

intestinal synthesis. We are presently unable to suggest any reason for the differences observed between males and females in their response to vit. B₁₂. It may be that there are sex differences in the requirement for some additional factor. This factor could be present in sufficient amounts to permit response on the part of males to vit. B₁₂ but so limit the growth of the females that no response to vit. B₁₂ supplementation was possible.

The effectiveness of hot-water insoluble liver residue in counteracting the growth depressing effects of thyroid toxicity has been previously observed by Ershoff (4,5). A preparation obtained as a by-product of the manufacture of liver extract (Armour and Company) was tested and found to give a positive response. This substance was more effective in males than in females, although the response with the latter was significant at the 5% level of probability. The hot-water soluble fraction of liver (Biopar A, Armour and

Company) was likewise more effective in males than in females but the number of animals was too small for reliability. Very preliminary efforts to fractionate the activity of the liver residue by exhaustive extraction with 70% ethanol yielded inconclusive results. Both the extract and extracted liver residue were moderately active for males and showed little activity in the case of the females. Again, however, the number of animals used has been very limited.

Summary. The growth depressant effect of toxic levels of iodinated casein in the ration of immature male and female rats was partially counteracted by the addition of fish solubles, liver extracts and a commercial APF supplement. Supplementation with crystalline vit. B₁₂ was more effective in males than in females, but maximum response was not obtained with this substance. The multiple nature of the "antithyrototoxic factor" is therefore suggested.

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Course of Experimental Tuberculosis in the Albino Rat as Influenced by Cortisone.* (18282)

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Previous studies in this laboratory have demonstrated certain quantitative differences in the cellular response of the rat and the rabbit to the intraperitoneal injection of mineral oil (1). It was shown that an oil-induced peritoneal exudate is strikingly different in the rabbit, naturally susceptible to tuberculosis, and in the rat, naturally resistant to the

disease. The rat responded to the intraperitoneal injection of mineral oil with an exudate richer in total cells, percentage of mononuclear cells, and in the absolute number of mononuclear cells than did the rabbit. The administration of cortisone depressed the total exudate in both species with the total and differential counts of the rat approaching those of the normal rabbit (2). These results suggested that tuberculosis in the naturally resistant rat might be altered by cortisone administration. The albino rat responds to infection with virulent human tubercle bacilli with a chronic, granulomatous, non-fatal pro-

* Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. 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.

1. Bloom, W. L., Cummings, M. M., and Michael, M., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1950, v75, 171.

2. Cummings, M. M., Michael, M., Jr., Bloom, W. L., in press.

cess in which caseation is inconspicuous(3). Indeed, in some strains no gross or microscopic lesions appear to develop, despite the fact that tubercle bacilli can be cultured from the tissues and blood as long as 120 days after inoculation(4).

We are reporting herewith the results of experiments designed to test the effect of cortisone on resistance to tuberculosis in the albino rat.

Materials and methods. Albino male Sprague-Dawley rats, whose average weight was 150 g, were used in the studies. These animals were free of bartonella and *P. pseudo-tuberculosis* infection. Two experiments were carried out at different times. In the first experiment, 15 rats were injected intraperitoneally with 2 mg of a young culture of *M. tuberculosis* (H37Rv). Ten of the animals were given cortisone, 5 mg daily in a single injection for the duration of the experiment. The remaining untreated animals in this group served as controls. Five uninfected animals received 5 mg of cortisone daily throughout the study. In the second experiment, the infecting dose of tubercle bacilli was 4 mg. Ten rats were given cortisone alone, 10 received cortisone plus tubercle bacilli, and 20 were infected only with tubercle bacilli. Of this latter group, 10 served as controls for survival times and the other group as controls for pathologic studies of the infected and treated animals.

The first experiment was terminated at 42 days and the second at 52 days. In the second study, with the death of each animal infected and treated with cortisone, a control infected animal was killed. Sections of lungs, liver, spleen, and adrenals were fixed in formalin and in Zenker's solution for staining with Ziehl Neelson and hematoxylin eosin.

Results. The survival time for all groups of animals is indicated in Table I. It will be noted that at the end of the first experiment 100% of the infected animals survived. One animal was killed at 24 days for pathologic studies. Of 5 animals given cortisone alone,

TABLE I.
Survival Times for All Groups of Animals in Each Experiment.

No. of animals	Group	Day of death	No. survivors at 42 days
Experiment 1			
5	TB	24*	4
5	Cortisone	24*,27	3
10	TB + Cortisone	16,18,19,21,25, 30,33,36,38	1
Experiment 2			
10	TB		at 51 days
10	Cortisone	14	10
10	TB + Cortisone	9,14,19,21,25 42,42	9
			3

* In Exp. 1 denotes animals killed at stated time for pathologic study.

80% survived. One rat was killed at 24 days. On the other hand, of the group infected and given cortisone, only 10% were living at the conclusion of the study period. In the second experiment, the results were almost identical. One hundred percent of the group given tubercle bacilli survived, 90% of the group given cortisone alone survived, whereas only 30% of those infected with tubercle bacilli and "treated" with cortisone survived the 52 days of the experiment. The control animals, *i.e.*, those receiving either cortisone alone or tubercle bacilli alone, were still living one month after the study was concluded. Combining survival times for both studies, the figures are as follows: tubercle bacilli alone, 100%; cortisone alone, 87%; and tubercle bacilli plus cortisone, 20%. This significantly higher mortality rate for the infected group receiving cortisone far exceeds that previously recorded for rats infected with tubercle bacilli.

GROSS LESIONS: Rats infected with tubercle bacilli: In all animals, the spleen was enlarged. Diffuse, small, pearly nodules were seen on the pleural surfaces of the lungs in the animals killed after the first month of infection. The lungs otherwise appeared grossly normal. Cultures made from the lungs of several animals in this group were positive for tubercle bacilli.

Rats uninfected, but given cortisone: The spleens were small and no pulmonary lesions were noted. In one animal from Exp. 1 killed on the 40th day, a draining abscess was

3. Cobbett, L., *The Causes of Tuberculosis*; Cambridge, Mass., 1917.

4. Ornstein, G. G., and Steinbach, M. M., *Am. Rev. Tub.*, 1925, v12, 77.

present, extending from the right inguinal region for 2 cm into the right flank. *Staphylococcus aureus* was cultured from this abscess. The adrenals of all rats in this group were quite small. Tubercle bacilli were not recovered in cultures made of the lungs of several animals in this group.

Rats infected with tubercle bacilli and given cortisone: The spleens of animals in this group appeared normal in size. The lungs of three rats in the first experiment (dying on the 19th, 25th, and 30th days) were studded with large, caseous masses involving whole lungs and even invading the chest walls in one instance. Similarly, 4 animals in the second experiment (2 being killed on the 51st day at termination and one each dying on the 25th and 42nd days) showed these lesions. We were unable to isolate *P. pseudotuberculosis* from any of these caseous masses. The lungs of the remainder of the rats showed scattered, purplish, gelatinous, atelectatic areas, at times involving an entire lobe, but usually remaining patchy. There was no correlation between duration of infection and appearance of the lesion. Cultures made from various organs of animals selected at random were positive for tubercle bacilli.

MICROSCOPIC LESIONS: *Rats infected with tubercle bacilli:* The lungs of these animals showed clusters of pale, mononuclear cells, surrounded in some areas by lymphocytes and with little or no alveolar exudate or surrounding edema. The whole picture was that of an orderly well-contained granulomatous process. Acid-fast stains showed a few organisms located chiefly within the mononuclear cells comprising the tubercles. The spleens contained small collections of epithelioid cells in the center of Malpighian corpuscles. Acid-fast bacilli were sparse, located principally within these cells. No lesions were noted in the liver and only small numbers of acid-fast organisms were found in the Kupffer cells.

Rats uninfected, but given cortisone: The histologic changes in this group were those expected from prolonged cortisone administration. The only microscopic findings in the lungs were areas of pulmonary edema and hemorrhage probably resulting from killing the animals by a blow on the head.

Rats infected with tubercle bacilli and given cortisone: In contrast to the lungs of the group infected, but given no cortisone, these animals had diffuse interstitial pneumonitis with profuse alveolar exudation containing large numbers of mononuclear cells, but a paucity of lymphocytes. There was no attempt at tubercle formation and all areas of lung tissue were involved with the pneumonitis. The lungs of the rats described as having caseous areas showed in addition to the described mononuclear lesions intense polymorphonuclear exudation in the alveoli. Acid-fast stains revealed numerous bacilli throughout the lung appearing in the exudate and in interstitial areas. The organisms were much more plentiful than in the group not receiving cortisone. The spleens showed the depletion of lymphoid elements; a few Malpighian corpuscles remained. Numerous acid-fast bacilli were found in the supporting stroma. The livers showed abundant acid-fast organisms lying principally within the Kupffer cells.

Discussion. Our previous studies have shown that cortisone depresses an inflammatory exudate which is characteristic of the cellular response to mineral oil. This exudate is quite similar to that stimulated by tubercle bacilli. Quantitative differences in this mineral oil inflammatory reaction can be demonstrated in the naturally resistant and naturally susceptible animal. Thus, a quantitative depression of the inflammatory response to lipids might reduce the natural resistance of the host to tubercle bacilli if it can be said that cellular defenses are important in this infection. This seems to be borne out by the present series of observations. The changes in the cellular response induced by cortisone may not only be suppression of the over-all capacity of the host response; but, also, a more specific influence on individual cells or on humoral mechanisms. The known specific effects of cortisone on lymphocytes(5), neutrophils(6), and fibroblasts(7), could disturb the orderly response to infection with tubercle bacilli and result in a more favorable

5. Dougherty, T. F., and White, A., *Am. J. Anat.*, 1944, v77, 1.

6. Roche, W. P., and Lathem, W., unpublished observations.

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 sug-

gestion 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 granulomas 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.

7. Ragan, C., Hornes, H. L., Plotz, C. M., Meyer, K., and Blunt, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1949, v72, 718.

8. Spain, D. M., and Molomut, N., *Ab. Rev. Tub.*, 1950, v62, 337.

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

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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 com-

pounds 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.

1. Long, D. A., and Miles, A. A., *Lancet*, 1950, v1, 492.

2. Harris, S., and Harris, T. N., *Proc. Soc. Exp. Biol. and Med.*, 1950, v74, 186.

3. Freeman, S., Ferthing, J., Wang, C. C., and Smith, L. C., *Proc. 1st Clinical ACTH Conference*, edited by J. R. Mote, Blakiston Co., Philadelphia, 1950, p. 509.

4. Favour, C. B., (quoted by Thorne, G. W., *et al.*), *New England J. Med.*, 1950, v242, 866.

5. Long, J. B., and Favour, C. B., *Bull. Johns Hopkins Hosp.*, 1950, v87, 186.