I. Bacteriology of Rat Leprosy: Electron Micrographs of Rat Lepromas and Cultures with Three Plates From the Instituto Oswaldo Cruz, Rio de Janeiro

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In 1936 I started working with three strains of lepromas of rats, from Berlin, London and Paris, obtained respectively through Professor Ficker, Professor Laidlaw and Professor Marchoux¹. With them I tried many times to isolate and cultivate the bacillus of Stefansky; but failed.

At the time I studied the two strains of acidfast bacilli obtained in the U.S.A. from rat leprosy, being culture N.367 'Rat 2' and culture N.368 'Rat McCoy', of the American Type Culture Collection (ATCC) isolated about 1910 and 1911².

In 1931 and 1932 Dr. Schuzo Asami obtained, in Japan, two strains of acidfast cultures from rat leprosy, which he named *Mycobacterium leprae muris cremeum* and *Mycob. leprae muris vitellinum*³, pathogenic for laboratory animals.

On 12th September, 1947, Doctor Roland Chaussinand kindly furnished to me, in Paris, his strain of rat-leproma of the Institut Pasteur. With this good strain I made 'S' lepromin and antigen for immunological researches and inoculated white rats, laboratorybred, obtaining from their lesions in 1948 two chromogenic strains (yellow, type smooth 'S' of pure acidfast bacilli) and in 1949 obtained also from experimental lesions of black mice, American race C-57, two other strains which were non-chromogenic (eugonic type 'R'). All four of these cultures were infective for rats and mice, always giving positive retrocultures. I exhibited these four cultures to the Vth International Congress for Microbiology, on 19th August, 1950, in Petropolis, completing my report with the following notes:

"... these two non-chromogenic cultures are more pathogenic than the chromogenic ones. Recently Dr. Laerte Andrade proved, by repeated tests, that the strains I and II (chromogenic) treated by auramine are fluorescent, and negative to the Dubos cytochemical test for virulence, while the strains III and IV (nonchromogenic) are positive for both⁴".

I sent to Dr. R. Chaussinand samples of all four original strains of rat leprosy, advising him that probably his rats were infected with an association of two *Mycobacteria*, perhaps the chromogenic being a symbiotic. I sent also these four cultures for study to Professor Pierre Hauduroy, Director of the International Centre of Type Cultures, Lausanne, Switzerland; Professor Giuseppe Penso, Director, Instituto Superiore di Sanità, Rome, Italy; Professor Charles M. Carpenter, Medical School, University of California, Los Angeles, Calif., U.S.A.; Dr. Frederick Eberson, Chief Pathologist, Veterans Administration, Memphis, Tennessee, and Dr. F. Johansen, Medical-in-Charge, National Leprosarium, of Carville, Louisiana, U.S.A.

Testing the bacteriostatic action of Hydrazide Schering, I mixed one part of a solution of this product with another of rat leproma, and inoculated batches of white rats and black mice, proving that the classical rat leprosy lesions were produced normally. From such experimental lesions I obtained, by Loewenstein and Petroff methods a mixed white and yellow culture, which I was able to separate by successive transplants in Loewenstein medium. These findings I communicated to the VIth International Congress for Microbiology, held in Rome, Italy, in September, 1953, finishing my paper with the following notes:

"Yellow strain: Dubos cytochemical test for virulence, negative (although pathogenic for murines). Fluoroscopy: positive, 1-plus. Stained by Ziehl-Neelsen: median size bacilli strongly a.a. fast; by 'Fontes': typical bacilli, most homogeneous with pale cytoplasm and dark-blue granules. *White strain:* Test of Dubos, positive 2-plus. Fluoroscopy positive 2-plus, very bright fluorescence of bacilli and granules, showing the aspects of fresh suspension of rat leproma. Stained by Z-N: mainly coccobacillary forms, strongly a.a. fast; by Fontes: all classical forms of Mycobacteria. These cultures seem to be the same previously isolated, many times, from the same source.."

Conclusions

1. Hydrazide did not change the morphology and staining properties of the Stefansky's bacilli and did not cure leprous rats nor delay the progress of the disease.

2. Hydrazide seems to have facilitated the isolation and culturing of the inoculated acidfast bacilli originating from rat leprosy⁵.

Some of the electronmicrographs of Plates 1, 2 and 3 are similar to those published by Bishop, Suhrland and Carpenter⁶ and others.

Acknowledgment

Many thanks are due to Professor Mario G. Malfatti, Chief, Laboratory of Electronic Microscopy, Navy Hospital of Buenos Aires, and Drs. Hans Muth and Penna Franca, of the Electronic Microscopy Laboratory, Instituto Oswaldo Cruz, Rio de Janeiro, who made the electronmicrographs of rat leproma and my cultures.

II. Bacteriology of Human Leprosy

Since 1928 I have been working in the bacteriology of leprosy. From 1930 till 1940 I made intensive studies with the fourteen classical

strains of 'Bacillus leprae' from the Lister Institute of London and six others of the ATCC, from the National Institute of Health of Washington. During this decade I tried many times to isolate and cultivate the bacillus of leprosy, from various kinds of human material, and failed. Only in October and November of 1941 I secured two absolutely identical strains of a chromogenic (goldenyellow) culture of a permanent acid-alcohol-fast bacillus, from close skin lesions of a seven-years-old boy (José F.) coming from the North of Brazil (State of Piauhy), son of leprous parents and having an elder sister also a lepromatous case and another with pulmonary tuberculosis, who died within one year of residence in Rio.

Such culture I baptised as strain 'José 1', which was used to infect guinea pigs, rats and mice, from whose lesions I recovered the culture.

The original strain was used in April, 1943 to prepare a kind of leprolin (Leprolina 'Souza-Araujo', Strain José, 1941), similar to that prepared in Burma, in 1904, by Major Dr. E. R. Rost, using his original acid-fast culture of leprosy bacillus. My leprolin is being employed in the treatment of leprosy and of some neurological diseases, and also in immunological studies.

From 1941 to 1958 I secured about fifteen new strains of acidfast bacilli from human and rat leprosy lesions, most of them already published.

In this article I will report only the electron microscopy of a few bacteria of said cultures. The complete material will be used in another future article, entitled: 'A review of 30 years work in bacteriology of leprosy'.

The electronmicrographs of this paper, in many occasions from fields selected by myself, were kindly made by Professor Dr. M. G. Malfatti, University of Buenos Aires, and Drs. Hans Muth and Penna Franca, of the Instituto Oswaldo Cruz, Rio de Janeiro, to whom I am most grateful.

Nota Bene: All electronmicrographs of Professor Malfatti were taken at 3,700x and enlarged as convenient. The morphology of bacilli is different according to solid or liauid state of the culture media.

Rio de Janeiro, 28th April, 1960, Instituto Oswaldo Cruz.

References

- 1. SOUZA-ARAUJO, H. C. de; A lepra dos ratos. Com 3 estampas. Memorias Instituto Oswaldo Cruz, T.33, F.2, Agosto 1938, pp. 297-318. 2. American Type Culture Collection. Catalogue of Cultures, Second Edition,
- 1928, p. 84.
- 3. ASAMI, SCHUZO; Lepra murium. Ueber Rattenlepra. Booklet in Japanese with summary in German. Tokyo, 1931-32.
- 4. SOUZA-ARAUJO, H. C. de; Four Strains of Cultures (Two Chromogenic and Two Non-Chromogenic) of Acidfast Bacilli Isolated from Rats and Mice Inoculated with Emulsion of Stefansky's Leproma, from Pasteur Institute of Paris. Fifth International Congress for Microbiology, Rio de Janeiro, 1950, Abstracts of Papers, pp. 58-59.



PLATE 1



PLATE 2



PLATE 3



PLATE 4



PLATE 5



PLATE 6



- 5. SOUZA-ARAUJO, H. C. de; Bacteriology of Rat Leprosy. New Cultures of Acidfast Bacilli obtained from Lesions Produced in Murines by a Mixture of Stefansky's Bacilli and Hydrazide. VI Congresso Internazionale di Microbiologia, Roma, 1953. Riassunti de le Comunicazioni, V. II, p. 654.
- 6. BISHOP, F. W., SUHRLAND, L. G. and CARPENTER, C. M.; A Comparative Study of Electronmicroscopy of the Morphology of *M. leprae* and Cultures of Mycobacteria, Internat. J. Lep., 16, 3, pp. 361-366, 1948.

PLATE 1

FIG. 1. Stef. 1 and Stef. II yellow cultures, 'S' type, isolated from white rat inoculated with Institut Pasteur strain in 1948; Stef. III and Stef. IV non-chromogenic, isolated in 1949 from black mice, type 'R'.

FIG. 2. A and B cultures in Loewenstein of strains III and IV, type 'R', cremeum, isolated from experimental lesion in black mice.

FIG. 3. (a) Yellow 'S' culture isolated from white rat inoculated with a mixture of suspension of rat leproma and solution of Hydrazide, after six months of incubation. (b) White culture isolated from white rat lesion produced by the above mixture of Hydrazide and rat leproma.

FIG. 4. (c) the original yellow culture from rat, 1948, in Loewenstein. (d) Yellow 'S' culture obtained from lesion of pectoral skin of a rhesus monkey infected with rat leproma. (f) The same above culture recovered from the pellicle produced in Dubos medium: yellowish, granulated, mutating to eugonic 'R' type. (Photos: Jose Mello)

PLATE 2. Electron Micrographs

FIG. 1. Fresh suspension of rat leproma, Inst. Pasteur strain, showing normal bacilli with bipolar condensations, or central, of the metachromatic granules. Increased from 3,700x. (Malfatti).

FIG. 2. Rat leproma suspension shaded by chromium vapour. (H. Muth).

FIG. 3. Emulsion of rat leproma: bacilli in mass circumscribed by neat gloea. 19,000x. (Penna Franca).

Fig. 4. Three Stefansky bacilli with their condensed metachromatic granules and gloea. 14,000x. (Penna Franca).

FIG. 5. Group of Stefansky bacilli from lesion of black mouse infected with leproma of a rhesus infected with rat leproma; with fringed large gloea. 18,000x. (Penna Franca).

PLATE 3

FIG. 1. Fresh suspension of rat leproma, 5 months incubation, showing the association of two mycobacteria. Two short ones with gloea. 18,000x. (Penna Franca).

FIG. 2. Another photo of the same suspension above confirming the association of two mycobacteria: one large bacillus with 3 nodules in its ends and 2 bars of condensations and membrane. 22,000x. (Penna Franca).

FIG. 3. Another photo of the same suspension showing a large mass of short

bacilli, with neat gloea. 19,000x. (Penna Franca). FIG. 4. Photo of Stef. II culture, chromogenic, similar to one of the 3 above made by Penna Franca. 3,700x. (Malfatti).

FIG. 5. Suspension in sterilised distilled water of culture strain Stef. III: large bacilli, some massive, some granulated. (Malfatti). FIG. 6. Culture Stef. III deposit of glycerine broth culture, suspended in distilled

water: short trapus bacilli with gloea. 18,000x. (Penna Franca).

FIG. 7. Suspension of culture of tumour of black mouse infected with material from skin lesion of a rhesus infected with rat leproma. Short and large bacilli with condensed nodules or bars, similar to those of Fig. 6.

N.B.—Fig. 5 is similar to Fig. 1 of Plate 2.

PLATE 4. Bacteriology of Human Leprosy FIG. 1. Suspension of strain 'Chaves 1', isolated from leprous skin. Bacilli shaded by aluminium. (Malfatti)

FIG. 2. Same strain 'Cl' recovered from experimental lesion produced in the same patient. (Malfatti)

FIG. 3. Same strain 'Cl' recovered from Mitsuda test of patient's wife (Maria D.), also a case of leprosy. (Malfatti)

FIG. 4. Same strain 'CI' recovered from experimental lesion produced in the patient 'Jesus', a case of suspected leprosy: large granulated and short homogeneous bacilli. (Malfatti)

FIG. 5. The same strain above, from 'Jesus': shaded. (Malfatti)

FIG. 6. The original strain 'CI': large bacillus with clear spaces simulating vacuoles and many condensed bars. (Malfatti)

FIG. 7. The same strain 'CI' cultured in glycerine-broth, a large beautiful bacillus with many condensed granules, sample recovered from 'Adelina', a female leprosy patient. 20,000x. (Penna Franca)

FIG. 8. The same strain 'Cl' culture in Loewenstein, sample recovered from 'Loureiro', a male leprosy patient. Large bacillus with 4 condensed bars and clear cytoplasma. 30,000x. (Penna Franca)

FIG. 9. The same strain 'CI' in pure culture after passage in a series of three rhesus monkeys. 22,000x. (Penna Franca)

PLATE 5

FIG. 1. Strain 'Emilia' obtained from leprous skin, bacilli with nodules. (Malfatti)

FIG. 2. The same strain 'E' recovered from skin lesion of Rhesus 'Sofia', after 95 days incubation. 19,000x. (Penna Franca)

FIG. 3. The same Rhesus sample with large bacillus with six metachromatic condensations. 22,000x. (Penna Franca)

Fig. 4. The strain 'Emilia' recovered from abscess of Mitsuda test in patient 16 of Pernambuco. 28,000x. (Penna Franca)

FIG. 5. Strain 'Hecke', non-chromogenic, in glycerine-agar: Bacilli with defined

granules and one with many small scattered granules. 21,000x. (Penna Franca) FIG. 6. Another aspect of the same 'Hecke' strain in 5% glycerine-broth: bacilli with condensed bars and thin gloea. 26,000x. (Penna Franca)

FIG. 7. Strain chromogenic from nasal mucus of the leprosy patient 'Maria Nascimento', in Loewenstein medium: homogeneous bacilli. (Malfatti)

FIG. 8. Strain chromogenic from nasal mucus of the leprosy patient 'Dalva', in Loewenstein: Mass of bacilli showing a neat gloea, similar to material of human leproma: one large and another short bacillus with condensed bars and clear cytoplasma. 22,000x. (Penna Franca)

PLATE 6

FIG. 1. Photo of strain 'CI', chromogenic, in Loewenstein medium, obtained

in September 1949 from skin lesion (7th biopsy) of José Chaves. Fig. 2. Photo of strain 'Maria D.', chromogenic (sub-strain of CI) recovered from biopsy of abscess on scar of Mitsuda test of left arm, in January 1950. Maria D. is the wife of Chaves, above. She was inoculated with her husband's strain 'CI'.

FIG. 3. Photo of strain 'H', non-chromogenic, in Loewenstein medium, obtained from biopsy of skin lesion of patient Hecke, in September 1949. FIG. 4. Photo of strain 'E' chromogenic, in Loewenstein medium, obtained

from leproma of left knee of patient Emilia, in March 1950.

Fig. 5. Photo of strain 'E' in Loewenstein (sub-strain recovered from abscess in scar of Mitsuda test in patient n.21 of Pernambuco, inoculated with the original strain 'E').

FIG. 6. Photomicrograph of smear of left ear leproma of patient Chaves, biopsy of 24th June, 1949, stained by Z-N. 1,000x.

FIG. 7. Photomicrograph of smear of the original strain 'Cl' obtained from skin lesion of the above Chaves (Fig. 6) stained by 'Fontes' method, 1,000x. The morphology of bacilli of Fig. 6 and Fig. 7 is absolutely equal.

FIG. 8. Photo of Rhesus I inoculated with culture in glycerin-broth of strain 'Cl' (Fig. 1), after 14 days incubation.

FIG. 9. Photomicrograph of section of the facial nodule of Macaca mulatta above, showing characteristic experimental leproma. 150x.

FIG. 10. Photomicrograph of section of facia nodule of a Ø Cebus fatuellus monkey infected with strain 'CI'. Leproma structure with many acid-fast bacilli. 1,000x. Stained by Z-N.

PLATE 7

FIG. 1. Culture of strain 'Cl' in 100 ml of broth with 5% glycerine, 20 days incubation at 37° C.: veil and deposit yellow. Culture ready for preparation of Leprolin for immunological studies and treatment of leprosy.

Fig. 2. Culture of strain 'H', non-chromogenic, recovered from rat, in 100 ml of broth with 5% glycerine, after 12 days incubation at 37° C. The 100 ml of this culture gave 1 litre of good Leprolin.