Response of Tuberculin Skin Test to ACTH and Cortisone in Tuberculous Guinea Pigs. (18218)

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It has been shown that adrenocorticotrophic hormone (ACTH) and compound E (Cortisone) may modify tuberculin cutaneous reactions in guinea pigs sensitized with tuberculin(1) or heat-killed human tubercle bacilli(2), or infected with BCG tubercle organisms(3). These observations would seem to demonstrate the need for testing the effect of these hormones on the tuberculin reaction in the presence of active infection by virulent organisms.

Methods. Fifty-one guinea pigs (average weight 200 g) were infected by the subcutaneous injection into the groin of a suspension containing 0.1 mg virulent tubercle bacilli. The organisms had been recently cultured from patients with active pulmonary tuberculosis. All animals subsequently showed definite evidence of the presence of tuberculous infection, manifested by localized or generalized lymphadenopathy (1+), buboes (2+), draining sinuses (3+), or ulceration in the groin (4+) (Table I); in all animals tested acid-fast organisms were found on smear of exudate discharged from lesions, and in all cases additional confirmation of tuberculous infection was discovered at necropsy (results to be reported separately). The animals were divided into 3 groups of 17 each. The animals in Group 1 received daily subcutaneous injections of 1 mg of Cortisone; injections were begun one day before infection by tubercle organisms and continued until the observations were completed. Animals in Group 2 were used to observe the effects of varying dosages of ACTH and Cortisone on the tuberculin reaction. The animals in Group 3 served as controls. Selected animals of the control group were challenged at intervals with intradermal injections of 0.005 mg of Purified Protein Derivative (0.1 cc of the 2nd strength) and 6 weeks after infection all animals in this group showed a strongly positive cutaneous reaction. Then the animals of Group 1 received the challenging intradermal test, and the animals of the second group were utilized in short term experiments. The tuberculin reactions were read 24, 48 and 72 hours after introduction of the test dose and classified as 1+ (erythema and induration), 2+ (erythema, induration and hemorrhage), and 3+ (erythema, induration, hemorrhage and necrosis). In representative cases photographs were made and the lesions excised for histopathologic study.

TABLE I. Effect of Cortisone on Tuberculin Reaction. 17 animals in each series. (1 mg daily from beginning of infection).

	Degree of infection*				Tuberculin reaction		
Group	1+	2+	3+	4+	1+	2+	3+
			Cortisone				
1	2	4	10	1	6	8	3
			Controls				
3	1	6	3	7	4	9	4

* See text for explanation of symbols indicating degree of reaction.

Results. The animals in Group 1 (which received 1 mg Cortisone daily) manifested tuberculin reactions not significantly different grossly and microscopically from those in the controls.

The histologic picture (Fig. 1) consisted in a severe inflammatory reaction in the epidermis, dermis and underlying muscle tissue. There was dilatation of small blood vessels with swelling of their endothelium, together with a large outpouring of exudate. This exudate was composed principally of neutrophiles,

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FIG. 1.
Reaction to tuberculin skin test after 6 weeks of cortisone (1 mg/day).

lymphocytes and macrophages, with only an occasional eosinophile being present. The exudate was diffusely disseminated but there was a notable tendency for perivascular and perineural accumulation. The subcutaneous connective tissue was edematous. Many of the collagen bundles in the dermis were fragmented and stained poorly. There was ulceration of the epidermis immediately overlying the more intense inflammatory zones and in these areas considerable necrosis was noted with almost complete destruction of the cutaneous appendages.

In Group 2, Cortisone was administered intramuscularly in divided doses twice daily to 3 pairs of animals. To 1 pair, 10 mg daily per animal was given for 3 days; the animals were tested on the 2nd day; both had strongly positive cutaneous reactions on the 4th day, identical in intensity (2+ and 3+) with results of control tests made in the same animals prior to administration of the hormone. A 2nd pair received 10 mg daily per animal for 4 days; the animals were tested on the 3rd day; both had strongly positive reactions (2+ and 3+) on the 5th day, the same result as prior to administration of the drug. The 3rd pair received 20 mg daily per animal for 3 days; the animals were tested on the 2nd day; both showed weakly positive cutaneous reactions (1+). In all animals the sections for biopsy were identical with those from the controls, with the exception of the last pair, 1 of which showed less inflammatory reaction (Fig. 2).

Two animals received 5 mg ACTH daily for 4 days, in the same manner as for Cortisone and were tested on the 2nd day. Both had strongly positive reactions (2+) on the 4th day, and sections for biopsy were not different from those of the controls. Five animals, having first been given control skin tests, received 10 mg ACTH daily (2.5 mg/6 hours) for 8½ days; the animals were tested on the 7th day, with the following results (Table II): in 1 animal the previously strong-

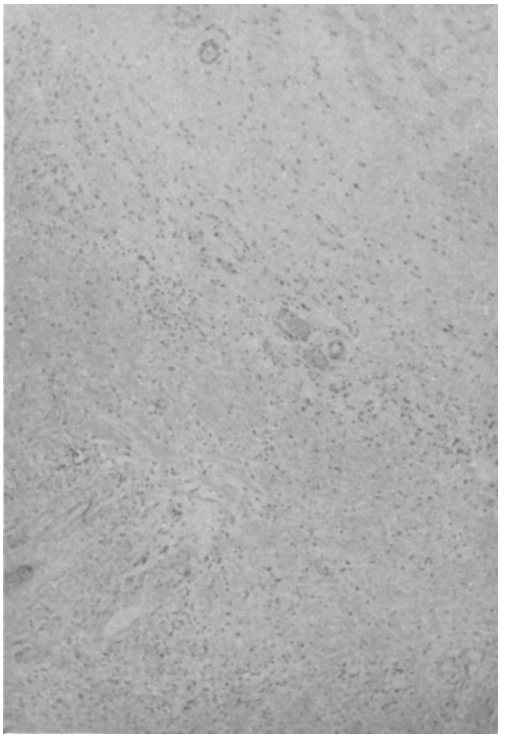

FIG. 2.
Reaction to tuberculin skin test after 3 days of cortisone (20 mg/day).

TABLE II. Effect of ACTH on Tuberculin Reaction. (10 mg daily for 8½ days).

Animal	Prior to ACTH		After ACTH	
	24 hr	48-72 hr	24 hr	48-72 hr
1	2+*	3+	3+	3+
2	3+	3+	1+	1+
3	1+	2+	1+	1+
4	1+	2+	1+	1+
5	3+	3+	(Died 40 hr after test)	

* See text for explanation of symbols indicating degree of reaction.

ly positive reaction was unchanged, 3 animals showed weakly positive reactions with intensity definitely less than prior to ACTH; and the 5th animal died 40 hours after administration of the challenging test with a negative reaction. Results of histologic studies of the specimens for biopsy were in accord with the gross observations, *i.e.*, sections from the first animal showed severe inflammatory reaction, from animals 2, 3 and 4 minimal inflammatory reaction, and from the 5th animal a normal appearance (Fig. 3). In those animals in which there was microscopic evidence of a less severe reaction the principal change was one of degree; if only a minimal inflammatory reaction was present, the exudate was composed principally of lymphocytes and macrophages with few neutrophiles.

Discussion. With respect to dosage, Hench and associates(4) suggested that 75 to 100 mg of Cortisone may represent the minimum effective daily dose in adult human beings (equivalent to 1.0 to 1.4 mg/kg); since our animals averaged 1/5 kg in weight, a dose of 1 mg a day (equivalent to 5 mg/kg) would represent a 4 fold increase over the minimum human dose, provided the dosage could be transferred to that species. A difference in species' response is indicated by the protection of rats against the "anaphylactoid reaction" to egg albumin by both ACTH and Cortisone(5), and the failure to protect guinea pigs against anaphylactic shock by ACTH* (6). Of the various dosages tried the only ones effective in modifying the tuberculin reaction were 10 mg ACTH daily (2.5 mg intramuscularly every 6 hours) for 7 days which modified the reaction in 4 of 5 animals and (possibly) 20 mg Cortisone (10 mg intramuscularly twice daily) for 3 days which apparently modified the response in 1 of 2 animals.

Numerous investigators(8) have shown that there is no correlation between the in-

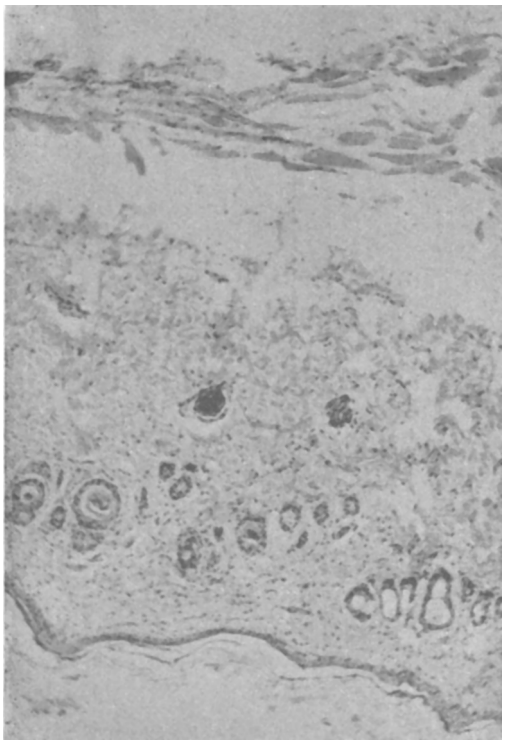

FIG. 3.
Negative reaction to tuberculin skin test.

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* At the time these experiments were undertaken we could find no information on the dose of the hormones in the guinea pig; and, although later studies by us and by others(7) have shown much larger doses are necessary, 1 mg Cortisone daily seemed to be a reasonable starting dose).

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8. Boquet, A., and Bretey, J., *Ann. Inst. Pasteur,* 1934, v52, 252.

tensity of the tuberculin reactions and the severity of the tuberculous infections, and that the tuberculin reaction appears after a variable latent period, rises sharply in intensity, levels off, and then diminishes, perhaps disappearing at the time of death of the animal; therefore, we are unable to attribute the absence of the reaction in the one animal (which died 40 hours after the test) to the administration of ACTH.

Summary. (1) ACTH and Cortisone when given in proportionately large doses modified but did not abolish cutaneous tuberculin reaction in guinea pigs infected with virulent tubercle organisms. (2) Following administration of ACTH and Cortisone the histopathologic changes consisted in a quantitative diminution in the inflammatory exudate rather than a qualitative change in its cytologic structure.

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Development of Embryonic Guinea Pig Mullerian Ducts in Anterior Eye Chamber Grafts.* (18219)

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The problem of the relation of sex hormones to sexual differentiation in mammals has received the attention of many investigators who have utilized fundamentally different types of approach in their studies. The injection of sex hormones into the pregnant female(1-5), the treatment of developing pouch young in marsupials(6-9), gonadectomy during development(10-13), and the transplantation of embryonic tissues to postnatal hosts in different sexual conditions(14, 15) have all contributed new information on the subject. However, unanimity of opinion regarding the necessity for sex hormone action for proper sex differentiation does not yet exist. In 1945 I began a study of the relation of sex hormones to uterine gland development in the guinea pig by comparing the conditions found in normal newborn and later stages with those subjected to ovariectomy at birth, and with others treated by estrogen injections(16). Although the uterus of the newborn guinea pig readily responded to treatment with estrogens, it became clear that critical stages occurred earlier in development since uterine glands were already present in the guinea pig at birth.

In order to examine the possibilities for the differentiation of the uterus, and especially of uterine glands, in the absence of all hormones secreted by sex glands, portions of the embryonic reproductive duct system were removed from embryos of timed development

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