environment for bacterial growth.

Though the mechanism is far from clear, it is apparent from these studies that tubercle bacilli in the rat under the influence of cortisone are able to multiply more rapidly, to produce more diffuse disease, to disseminate, and to result in the death of the animal. It is obvious that findings in an animal resistant to tuberculosis cannot be carried over *in toto* to man, naturally susceptible to tuberculosis. However, the recently recorded(8) adverse effects of cortisone on tuberculosis in the guinea pig, a species naturally susceptible to the infection, would lend support to the suggestion that caution be exercised in considering the administration of cortisone to humans with active tuberculosis.

Summary. 1. Rats injected with virulent tubercle bacilli did not die of tuberculous infection. A majority of rats similarly infected with tubercle bacilli but treated with cortisone died of tuberculosis. 2. Striking histologic differences were noted. Localized granulomatas were seen in the organs of rats injected only with tubercle bacilli, whereas the lesions in the animals also receiving cortisone were diffuse and contained fewer inflammatory cells. 3. The influence of cortisone on experimental tuberculosis appears to be a lowering of the host's natural resistance. The possible modes of action resulting in this are discussed.

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Failure of ACTH or Cortisone to Suppress Tuberculin Skin Reactions in Tuberculous Guinea Pigs.* (18283)

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(Introduced by J. V. Warren)

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Some recent reports indicate that tuberculin skin reactions are either obliterated or suppressed following the administration of ACTH or cortisone(1-5). From these observations it has been concluded that the compounds interfere directly with the delayed type of antigen-antibody reaction. Observations made in our laboratories did not confirm the findings reported by others. Therefore, the influence of ACTH or cortisone on the tuberculin skin reaction was tested experimentally.

Material and methods: Fifteen male albino guinea pigs with an average weight of 375 g were injected intradermally with 0.1 ml of 10% Old Tuberculin and were found to be tuberculin negative. The animals were then inoculated subcutaneously in the groin with 0.1 mg of a young culture of human virulent tubercle bacilli (H37 Rv). One month after infection the guinea pigs were divided into 3 groups. Five animals received 2 daily intramuscular injections of 2 mg of ACTH (Armour) for 6 days. Another 5 animals were injected subcutaneously 2 times daily with 2

* The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

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TABLE I.

Exp. group	G.P. No.	Tuberculin reaction 0.1 ml 10% O.T. 24 hr Diam. mm	24 hr Central blanching	48 hr Diam. mm	48 hr Central blanching
ACTH 4 mg	582	15	2+	*	*
	589	10	3+	*	*
	584	15	+	10	±
	579	10	3+	5	±
	587	10	2+	5	±
Corti-sone 4 mg	586	12.5	3+	*	*
	588	12.5	3+	*	*
	596	10	3+	10	±
	591	7.5	2+	5	±
	598	10	+	5	±
Control 0.5% phenol	576	15	0	*	*
	597	20	2+	*	*
	578	20	0	10	0
	600	10	0	15	0
	580	20	2+	10	2+

* Sacrificed after 24 hr.

mg of cortisone (Merck) for the same period of time. The remaining 5 guinea pigs served as controls and for six days were given twice daily intramuscular injections 0.2 ml of 0.5% phenol in aqueous solution. On the fourth day of hormone administration tuberculin skin tests were repeated in the same manner as described above. After 24 and 48 hours the diameter and the general characteristics of the skin response were recorded and special attention was given to erythema, induration and central necrosis.

Twenty-four hours after tuberculin skin testing, 2 animals from each group were sacrificed by a blow on the neck. The remaining guinea pigs were killed in the same manner after 48 hours. The site of the skin reaction was delineated and excised in a manner to include the adjacent grossly normal tissues. An autopsy was performed and blocks from the excised skin as well as from lung, liver, spleen, lymph nodes and adrenals were fixed in Zenker's fluid with 5% glacial acetic acid and in 10% USP formaldehyde. Sections were stained routinely with phloxine-methylene blue. Sections from the adrenals were also stained with Scarlet Red.

Results. The results are summarized in Table I. All animals receiving either ACTH or cortisone reacted to tuberculin with definite erythema, induration and central blanching. The blanching was more pronounced in the hormone treated animals than in the controls although these showed unmistakable reactions to tuberculin in every instance. The size of the tuberculin reaction as well as the degree of erythema and induration appeared to be less in the animals receiving hormone.

At autopsy all guinea pigs showed widespread active tuberculous infection with marked involvement of lungs, liver, spleen and lymph nodes.

All animals receiving ACTH or cortisone revealed marked lymphoid depletion of the spleen and lymph nodes. The adrenals of the animals treated with cortisone were about one-third normal size and histologic examination revealed marked cortical atrophy. On gross and histologic examination no obvious difference was noted in the size of the adrenals in the controls as compared with the group treated with ACTH. Histologic studies of the skin at the site of the tuberculin reaction revealed distinct differences in the degree of tissue response between the hormone-treated animals and the controls. Proliferation of fibroblasts and endothelium was less active in the lesions of the former. The suppression of the repair process was noticeable at 24 hours and had become distinct at 48 hours. Differences in the degree of polymorphonuclear leukocytic infiltration were not appreciable.

Discussion. The occurrence of an unmistakable positive tuberculin reaction in all guinea pigs receiving either ACTH or cortisone corresponds with the observations reported by Spain and Molomut(6). Our results differ from the clinical observations and experimental findings of others who have reported obliteration and suppression of the tuberculin reaction following hormone administration. The presence of active progressive tuberculosis in our animals might in part explain this difference since the experimental findings of others were made on animals which were not infected with tuberculosis but merely sensitized with B C G(1,2). Similarly a recent clinical study of the influence of ACTH or cortisone on the tuberculin reaction was

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carried out on patients with diseases other than active tuberculosis(5). We cannot explain the negative tuberculin skin tests reported in patients with active tuberculosis following the administration of ACTH(3,4).

The daily dose of ACTH or cortisone given to the animals in this experiment corresponds to 10.6 mg per kilo body weight. The morphologic changes in the adrenal cortex, the lymphoid tissue and other organs indicate the effectiveness of the injected hormones. Larger doses of cortisone gave similar results. In a different experiment positive tuberculin skin reactions were always present in 20 guinea pigs with active progressive tuberculosis which received daily 5 mg of cortisone for 50 days. In these animals the tuberculin skin reactions were obtained at 30 and 45 days after the beginning of hormone administration.

In our experiments ACTH or cortisone did not obliterate the tuberculin skin reaction but appeared to diminish its intensity. We believe that the hormones do not interfere primarily with the immune mechanism of the delayed type of antigen-antibody reaction. The apparent modification of the reaction might result from the inhibitory effect of adrenal corticosteroids on the acute inflammatory process(7).

Summary. Guinea pigs with active progressive tuberculosis were given ACTH or cortisone for 6 days. A definite tuberculin skin reaction was obtained in all animals during the period of hormone administration. The reaction appeared less marked in the treated animals than in the controls. It is suggested that ACTH or cortisone do not interfere primarily with the immune mechanism of the tuberculin skin reaction but decrease the associated acute inflammatory process.

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The Relationship Between Aureomycin, Chloramphenicol, and Terramycin (18284)

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Herrell *et al.*(1), working with aureomycin, chloramphenicol and terramycin, found, for a number of strains of *Escherichia coli* and *Aerobacter aerogenes*, that when resistance developed against one of the 3 antibiotics there was a parallel increase in resistance to the other 2.

In the present study, strains of *E. coli* and *Micrococcus pyogenes* var. *aureus 209P* were made resistant to aureomycin or chloramphenicol by subjection to repeated increasing doses of the antibiotics. Their resistance to aureomycin, chloramphenicol and terramycin was then determined. The minimal inhibiting concentrations (M.I.C.) were determined in Difco Penassay Broth according to the method described earlier(2). The results obtained are presented in the accompanying table.

It will be noted that with both organisms, a rise in resistance to either aureomycin or chloramphenicol is accompanied by an increased resistance to the other 2 antibiotics. Thus, after 7 passages in the presence of chloramphenicol, *E. coli* D56 shows a 21-fold increase in resistance to this antibiotic, accompanied by an 11-fold increase towards aureomycin and a 6.5-fold increase to terramycin. After 8 passages in the presence of aureomycin-hydrochloride, a 31-fold increase in resistance is noted, accompanied by 27-fold and 12-fold increases in resistance to chloramphenicol and terramycin-hydrochloride, respectively. The case with *M. pyogenes* var. *aureus 209P* is much the same although re-

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