PATHOGENICITY OF ACID-FAST BACILLI ISOLATED FROM HUMAN LEPROSY BY MIGONE*

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Since 1927 I have collected cultures of acid-fast bacilli isolated from human leprosy and rat leprosy. In this collection are all of the cultures carried by the Lister Institute of London, the National Health Institute of Washington, and the American Type Cultures Collection of Chicago. Among others are some received from Carville, sent by Hasseltine, and those cultivated in Japan by Ota and Sato, in Germany by Deycke, in Honolulu by Badger, in Colombia by Lleras Acosta and in Paraguay by Migone.

In 1928 I isolated an actinomyces (*A. lepromatis*) from a human leproma. That organism, upon being transplanted into special media according to Lieske's advice, mutated into a facultative acid-fast bacillus. I do not know, as yet, its relationship with leprosy—it was not pathogenic for laboratory animals at the time—but that isolation represents a partial repetition of results of previous works of others, and was later confirmed by Walker (1929).

I consider of great importance the isolation, from cases of leprosy, of any cultures of "permanent" acid-fast bacilli, whether they are chromogenic or not. Those acid-fast bacilli are, in general, pathogenic for laboratory animals, and they serve well as antigens for serological reactions or for skin tests. Since 1933 I have been carrying on various studies with some of the above-mentioned cultures, with results that have been published from time to time.

EXPERIMENTS WITH MIGONE'S CULTURES

Recently I have received from Dr. L. E. Migone, of Asunción, Paraguay, three cultures of acid-fast bacilli isolated by him from human leprosy. I had already tried to infect animals with

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strain I, in 1933 and 1934, without much result. With strains II and III results have been obtained that are worthy of consideration.

RESULTS WITH STRAIN II

This organism was isolated from cutaneous lesions of leprosy. For purposes of inoculation I triturated a growth on glycerinated potato in a sterilized mortar with saline solution. The emulsion obtained, which contained only acid-fast bacilli, was inoculated into five white rats (lot. 5) on June 9, 1939, 1 cc. being given subcutaneously in the right axilla and 1 cc. intraperitoneally.

On June 15, one rat died. No lesions were found. Smears from the spleen, liver, lung, kidneys and testicles showed a few acid-fast bacilli. On July 10, the 31st day after inoculation, another one died. Autopsy showed lesions in the liver. Smears of this organ were rich in bacilli, grouped in bundles and globies, which forms are found uncommonly or very rarely in experiments of this kind. Smears from the spleen, kidneys and feces were also positive.

Passage.—On July 10 an emulsion was prepared from fragments of the liver and spleen of the animal last mentioned, and inoculated it into four white rats (Lot 5A), 1 cc. each, subcutaneously in the right axilla. On August 4th one rat died, and another on August 11th. On autopsy they showed no lesions. Smears from the viscera were negative. On December 26th, the 169th day after the inoculation, another one died. No macroscopic lesions were found. Smears taken from liver, spleen, lung and kidneys were positive, with a few bacilli; none in smears from the testicles.

On January 5th, 1940, the last rat was found to have scattered zones of alopecia, and a nodule in the axilla. On March 7th (241 days after inoculation) it presented a tumor covering the entire chest, from axilla to axilla, like a "plastron," most developed in the right axillary region where the skin was almost deprived of hairs. Heart puncture showed bacilli in the blood.

The animal was sacrificed with ether. The aspect of the granuloma was identical with that of a rat leproma, but in autopsies of more than 100 rats with tumors produced by Stefansky's bacillus I have never encountered one that adhered to the skin and penetrated the muscles like this one (see Plate 20, Figs. 1 to 3).

Smears taken from a piece of this granuloma were surprisingly rich in acid-fast bacilli in bundles, masses and globies. Smears from the liver and spleen were also very rich in intracellular globies; those from the kidneys and testicles were positive. An emulsion of the tumor was subinoculated to other animals, including guinea-pigs for exclusion of tuberculosis; they are under observation.

Histopathology.—Fragments of the tumor and organs were sent for examination to Dr. C. Magarinos Torres, chief of the section of anatomo-pathology of this institute. The following is from his report.

Chest muscle: Considerable infiltration of the perimysium and the connective septa (endomysium) by large mononuclear cells with apparently normal nuclei, containing in their cytoplasm large numbers of acid-fast bacilli, the whole presenting a histologic aspect similar to the granulomatous infiltration of rat leprosy. These macrophages lie side by side, forming a tissue of uniform aspect which may be seen in successive microscopic fields. In certain fields the parallel disposition of the cells and their elongated nuclei remind one of the aspect of fibroblasts. In the primitive fasciculi, in which the endomysium is invaded by the granulomatous infiltration, there is atrophy and disappearance of muscle fibers (Figs. 1 to 3). There are also giant cells, not of the Langhans type. There is nothing resembling the follicles of tuberculosis, and no necrosis or caseation. Sometimes, in small areas, there are seen large numbers of nuclei in karyorrhexis (circumscribed infiltration by heterophiles?). The uniformity of the granulomatous infiltration is scarcely disturbed by small and rare foci of perivascular lymphocytic infiltration. Presence of rare sarcosporidia.

Lung: bronchopneumonia. Testicle: small calcified nodules. Liver: hyperemia. Spleen: congestion; pigmented cells in the red pulp. Kidneys: congestion. Heart: hyperemia.

RESULTS WITH STRAIN III

Ten white mice (lot 7) were inoculated on March 26, 1940, with a homogeneous emulsion of a culture of this strain, an acid-fast bacillus isolated from skin lesions of human leprosy. The culture had been grown for seven months on the surface of glycerinated water of a potato medium (Fig. 6). The inoculations were made subcutaneously in the groin. The dosage, 1 cc., proved to be too heavy, because it killed three animals on the fifth day and two on the sixth.

The last two mice were autopsied. One showed no lesions, but smears from the viscera were positive. The other one showed a caseous nodule in the injected groin, hypertrophy of the inguinal lymph nodes and abscesses in the liver. Smears taken from the caseous nodule, lymph nodes, liver and spleen were strikingly

rich in acid-fast bacilli. Many masses and globies were seen inside cells, evidence of the multiplication of the bacillus inoculated. Smears from the kidneys and testicles were much less rich in bacilli.

On April 4th, the 10th day after inoculation, another mouse died. Autopsy showed a caseous nodule in the right groin (site of inoculation) and enlargement of the inguinal and axillary lymph nodes. Smears from the caseous mass were rich in acid-fast bacilli, as is seen in Fig. 7. Smears from the liver, lung, lymph nodes, spleen, kidneys and testicle were all rich in bacilli.

The caseous nodule was emulsified, together with fragments of the liver and spleen, and inoculated into five mice (Lot 7A). Four mice of Lot 7 were left under observation.

Histopathology.—A portion of the liver of the mouse last described was submitted to Dr. Torres, who reported as follows:

> Sections of various parts of this fragment show, besides hyperemia, foci of macrophagic infiltration. The granuloma is situated in the interior of the liver lobe itself. There is stasis of cells in the lumen of capillaries, the mononuclear type predominating. In one of the liver fragments the inflammatory granuloma, more extensive, shows caseation necrosis.

Passage.—Five mice (lot 7A) were inoculated with an emulsion of the tissues of a mouse of lot 7. Dosage 1 cc., subcutaneously, in the groin. On the next day one died; aspect normal, few bacilli in the spleen. On the second day two others died, both with enlarged inguinal lymph nodes and caseous nodules at the points of inoculation. On the fifth day the fourth mouse died, leaving one. Autopsy showed a nodule of tumor consistency in the right groin. Smears from the nodule, liver, lung and kidneys were positive for acid-fast bacilli.

Histopathology.—Dr. Torres reported on an examination of the liver of this fourth mouse, as follows:

> Sections of different parts of the organ show an inflammatory granuloma consisting of macrophages distributed in small foci in the interior of the liver lobes. There are also extensive and irregular areas of coagulation necrosis of the hepatic tissue.

CONCLUSIONS

1. Two new strains of acid-fast bacilli isolated from skin lesions of human leprosy by Professor Luis E. Migone, of Paraguay, have been studied.

2. These cultures have proved to be pathogenic for rats and mice, producing systemic infection and typical granulomata

identical with those obtained with fresh emulsions of human lepromata.

3. The characteristic granuloma so produced is a skin-muscle tumor differing from those obtained by artificial infection of rats with the Stefansky bacillus.

4. Smears from these granulomata, or from viscera of the infected rats or mice, were very rich in clusters or "globies" forms of the Hansen bacillus, very seldom obtained in experiments with cultures.

5. The experiments with Migone's cultures will be continued in comparison with other cultures of so-called *"Bacillus leprae"* cultures.

ADDENDUM

Further passages, Migone strain II.—(*a*) With an emulsion of the granuloma found in the last rat of lot 5A, described in the foregoing, two guinea-pigs were inoculated on March 18, 1940, in the right groin. One died on June 16th, presenting a caseous nodule in the region injected. This nodule was rich in acid-fast bacilli, as were also the inguinal axillary lymph nodes, an abscess of the testicle, and the spleen, kidneys, liver and lung.

Histopathology (Dr. Torres, June 24, 1940).—Spleen: nodules composed of epithelioid cells, with extensive central caseation (tubercles?). Liver: small nodules (tubercles?) of mononuclear (epithelioid?) cells, with occasionally giant cells; hyperemia. Testicle: extensive areas of caseation circumscribed by epithelioid and fusiform cells (tuberculosis?). Lung: hyperemia.

On June 28th the second guinea-pig, which had a caseous nodule in the groin, was sacrificed. Smears of the nodule and of the heart blood were positive.

(*b*) Ten rats were inoculated with the same kind of material on March 18th. Between August 13 and September 19, 1940, five died. All of them had systemic infection, and four had large axillary tumors.

(*c*) Five guinea-pigs were inoculated (August 15, 1940) with an emulsion of the tumor of a rat of lot 4 inoculated with the Migone culture II. On September 2 one died, presenting hypertrophy of the lymph nodes. Smears of the inguinal nodes were strongly positive (clumps and globies); smears of the liver, spleen, kidneys and lung, were also positive, with fewer bacilli. On September 10 another died; it presented a normal aspect, but smears of inguinal lymph nodes, spleen, kidneys, liver and lung were positive and very rich in bacilli.

Histopathology.—Organs of the last guinea-pig were examined (Dr. Torres; September 19, 1940). Spleen: many giant cells in the red pulp; also epithelioid cells and areas of caseation, strongly modifying the structure of the tissue. Absence of follicles of typical tubercle structure. Liver: small nodules composed of large mononuclear cells with vacuolate cytoplasm, inside the liver lobes; absence of tubercle follicles or caseation; hyperemia. Lung: hyperemia, edema, and hemorrhage. Kidney: in one fragment an anesmic infarct; hyperemia.

(*d*) Another lot of five guinea-pigs, third passage of Migone II, was inoculated on August 26, 1940. On September 28 two of them died. One was negative, the other positive (systemic infection). Organs of this guinea-pig were examined histologically.

Histopathology (Dr. Torres, October 4, 1940).—Kidneys and liver: hyperemia. Spleen: pigmentation. Lung: hemorrhage and hyperemia. Absence of caseation necrosis. Absence of tubercle follicles.

On September 30 another of these guinea-pigs died. Smears of its hypertrophied lymph nodes were strongly positive. Smears of the spleen, liver, kidneys and lungs were positive, but with few bacilli. The other animals inoculated continue under observation.

DESCRIPTION OF PLATES

PLATE 20

FIG. 1. Photomicrograph of a granuloma of the chest muscle of a rat (lot 5A) produced by the Migone II culture. This granuloma is identical with others produced by inoculations of human leproma material.

FIG. 2. The granuloma penetrating the chest muscle of a white rat, occurring as small islands separated by normal muscle fibers.

FIG. 3. Another aspect of the same granuloma, showing muscle fibers cut transversely.

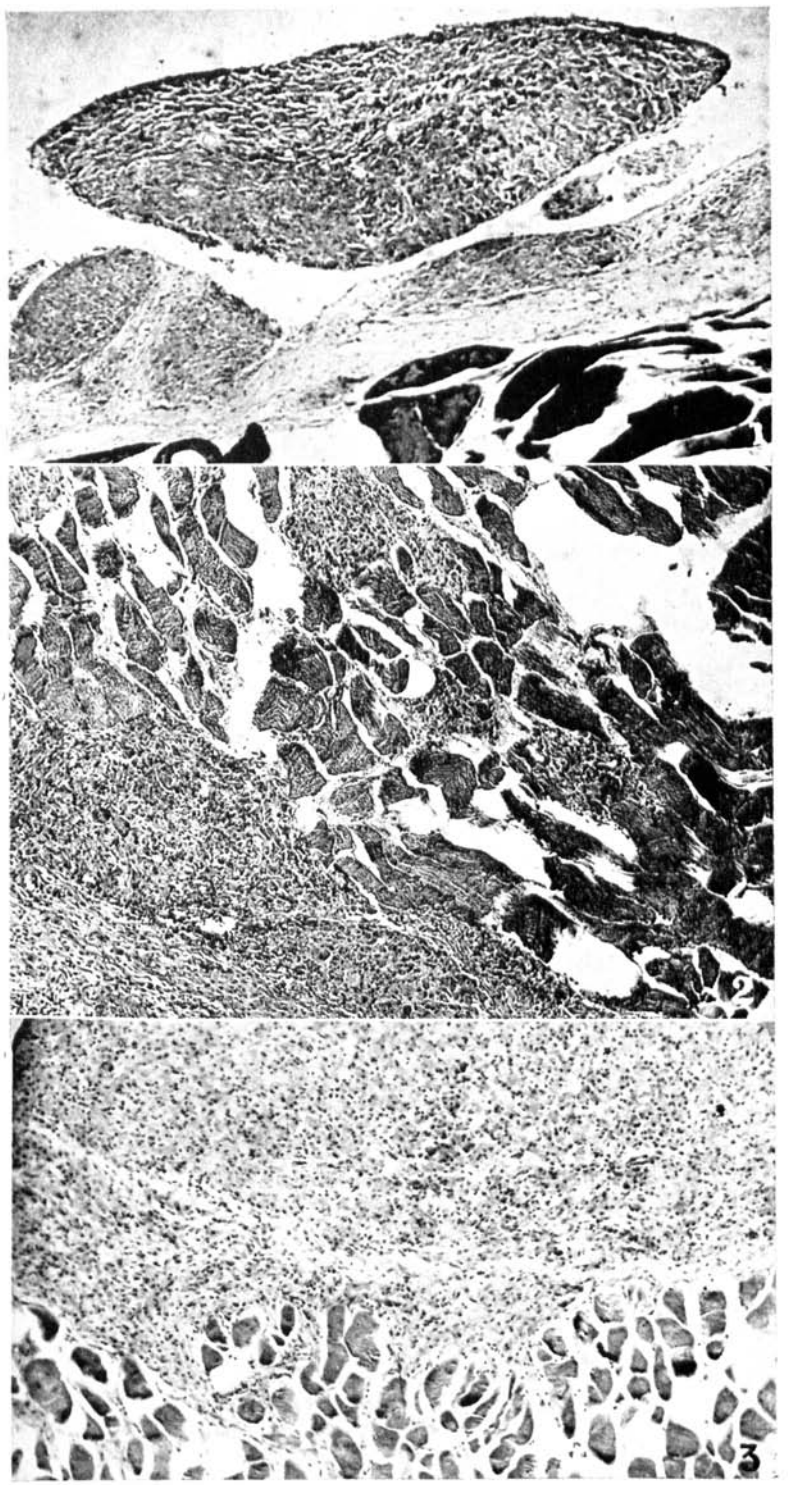

Plate 20

PLATE 21

FIG. 4. Compression smear from a fragment of the granuloma produced by the Migone II culture, showing clusters of acid-fast bacilli.

FIG. 5. Smear from an emulsion of the leproma, prepared for passage into other animals.

FIG. 6. A seven-months culture on glycerinated potato, of Migone III acid-fast bacillus, isolated from skin lesions of human leprosy.

FIG. 7. Smear from granuloma of a mouse (lot 7) inoculated with emulsion of the Migone III culture.

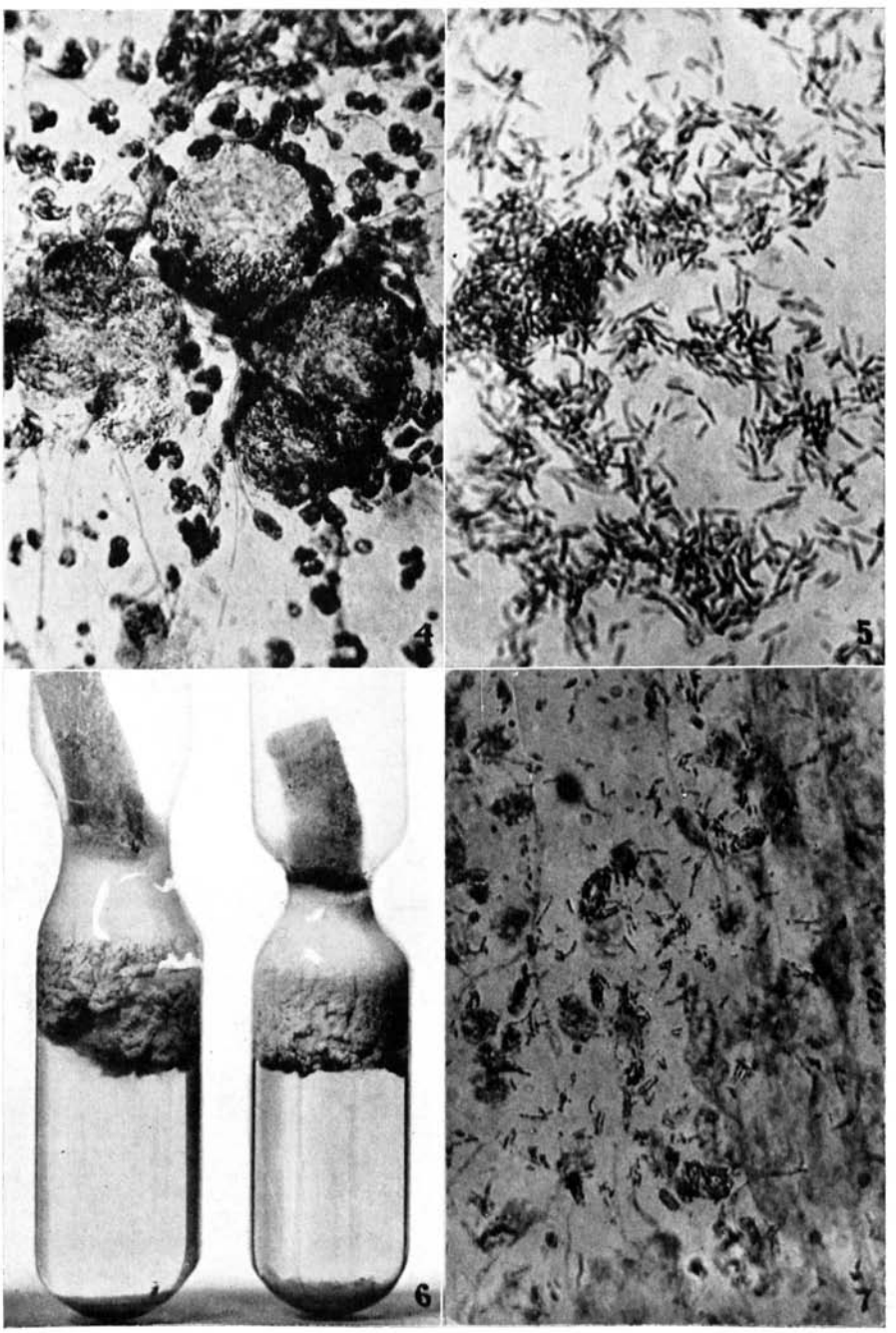

PLATE 21