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SUPPRESSION BY CORTISONE OF GRANULOMA FORMATION AND ANTIBODY IN GUINEA PIGS RECEIVING EGG ALBUMIN WITH FREUND ADJUVANTS

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CORTISONE and corticotropin have been found to inhibit antibody formation in rabbits after repeated injection of antigen intracutaneously or intravenously.^{1, 2} The inhibition is manifested when cortisone is administered during the primary response, after immunization is well established, and still later on elicitation of the secondary response.³ A striking diminution of lymphoid tissue and disruption of the normal architecture of the lymphoid organs are associated with the administration of adrenal cortical hormone.^{4, 5}

The development of acute disseminated encephalomyelitis in animals receiving homologous brain emulsions with Freund adjuvants has been inhibited with corticotropin⁶ and cortisone.⁷ In the latter study with monkeys receiving large doses of cortisone, the suppression of encephalomyelitis correlated strikingly with the suppression at the inoculation sites of the granulomatous response to the dead tubercle bacilli contained in the antigen-adjuvant emulsion.⁷ A suppressive effect of cortisone on antibody formation in guinea pigs injected with egg albumin in the Freund adjuvants was reported by Germuth and associates.⁸

Antibody production with the use of adjuvants depends in large part on inflammation and granuloma formation at the site of antigen-adjuvant depot.⁹⁻¹¹ When a potent stimulus, dead tubercle bacilli, is omitted from the antigen-adjuvant mixture, granuloma formation does not occur and, concurrently, antibody formation in guinea pigs is markedly diminished.^{12, 13}

The purpose of this study was to determine the effect of cortisone at two dosage levels on antibody production in guinea pigs, when the antigen is administered with the Freund adjuvants. Antibody formation was found to be

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markedly suppressed by the larger dose of cortisone and this was accompanied by the absence of local or systemic granuloma formation.

EXPERIMENT

Four-times-recrystallized egg albumin was used as antigen. The antigen-adjuvant emulsion was made by mixing the ingredients in a mortar in the following proportion: 500 mg. egg albumin dissolved in 25 ml. saline, 25 ml. Aquaphor, and 50 ml. of paraffin oil containing 64 mg. of dried heat-killed tubercle bacilli. Guinea pigs weighing between 400 to 600 grams were distributed according to sex and weight in an attempt to achieve comparable control and cortisone-treated groups. Three injections of 1 ml. of antigen-adjuvant mixture were given intramuscularly at weekly intervals at different sites in the following order: right shoulder girdle, left shoulder girdle, and midsacral area. Animals were bled by cardiac puncture at 2 to 4 weeks after the third injection. Cortisone (Cortone:Merck and Company, Inc.) was administered in two dosages. Guinea pigs of Group I received 5 mg. per day and those of Group II received 25 mg. per day, intramuscularly. Cortisone administration was started three days before the first antigen-adjuvant injection and continued for the duration of the experiment, a total of about 4½ weeks. Complete autopsies were performed at the end of the experimental period.

Sera were allowed to remain in the refrigerator at 4° C. until hemolytic complement activity had disappeared. Analyses for antiegg albumin nitrogen were carried out in duplicate by the quantitative precipitin method of Heidelberger and Kendall¹³ with the Heidelberger and MacPherson modification¹⁴ employing the Markham apparatus¹⁵ since only small samples of blood were obtained. Details of the procedure have been summarized.¹²

RESULTS

The antibody nitrogen content of sera at 2 and 4 weeks after the last of three weekly antigen-adjuvant injections is presented graphically in Fig. 1 and summarized in Table I. The distribution of antibody nitrogen levels in the control group is extremely varied over a wide range with no suggestion of a normal distribution curve. Therefore, the calculation of a mean titer or standard deviation has little significance. The distribution can be characterized graphically (Fig. 1) or less definitively by comparing ranges and median values. At two weeks after the last antigen-adjuvant injection, the 49 control guinea pigs had a range of 0 to 428 μg antibody nitrogen per milliliter serum (μg AbN./ml.) with a median of 123, while at 4 weeks the range of 42 control animals was substantially the same, from 1 to 330 μg AbN. per ml. with median values of 100 and 106 μg AbN./ml. The antibody levels of guinea pigs given 5 mg. cortisone daily are also widely distributed. At 2 weeks the range for 11 animals was 5 to 202; median 86 μg AbN./ml., and at 4 weeks 5 animals varied from 16 to 203 μg AbN./ml. with a median of 113. In contrast to these groups, the antibody levels of guinea pigs given 25 mg. daily are decidedly localized at the extremely low levels. At 2 weeks, 11 guinea pigs had a range of 0 to 23 μg AbN./ml., median 7; and at 4 weeks, the range of 6 animals was 0 to 24, with median values of 1 and 7 μg AbN./ml.

In the control group, granulomatous lesions were found at the inoculation sites, regional lymphoid tissue and lungs, as has been described in animals treated with similar adjuvants.^{9-12, 16, 18} In addition, there was hyperplasia of lymphoid tissue generally as has been described with intensive immunization.¹⁷ The group receiving 5 mg. cortisone was not distinguishable in these respects from the untreated control group. In the group receiving 25 mg. of cortisone, however, there was very little reaction grossly at the sites of antigen-adjuvant injection and the histologic picture was similar to that described by Kabat and associates⁷ in monkeys treated with cortisone and injected with brain-adjuvant emulsion. The appearance of the tissues also resembled that present in animals which had received adjuvant emulsions lacking tubercle bacilli^{12, 18} with the exception that there was somewhat more polymorphonuclear leukocytic infiltration at the local sites of injection of antigen-adjuvant emulsion in the cortisone-treated animals. The lymphoid tissue of the animals receiving 25 mg. cortisone showed a marked degree of atrophy, as has been frequently observed previously.^{4, 5}

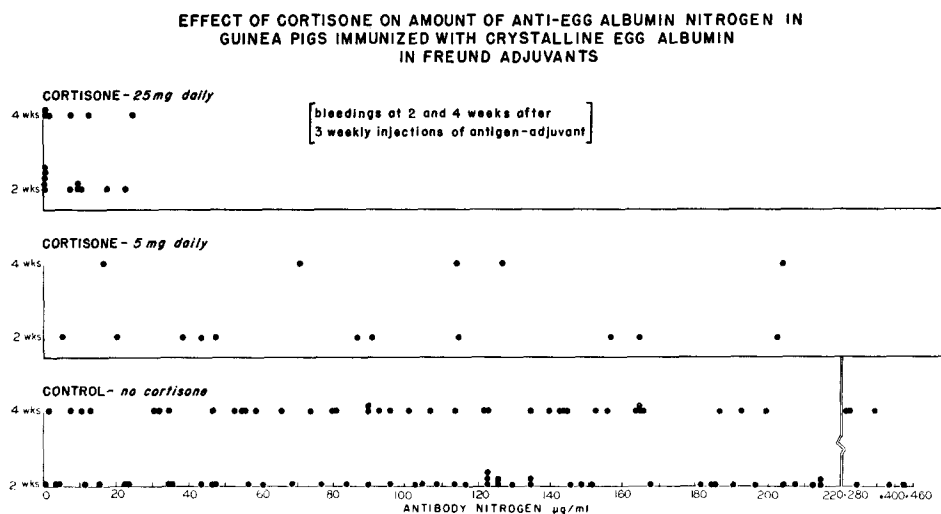


Fig. 1.

During the experimental period there was considerable mortality among the guinea pigs, particularly those receiving the larger dose of cortisone. Several animals in all groups died of unknown cause, some even before injections of antigen-adjuvant emulsion or of cortisone. It is difficult to estimate mortality rates attributable to cortisone (or to secondary infection) since repeated trauma due to injections and cardiac puncture created too many variables. In one group of guinea pigs receiving 25 mg. of cortisone daily, there were 11 deaths among 36 animals during the interval between the twelfth and twenty-fifth days of cortisone treatment, during which time intramuscular injections of antigen-adjuvant were being given but no cardiac punctures were done. In the untreated control group, 2 of 24 guinea pigs died. At another time, when controls were being immunized concurrently with the group receiving 5 mg. cortisone

daily, there were no deaths among 16 animals between the twelfth and twenty-fifth days of cortisone treatment, but during the corresponding time in the control group, 3 of 14 animals died.

Weight changes occurred in both control and cortisone-treated groups. About one-half the untreated control group lost some weight during the 4 weeks after the initial antigen-adjuvant injection. All but one of the group lost considerably more weight in the subsequent 2 weeks (between the first and second bleeding).

In the Group II animals receiving 25 mg. cortisone, 7 of 11 animals lost weight in the first 4 weeks, and all the survivors lost weight in the subsequent 2 weeks. In previous studies in rabbits,¹ it was concluded that there was no obvious correlation between weight loss or morbidity and antibody production under the conditions of the experiment. In the present study, there was no correlation between change in weight and antibody levels within the control group. Neither was there a correlation within the group treated with 25 mg. cortisone, although this was to be expected since all animals had substantially the same range of antibody. The effect of cortisone on antibody production without significant variation in body weight was adequately demonstrated by suppression of the specific anamnestic response in an experiment in which the duration of cortisone administration was not prolonged.³

The optimal dosage of cortisone in different animal species is a variable, apparently independent of body weight, as it also appears to be within one species—man (cf.^{19, 20}). In guinea pigs, the daily dose of 25 mg. was the same as that found to be necessary to prevent wound healing and the inflammatory response in guinea pigs²⁰ and to protect guinea pigs from the development of adrenal lesions due to diphtheria toxin.²¹ In both instances, the reactions were not inhibited by daily doses of 10 mg. or 5 mg. cortisone.^{20, 22}

DISCUSSION

Cortisone inhibits antibody production when the antigen is deposited locally with Freund adjuvants as had previously been found when the antigen was administered intravenously or intracutaneously.^{1,2, 8, cf. 19} Germuth and associates⁸ found that smaller doses of cortisone (5 mg. per day for 18 days, then 10 mg. per day for 12 days) reduced antibody production in guinea pigs. The 5 mg. dose employed in the present study had no such effect. A complicating factor in their experiment was the introduction, on four occasions following injection of antigen with adjuvants, of a solution of egg albumin intracutaneously so that antibody levels at the time of bleeding represent the combined result of two methods of immunization. Cortisone had previously been shown to suppress antibody production when the antigen was introduced intracutaneously without adjuvants.² In their work, the mean titer of the control group (109 ± 17 μ g AbN./ml.) is comparable to the median titers of the control group and of Group I in the present study (Table I). The mean titer of their cortisone-treated group (26 ± 7 μ g AbN./ml.) is somewhat higher than the median titer of Group II animals in this study. With the amount of cortisone employed by Germuth and his co-workers,⁸ a reduction in the weight of spleen and

adrenal glands was observed; microscopic examination and findings at the inoculation sites were not described.

TABLE I. EFFECT OF CORTISONE ON AMOUNT OF ANTIEGG ALBUMIN NITROGEN (AbN./ML.) IN GUINEA PIGS IMMUNIZED WITH CRYSTALLINE EGG ALBUMIN IN FREUND ADJUVANTS

TREATMENT	TIME AFTER THE LAST OF THREE WEEKLY INJECTIONS OF ANTIGEN-ADJUVANT					
	2 WEEKS			4 WEEKS		
	NO. GUINEA PIGS	μ G AbN./ML.		NO. GUINEA PIGS	μ G AbN./ML.	
		RANGE	MEDIAN		RANGE	MEDIAN
<i>Group I</i>						
Cortisone—5 mg.	11	5-202	86	5	16-203	113
<i>Group II</i>						
Cortisone—25 mg.	11	0-23	7	6	0-24	1-7
<i>Control</i>						
No cortisone	49	0-428	123	42	1-330	100-106

Intensive immunization is followed by hyperplastic changes in lymphoid tissue.¹⁷ Large doses of cortisone result in striking suppression of lymphoid tissue.^{4, 5} Antigen deposited locally with Freund adjuvants results in local granuloma formation and granulomatous changes in the regional lymph nodes, lungs, spleen, and elsewhere,⁹⁻¹¹ together with a peculiar "halo" of fibrous tissue around the malpighian corpuscles.¹² With the larger dosage of cortisone in the present study, diminution of antibody formation was strikingly correlated with absence of the granulomatous response. The low antibody levels to egg albumin and the absence of a granulomatous response also occurred when tubercle bacilli were omitted from the antigen-adjuvant emulsion.¹² In these respects, therefore, it appears that cortisone administration inhibits the inflammatory reaction due to tubercle bacilli. In both experiments, it appears that the degree of granulomatous reaction has an intimate relationship to the development of antibody. Furthermore, the development of experimental encephalomyelitis is inhibited by either omission of tubercle bacilli from the brain-adjuvant emulsion^{18, 23, 24} or by the administration of corticotropin⁶ or cortisone⁷; omission of the tubercle bacilli^{18, 23, 24} or treatment with cortisone⁷ strikingly reduced granuloma formation at the inoculation sites. These findings furnish additional evidence compatible with the hypothesis that experimental disseminated encephalomyelitis involves an immunologic mechanism.

SUMMARY

The administration of large dosages of cortisone to guinea pigs receiving crystalline egg albumin with the Freund adjuvant resulted in marked reduction of antibody levels and in marked diminution of granuloma formation at the site of the antigen-adjuvant injection. The relationship of granuloma formation to antibody production is discussed in the light of these studies together with previous work on the omission of tubercle bacilli and on experimental encephalomyelitis.

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