Studies upon Leprosy

I. Transmission of Human Leprosy to the white Mouse (*) (Preliminary note, with 7 figures).

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(Reprinted)

On perusing again literature about experimental transmission of human leprosy and murine leprosy to laboratory animals, I verified the frequent mistaking the "Hansenian infection" and "provokind leprosy", which are far from being synonymous, for, in my opinion, nobody has still obtained the transmission of "human leprosy" to white mouse.

LITERATURE

In 1902, T. SUGAI (1) inoculated human leper material to Japanese dancing mice, and obtained, in about 20 of them, an infection followed by lepromatous infiltration of the viscera, containing numerous bacilli. The author reports that, the death of the animals took place between the 19th and the 104th day of the inoculation, but does not describe cutaneous lesions.

CHARLES W. DUVAL (2) succeeded likewise in infecting this variety of mouse, either with his culture of the bacillus of leprosy, or with leproma emulsion. The incubation stage lasted from 4 to 6 weeks; DUVAL states he reproduced "the disease in the Japanese dancing mouse", which animal he considers as a test material. Nor does he describe any cutaneous lesion. As to the white mouse, white and grey rats, etc., his renewed attempts to infect such remained unsuccessful.

W. J. KEDROWSKY (3) made use of his culture known as "bacillus of leprosy" after a passage through Guinea pig, and succeeded in infecting three white mice. KEDROWSKY affirmed reservedly, before the Third International Conference of Leprosy (1923), basing himself on this preliminary attempt of 1903 or 1904, which remained pending, owing to the Russo-Japanese War, he had been successful in inoculating leprosy to rabbits and mice (J'ai réussi à inoculer la lèpre aux lapins et aux souris, Page, 32, Rapport). In this author's opinion, the best infection way for rats and mice is the ocular mucosa.

MARCHOUX and SOREL (4) verified that the white mouse *can be* infected with murine leprosy, without generalization of the bacilli, as observed in rats.

With material from the corpse of a man who had died from murine leprosy, showing granulous bacilli in the viscera, MARCHOUX obtained

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the infection of 5 out 6 white rats he had inoculated. MARCHOUX proved therefore that granulous bacilli are not "cadavers" as some leprologists would pretend, and that murine leprosy can, occasionally attack man. Anyhow, he did never succeed in transmitting human leprosy to rats or mice.

Struck with this fact, MARCHOUX (6) undertook in 1925, numerous researches, inoculating white rats and Guinea pigs with fresh material of human leprpsy, with a view to make certain the bacilli of leprosy are and the same in both diseases. He did not come, nevertheless, to any result.

K. HARADA (7) using his own culture of the bacillus of leprosy, told me he had obtained the infection in rats, the result being : death after the 30th day. I have no idea, what kind of rats he used for his experiments.

MUIR, HANDERSON and LANDEMAN (8) obtained experimental infection in 97.7 o/o of the white rats and decumanus inoculated with *Bacillus leprae murium,* with formation of abdominal tumors, meanwhile MUIR and HANDERSON (9) came to negative results in inoculating material ef human leprosy to Japanese dancing mice, white rats and Chinese hamster (*Cricetulus griseus*).

ANGELO H. ROFFO (10) had no better success with 100 rats he inoculated in 1927 with leproma emulsions, from various patients, rich in bacilli.

This summary of the literature on the subject is necessary for a better appreciation of some points of my own experiments.

MY EXPERIMENTS

With an emulsion of a leproma scratched out from the back of the hand of Dr. J. P. A. (of Minas Geraes) (see figs 1 and 2) rich in bacilli, I inoculated on the 6th of June of 1928 :

1) One monkey *Pseudo·cebus* with
   1 c.c. in the malar region, subcutaneously, and
   1 c.c. intraperitoneally ;
2) One white rat with
   1 c.c. intraperitoneally ;
3) Three white mice each of them with
   1/2 c.c. intraperitoneally.

Results after 23 days, say, on the 29th of June 1928:

a) The monkey shows a nodule in the face. The same monkey had undergone a former inoculation with leproma from another patient, on the 3rd February, in the face and peritoneum. On the 18th of March, he showed two small nodules on his forehead, these having disappeared after one month. The first infection was patent after 45, the second after 23 days.

b) The white rat does not show abnormality whatever.

c) Of the two mice, one died on the day next to the inoculation. The other showed on the 23rd a fistula between the lower right ribs. The liquid gathered herefrom with a platinum rod, after disinfection of the spot, was very rich in acid-fast bacilli and globoid masses (see fig. 3). The animal

who grew rather ill, was then killed, and I found an *abdominal tumor*, one centimetre long, half as wide, sticking on the spleen, affecting the peritoneum and the lumbar musculature. Smears of this tumor, of the abdominal ganglia, of the spleen and liver, showed many acid-alcohol-fast bacilli, most of which in fasciae, also in the form of globoid masses. Macroscopically, the liver appeared normal, though being the most infected organ. Sections of this tumor—of lymphoid constitution—of the spleen and kidneys, which I am indebted for to the kindness of Dr. BURLE DE FIGUEIREDO, revealed to my examination, in larger extent the former two, abundance of acid-fast bacilli in fasciae, of normal appearance.

Here is a case of generalized "Hansenian infection".

The presence of globoid masses (zoogleic masses, the "globies" of MARCHOUX, *specific of human leprosy) in the liver, spleen and ganglia, proves the multiplication of the bacillus in situ, according to* WADE's *affirmation, and this is not a case of murine leprosy.*

With an emulsion of viscera of this animal, I inoculated three mice one Guinea pig and one white rat. Two of the former died accidentally, the other one is well, the same as the Guinea pig.

The rat showed, 18 days afterwards, an abdominal subcutaneous nodule which grew up to the size of an olive. On the 24th day, I decided to extirpate this tubercule for culture purposes and found it caseified. Smearings of the pus and of the abdominal scratches showed some acid-alcohol-fast bacilli, pigmented, with the appearance of real degeneration, as also a big globoid mass, with granulous bacilli.

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On the 3rd of July, I extracted, without skin, two more lepromas from the same patient Dr. J. P. A. After preparing the emulsion of same, which was rich in bacilli, I repeated the culture in various media, and inoculated 9 white mice and one Guinea pig intraperitoneally. Half 1/2 was injected to the mice, and one 1 c.c. to the Guinea pig. On the 17th of same month, one of the mice showed a dermic nodule on its back and another in the right scapula, both of the size of a pea; also a smaller one on the abdomen. There was a complete alopecia on both former elements. (See Photo n. 6).

On the 20th, these nodules showing tendency to diminish, I then decided to extirpate the dorsal one for culture purposes, but found it caseified. A summary examination of the pus did not prove this to be bacillipherous. Being the animal killed, I found the liver and spleen hardly congested and increased in size. Smears from the liver, spleen and kidneys showed some acid-alcohol-fast bacilli, scattered into groups of 2 to 4, segmented or granulous, proving an advanced degeneration.

The production of dermic nodules, similar to lepromas, accompanied with complete alopecia in loco, induces me to consider the case one of transmission af human leprosy to the white mouse.

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On the 5th of July, I extracted two blooming lepromas from Miss H. S. (of Minas Geraes). With the emulsion of one of them, I inoculated, intraperitoneally, 6 white mice. Dose for each of them: half c.c. I left the other leproma to stay in pure alcohol, for 36 hours, after which I triturated it and passed it into an emulsion, inoculating then three other mice (witnesses), with the same dose and in the same way as the former. The emulsion of leproma treated by alcohol was also rich in bacilli, of a morphological appearance equal to that of fresh material. On the 27th in the morning, one of the first 6 mice happened to have died. Necropsy revealed: liver and lungs covered with micro-abscesses (aspect of granul a); the other viscera with normal appearance. (See fig. 7). Smearings from lungs, liver, spleen, abdominal ganglia, kidneys and testicle showed acid-alcohol-fast bacilli, single and in fasciae. The kidneys showed masses of bacilli. Smears from the micro-abscesses showed apparent heaps of acid-alcohol-fast bacilli with microscopic fields of 3 to 5 "globies" (see fig. 4).

I decided to keep the piece intact for the Museum of the Institute.

Examining again to-day all the other inoculated animals—about 30 in all—I found no case of apparent illness.

CONCLUSIONS

1) The inoculation of emulsion of lepromata to white mouse, intraperitoneally, produces either a mere generalized infection, causing death within 3 or 4 weeks, or an infection characterized by a tumor, micro-abscesses or dermic nodules.

2) The presence of "globies" in the tumor, micro-abscesses and nodules in the viscerae, proves a real multiplication of Hansen's bacillus in the animal.

3) The transmission of the infection from the white mouse to the white rat was positive.

4) The verification of the infectableness of the white mouse through Hansen's disease, has opened a wide field of experimentation for the study of the etiopathogeny of leprosy.

Manguinhos, 31st of July 1928

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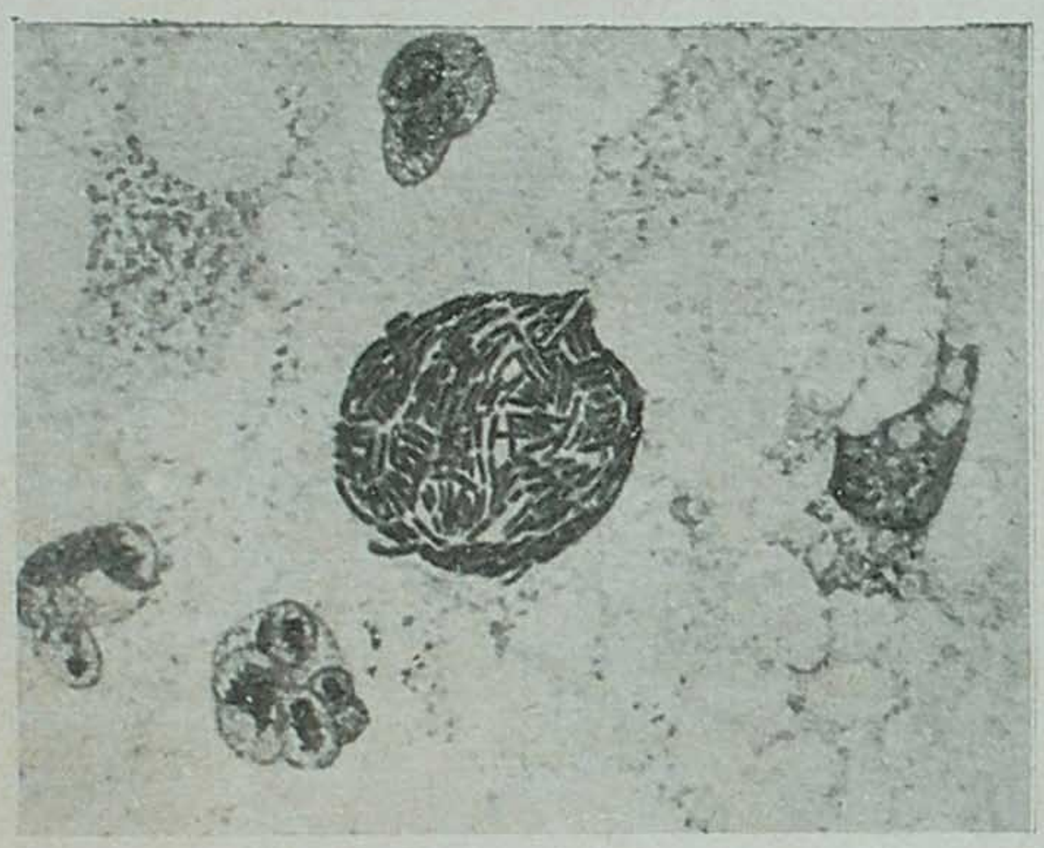

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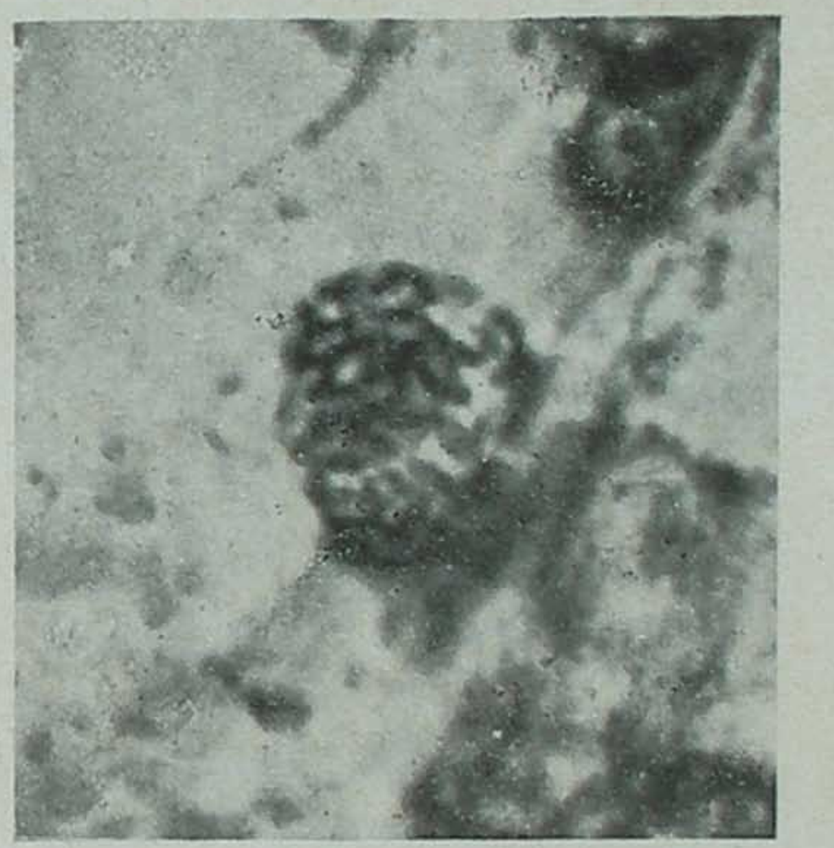

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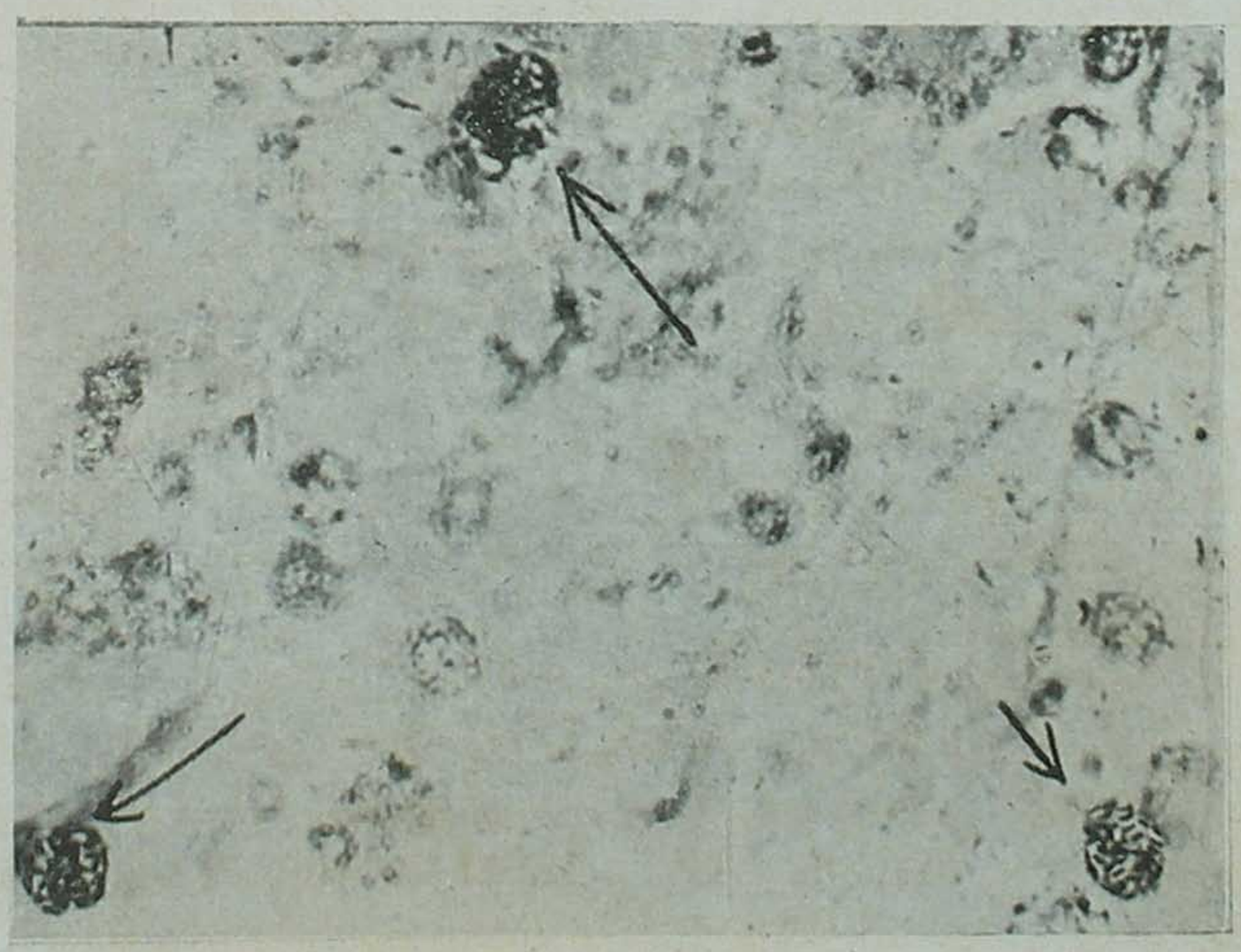

5 Phots J. Pinto

Fig. 1—J. P. A., a leper since 26 years, who provided material for infecting a monkey, [white mice, and a white rat (the latter by passage).

Fig. 2—Design of smearings of the inoculated leproma emulsion. (Immersion 1/12 and ocular 15).

Fig. 3—A "globie". Design of the smearings of the secretion of the fistula in the first infected mouse. Largely magnified.

Fig. 5—Globoid masses. Microphotograph of smearings of micro-abscesse of the third infected mouse.

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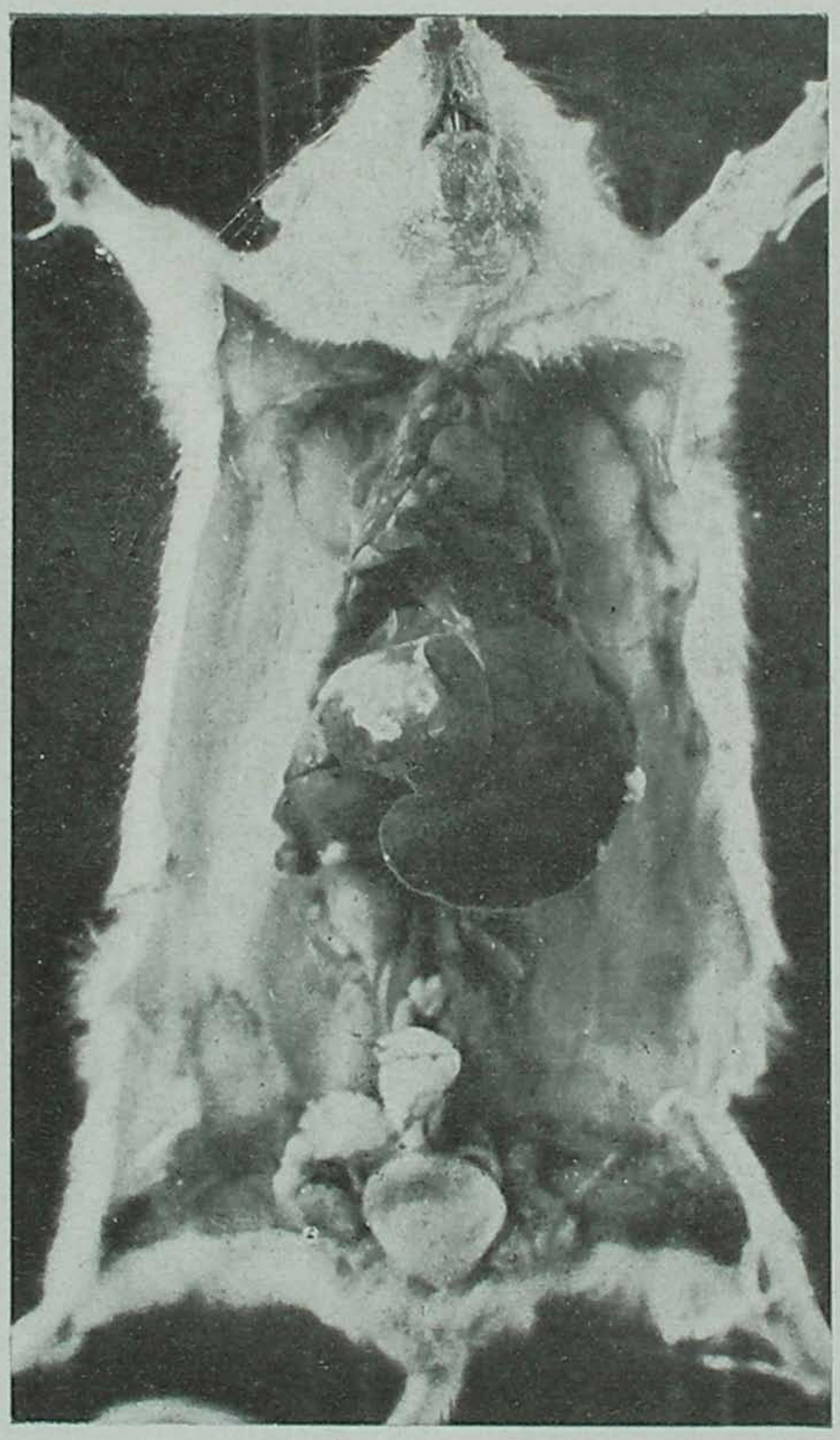

7 Phots J. Pinto

Fig. 6--Second mouse infected with material from the patient J. P. A. Nodules on the back, right scapula and abdomen. Acid-alcohol-fast bacilli in the viscera.

Fig. 7—Third mouse infected with human leprosy. The micro-abscesses of the lungs and liver were rich in globoid masses.

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