until about the twelfth day followed by a gradual decline. 2. Extracts of mammary glands from normal male and female rats, as well as castrate animals, assayed by the spreading method failed to exhibit any spreading effect at the dilution employed. 3. Turbidimetric assays of mammary gland extract showed that the spreading factor did not hydrolyze the substrate, hyaluronidate. 4. Tests showed that after heating at 60°C for 10 minutes, the spreading factor was still active but inactive after heating at 70°C for 10 minutes.

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Action of Adrenocorticotropic Hormone (ACTH) in Experimental Allergic Encephalomyelitis of the Guinea Pig. (18207)

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Recent observations in the experimental (1,2) and clinical(3) fields suggest that administration of ACTH inhibits allergic reactions. The type of encephalomyelitis elicited in the guinea pig by the injection of brain emulsion with adjuvants(4-7) provides a convenient laboratory example of an allergic condition since it can be produced easily with a single inoculation of antigen, follows a regular pattern, and shows distinctive clinical and pathological features. Therefore, it was considered of interest to investigate the effect of ACTH upon the occurrence and severity of this type of experimental allergic encephalomyelitis.

Material and methods. Eight groups of guinea pigs, each weighing 300-350 g were given a single injection of rabbit-brain emulsion with adjuvants in the manner previously described(5). Two groups served as controls and received no further treatment, whereas the other 6 groups received injections of ACTH. A purified hormone preparation was used which was assayed for ACTH potency by the ascorbic acid depletion method of Sayers(8). A typical assay of potency on this

TABLE I. Assay of Potency of ACTH Preparation

Dose inj. per 100 g rat body wt, γ	Decrease in ascorbic acid mg/100 g adrenal tissue
0.5	43
2.0	112
8.0	158

1. Selye, H., *Canad. Med. Assn. J.*, 1949, v61, 553.

2. Berthrong, M., Rich, A. R., and Griffith, P. C., *Bull. Johns Hopkins Hosp.*, 1950, v86, 131.

3. *Proceedings of the First Clinical ACTH Conference*, Ed. by J. R. Mote, Blakston Co., New York, 1950.

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

5. Jervis, G. A., and Koprowski, H., *J. Neuropath. and Exp. Neurol.*, 1948, v7, 309.

6. Cazzullo, C. L., and Ferraro, A., *Arch. Path.*, 1949, v48, 316.

7. Alvord, E. C., *J. Immunol.*, 1949, v61, 355.

8. Sayers, M. A., Sayers, G., and Woodbury, L. A., *Endocrinology*, 1948, v42, 379.

TABLE II. Effect of ACTH on Experimental Allergic Encephalomyelitis Induced in Guinea Pigs.

Group No.	Admin. of ACTH after inj. of brain emulsion: No. of 5 mg doses	Total dose, mg	Days of inj.	No. of animals	No. of guinea pigs that: Remained normal	Showed paralysis: Died	Recovered	Sacrificed	Pathologic lesions
468	13	65	1st-14th	10	10*	—	—	—	(See text)*
512	12	60	1st-13th	10	6	—	2	2	Minimal
511	4	20	5,8,11,15th	10	7	2	1	—	Mild
509	2	10	1st & 2nd	10	5	1	1	3	Mild to moderate
510	2	10	5th & 10th	9	6	—	—	3	Moderate to severe
508	2	10	10th & 11th	8	4	1	1	2	" " "
Controls									
469				9	1	1	2	5	Severe
513				11	2	3	4	2	"

* 5 animals were sacrificed for histologic examination; 2 showed no apparent lesions, in the other 3 very small subpia lesions and few single perivascular infiltrations were observed.

material is shown in Table I. It should be mentioned that the presence in this preparation of small amounts of pituitary hormones other than ACTH could not be excluded. The ACTH was administered by the subcutaneous route in a 5 mg (0.5 ml) dose, according to the schedule shown in Table II. The animals were kept on normal laboratory diet and examined daily for evidence of illness or paralysis. From each group 2 or more animals were examined histologically. With the exception of Group 468, those animals in each group which showed the most severe clinical manifestations of involvement of the central nervous system were selected for examination. The entire brain and spinal cord were fixed in formalin, embedded in paraffin, and cut serially at 25 μ. Each tenth section was stained with Einarson's gallocyanin method(9). A series of some 200 sections was thus examined in each case. This procedure enabled an adequate appraisal of the intensity and distribution of the lesions.

Results. It may be seen from the results summarized in Table II that high dosage of ACTH (total 65 mg), started on the day after the injection of brain emulsion and continued until the time when usually symptoms are apparent, protected all of the animals in Group 468, none of which developed clinical signs of the disease. Five animals from this group were sacrificed and studied histologically. Two showed no apparent lesions; in the other 3 very small subpia lesions and a very few single perivascular infiltrations were found which showed little tendency to spread into the surrounding tissue (Fig. 1a). It should be noted that in the specimens from the control animals almost every section showed multiple lesions (Fig. 1b). The contrast between treated and untreated animals was striking, from a pathological point of view. In the second group (512) of guinea pigs which had been given a similarly high dose of ACTH (total 60 mg), 4 developed paralysis; 2 of these recovered and 2 were sacrificed. The lesions seen were of about the small size as those observed in the preceding group except that they were more frequent in number. That a smaller dose (total 20 mg) still affords significant protection is shown by Group 511 in which 7 out of 10 animals showed no signs of the disease. The 2 animals of this series, sacrificed when paralyzed, showed small perivascular infiltration, more diffuse in distribution than in Group 512.

In an attempt to establish the minimal protective dose, and the time at which the hormone becomes effective, 3 experiments were carried out. Group 509 showed that some protection is obtained from small doses (total 10 mg), provided that the hormone is given early after the injection of antigen, as evidenced by the difference in the pathologic lesions seen in Groups 510 and 508 and those in Group 509. Although the number of guinea pigs affected in these 3 groups was not

9. Einarson, L., *Am. J. Path.*, 1932, v2, 295.

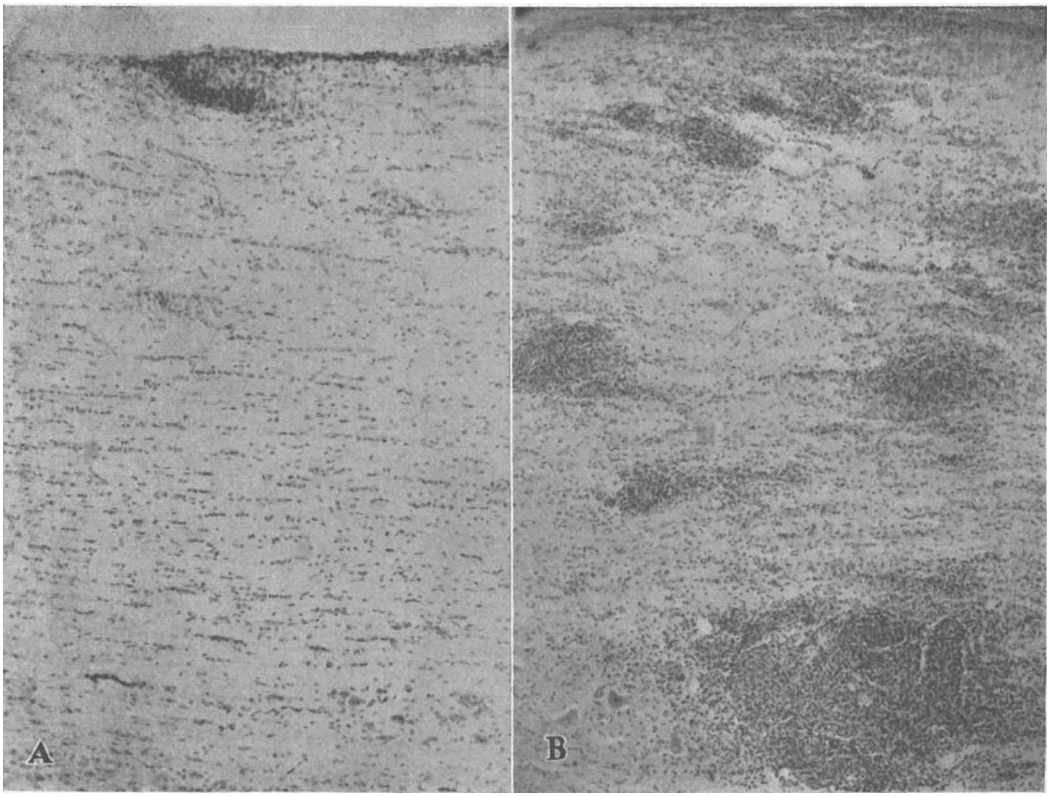

FIG. 1.
White matter of spinal cord—Einarson's stain low power. (a) Animal treated with ACTH showing only a small perivascular lesion. (b) Control animal showing extensive encephalitic lesions.

strikingly different, the pathologic lesions were much more severe and diffuse when the administration of ACTH was delayed until the 10th day after the injection of brain emulsion. Additional experiments are in progress, aiming at a more exact determination of effective dosage and time of administration of the hormone.

Comments. The available evidence on the biological effects of ACTH being still scanty and contradictory, it is premature to offer a satisfactory explanation of the mechanism of protection showed by ACTH in experimental encephalomyelitis of the guinea pig. It may be pointed out, however, that since allergic encaphalomyelitis is presumably associated with the formation of brain antibodies(10), the protective action may be due to the inhibitory effect of ACTH on antibody formation, as suggested by Fischel(11). In this connection it is of interest to note that other agents, such as nitrogen mustard(12) and salicylates(13), which are known to block antigen antibody reactions, offer some protection against the development of experimental encephalomyelitis of the guinea pig. Additional evidence in favor of the inhibiting action of the hormone on allergic phenomena is reported by Selye(1) who demonstrated that ACTH inhibits the anaphylactoid reaction brought about by the parenteral administration of egg white, and by Berthrong *et al.*

10. Koprowski, H., and Jervis, G. A., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 472.

11. Fischel, E. E., *Bull. N. Y. Acad. Med.*, 1950, v26, 255.

12. Koprowski, H., Jervis, G. A., and Black, J., Unpublished experiments.

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

(2) in experiments on vascular hypersensitivity.

On the other hand, the mechanism of the action of ACTH in allergic encephalitis might be purely at a vascular level. It has been shown by Soffer *et al.*(14) that ACTH suppresses the Schwartzmann phenomenon when administered prior to the provocative injection. This phenomenon is admittedly not anaphylactoid in nature, and its cause is to be found in a profound alteration of vascular reactivity(14). Pathologic studies of allergic encephalomyelitis(5) show that the lesion begins in and around the blood vessels from which it spreads into the nervous parenchyma. Therefore, it is possible that by modifying the vascular reactivity in a manner similar to that observed by Soffer(14), ACTH protects the nervous tissue against the development of encephalitis lesions. This hypothesis is, at present, the object of further investigation in guinea pigs and in monkeys.

Although the relation of experimental encephalomyelitis in animals to human disease is still controversial, it would be of interest to investigate the action of ACTH, or of cortisone, in the prophylaxis of post-vaccination encephalitis and in the prevention of relapses of certain acute forms of multiple sclerosis, two types of human disease in which pathogenic factors similar to those operating in the experimental encephalomyelitis of the guinea pig are presumably playing a role.

Summary. Timely administration of adequate doses of ACTH shows an inhibiting action upon the occurrence of clinical manifestations and the development of pathological lesions in experimental allergic encephalomyelitis of the guinea pig.

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The Determination of Radioactive Iodine in Biologic Material.* (18208)

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Most of the methods for the determination of radioiodine in the presence of organic material are insensitive and time-consuming. The purpose of this paper is to describe a method which obviates to an appreciable extent these disadvantages.

Reagents and apparatus. Sulfuric acid, 18 N; potassium permanganate; oxalic acid, saturated aqueous solution; lithium arsenite, a solution containing 0.74 g of arsenious oxide (As_2O_3) and 1.20 g of lithium hydroxide (LiOH) per 100 cc of solution; modified Chaney distillation unit(1,2); Geiger-Müller beta ray counter, with appropriate scaler.

Experimental. The material (blood, urine or tissue) to be analyzed is placed in a 500 cc pyrex digestion flask to which is added 1 g of potassium permanganate and 18 cc of 18 N sulfuric acid for each 50 mg of organic material. (Caution: The addition of undiluted concentrated sulfuric acid to permanganate is dangerous.) As carrier, 1 cc of a solution containing 1 mg of sodium iodide per cubic centimeter is added to the flask. The flask is heated by either a gas flame or an electric heater until dense white fumes form (186°-190°C). The flask is then cooled, 25 cc of distilled water is added and

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2. Talbot, N. B., Butler, A. M., Saltzman, A. H., and Rodriguez, P. M., *J. Biol. Chem.*, 1944, v153, 479.