Studies upon Leprosy

III. Transmission of Human Leprosy to with Mouse (*)

(2nd Note with 6 figures)

By H. C. de SOUZA-ARAÚJO, M. D., Dr. P. H.

I shall have the honor to read before this Academy my second note upon experimental leprosy. It is a summary of the results of inoculations in 33 white mice with virulent leprotic material. In a further note, I shall deal with the results of inoculation of mice with avirulent leprotic stuff, as also with inoculation of other laboratory animals, less sensible to the infection.

Technique. The lepromas, extirpated without any skin, are triturated in a sterilized mortar, with sand and saline solution. The emulsion thus obtained is then diluted in the same solution up to a convenient concentration, which is verified by means of the microscope, filtrated afterwards on cotton, and inoculated as quickly as possible. Intraperitoneal injection was the preferred way, the dose for each mouse being half a cubic centimetre, this inoculation being allowed to be repeated after two or three weeks.

Results. The three mice referred to in my previous note (2. VIII. 1928) belonged to the lots 3, 4 and 5.

Lot 4 consisted of 9 mice, inoculated on the 3. VII. 1928, with ANDRADE's material. The No. 2 of the first note, having been withdrawn, there remained 8 mice, of which 4 were inoculated again, on the 26. VII. 1928 with the same dose and the same material of the same patient.

The sort of the four primitive mice was the following: one died on the 9. VIII. (after 37 days inoculation), showing micro-abscesses in the lungs, rich in acid-alcohol-fast bacilli, and caseated mesenteric ganglia, with but few bacilli. Smears of the liver, kidneys and spleen remained negative.

Another died on the 21. VIII. (49 days) showing hardly some scarce bacilli in kidney and a "globie" in the peritoneal fluid.

The 3rd, killed in the agonic period, on the 26. VII. (53 days) showed white nodules sand-stone-like or similar to granulations of mycetoma, sticking to the mesentery, to the epiploon and the abdominal viscera, being squash-proof and formed with masses of acid-alcohol-fast bacilli, and very scarce cellular detritus.

The fourth, killed and necropsied on the 15. XII. (165) showed three micro-abscesses in the liver, identical to those of the third mouse

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stated in my previous note, two white nodules equal to those described above, between the pancreas and the spleen and a third one sticking to the epiplon.


The presence of these nodules appeared to me of such high interest, that I wished my colleagues of the Oswaldo Cruz Institute to see them in loco, and upon my invitation to do so, Dr. CHAGAS, Dr. GODOY, Dr. FARIA, Dr. FONSECA and others came to my laboratory. Since then, Dr. LUTZ began to show interest for my experiments, and I greatly availed myself of his valuable advices.

Of the 4 re-inoculated mice, one died 114 days later (25. X.) without showing either lesions or bacilli.

Another, which died on the 5. XII. (154 days), though not showing any macroscopic lesion, revealed in great abundance, bacilli in bundles and "globies" in rubbings of viscera.

The third, killed on the same day (5. XII.) showed *minim nodules* in the liver, between the square lobe and the SPIEGELII, one bigger nodule (small tumor) sticking to the right lobe (See microphotographs 2 and 3) and others of various sizes sticking to the mesenteric folds and to the epiplon. Sections of the same liver revealed granulomae of peripheric origin.

Sections of the tumor show necrotic foci with spaces left by crystals of cholesterin.

One of the biggest nodules of the liver, being cut and squashed on a glass platen, appeared like a cystic swelling full with bacillary masses, a kind of giant "globies" which were never to be seen, either in human or experimental material.

The 4th, killed and necropsied on the 15. XII. (164 days) also showed such granules in the liver, just below the square lobe. Smears of abdominal viscera of the last two animals, colored by ZIEHL-NEELSEN, on a first rather quick examination, did not reveal acid-alcohol-fast bacilli.

These encouraging results demonstrate the convenience of re-inoculations, a matter which has been much discussed about at the 3rd International Conference of Leprosy (Rapport, 1924, p. 117).

Lot 5 consists of 6 mice, inoculated on the 5. VII. 1928, with HILDA's material, one of which named as N. 3 in my previous note. The 2nd, which died on the 12. VIII. (38 days) and the 3rd on the 19. IX. (76 days), showed acid-alcohol-fast bacilli in rubbings of the main abdominal organs, as also, with regard to the second, in the mesenteric lymph-nodes.

The 4th, killed when in agony and necropsied on the 9. X. (97 days) showed a slight tumor between the liver and the pancreas, and micro-abscesses in the spleen. Rubbings of this tumor, of the liver, of the spleen and of the lung were rich in acid-alcohol-fast bacilli.

The fifth, died on the 24. XI. (172 days) showed a small tumor in the pancreas (See microphotograph n. 4) and 8 minim nodules adhering to the mesenteric folds and to the epiplon.

The inoculation of these nodules on butter-agar resulted negative.
The 6th, killed and necropsied on the 23rd of January 1929 (after 202 days from the inoculation) showed some necrosed spots in the liver, with nodules adhering to the liver capsule, and other scattered about in the abdominal cavity. These nodules were taken off, triturated in a mortar and the emulsion injected into two mice. The result was negative.

The tumor of the pancreas above referred to was examined and described by Dr. MAGARINOS TORRES, who judged it most interesting, owing to the presence of spaces left by crystals of cholesterin. Dr. AMADEU FIALHO who was also applied to in this respect, is of the opinion that this discovery is a real histopathologic curiosity.

The 6 mice of the lote 7, were inoculated on the 26. VII. 1928 with ANDRADE's material. One of them died on the first month and was not examined; another died on the 15. XI. (1/2 days) showing one nodule in the liver, with big masses of acid-alcohol-fast bacilli, and, in the spleen, zones of necrosis with but few bacilli.

The other four were killed and necropsied on the 15. XI. (142 days) appearing as in normal condition. In 3 of them, there were nodules absolutely alike to those already described.

All the rubblings of abdominal viscera proved negative.

The 3 mice of lot 13, were inoculated on the 23. X. 1928 with material of F. LOPES. As the emulsion was of a weak concentration, I increased the dosis to 1 c.c. for intraperitoneal injection and gave more 1/4 of a c.c. subcutaneously.

With the purpose of a demonstration to Dr. BORZONE, the Director of the Bacteriological Institute, Santa Fé, Argentine Republic, I killed these three animals on the 24th of January 1929 (92 days after inoculation).

The first autopsy allowed us to see innumerable granules scattered about and sticking to the mesentery, to the epiploon and to the liver.

The latter gland showed generalized micro-abscesses.

The rubblings of the granules, of the liver and the spleen, revealed a huge quantity of acid-alcohol-fast bacilli in bundles, in typical "globies" and in masses. Dr. BORZONE took the whole material of this mouse with himself for studying purpose, on his way back to Argentine.

The second we autopsied showed: a few nodules, one tumor sticking to the spleen and to the abdominal wall, one abscess in the left lobe of the liver and innumerable small nodules just below this organ, adherent to the fan-shaped fold of peritoneum and other scattered in the intestine, etc.

Both rubblings and cuts of this tumor proved to be rich in bacilli of leprosy.

The third and last one allowed us to observe also granules, in smaller quantity, scattered in the abdominal space. The rubblings of the granules, of the spleen and the liver were positive.

The 6 mice of lot 14 were inoculated, on the 27. XI. 1928, with 1 c.c. of the material of A. PARIS. Two of them died on the first and 4th December, and were not examined. Two other, died on the 6. XII. (9 days after inoculation) one of them showing a few acid-alcohol-fast bacilli in the spleen, the other in the spleen, kidney, testicle and peritoneal fluid. No
nodule or ganglia were to be observed with bare eye. The 5th, died on the 10. XII. (13 days) and showed one mesenteric ganglion rather increased. Viscera examined macroscopically appeared as normal. Rubbings of ganglia, spleen, kidneys and liver were rich in acid-alcohol-fast bacilli, which was probably a product of absorption (?). No nodules were observed.

The 6th and last, killed on the 25. I. 1929 (59 days) showed rare parasite granules and one mesenteric ganglion very enlarged, with a few acid-alcohol-fast bacilli.

Lot 17 comprised 5 mice, inoculated on the 22. II. 1929 with a new and filtrated emulsion of lepromas which I had extirpated from 5 patients at the St. Sebastian Hospital. The dose was 1/2 c.c.

The first of these died on the 10. III. 1929 (16 days) showing a small tumor close to the liver and sticking to the musculature of the front wall of the abdomen (see microphotographs 5 and 6), liver discolored, one mesenteric ganglion and another groin ganglion were enlarged.

Sections of the tumor proved to be rich in acid-alcohol-fast bacilli, intra and extra-cellular. Rubbings of the liver, kidney, lung and of a intestinal ganglion were positive, those of the spleen proving negative.

The 2nd was killed and on the 15. V. (52 days) when it showed a large crusted ulceration in the hips. We observed in the viscera bacilli and also abdominal nodules.

The last three mice were killed and necropsied on the 27th May 1929 (94 days). I saw in the first one various white granules in the folds of the mesentery and under the liver between the square lobe and the lobe of SPIEGEL, also a small abscess in the liver. The microscopical examination of the granules and organs gave a positive result, equal to that described repeatedly here above. The second mouse had a few nodules, one of which sticking to and binding two lobes of the liver, this organ as also the spleen being congested and increased. The rubbings of the liver were rich in bacillar masses of gigantic size. The last third showed hardly few bacilli in the liver.

The new series of inoculations consisted of 35 animals, injected on the 28th of May with an emulsion of lepromas from a number of patients of the leprosarium at Curuçá, Jacarépaguá, which were kindly handed to me by Dr. THEOPHILÔ DE ALMEIDA.

As is patent, our experiments are being carried on in a larger scale.

Summary. Of the 33 inoculated mice, 3 were not examined, on account of having died too early or during holidays; 2 remained negative and 28 positive, that is, showed acid-alcohol-fast bacilli in either organ.

Of the 28 positive, 17 had nodules, 5 had tumors, and 4 had micro-abscesses. Some ones had tumors with micro-abscesses, others nodules with tumors. A noteworthy fact is the coincidence between the results of lots of animals which were inoculated with material from various patients (in the whole 9) and coming from different states.

Passages. The inoculations of mice with emulsions of bacilliferous organs and of nodules and lymph-node extractions from infected mice
have remained negative. I must state, in fact, these passages were but very few (11 animals) owing to scarcity of material, which was either reserved for the museum, or and always for cuts, rubbings and attempts of cultures.

*The nodules.* I observed nodules or granules of two classes: some ones white, highly resistant, real bacillary masses, with some resemblance with grains of myceloma, of varied size, either loose, or sticking to the abdominal viscera; others, ash grey, softer, sometimes friable, adhering to the mesentery or the epiploon, containing less bacilli and a few cells or detritus: these should be mesenteric or epiploic ganglia very hypertrophied.

In normal mice, such ganglia are not likely to be seen with bare eye. The granulomas of the liver seem to me as of peripheric origin, in view of the proliferation of one of these nodules which are strongly sticking to the liver capsule. No cirrhosis of the liver nor degeneration of hepatic cells has been found in the cases I studied.

*Tumors.* Small neo-formations have been found sticking to the spleen the pancreas, the liver and the abdominal walls. At first, it seems these would be fibroblastomae, afterwards, lymphoid tumors, always rich in acid-alcohol-fast intra and extra-cellular bacilli. Later on, other types of cells appear all around the foci of caseating necrosis, with in their centre, spaces left by crystals of cholesterin.

According to Dr. MAGARINOS TORRES, the structure of one of these tumors, one of the pancreas, the only one he examined, did not show "the histologic aspect of typical tuberculous follicles".

Cholesterol was verified in tumors of the liver and the pancreas.

We know this monoatomic alcohol is provenient mostly from the internal secretion of bile the adrenals, being eliminated by the bile; also that its defensive action against external intoxications and infections is a powerful one; and that it exists in excess in the blood of active lepers.

What then may account for the presence of this stuff in the tumors of mice in our experiments?

Only further studies will enable us to throw some light hereon.

Bringing this before the Academy, I confined myself in exposing concrete facts, which I deem of a certain importance.

Future only may enable to draw conclusions.

Manguinhos, 31st of May 1929.