TABLE I.
Summary of Experimental Results.

No. of rabbits	Avg wt, kg	Treatment: Estradiol benzoate μg	Treatment: Progesterone mg	Results: Ovulated	Results: Failed to ovulate	Results: % ovulated
10	3.5	2 × 85	—	0	10	0
10	3.4	—	2	0	10	0
10	3.2	2 × 85	2	4	6	40

been effective in facilitating ovulation have all been spontaneous ovulators, though at least in the rat a neurogenic timing factor is involved in the LH-release mechanism (13,14,8). The rabbit is the first form in which, to our knowledge, a species normally requiring a coital stimulus has been converted to a "spontaneously ovulating" type by hormone administration. In this respect it resembles the persistent-estrous rat, in which spontaneous ovulation can be restored by progesterone treatment(6). To what extent in the rabbit progesterone operates through nervous mechanisms requires further study. Addition of this animal to the growing list of species in which progesterone stimulates ovulation, increases the likelihood that progesterone facilitation may be a fairly general phenomenon.

Summary. Following treatment with estrogen and progesterone 4/10 rabbits ovulated spontaneously, *i.e.*, without the coital stimulus normally required to induce LH release from the hypophysis in this species.

13. Sawyer, C. H., Everett, J. W., and Markee, J. E., *Endocrinology*, 1949, v44, 218.

14. Everett, J. W., Sawyer, C. H., and Markee, J. E., *Endocrinology*, 1949, v44, 234.

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Effect of Cortisone on Some Reactions of Hypersensitivity in Laboratory Animals.* (17849)

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The effects of ACTH and cortisone which have been reported in the case of a number of different disease states known or thought to involve hypersensitivity of tissues(1-3) have suggested that these agents may influence the manifestations of antigen-antibody reactions *in vivo* or of bacterial hypersensitivity. Accordingly, a study was instituted of the effect of cortisone on a number of these reactions of immunity and hypersensitivity in experimental animals.

Procedure. 1. *Effect of cortisone treatment on the tuberculin skin reaction in sensitized rabbits and guinea pigs.* a. A total of 18 rabbits of approximately 2.2 kg were infected intradermally with 0.15 mg of tubercle bacilli (BCG). After 3 weeks it was established that cutaneous sensitivity to tuberculin (OT) and to cytoplasmic particles (CP)

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1. Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F., *Proc. Staff Meet. Mayo Clinic*, 1949, v24, 181.

2. Hench, P. S., Slocumb, C. H., Barnes, A. R., Smith, H. L., Polley, H. F., and Kendall, E. C., *Proc. Staff Meet. Mayo Clinic*, 1949, v24, 277.

3. Mote, J. R. (Editor), *Proc. First Clinical ACTH Conference*, The Blakiston Company, 1950.

TABLE I.
Tuberculin Test in Rabbits Sensitized with BCG.

Test period	Group	No.	Skin test with OT Volume in mm³—48 hr				Skin test with CP Volume in mm³—48 hr			
			Neg.	to 200	201-400	>400	Neg.	to 200	201-400	>400
Preliminary	Control	10	1	4	2	3	1	4	4	1
	Cort.	8	0	4	2	2	0	5	1	2
Test	Control	10	0	7	3		0	4	4	2
	Cort.	8	8	0	0		8	0	0	0

prepared from BCG organisms by high speed centrifugation(4) was present in all of the rabbits. Cortisone was injected intramuscularly into 8 rabbits twice daily at a total daily dose of 34 mg per rabbit. A fall in the total number of circulating lymphocytes was noted in all 8 rabbits within 1 day after beginning the injections. After 3½ days of cortisone treatment all the rabbits were tested by intradermal injections of 0.1 cc of OT 1:10 and CP (1 mg/cc). Readings of the volume of the intradermal inflammation (5) were made after 48 hours, after which the cortisone was discontinued. It was found (Table I) that the rabbits receiving cortisone did not exhibit the cutaneous evidence of sensitivity to proteins of the tubercle bacillus which they had shown in the preliminary tests.

b. Guinea pigs of approximately 360 g were infected intradermally with 0.15 mg of tubercle bacilli (BCG). After 3 weeks skin sensitivity to OT and CP was established. Twenty of these animals were given cortisone totalling 11 mg per day, injected intramuscularly twice daily for 6 days. Blood eosinophile counts made before and during administration of cortisone showed a sharp fall in the total number of these cells. On the third day of cortisone treatment all the guinea pigs were retested. Since the guinea pigs were multicolored the area of the dermal reaction could not be read with satisfactory accuracy. The intensity of the inflammatory response is therefore presented in terms of mm of thickening of the skin at the site of injection. These results (Table II) showed that of 20 guinea pigs under the influence of cortisone 17 and 18 failed to show positive reactions to OT and CP respectively, and 3 and 2 showed slight reactions. The entire group was again tested with the same dilutions of OT and CP 3 to 6 days after the last injection of cortisone, and dermal reactions now appeared again at the sites of the new tests. Some days after the cessation of cortisone therapy ulcers appeared in a number of the cortisone-treated guinea pigs at the sites of skin tests made during the course of cortisone treatment, and which had been negative at that time.

c. Seventeen guinea pigs sensitized with tubercle bacilli, 8 on their fourth day of treatment with cortisone and 9 controls, were given intraperitoneal injections of 0.6 cc of a preparation of OT which had been dialyzed, lyophilized and reconstituted to 5 times its original concentration. Within a few hours of this injection all the guinea pigs which had not received cortisone appeared to be ill, showing diarrhea, listlessness, lowering of body temperature and drooping of the eyelids so that the palpebral fissure was only 3 or 4 mm. The guinea pigs receiving cortisone remained well and active. Within 48 hours 7 of the 9 guinea pigs of the control group were dead, whereas the 8 cortisone-treated animals were alive and well. The adrenal glands of the control animals were removed at autopsy and were found grossly to be hemorrhagic in varying degrees, from a few small, distinct hemorrhages to a confluent hemorrhagic mass. The 2 remaining control animals were sacrificed and their adrenal glands also showed gross hemorrhages. For histologic study 2 guinea pigs of the cortisone group were sacrificed after 48 hours and their adrenal glands found

4. Boltjes, B. H., and Harris, T. N., in preparation.

5. Lurie, M. B., *Am. Rev. Tuberculosis*, 1941, v44, Supp.

TABLE II.
Tuberculin Test in Guinea Pigs Sensitized with BCG.

Test period	Group	No.	Intensity of reaction—thickening of skin in mm									
			OT (1:20)				CP (2 mg/cc)				OT (1:10)			
			Neg.	to 1	1-2	>2	Neg.	to 1	1-2	>2	Neg.	to 1	1-2	>2
Preliminary	Control	20	4	8	7	1	0	5	14	1				
	Cort.	21	5	8	7	1	1	8	12					
Test	Control	19	1	6	7	5	0	7	9	3	0	5	6	8
	Cort.	20	17	2	1		18	2			18	1	1	

grossly to be small and free of hemorrhages. The remaining animals of the cortisone group were sacrificed after 4 more days of apparent good health. Their adrenals were found to be normal on gross examination.

II. *Arthus reaction in rabbits.* Normal horse serum (NHS 1.0 cc) was injected subcutaneously into 19 rabbits of approximately 2.2 kg at weekly intervals for 4 weeks. After the fifth injection of NHS a local lesion appeared in most of the animals at the site of the injection, and an inflammatory reaction could be elicited after the injection of 0.1 cc of NHS intradermally. This latter reaction usually reached its height in 24 hours. Part of the group of rabbits was then given 34 mg of cortisone daily in 2 injections for a total of 5 days. On the third day after cortisone treatment was begun, another subcutaneous injection of NHS was given to all the rabbits and the thickness of the local lesion which appeared subsequently at the site of injection was measured, as well as the reaction to a skin test of 0.1 cc of NHS placed on the opposite side of the animal. Neither the extent of the local Arthus reaction nor the presence or intensity of the skin test reaction appeared to be affected by the administration of cortisone (Table III).

III. *Antibody production.* All of the rabbits involved in the tuberculin sensitivity experiment were injected subcutaneously with 2 cc of a mixture of sheep erythrocytes (50% suspension) and *Shigella paradysenteriae* (10 mg) one day after the injection of cortisone had begun. Blood was drawn from these animals after 3, 4, 6 and 8 days, and the serum tested for sheep red cell hemolysins and dysentery agglutinins. The rising curves of serum antibody titers against both antigens did not show significant differences between the group of cortisone-treated and control animals, although there was a tendency for higher titers to appear in the sera of the control group.

IV. *Anaphylaxis.* In one experiment guinea pigs received semi-weekly subcutaneous injections of 0.5 cc NHS for 4 weeks. Of the 14 animals 9 were then given varying doses of cortisone twice daily for 2½ days. Four hours after the last injection of cortisone all

TABLE III.
Arthus Reaction in Cortisone-Treated Guinea Pigs and Controls.

Test period	Group	No.	Skin test readings Volume in mm³—24 hr				Arthus reaction in mm —24 hr			
			Neg.	1-200	201-400	>400	Trace	1-4	5-8	>8
Preliminary	Control	9	0	6	2	1		1	5	3
	Cort.	9	0	2	3	4	1	1	4	3
Test	Control	9	0	6	3			1	3	5
	Cort.	9	1	3	4	1		6	3	

the animals were given 1.5 to 2.0 cc of NHS by intracardiac injection. Shock occurred in all of the animals, and the number of deaths in the respective groups was not significantly different. In this experiment, which was the first one of this study, the range of dose of cortisone was considerably lower than that adopted later.

Discussion. The data presented here indicate that cortisone can suppress the manifestations of some of the reactions of hypersensitivity in experimental animals. Under the conditions of these experiments, in the case of rabbits and guinea pigs sensitized to tubercle bacilli, the cutaneous sensitivity of both species to OT, and the systemic shock of guinea pigs, were suppressed while the animals were under treatment with cortisone. This effect was not demonstrated in the case of anaphylaxis in the guinea pig nor of the local Arthus reaction in the rabbit. The latter findings are consistent with observations made by Leger(6) in anaphylaxis and Fischel(7) in the Arthus reaction in animals treated with ACTH. These contrasting results again point to some difference in the mechanism of the antigen-antibody reactions *in vivo* such as the Arthus phenomenon and anaphylaxis on the one hand, and the bacterial type of hypersensitivity such as the tuberculin reaction on the other hand. The mechanism of the action of cortisone in suppressing these reactions is not understood. In the cortisone-treated animals no reaction had been observed 24 or 48 hours after the intradermal injection of skin test doses; however, at some of these sites of injection ulcers were noted some days after the cessation of cortisone therapy. There would seem to be two alternative hypotheses for this sequence of events; first, that the cortisone does not actually prevent injury of the hypersensitive cell, but merely delays the objective manifestation of that injury and, second, that residual antigen remaining at the site 72 hours after the injection (a day after the last injection of cortisone) could then proceed to act as in the control animals.

Summary. In rabbits and guinea pigs sensitized with tubercle bacilli (BCG) dermal reactivity to Old Tuberculin and to cytoplasmic particles from tubercle bacilli and systemic shock produced by intraperitoneal injection of OT could be suppressed by treatment with cortisone. Four days after cessation of treatment the skin test again became positive.

Cortisone did not prevent anaphylactic shock in guinea pigs sensitized with normal horse serum nor did it exert any influence on either the appearance of the Arthus reaction in rabbits injected with normal horse serum or on the skin test reaction to an intradermal injection of normal horse serum in these rabbits.

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6. Leger, J., Leith, W., and Rose, B., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 465.

7. Fischel, E. E., *Am. J. Med.*, 1949, v7, 772.