vealed morphological details.

The extremely short shadows indicate that the organisms were flattened by the drying process. Considerable variation in length was seen in all strains, ranging from 6 to 15 μ, occasional specimens being too long to be included in a single microscope field. General morphological features were common to all strains, such as a spiral, or cork screw, pattern. Shadowing revealed a fine filament entwined about the main structure of the organisms which often extended beyond one or both ends of the leptospirae.

The accompanying figures are typical electron photomicrographs of *L. autumnalis,* Akiyama A, *L. hyos, L. pomona, L. canicola,* and *L. mitis,* Johnson. Electron photomicrographs of *L. bataviae,* Swart van Tienen, *L. bovis,* Palestine, and *L. icterohemorrhagiae,* Wijnberg showed organisms that were indistinguishable morphologically from those illustrated in the figures.

Discussion. Length and diameter observed agree with the findings of Morton and Anderson(1). Shadowing with chromium precluded study of internal structure. In contrast to the observations of Morton and Anderson(1) and van Thiel and van Iterson(2) an axial filament entwined uniformly along the central cell mass was demonstrated in each leptospiral strain examined.

Summary. Eight strains of leptospirae were examined by electron microscopy. No morphological differences were found between strains. All strains possessed an axial filament. Diameter of the strains was approximately 0.1 μ, lengths varied from 6 to more than 15 μ.

1. Morton, H. E., and Anderson, T. F., *J. Bact.,* 1943, v45, 143.

2. van Thiel, P. H., and van Iterson, W., *Proc. Kon. Ned. Akad. v. Wet.,* 1947, v50, 823.

3. Schuffner, W., and Mochtar, A., *Proc. Kon. Ned. Akad. v. Wet.,* 1927, v30, 25.

Received May 13, 1952. P.S.E.B.M., 1952, v80.

Influence of Cortisone on Experimental Hypersensitivity and Circulating Antibody in the Guinea Pig. (19565)

FREDERICK G. GERMUTH, JR.,* BARBARA OTTINGER, AND JIRO OYAMA.
(Introduced by Samuel Bukantz.)

From the National Institutes of Health, Microbiological Institute, Bethesda.

Previous studies(1,2) have demonstrated that cortisone suppressed the Arthus reaction and reduced the concentration of serum antibody in rabbits sensitized with egg albumin. This report summarizes the results of a study of the effects of cortisone on antibody-antigen reactions and circulating antibody in the guinea pig. The observations indicate that administration of large doses of this hormone during the course of immunization of guinea pigs with egg albumin modifies the Arthus reaction and effects a significant reduction in circulating antibody. In confirmation of results of other investigators(3,4), pretreatment with cortisone fails to alter either active or passive anaphylactic shock.

* Present address: Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

Materials and methods. Cortisone acetate (Merck and Co.) was administered. Crystalline hen's egg albumin was prepared by sodium sulfate precipitation(5). A pooled rabbit anti-ovalbumin serum containing 0.77 mg of antibody nitrogen per ml was employed for passive sensitization. Guinea pigs weighing 200 to 300 g were injected intravenously with 0.5 ml of a 1:10 dilution of the serum, equivalent to 39 μg of antibody nitrogen. This amount of antibody was sufficient to cause anaphylactic death of 90% of control animals challenged intravenously 48 hours later with 1 mg of egg albumin. For active sensitization, animals were given 2 subcutaneous injections of 10 mg of egg albumin on alternate days. Fifteen days after the first injection, they were challenged intravenously

TABLE I. Effect of Cortisone on Passive Anaphylaxis in Guinea Pigs.

Cortisone, mg/inj.	Total	Treatment before challenge, hr	No. of pigs	No. of deaths	No. of survivors
1	1	2	2	2	0
		4	3	2	1
		6	3	2	1
		8	2	2	0
		24	3	2	1
4	4	4	2	1	1
8	8	4	8	7	1
	8	8	7	7	0
	8	24	8	7	1
	24	21, 52, 69	2	2	0
	32	4, 21, 52, 69	8	8	0
Total cortisone-treated			48	42 (88%)	6
" controls			40	36 (90%)	4

with 1 mg of ovalbumin. In the study of the effect of cortisone on the Arthus reaction and on circulating antibody, a group of 60 male white guinea pigs weighing 500 to 600 g were inoculated subcutaneously with 0.5 ml of an emulsion prepared by blending 100 mg of heat-killed tubercle bacilli, 40 ml of "Falba," 1 g of egg albumin dissolved in 20 ml of saline, and 20 ml of paraffin oil(6). A second injection of 0.5 ml of the emulsion was administered 4 days later. Four, 10, 17, and 23 days after the second injection, the guinea pigs were challenged intracutaneously with 0.2 ml of an antigen solution containing 0.06 mg of egg albumin nitrogen. At the time of the last skin inoculation, a test for tuberculin hypersensitivity was performed with 0.1 ml of a 1:20 dilution of O.T. prepared by the Bureau of Animal Industry, U. S. Department of Agriculture. Three days later or 30 days following the first sensitizing injection, the animals were bled from the heart and killed with chloroform. Spleen, liver, and adrenals were weighed. Antibody nitrogen content of the sera was determined by the quantitative precipitin method of Heidelberger and Kendall and the micro-Kjeldahl technic(7).

Experimental results. A. *Effect of cortisone on passive anaphylactic shock.* Thirteen passively sensitized guinea pigs were treated with a single intramuscular injection of 1 mg of cortisone at 2, 4, 6, 8, or 24 hours before challenge. Thirty-three others were given injections of 8 mg of the hormone at intervals ranging from 4 to 69 hours prior to receiving the antigen. Since the interval between passive sensitization and challenge was 48 hours, those animals which received cortisone 69 hours before challenge were treated with cortisone prior to sensitization. Following the intravenous injection of antigen, all animals developed symptoms typical of anaphylactic shock; no significant differences were observed between the cortisone-treated group and the controls (Table I).

B. *Effect of cortisone on active anaphylaxis.* Fifteen guinea pigs undergoing active sensitization were treated daily with a single intramuscular injection of 1 mg of cortisone beginning on the first day of sensitization. Another 15 were given 2 mg of cortisone daily; 10 others received 5 mg of cortisone for 4 days prior to sensitization and for the next 10 days, after which time the dosage was reduced to 2.5 mg per day because of weight loss and deaths. Fifteen days after the first sensitizing injection of antigen, while still under treatment, the animals were challenged intravenously with 1 mg of egg albumin. As shown in Table II, treatment with cortisone neither inhibited sensitization nor modified the symptoms of anaphylactic shock, and deaths in all groups occurred within 6 minutes after receiving the antigen.

C. *Effect of cortisone on the active Arthus reaction,* on circulating antibody, and on the tuberculin skin test. Twenty of 60 guinea pigs sensitized with antigen incorporated in Freund's adjuvant mixture were treated daily from the onset of sensitization with 5 mg of cortisone for 18 days and then with 10 mg of the hormone for the next 12 days. The hor-

TABLE II. Effect of Cortisone on Active Anaphylaxis in Guinea Pigs.

Cortisone, mg/inj.	Total	No. of pigs	No. of deaths	No. of survivors
1	15	14	14	0
2	30	14	12	2
2.5–5	72.5	5	4	1
Total cortisone-treated		33	30	3
" controls		34	32	2

TABLE III. Effect of Cortisone on Active Arthus Reaction and Circulating Antibody in Guinea Pigs Sensitized with Crystalline Egg Albumin.

No. of pigs	Treatment	Skin reaction	Serum antibody conc. in μg of Ab N/ml, mean $\pm$ S.E.
17	Cortisone, 5 mg/day, 0-18th day; 10 mg/day, 18-30th day	Avg = .5 r* 3 hemorrhagic 9 red 5 pink	26 $\pm$ 7 3 of 16 with conc. >40
32	0	Avg = 4 h 18 hemorrhagic 12 red 2 pink	109 $\pm$ 17 21 of 28 with conc. >40

* Product of length, width and height measured in cm.

mone was administered in 2 divided doses given 12 hours apart. These dosages of 10 to 20 mg of hormone per kg of body weight, which are approximately 5 to 10 times greater than that generally employed in the treatment of man (100 mg per 50 kg), did not markedly affect the nutritional status of these larger guinea pigs. Most of the treated animals gained weight during the experimental period, although not as rapidly as did the 40 controls. Three of 20 treated animals and 8 of 40 controls died before the termination of the experiment.

Eight days after the first sensitizing injection of egg albumin in adjuvant, most of the animals produced a slight pink skin reaction to an intradermal test dose of antigen. With this first skin test, no differences were observed in the responses of the cortisone and control animals. Although the control animals responded with more intense Arthus reactions to succeeding skin tests, the reactions of the cortisone treated animals failed to increase in intensity. In Table III are presented the average skin responses to the last or fourth injection of egg albumin. Eighteen of 32 control animals or 56% produced a hemorrhagic Arthus reaction, whereas only 3 of 17 treated guinea pigs or 18% responded in a similar manner. By the χ^2 method, this difference could occur by chance alone in approximately once in 50 times. It is, therefore, probable that treatment with cortisone produced a significant inhibitory influence on the Arthus reaction.

The effect of cortisone on circulating antibody was striking. As shown in Table III, the mean serum antibody nitrogen concentration of the treated animals was less than one-fourth that of the controls (26 and 109 μg of antibody nitrogen per ml of serum, respectively). The probability of this result occurring by chance alone is approximately 1 in 1,000 as determined by "Student's" T test. A rough correlation similar to that previously observed in rabbits(2), between the serum antibody concentration and the severity of the skin response, was encountered. Only one of 3 treated animals and one of 15 controls which responded maximally to the antigen had less than 40 μg of circulating antibody nitrogen per ml of serum.

Since the guinea pigs in the present study were sensitized with antigen in Freund's adjuvant containing heat-killed tubercle bacilli, an opportunity for study of the effect of cortisone on the development of the tuberculin reaction arose. The average tuberculin reactions present 48 hours after an intradermal injection of 0.1 ml of a 1:20 dilution of "Old Tuberculin" are given in Table IV. It is apparent that treatment with cortisone diminished the tuberculin response. The skin le-

TABLE IV.
Effect of Cortisone on Tuberculin Skin Test.

No. of pigs	Treatment	Tuberculin reaction
16	Cortisone, 5 mg/day, 0-18th day; 10 mg/day, 18-30th day	Avg = .1 p* 3 red 13 pink
31	0	Avg = 1 r 13 red, necrotic center 11 red 7 pink

* Product of length, width and height measured in cm.

TABLE V. Effect of Cortisone on Weights of Spleen, Liver and Adrenals.

No. of pigs	Treatment	Wt of following organs in g, Mean ± S.E. Spleen	Liver	Adrenals
17	Cortisone, 5 mg/day, 0-18th day; 10 mg/day, 18-30th day	.77±.05	27.7±1	.20±.01
26	0	1.22±.05	24.4±1.1	.27±.01

sions of the treated animals were smaller and less severe than those of the controls. Furthermore, none of the lesions of the treated animals became necrotic, whereas one-third of the control lesions showed necrotic centers.

The organ changes produced by cortisone (Table V) were similar to but less extensive than those which have been previously observed in the rabbit(8). The spleen and adrenals were distinctly smaller than those of the controls, weighing respectively two-thirds and three-fourths as much. The liver was normal in size and appearance and no lipemia was visible.

Summary. 1. Large doses of cortisone failed to protect guinea pigs against active or passive anaphylactic shock. These results are in agreement with those of other investigators(3,4). 2. Treatment with this hormone during period of active sensitization diminished the intensity of the Arthus reaction and the quantity of circulating antibody produced. Neither of these effects was as extensive as those observed in rabbits following the administration of comparatively smaller doses of cortisone(1,2). Similarly, the anatomic changes produced by cortisone were less striking in the guinea pig than in the rabbit (8). These findings suggest that the guinea pig is considerably more resistant to the action of cortisone. The failure of cortisone to abolish the active Arthus reaction and possibly its failure to alter active anaphylactic shock might have been related to the incomplete suppression of circulating antibody under the experimental conditions employed.

1. Germuth, F. G., Jr., and Ottinger, B., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 815.

2. Germuth, F. G., Jr., Oyama, J., and Ottinger, B., *J. Exp. Med.*, 1951, v94, 139.

3. Fischel, E. E., *Bull. New York Acad. Med.*, 1950, v26, 255.

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

5. Kekwick, R. A., and Cannan, R. K., *J. Biochem.*, 1936, v30, 227.

6. Freund, J., and McDermott, F., PROC. SOC. EXP. BIOL. AND MED., 1942, v49, 548.

7. Kabat, E. A., and Mayer, M. M., *Experimental Immunochemistry*, Springfield, Ill., Charles C. Thomas, 1948, 133.

8. Germuth, F. G., Jr., Nedzel, G. A., Ottinger, B., and Oyama, J., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 177.

Received March 31, 1952. P.S.E.B.M., 1952, v80.

A Phylogenetic Study of the Milk "Let-Down" Hormone. (19566)

W. G. WHITTLESTONE, E. G. BASSETT, AND C. W. TURNER.*

From the Ruakura Animal Research Station, Hamilton, N. Z., and the Missouri Agricultural Experiment Station, Columbia.

Mammals are divided into 3 divisions. The monotremata or egg-laying mammals are the most primitive derivatives from reptilian stock. Their mammary glands open on the skin in association with hairs but are without

* Contribution from the Department of Dairy Husbandry, Missouri Agric. Exp. Sta., Journal Series No. 1307.