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Electron Microscopy of Leptospiral Strains. (19564)

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Although several studies(1,2) have been made on the morphology of leptospirae, additional information about structural details of these organisms is desirable. The following strains of leptospirae currently under study in this laboratory were therefore prepared for electron microscopy: *L. icterohemorrhagiae,* Wijnberg obtained from the University of Tropical Medicine, Leiden, The Netherlands; *L. autumnalis,* Akiyami A, *L. bataviae,* Swart van Tienen, *L. pomona,* and *L. mitis,* Johnson obtained from the National Institutes of Health, Bethesda, Maryland; *L. hyos* obtained from the Malbran Bacteriological Institute, Buenos Aires, Argentina; *L. bovis,* Palestine obtained from the Hebrew University, Jerusalem, Israel; and *L. canicola* isolated at the Veterinary Division, Army Medical Service Graduate School, Washington, D. C.

Materials and methods. Five-day-old cultures of leptospirae grown in Schüffner's modification of Verwoort's medium(3) were employed. Cultures were centrifuged at 20,000 g in the International PR-1 refrigerated centrifuge for 10 minutes at 5°C. The supernatant fluid was discarded and the leptospirae washed in M/15 phosphate buffered

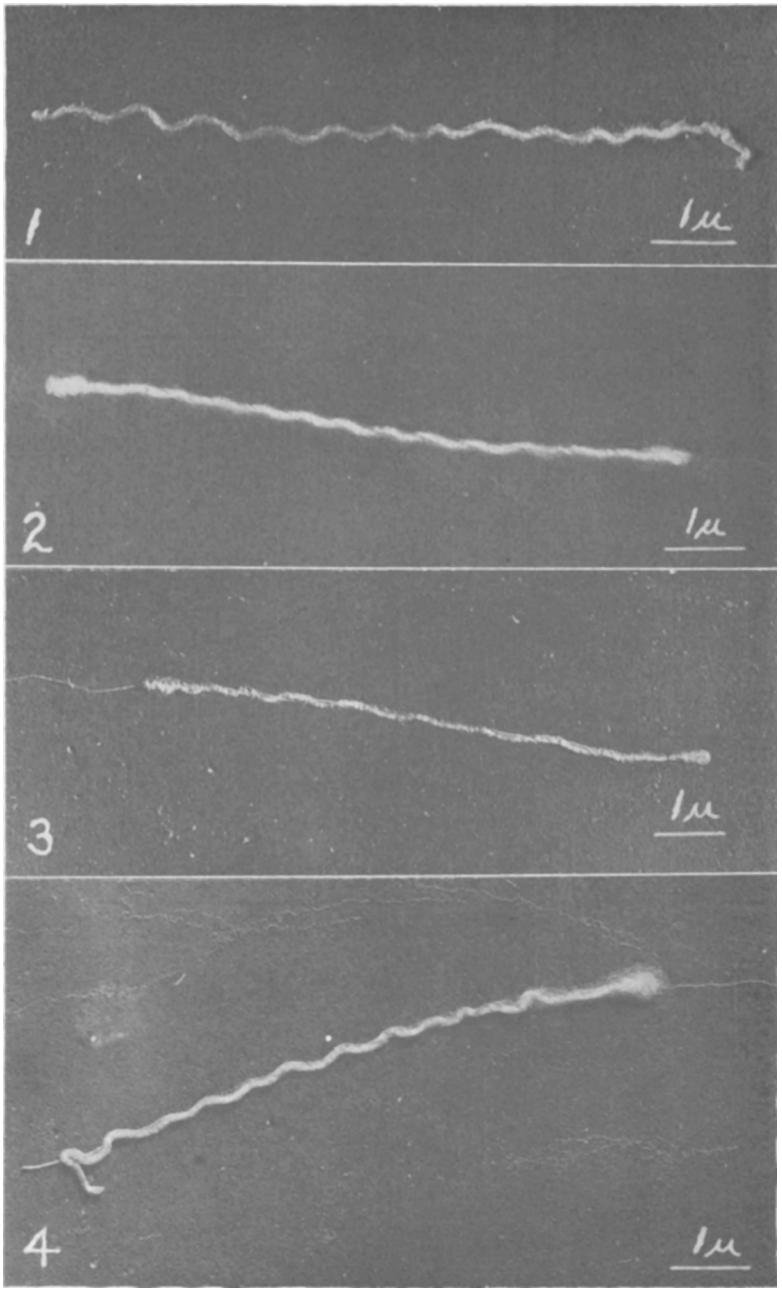

FIG. 1. *L. autumnalis,* Akiyami A, chromium shadowed, magnification 10,750 ×.
FIG. 2. *L. autumnalis,* Akiyami A, " " " 10,750 ×.
FIG. 3. *L. hyos,* chromium shadowed, magnification 9,200 ×.
FIG. 4. *L. hyos,* " " " 10,600 ×.

saline at pH 7.0. The suspensions were centrifuged again at 20,000 g for 10 minutes and the supernate discarded. Final suspension was made in the same buffered saline to approximately half the original volume. A drop of each of the freshly prepared suspensions was placed on standard stainless steel specimen grids which had been previously coated with a parlodian membrane. Excess solution was removed with a micropipette and the

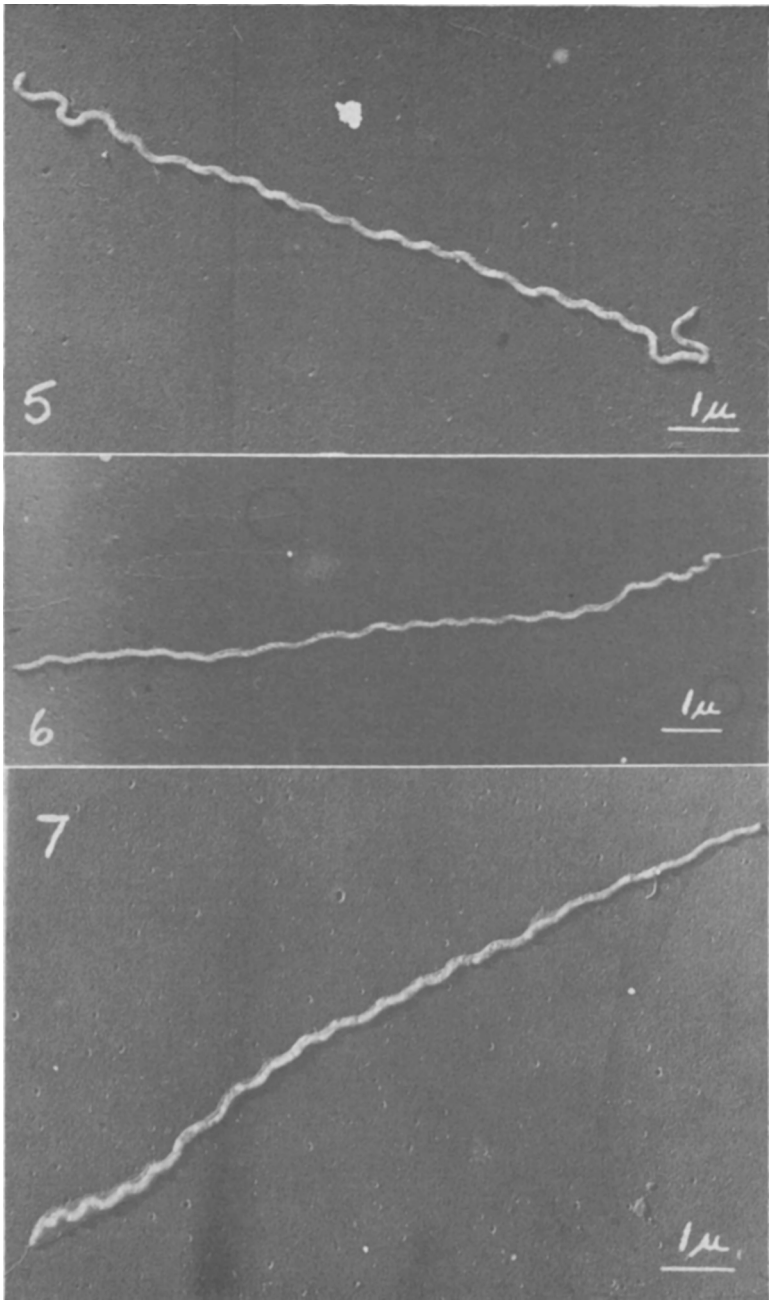

FIG. 5. *L. pomona,* chromium shadowed, magnification 9,200 ×.
FIG. 6. *L. canicola,* " " " 7,500 ×.
FIG. 7. *L. mitis,* Johnson, chromium shadowed, magnification 9,200 ×.

specimens dried in air. The screens were shadowed with 23 mg of chromium at an angle of 11°. An RCA model EMU-2B electron microscope was used. Specimens of each strain were examined and representative organisms photographed.

Results. Leptospirae were evenly distributed on the screens. Extended rather than curled specimens were chosen for electron photomicrography since they more clearly re-

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.

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

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(Introduced by Samuel Bukantz.)

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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.

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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