

RAT LEPROSY: SUSCEPTIBILITY OF THE BLACK MOUSE
(AMERICAN RACE) TO THE STEFANSKY BACILLUS

PRELIMINARY REPORT

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On March 15, 1949, with the assistance of Dr. Bueno de Mesquita of Surinam, I inoculated nine black mice (*Mus musculus*, black race C-57, Rockland Farms, U. S. A.), with a fresh suspension of lepromas from white rats inoculated six months previously with Stefansky bacilli supplied by Dr. R. Chaussinand of the Institut Pasteur, Paris. The inoculations (0.4 cc. of the suspension) were made subcutaneously, in the right axilla or groin of the animals, which weighed from 20 to 30 grams.

On April 6, 22 days after inoculation, four of the nine mice presented areas of alopecia in various parts of the body, and small ulcerations at the points of inoculation. They were thin and seemed very sick. All four were killed with gas. Necropsies showed that three of them had enlarged lymph nodes, two had groin tumors, and one had an abdominal tumor. Suspensions of these tumors and lymph nodes were similar to ordinary rat leproma suspensions, rich in acid-fast bacilli. All of the viscera and testes were positive for acid-fast bacilli, but the smears were poor.

The suspensions of the tumors were inoculated onto two groups of 10 tubes of Loewenstein medium. After 30 days incubation at 37°C. there developed a pearl-like granular, creamy growth in one of these 20 tubes. Smears of this culture (strain No. 3) stained by the Ziehl-Neelsen method proved that it was a pure growth of acid-fast bacilli. They grow very well, although slowly, on Loewenstein medium and glycerin-agar. In the third generation this culture became slightly yellow. The aspect of its smear stained by carbol-fuchsin is represented in Fig. 4.

On May 3, the 48th day after inoculation, the fifth mouse was killed. It presented a large plaque of alopecia around the point of inoculation (Fig. 1). Necropsy showed a large tumor below the area of alopecia, involving the skin and the pectoral muscles. The skin with a part of the tumor was examined and reported on by Dr. C. Magarinos Torres, chief of the Pathological Anatomy of this Institute, as follows:

Skin of black mouse: Inflammatory granuloma in the dermis, predominantly formed by macrophages but also presenting lymphocytes and plasmocytes, without tendency to necrosis. Section stained by Ziehl-Neelsen showed a great number of acid-fast bacilli within the cytoplasm of the macrophages (see Figs. 2 and 3).

This Stefansky leproma was formed in 48 days, proving that this kind of mouse is much more susceptible to the Stefansky bacillus than any other laboratory animal.

A suspension made of a part of this tumor was treated with 10% NaOH (Petroff method), washed three times with sterile distilled water, centrifuged each time. The sediment was inoculated onto 11 tubes of Loewenstein medium, which were incubated at 37°C. After 30 days (June 3) a growth appeared in one of these tubes, granular and creamy, composed of acid-fast bacilli contaminated with blue-staining bacteria. Treatment of this culture by Petroff's method failed to purify it.

On May 13, 58 days after inoculation, the sixth mouse died with a groin tumor. Smears from the organs were rich in acid-fast bacilli. The tumor was made into a suspension with which three tubes of old, dried medium of Loewenstein were inoculated. No growth was obtained.

The last three mice of the group were reinoculated with this suspension of rat leproma. The seventh mouse died on June 8 but was not examined. The last two died on July 12, the 89th day after the first inoculation. The organs of both were positive for acid-fast bacilli, and one had enlarged lymph nodes and a tumor.

On the next day a suspension of the lymph nodes, treated by the Petroff method, was inoculated onto tubes of Loewenstein medium. After four weeks (August 19) one of these tubes showed a growth, a very thin layer of brilliant-yellowish color which microscopic examination (September 3) showed to be composed entirely of acid-fast bacilli, mostly of coccoid form (strain No. 4). Transplants were made on Loewenstein and other media. On September 15 the original culture had not grown much, covering only one-half of the surface of the medium; it was cream-yellowish and of dry aspect. The second generation was growing normally on suitable media.

A study of these two cultures derived from mice will be made comparatively with two other strains that were obtained from white rats inoculated with the Parisian strain of the Stefansky bacillus, and will be presented to the Section of Mycobacteriaceae of the Fifth International Congress for Microbiology, to be held in Rio de Janeiro in August 1950. The less chromogenic of the

cultures obtained from white rats referred to is highly pathogenic for rats and the black mouse, and subcultures can easily be recovered from animals inoculated with this strain.

SUMMARY

The black mouse used, inoculated with Stefansky bacillus material, has developed typical lesions of rat leprosy in a very short time, showing itself to be more susceptible than any other animal used.

From lesions induced in the black mice inoculated with such material, two cultures of acid-fast bacilli have been obtained.

DESCRIPTION OF PLATE

PLATE 4.

FIG. 1. Photograph of a black mouse inoculated with a suspension of a rat leproma, Paris strain of the Stefansky bacillus, on March 15, 1949, killed on May 3rd, 48 days after inoculation. The area of alopecia overlies an extensive granuloma.

FIG. 2. Photomicrograph of a section of the skin showing the Stefansky granuloma.

FIG. 3. Photomicrograph showing masses of Stefansky bacillus into the granuloma. X 200.

FIG. 4. Photomicrograph of a smear of the first culture obtained from mouse granuloma.

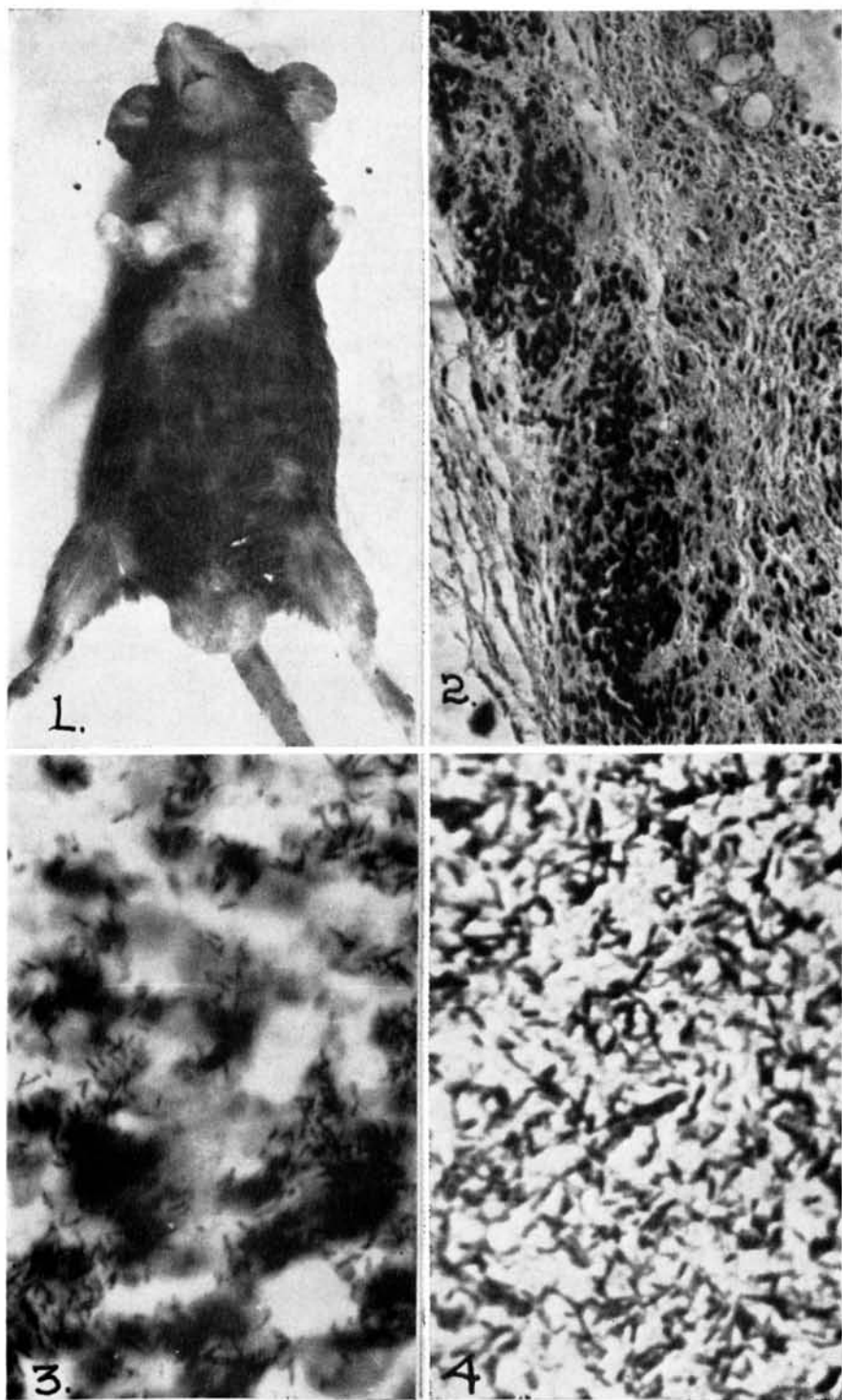


PLATE 4.