

Effect of Cortisone and ACTH During Sensitization on Eosinophils and Anaphylaxis in Guinea Pigs. (18704)

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Leger, Leith and Rose(1) have shown that the administration of ACTH to sensitized guinea pigs 6 to 8 hours prior to the giving of a challenging injection of the antigen does not alter the anaphylactic reaction. This observation has been confirmed by Friedlaender and Friedlaender(2) and by Dworetzky, Higgins and one of us (Code)(3). It has also been demonstrated that treatment with cortisone just prior to the administration of the antigen likewise has no observable effect upon the ensuing reaction(3,4). While these investigations clearly showed that cortisone and ACTH are without visible effect upon the anaphylactic reaction in fully sensitized guinea pigs, the possibility remained that ACTH and cortisone might have some influence upon the development of the hypersensitive state if they were administered with the sensitizing injections. This possibility was further heightened by the observations of Germuth and Ottinger(5), that the administration of cortisone and ACTH during the administration of sensitizing injections to rabbits inhibits the development of the Arthus phenomenon and the appearance of free antibodies in the blood.

Methods. Two series of guinea pigs were studied. In both series there were 3 groups of animals, each composed of an equal number of males and nonpregnant females. The initial body weights of all the animals ranged from 350 to 490 g and there was no significant

difference between the initial mean body weight of the animals in the different groups. All the animals were sensitized with diluted fresh egg white. This was prepared by mixing one part of the whites of fresh eggs with 4 parts saline solution and discarding any insoluble material. The schedule of injections was identical in both series. Commencing on the third or the fourth day after the administration of cortisone or ACTH, each guinea pig was given 4 successive daily subcutaneous injections of 0.5 ml. The sensitivity of the animals to the antigen was tested three to seven weeks later by the intravenous administration of 0.2 ml or less of the diluted egg white.

In Series 1 the 3 groups were composed of 12 guinea pigs each. The animals in the first group served as controls and were not given injections of either hormone. Those in the second group were given 25 mg of cortisone in a single injection, and then 4 days later 5 mg and thereafter 5 mg every other day for 27 days. The sensitivity of 4 of the pigs to egg white was then tested. Four of the remaining 8 animals continued to receive cortisone, 4 were untreated and at the end of 14 more days the sensitivity of these pigs was tested. The animals in the third group received ACTH.* Initially, each was given an injection of 6.25 mg repeated 12 hours later and thereafter 1.25 mg every 12 hours for 27 days, at the end of which the sensitivity to egg white of 4 of the group was tested. The remaining 8 received no further treatment and their sensitivity to egg white was tested 14 days later. The sensitivity of 4 of the control pigs was also tested at 27 days and the remainder 14 days later. Series 2

1. Leger, Jacques, Leith, W., and Rose, Bram, *PROC. SOC. EXP. BIOL. AND MED.*, 1948, v69, 465.

2. Friedlaender, Sidney, and Friedlaender, A. S., *J. Allergy*, 1950, v21, 303.

3. Dworetzky, Murray, Code, C. F., and Higgins, G. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1950, v75, 201.

4. Harris, Susanna, and Harris, T. N., *PROC. SOC. EXP. BIOL. AND MED.*, 1950, v74, 186.

5. Germuth, F. G., Jr., and Ottinger, Barbara, *PROC. SOC. EXP. BIOL. AND MED.*, 1950, v74, 823.

* The ACTH used throughout this study was kindly provided by Armour Laboratories, Chicago, Ill. It had a biologic potency of 100% of Armour's La-I-A standard.

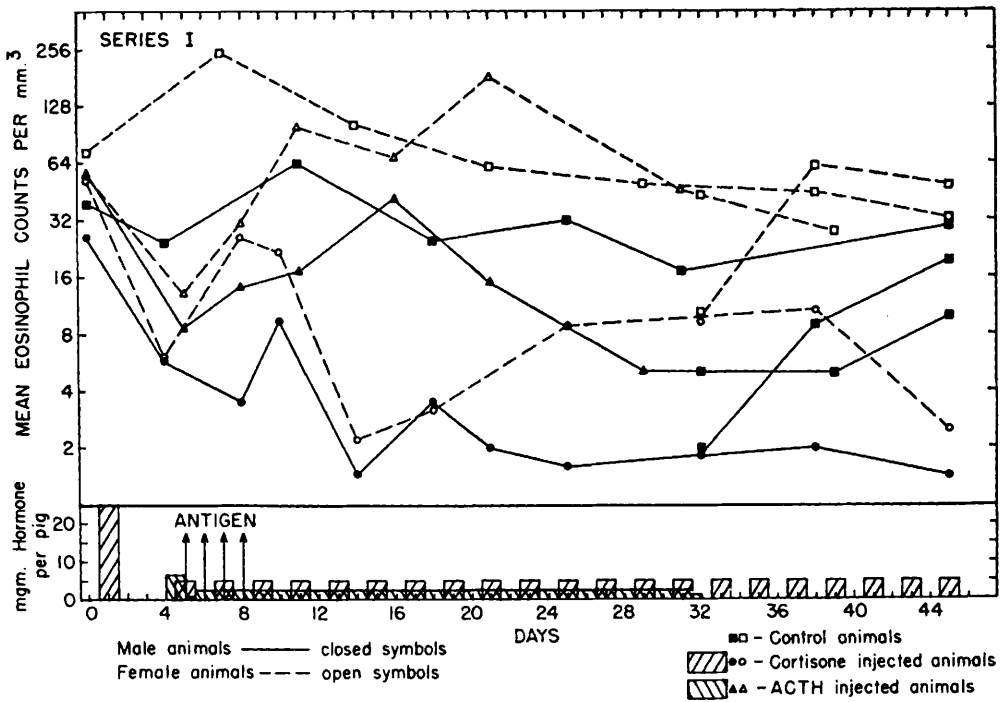


FIG. 1

Effect of the prolonged administration of ACTH and cortisone on eosinophils in guinea pigs in Series 1 of this study.

likewise consisted of 3 groups, each composed of 8 guinea pigs. Half of the control group received 0.2 ml of saline solution twice a day during the injection period. The remainder of the controls were untreated. The cortisone-treated group received 10 mg daily for 14 days. Those receiving ACTH were given 2.5 mg every 6 hours for 6 days; then 5 mg every 6 hours for 6 days. The sensitivity of all of the guinea pigs in series 2 was tested by an intravenous administration of the antigen 53 days after the first sensitizing injection.

As measures of the action of ACTH and cortisone, the number of eosinophils in the blood and the body weights of the animals were determined periodically. The eosinophils were counted by the modification of Dunger's (6) method which was developed by Thorn, Forsham, Prunty and Hills(7).

Results. Body weights. While the animals

were receiving injections, all but 2 gained weight. In series 1 the control animals gained, on the average, 143 g; and those receiving cortisone or ACTH gained 92 g. During the shorter injection period in series 2, the uninjected control animals gained, on the average, 75 g, the injected controls 40 g, those receiving cortisone 20 g and those given ACTH 15 g.

Eosinophil counts. A single control eosinophil count was made in the majority of animals prior to the injection of saline solution or hormone. The mean number of eosinophils per cubic millimeter of blood in the males was 32 ± 4.9 and 56 ± 7.3 in the females. The probability of a difference as great as that between these means occurring by chance is less than 1 in 100. When 1.25 mg of ACTH was given every 12 hours, as in the third group of series 1, no decisive change was observed in the number of eosinophils in the peripheral blood. When, however, larger doses (2.5 g) were given every 6 hours, as in series 2, a decisive and sustained reduction

6. Dunger, Reinhold, *München. med. Wchnschr.*, 1910, v57, 1942.

7. Thorn, G. W., Forsham, P. H., Prunty, F. T. G., and Hills, A. G., *J.A.M.A.*, 1948, v137, 1005.

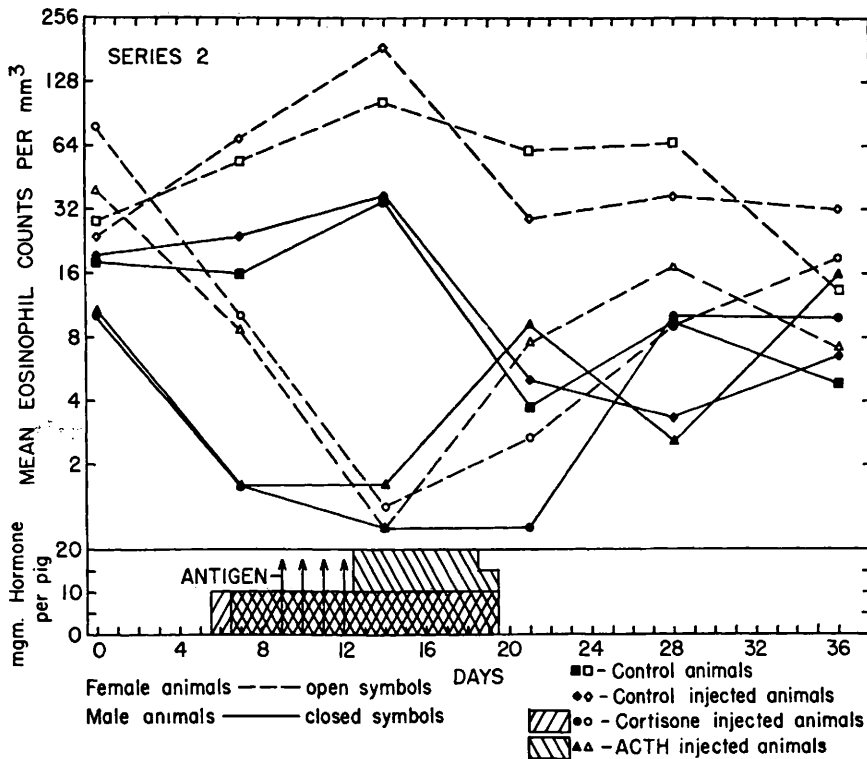


FIG. 2

Effect of repeated large doses of ACTH and cortisone on eosinophils in guinea pigs in Series 2 of this study.

in the number of circulating eosinophils occurred in both males and females (Fig. 1 and 2). Cortisone produced a pronounced eosinopenia in both series of animals (Fig. 1 and 2).

Anaphylactic shock. All the animals in both series exhibited severe reactions immediately after the antigen was given intravenously. The symptoms in every instance were characteristic of anaphylactic shock. Not more than 1 animal in any group survived the immediate effects of the reaction. All those which died promptly, exhibited the voluminous emphysematous lungs so characteristic of anaphylaxis in guinea pigs.

Comment. The observation of Dworetzky, Higgins and one of us (Code)(3) that the number of eosinophils in the blood of female guinea pigs is higher than in males has been confirmed. Likewise, their finding that both cortisone and ACTH produce eosinopenia in male and nonpregnant female guinea pigs has been verified.

Neither cortisone nor ACTH had any observable effect upon the anaphylactic reaction in guinea pigs when these hormones were given during the period of sensitization. The conclusion may be drawn that guinea pigs sensitized by subcutaneous injections show a different response to injections of ACTH and cortisone than rabbits sensitized by intracutaneous injections, as in the experiments of Germuth and Ottinger(5). Likewise, there is apparently a species difference between the guinea pig and the mouse, since Nelson, Fox and Freeman(8) have shown that the administration of cortisone to sensitized mice eighteen hours before the intravenous injection of the challenging dose of antigen prevents or diminishes the severity of the reactions. No satisfactory reason for these

8. Nelson, C. T., Fox, C. L., Jr., and Freeman, Elizabeth B., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 181.

species differences can be offered on the basis of the results of these experiments.

Summary. Two series of 36 and 24 guinea pigs were studied. Cortisone was given to a third of the animals, ACTH to a third and the remaining third served as controls. The administration of the hormones, in moderate or large doses, was started before giving the sensitizing injections of egg white, and was continued while they were being given and

for varying periods after their completion. No treatment had any effect on the severity of anaphylactic reactions induced by the subsequent intravenous injection of the antigen. Used in adequate amounts, both hormones produced pronounced and sustained reductions in the number of circulating eosinophils in the blood of male and nonpregnant female guinea pigs.

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Negative Effects of Vitamin B₁₂ on Blood of Healthy Individuals, with Special Reference to Eosinophils.* (18705)

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While examining blood smears from patients ill with pernicious anemia, who responded to therapy with vit. B₁₂, we were surprised to find that there had been no specific increase in eosinophilic polymorphonuclear leucocytes. We had expected to find a rise in eosinophilic cells since it is well known that oral or intramuscular therapy with whole liver or with liver extracts commonly produces an eosinophilia in patients ill with pernicious anemia or in healthy individuals(1-9), which may reach as high as 75% of the total white

blood cell count. Because of this, observations were made on the effects of therapy with relatively large amounts of vit. B₁₂ given, either by mouth or by injection, to 3 healthy young adults and to 5 healthy children.

Methods. Three adult males and 5 school children served as subjects. They were healthy and none of them gave a history of previous allergic disease, anemia, or blood dyscrasia. In the adults, studies of their blood were made 3 times a week; during a control period of 2 weeks, during a 28 day vit. B₁₂ therapy period, and during the 2 weeks of the post-therapy period. Blood from the children was observed twice during the pre-therapy period and at weekly intervals during the therapy and post-therapy periods. Fasting blood was collected in heparinized tubes and from these determinations of hematocrit, hemoglobin, and red blood cell counts were made by standard technics. Absolute eosinophil counts and white blood cell counts were made by a modification of Randolph's technic using 3 parts of phloxine to one part of methylene blue(11). Differential counts of stained blood were made by standard technics using fresh blood cover-glass smears and counting 200 white blood cells.

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11. Randolph, T. G., *Allergy*, 1944, v15, 89.