of significance but is difficult to explain on the basis of our present knowledge.

Summary. Growth of the mammary gland of the rat during normal pseudopregnancy was only 18% of the growth found at day 18 of normal pregnancy as measured by DNA. This suggests that the corpora lutea of pseudopregnancy function at approximately one-fifth the level of those in pregnancy. Hysterectomy, which resulted in a 6-day extension of pseudopregnancy, was without effect in the enhancement of mammary gland growth over normal pseudopregnancy. However, the ratio of RNA to DNA in the mammary glands of these animals was raised significantly. The production of deciduomata concomitant with pseudopregnancy resulted in a 9-day extension of pseudopregnancy and a slight increase in mammary gland growth over that of normal pseudopregnancy. It was only 30% of that at day 18 of pregnancy and 76% of that at day 12 of pregnancy when DNA was standardized to body weight.

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Mycobacterial Suppression of Delayed Hypersensitivity in Experimental Allergic Encephalomyelitis (32981)

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The theory that delayed hypersensitivity is involved in the induction of experimental allergic encephalomyelitis (EAE) is supported by the good correlation between delayed skin reactivity to the encephalitogenic protein 10 days after challenge and the subsequent development of disease (1–4). Shaw *et al.* (2) have suggested that the skin test is sufficiently reliable in its prediction of illness to provide a valuable "shortcut" in the testing of unknown fractions. In their analysis of delayed hypersensitivity in EAE, these authors also reported that both manifestations of delayed hypersensitivity (positive skin test and induction of disease) were suppressed by multiple injections of basic protein (BP) in Freund's incomplete adjuvant (whether the injections were made before challenge or in the interval between challenge and development of clinical signs).

The mechanism of disease suppression by injection of basic protein in incomplete adjuvant has not been clarified. It is possible that circulating antibody interferes somehow with the sensitization or proliferation of specific cells. This has been difficult to evaluate in the past because of the inadequacy of the methods available for detection of antibodies to encephalitogenic BP. Falk *et al.* (5) have recently shown that antibody to homologous

TABLE I. Effect of Concentration of Mycobacteria in Challenge Injection on the Induction of Delayed Hypersensitivity to Myelin Basic Protein (BP).

Strain of mycobacteria (mg)	Skin reaction at 10 days (mm ± SE)		EAE No. positive[a] /no. tested
	vs BP	vs PPD	
M. butyricum, 0.1	7.3 ± 0.5	13.5 ± 0.8	8/10
M. tuberculosis, 0.1	7.6 ± 0.6	15.8 ± 1.3	4/5
M. butyricum, 2	1.6 ± 0.7	12.8 ± 1.5	0/5
M. tuberculosis, 2	1.2 ± 0.7	14.4 ± 0.8	1/5
Normal controls	0 ± 0	0 ± 0	0/5

[a] Animals with a disease index of 4 or greater; range 0–10 (13).

BP detected by passive cutaneous anaphylaxis (PCA) in the guinea pig is present in high titers in some but not all sera of hyperimmunized (EAE suppressed) guinea pigs. It seems likely, therefore, that this type of antibody is not necessary for disease suppression. It is important to note that the PCA technique detects only 7S-γ_1 globulins when the test is applied to guinea pig sera in the guinea pig.

The present study was undertaken to evaluate delayed hypersensitivity and circulating antibody production under experimental conditions which achieve suppression of EAE without pretreatment with encephalitogen. It has been reported that EAE can be prevented by pretreatment of guinea pigs with Freund's complete adjuvant (6–8) or by the use of excessive amounts of mycobacteria in the challenge injection (9,10). The latter is particularly effective when purified BP rather than whole central nervous system (CNS) is utilized as the encephalitogenic agent (10).

The method chosen for detection of antibodies in this study was direct radioautography of ^{125}I-labeled BP–antibody complexes which has been shown to detect all of the major antibody types (11,12).

Methods. Hartley albino male guinea pigs weighing 350–500 gm were challenged with 20 μg of guinea pig BP emulsified in oil–Aquaphor mixtures containing the amount and type of mycobacteria stated in Table I. Skin tests were performed on the tenth day after challenge and read 24 hours later. The solutions used for skin test were: 15 μg of BP in 0.1 ml of physiologic saline; 5 μg of PPD (0.1 ml second strength); and 0.1 ml of physiologic saline. Normal unchallenged guinea pigs were used as controls.

To evaluate the effect of pretreatment with adjuvant on delayed hypersensitivity and antibody production, individual guinea pigs were injected in an anterior foot pad with 0.1 ml of complete Freund's adjuvant containing 0.1 mg *M. butyricum* in 1 part saline, 1 part Aquaphor, and 2 parts mineral oil. One month later each animal was challenged with 20 μg of guinea pig BP incorporated in 0.1 ml of the same adjuvant emulsion injected intracutaneously over the sternum. At the same time, a group of normal guinea pigs also received the challenge injection. These 2 experimental groups were skin tested on the tenth day after challenge. Clinical signs, histological studies, and disease index were noted according to the method of Alvord and Kies (13).

Serum from guinea pigs pretreated with mycobacteria was obtained 8 days prior to challenge (23 days after the injection of 100 μg of mycobacteria in an oil–Aquaphor emulsion). All animals were bled on days 5 and 12 after challenge and, if they were still alive, on days 19 and 26. Serum was also obtained from sick animals at time of sacrifice (14–19 days after challenge). Circulating antibody to homologous encephalitogenic basic protein was determined by radioimmunodiffusion and radioimmunoelectrophoresis as previously described (11,12,14–17). Sera from animals protected by pretreatment with homologous BP in incomplete Freund's adjuvant known to have circulating antibody (5,16,17) were used as positive controls and normal guinea pig serum served as a negative control. All

TABLE II. Effect of Pretreatment with Freund's Adjuvant on Skin Reactivity and Disease Induction.

Freund's adjuvant pretreatment	Skin reaction at 10 days (mm ± SE) vs BP	vs PPD	EAE No. positive[a] /no. tested
+	3.4 ± 0.5	19 ± 1.1	0/9
—	7.2 ± 0.6	10 ± 0	5/5

[a] Animals with a disease index of 4 or greater; range 0–10 (13).

radioautographs were coded and read by 2 investigators.

Results. As summarized in Tables I and II, pretreatment with complete Freund's adjuvant or use of relatively large amounts of mycobacteria in the challenge with purified encephalitogen inhibits both disease induction and development of delayed hypersensitivity to myelin BP.

In none of the experimental groups was any serum antibody to BP detected—active disease induction with effective combinations of BP and mycobacteria, EAE prevention by pretreatment with mycobacteria, or disease suppression by incorporation of large amounts of mycobacteria in the challenge. In contrast, antibody to BP is often detected in sera from guinea pigs suppressed with BP in incomplete adjuvant either with or without subsequent challenge (Fig. 1).

Discussion. The present report deals with two immunologic phenomena frequently associated with EAE and thought by some investigators to be causally related either to disease induction or suppression: (i) Delayed hypersensitivity as demonstrated by the presence of a delayed skin reaction to basic protein, and (ii) formation of circulating antibody to BP.

It has been reported (2) that delayed hypersensitivity to BP and susceptibility to EAE are abolished simultaneously by hyperimmunization of guinea pigs with BP in incomplete adjuvant (a nonencephalitogenic emulsion). Whether or not the two phenomena are dependent upon the presence of circulating antibody which could be demonstrated in a high percentage of sera from these hyperimmunized animals was open to question. We have now succeeded in abolishing delayed hypersensitivity to BP and susceptibility to EAE without induction of detectable serum antibody to BP.

In addition to the demonstration that EAE can be suppressed without concurrent induction of circulating antibody, it should be pointed out that the present data support the observation of Shaw *et al.* (2) that delayed hypersensitivity to BP correlates with EAE induction. This is in contrast to two other reports which failed to confirm the correlation (18,19). In one of these (18), the skin reactions were probably negative because the tests were made during active disease with insufficient antigen in the test solution. The second report (19) described a positive skin reaction in protected guinea pigs. In these experiments, both suppression and challenge injections contained whole CNS (in place of BP) and the solution used for skin test was not adequately defined. The significance of the delayed hypersensitivity reported was thus not clear and may not have involved reactivity to BP.

The inhibition of EAE and delayed hypersensitivity to BP by the large dose of mycobacteria used in these experiments is not a function of the amount of mycobacteria *per se* since Falk *et al.* (20) obtained positive skin tests to BP in guinea pigs challenged with 2.5 mg of mycobacteria combined with an equally massive dose of whole wet cord (125 mg). This challenge is uniformly effective in inducing a positive skin reaction to BP 10 days after challenge followed by severe disease in either NIH Hartley, or Strain 13 guinea pigs.

Lack of circulating antibodies in guinea pig sera following induction of EAE with homologous BP extends the results of Falk *et al.* (5) who failed to detect any anti-BP in the 7S-γ_1 globulin fraction of guinea pig sera following EAE induction. Alvord *et al.* (21) were also unable to detect precipitating antibody to BP following homologous EAE induction in guinea pigs. The method used for antibody detection in the present paper is a more inclusive technique than the PCA or precipitin reaction. However, the possibility remains that failure to detect serum antibody

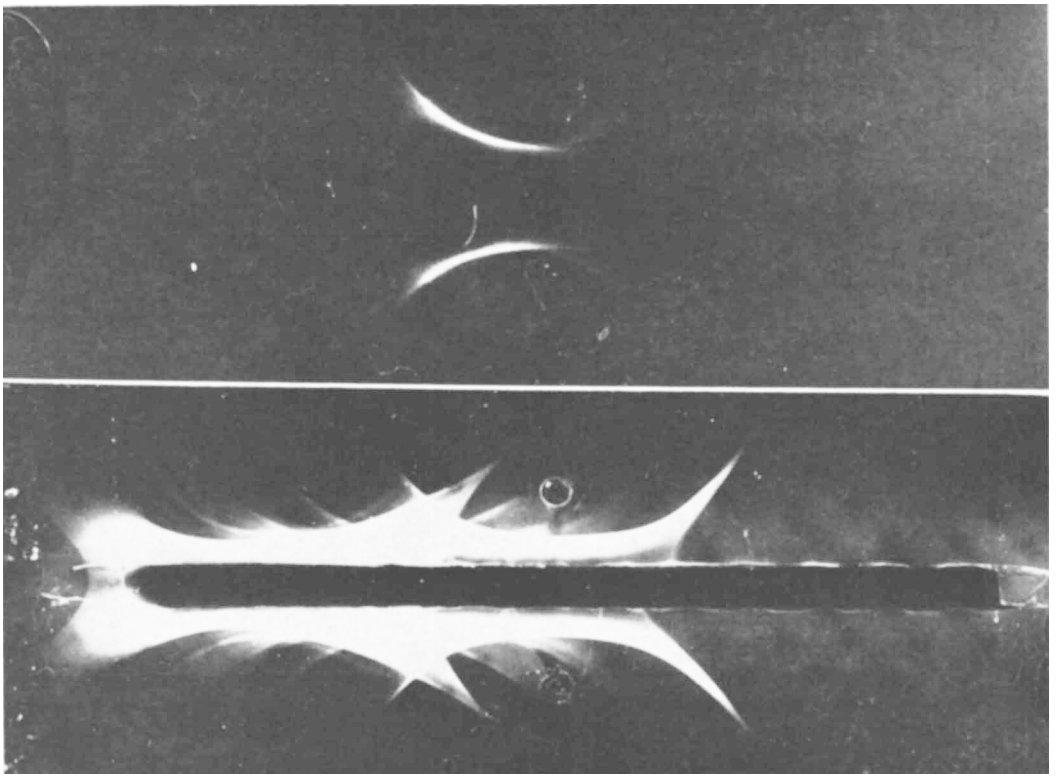

FIG. 1. Radioimmunoelectrophoresis of guinea pig antiguinea pig basic protein serum incubated with ^{125}I-labeled basic proteins. A:(*top*) Stained immunoelectrophoresis pattern. B:(*bottom*) Autoradiograph. (L) well: serum from a hyperimmunized protected animal 7 weeks after beginning immunization, 4 weeks after encephalitogenic challenge, incubated with ^{125}I-labeled guinea pig basic protein. (R) well: same serum as (L) well incubated with ^{125}I-labeled bovine basic protein. Trough: Rabbit antiguinea pig serum.

stems from the fact that antibody may be continuously taken up by the CNS and, therefore, not detectable (22,23). During active disease induction, when damage to the blood brain barrier is known to occur (24,25), CNS fixation of antibody is possible though it has never been demonstrated. When EAE is suppressed and there are no CNS lesions, the possibility that serum antibody is being taken up by the CNS is less probable than during active disease.

Summary. Experimental allergic encephalomyelitis, induced by injection of myelin basic protein in Freund's adjuvant, can be suppressed either by pretreatment with Freund's adjuvant alone (heat-killed mycobacteria in an oil–Aquaphor emulsion) or by addition of extra heat-killed mycobacteria to the encephalitogenic emulsion. Suppression of disease is accompanied by disappearance of skin reactivity to myelin basic protein. No circulating antibody to BP was detected in the sera of the suppressed animals prior to or at any time up to 26 days after challenge. Thus the suppression of delayed hypersensitivity noted in the present experiments cannot be attributed to presence of circulating antibody to myelin basic protein. Simultaneous suppression of skin sensitivity and CNS lesions strengthens the hypothesis that the two phenomena are related manifestations of delayed hypersensitivity.

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Immunofluorescent Localization of Parathyroid Hormone in Extracellular Spaces of the Bovine Parathyroid Gland* (32982)

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Various investigators have described intercellular spaces in the parathyroid glands of several species. A significant role for these spaces in the physiology of the gland has not previously been shown. Allara (1), using light microscopy, described spaces between parenchymal cells in human parathyroid glands. He pointed out that these spaces were surrounded by two thin membranes. Ham (2) noted that clumps of chief cells may form follicles in the parathyroid glands of older people and suggested that these follicles might represent locations for the storage of secretions. Trier (3), using light microscopy, demonstrated the presence of intercellular spaces in the parathyroid glands of the macaque monkey. He found that the membranes surrounding the spaces stained intensely with periodic acid Schiff, and that material within the spaces stained only faintly. In electron microscopic studies, Lever (4) identified subendothelial and parenchymal spaces and described the presence of an amorphous material within these membrane limited spaces. Munger and Roth (5) have also described connective tissue spaces in the parathyroid

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