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Leptospiral Antigen Demonstrated by the Fluorescent Antibody Technic in Human Muscle Lesions of *Leptospirosis icterohemorrhagiae*.* (20578)

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Leptospiral antigen was demonstrated with the fluorescent antibody technic in the muscle lesions of a patient with *L. icterohemorrhagiae* infection.

Materials and methods. A 1 cm square piece of the left gastrocnemius muscle was excised from a 42-year-old Negro construction worker on the 9th day of an acute febrile illness, associated with icterus, azotemia, muscle pain which was particularly marked in the calf, headache and malaise. Half the specimen was immediately quick frozen in dry ice and alcohol and stored at —20°C. The remaining tissue was divided and fixed in Zenker's fluid with 5% glacial acetic acid and in 10% formalin. Routine histologic preparations revealed the typical lesions of acute Weil's disease (Fig. 1). These lesions consisted of focal areas of necrosis of muscle fibers with slight proliferation of sarcolemmal nuclei and minimal inflammatory cell infiltration(1). Sections stained by the Whartin-Starry method failed to reveal any organisms. Agglutination tests were positive for *L. icterohemorrhagiae* with titres of 1:6400 on the 16th day of illness and 1:12800 on the 31st day. Both serum samples were also positive for *L. mitis* at a titre of 1:640 and for *L. canicola* at a titre of 1:200. During convalescence, 31 days after the onset of the illness, a biopsy of the right gastrocnemius muscle was taken. This tissue was prepared as described above. Routine histologic preparations of this specimen revealed advanced healing by regeneration. Silver stains for leptospira again showed no organisms. Frozen sections of unfixed frozen muscle were prepared following the technic of Linderstrøm-Lang as modified by Coons *et al.*(2). Preliminary to staining, the mounted sections were fixed either by dipping briefly in 70% ethanol and rinsing with buffered saline or by covering with acetone for 15 minutes with subsequent evaporation of the acetone in an incubator at 37°C. These 2 types of fixation had been arrived at while adapting the fluorescent antibody technic to the study of experimental leptospiroses in Syrian hamsters.

The sections were stained for 30 minutes with the specific conjugate prepared according to the method of Coons and his associates(3). Conjugates with fluorescein isocyanate were prepared from immune sera obtained from rabbits injected with either *L. icterohemorrhagiae* or *L. canicola*.† The agglutination

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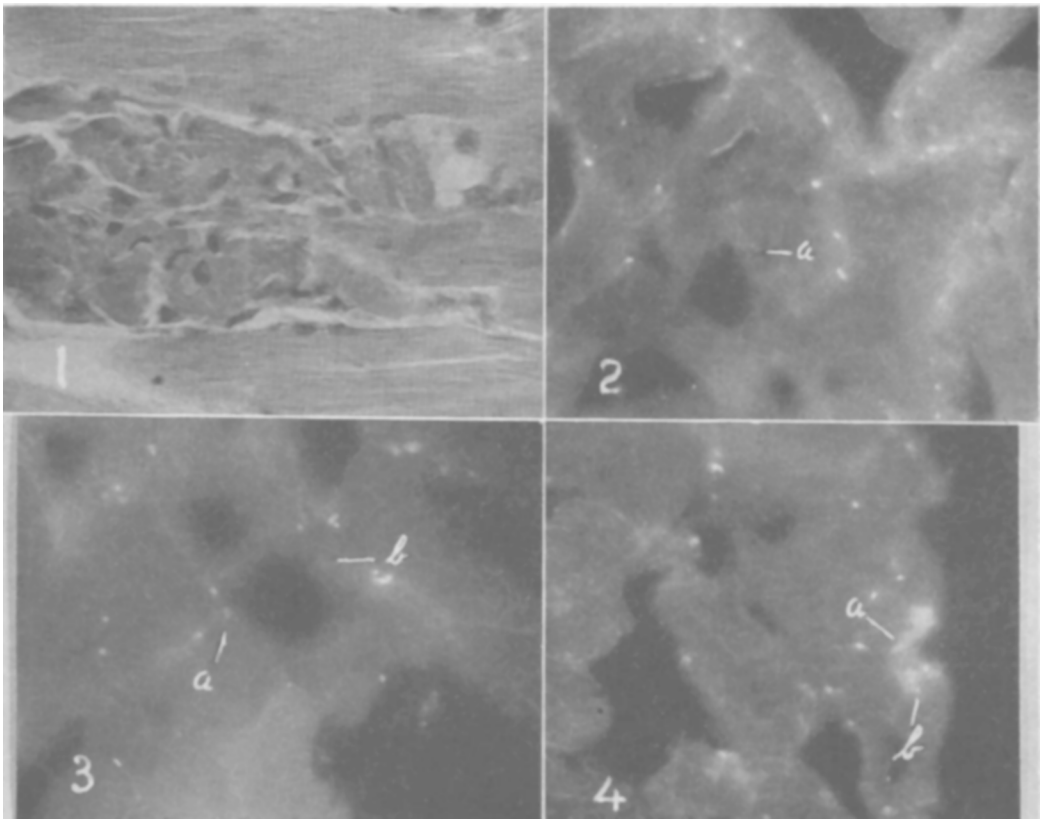

FIG. 1. Areas of focal necrosis in 2 adjacent striated muscle fibers. Hematoxylin-Phloxine. × 370.

FIG. 2. Fluorescence photomicrograph showing minute specifically fluorescing granular deposits (a) in sarcoplasm. × 370.

FIG. 3. Fluorescence photomicrograph showing 2 specifically fluorescing deposits in muscle. The larger deposit (a) is granular, the other (b) shows a delicate and slightly curved structure resembling a leptospira. × 370.

FIG. 4. Fluorescence photomicrograph showing area of muscle necrosis (a). A minute deposit of specifically fluorescing material is visible at the edge of the necrotic area (b). × 370.

titre of each serum was 1:6400. Before use for staining, the conjugate was absorbed twice with human liver powder. This procedure, however, did not eliminate the non-specific staining of human neutrophilic polymorphonuclear leucocytes, which was observed in blood smears of this patient and normal humans. It was found that the non-specific staining of these cells, which was observed also in other mammalian species could be eliminated in human as well as in rabbit tissue by absorbing the conjugate twice with acetone-dried rabbit bone marrow powder. Controls consisted of: 1. inhibition tests in which paired frozen sections were covered with unconjugated normal or specific immune serum preliminary to the staining with the specific conjugate, and 2. staining with *L. canicola* conjugated antiserum.

Results. Under the fluorescence microscope a few widely scattered deposits of specifically fluorescing green material were found. The deposits were located in a vacuolated appearing area within the sarcoplasm (Fig. 2). Only in a few instances were they encountered at the periphery of areas of muscle necrosis. They were occasionally seen at the periphery of a muscle fiber, but it could not be determined if they were located in the sarcoplasm, within the sarcolemma sheath or within the perimysium. The deposits appeared as minute granules but in rare in-

stances single elongated and curved structures resembling intact leptospirae were seen (Fig. 3). The foci of muscle necrosis were clearly visible and in general showed yellow fluorescence against the grayish-blue background (Fig. 4). Occasionally the yellow color showed a greenish fluorescing tinge. No antigen was found in the interstitial connective tissue or in the nuclei.

Fluorescent deposits were not found in sections stained with *L. canicola* conjugate or subjected to the inhibition test by treatment with unconjugated specific immune serum before staining with the specific conjugate.

Leptospiral antigen was not seen in the biopsy taken on the 31st day of illness.

Comments. The specificity of muscle lesions in Weil's disease has been questioned by Adams, Denny-Brown, and Pearson(4) since even in severe infections with florid lesions it is extremely difficult with the conventional histologic methods to identify with certainty leptospirae in the muscle lesions(5). I have studied the muscle lesions obtained by biopsy or at autopsy of many patients with *L. icterohemorrhagiae* infection. Only once have I encountered in silver impregnated sections from muscle biopsy structures which I felt certain were leptospirae. These structures were few in number and were found at the periphery of the muscle lesions. They were not present in the necrotic debris. The presence of leptospiral antigen in the muscle and its lesions seems to indicate that the lesions are produced by the organism.

The amount of demonstrable antigen in this patient was sparse. It was generally seen in the sarcoplasm not associated with well developed lesions and was found only rarely at the periphery of the foci of muscle necrosis. Antigen was not identified in the nuclei, interstitial tissues or among the debris of the lesions, although the necrotic material sometimes showed a faint greenish-yellow fluorescence. It is possible that the paucity of demonstrable antigen is due to its saturation or destruction by endogenous specific antibody since the biopsy was taken on the 9th day of illness, at which time there is a significant antibody titre in Weil's disease. The presence of a green fluorescing tint in some of the otherwise yellow fluorescing areas of necrosis suggests the diffusion of antigen among the necrotic material.

The non-specific staining of polymorphonuclear leucocytes of normal humans and other mammalian species is of interest. This staining appeared even in blood smears fixed with either alcohol or formalin and after treatment with fluoride. The suppression of the non-specific staining of these cells by absorption of the conjugate with bone marrow powder seems useful when the fluorescent antibody technic is applied to lesions associated with inflammatory cell infiltration.

Summary. Leptospiral antigen was demonstrated with the fluorescent antibody technic in the muscle lesions of a patient with *L. icterohemorrhagiae* infection. This observation indicates that the muscle lesions in Weil's disease are produced by the leptospirae. It has also been shown that the non-specific staining of human polymorphonuclear leucocytes can be eliminated by absorption of the conjugate with rabbit bone marrow powder.

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