A NEW CULTURE OF AN ACID-FAST BACILLUS ISOLATED FROM AN EXPERIMENTAL *TRIATOMA INFESTANS* INFECTION IN A LEPROSY PATIENT

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Introduction

When doing a leprosy survey with DR. ORESTES DINIZ in December, 1942 in Bambuhy in Minas Gerais State, in the bed and trunks of two leprosy patients living in an adobe house I discovered a few triatomata (*Triatoma infestans*, KLUG, 1834) infected by acid-alcohol-fast bacilli presumably of leprosy. At my suggestion DR. VANDICK DEL FAVERO searched dwellings of leprosy patients at St. Gothardo in the same region and found another species of triatoma (*Panstrongylus megistus*, BURM, 1835) which I examined and found also to be infected by acid-alcohol-fast bacilli.

This discovery was of great importance, as leprosy is a grave rural scourge in Minas Gerais State.

In Rio City in 1943 I made a large series of experiments in leprosy patients with the above two species, and also with *Triatoma sordida* (STAL, 1859) and *Rhodnius prolixus* (STAL, 1859), all bred in the laboratories of the Instituto Oswaldo Cruz, and I arrived at the conclusion that *Triatoma infestans* was the best for such experiments.

On 13th February, 1943, in my consulting rooms in Rio, with the assistance of DRS. SOUZA CAMPOS, SOUZA LIMA, and JOIR FONTE, I applied eight larvae of *Triatoma infestans* in one Borrel tube covered with gauze, to the lepromatous lesion of the right thigh of a patient (J. Carlos), who was a young law student of São Paulo who came to me with the diagnosis of ozaena. In a few minutes seven of the larvae finished their blood meal. I smeared the intestinal contents of three of them on to a batch of tubes of Loewenstein medium. After incubating at 37°C for 56 days one only of the tubes showed 11 slightly raised small yellow colonies. These on examination on 22nd April proved to be pure acid-alcohol-fast organisms. Transplants of these colonies grew well in various media and produced a veil and deposit in glycerin broth. It was used to make leprolin, which caused a very strong reaction in the patient, who absconded.

New experiments were done on another lepromatous case (F. Chagas, of white race, aged 62, of Ceará in NW Brazil). From 14th to 28th September, 1943 I bred nymphs and larvae of *T. infestans* in my laboratory which were applied to various skin lesions of this patient. Many of them sucked his blood and their intestinal

contents were treated by the Petroff method and smeared on Loewenstein medium in three large series of tubes. After incubation at 37°C for 51, 53, and 27 days respectively, I obtained in one tube of the first series of sowing, and in two of the 2nd and one of the 3rd series, three identical strains of acid-alcohol-fast bacillary cultures. By the Fontes method I obtained a very beautiful preparation of granular bacilli with the classical morphology of pathogenic mycobacteria. The fact that the cultures were obtained from three feeds on this patient suggests that these bacilli were those responsible for the leprosy infection of that patient.

This patient Chagas was used on 7th January, 1944 to provide a demonstration of my experiments to DR. MALCOLM H. SOULE, Professor of Bacteriology in the University of Michigan, to whom on that occasion I gave a set of my leprosy cultures for his study. (Ref. Memorias Inst. Osw. Cruz **40**, 1, 1944, 17–26, with colour plate).

The New Culture. (Weinstein strain, 1956). From 13th September, 1940 a new leprosy patient was studied. He was C. Weinstein, a white man 46 years of age, type L_2 N_1 positive for bacilli. In 1941 he infected his wife, and his elder son 10 years later. In 1948 he became L_3 case and began Diasone treatment. In 1953 he had three positive serological readings. In 1955 lepromin tests were negative. During strong lepra reaction he was given treatment for a short time with Liosulphone. During the early part of 1956 he had severe lepra reaction when I tried to infect *Triatoma infestans* from his skin, and I obtained good results in the third experiment using larvae and nymphs bred in the laboratory. Sowing material from these insects I obtained in June 1956 a thin layer of creamy culture of acid-alcohol-fast bacilli on Loewenstein medium. On 14th November, 1948 transplants at 25°C on Loewenstein tubes showed fairly exuberant clear yellow type S culture. New transplants were also kept at room temperature until 11th May, 1960, when the culture mutated to eugonic R type, with non-chromogenic warty colonies of pure alcohol-acid-fast bacilli producing 1 cm. elevated white pellicle in 5% glycerin broth (See photos). In 1959 the patient at 59 years of age was much improved by treatment with intravenous DDS.

Two slides with smears of this culture were sent with this paper to London for examination. One slide is stained by ZN and the other Fontes.

(A sample of the culture in glycerin-agar was sent to Mr. Rhodes-Jones, Senior Technologist of the East African Leprosy Research Centre, Alupe, Kenya.

Reports were as follows: The two slides were seen by Dr. S. W. A. Kuper of the Pathology Dept. of the Brompton Hospital who thinks the bacilli seen "are morphologically very like diphtheroids".)

Pathogenicity of the Culture. After trituration and homogenisation of the veil and the deposit of the culture in glycerin broth, the

product was inoculated subcutaneously in two batches of murines, one of seven white-yellow rats three months old, bred on a special diet in my laboratory, and five American black mice. On the fifth day of incubation all 12 murines showed small nodulations in their groins. One of each lot was sacrificed for bacteriology and histopathology, and it was found that smears of the nodules stained ZN show intra- and extracellular alcohol-acid-fast bacilli and some threads of six or more acid-fast granules. Rat material was treated and sowed in culture, and after incubation at 37°C for 11 days the culture was recovered in a pure state. Perhaps this retroculture would be more suitable for inoculations. The histopathological report of DR. EITEL DUARTE stated that both lesions were abscesses circumscribed by histiocytic inflammatory reactions. Sections of the skin stained by the Potz method showed many acid-fast bacilli.

After incubation for three weeks the nodules of both murines regressed, and some ulcerated, these results being quite different from those obtained with other acid-fast cultures. The "Weinstein strain" seems to be too virulent. The culture in glycerin bouillon will be inoculated intra-peritoneally in small doses. The bacteriological study of the culture is in progress. It has been found that the experiments with *Triatoma infestans* are very easy, clean, and elegant, but in general the insect sucks few bacilli because its proboscis does not reach the subcutaneous tissue where the leprosy bacilli multiply abundantly.

Summary

In 1942 the author found two species of Triatomidae in the jungle of Minas Gerais State in Brazil namely *Triatoma infestans* and *Panstrongylus megistus*, and also in the beds and trunks of leprosy patients. These insects were infected by acid-fast bacilli presumably *M. leprae*. The author began in Rio City a long series of experiments with the aim of infecting from lepromatous leprosy patients these two and other species, and found that *Triatoma infestans* was the most suitable for these experiments. In February 1943 by applying larvae of the said species, bred in the laboratory on the skin lesions of an active case of leprosy, the author succeeded in obtaining a pure culture of acid-fast bacilli which were pathogenic for laboratory animals, and suitable for the preparation of leprolin. In September 1943 he applied larvae and nymphs of *T. infestans* which had been bred in the laboratory to lepromatous skin lesions of another patient who had just come from the north of Brazil and had not been given antileprosy treatment. In this case the author obtained three new acid-fast strains and as the strains were similar and obtained through the feeding of the same haematophagous insect the author concluded that the acid-fast bacilli obtained were those responsible for the leprosy of the patient.

In June 1956 in the third series of experiments the author obtained another pure acid-fast strain from larvae and nymphs of *T. infestans* which had been infected on the skin lesions of a L_3 case under severe lepra reaction. The first culture, obtained on Loewenstein medium was slightly pigmented, and smooth, and when transplanted to different media and kept at room temperature (about 25°C) for three to four years mutated to eugonic R type, colourless in glycerin agar and slightly pink on Loewenstein. This culture proved to be pathogenic for rats and mice, producing nodular lesions from which the culture was easily recovered, and its biological properties are being studied.

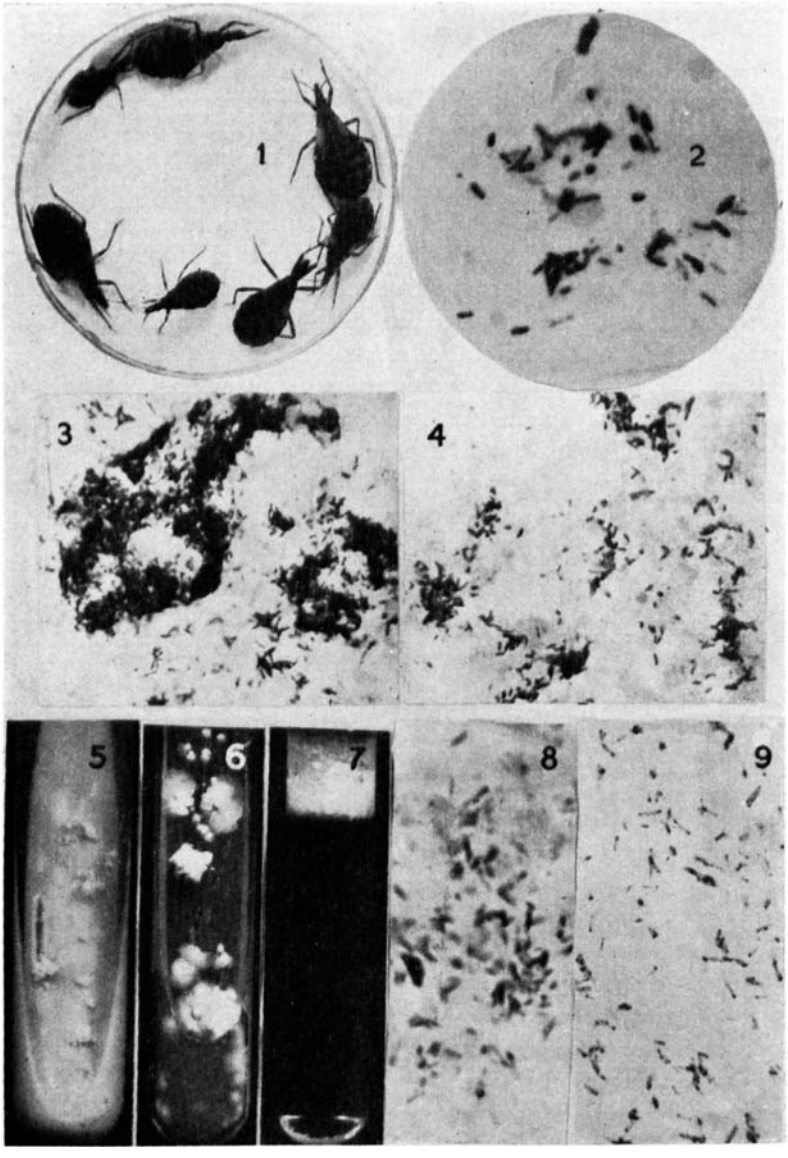

FIG. 1 Nymphs and larvae of *Triatoma infestans* bred in the laboratories of the Instituto Oswaldo Cruz.

FIG. 2 Microphotograph of smear of the intestinal contents of one nymph infected from the skin of a leprosy patient. × 1,200: stained ZN

FIG. 3 Smear of the original culture of acid-fast bacilli obtained from *T.infestans* infected from the skin of the leprosy patient Chagas. × 1,000: Stain ZN

FIG. 4 Microphotograph of smear of the 2nd generation of the same Chagas culture.

FIGS. 5, 6 & 7 Photos of the Weinstein culture in Lowenstein medium, glycerin agar, and glycerin broth, showing beautiful white veil and limpid fluid.

FIG. 8 Smear of the Weinstein strain. × 1,000: stain ZN

FIG. 9 Smear of the same culture stained Fontes showing all the classical morphology of pathogenic mycobacteria.