Case of acute malignant leprosy, with infection of the consort within three months of matrimonial life. Isolation from a cutaneous lesion of the same patient of an acid-alcohol fast bacillus (Chromogenic culture) pathogenic for rats, mice, Macacus rhesus and man.

by

H. C. de Souza-Araujo, M. D., Dr. P. H.

Clinical history

June 24. 1949. Under the guidance of a nurse and following instructions from Doctor Edgard Tostes, head of the Medical Department of Panair of Brazil, Mr. J. L. Chaves N., radio operator of that company for 6 years and a former telegraph operator of the Department of Posts and Telegraph, came to our office at the Instituto Oswaldo Cruz, for medical advice and care.

Chaves, a 30 years old white man, 61 kg and 1.56m., was born in Barra do Corda, Maranhão. His father died in 1931 from an hearth ailment. His mother (Neusa Nava) is now 52 yrs old and healthy. He has 5 brothers, all healthy adults living in Maranhão, and a sister (Edith), 31 years old, living with him, in Rio.

While in Maranhão, two and an half months ago (April 9. 1949), Chaves married a 18 years old girl, Maria Domingas.

Disease history — Chaves reports that in July 1946, while stationed in the Panair base of Turiacú, Maranhão, he noticed a small alopecial area on the right thigh, about 10 cm, above the knee, which became anaesthetic. The patient denies any leprosy history or contact with lepers, an information hardly to be accepted as reliable as far as the interior of the State of Maranhão is concerned since leprosy is widespread in that region. In this case we must apply the Mark Boyd’s rule that most leprosy patients are infected in unknown focuses. On December 1948, while travelling horseback in the hinterland of the same State, the patient felt, in the internal surface of the left heel, a sudden, acute pain which still subsists, in spite of the fact that the
external surface is normal. Two months later, above this painful spot, on the malleolar region, a small macula made its appearance, which looked to the patient as just a plain tetter. The patient insists that the lesions now existing are only three months old and that they appeared without any preliminary signs, either fever, cephalaea or depression.

A dermatological examination showed: many acute nodules on the ears; moderate infiltration and cyanosis of the front and malar regions; many scars from old acne crateriform lesions on the back; small roseoliform maculae in the skin of the abdomen, posterior surface of the arms, buttocks and thighs; in the skin of the abdomen, corresponding to the appendicular region, a circular, infiltrated patch, about 10 cm in diameter, with a depressed and dyschromic center; inguino-crural glands apparently normal; on the antero-external view of the left thigh a 6 by 12 cm patch, with a lepromatous appearance, in which a biopsy has been performed, 15 days ago, by Doctor Hildebrando Portugal; on the right thigh a circular, erythematous macula, located near the groin, besides two hard, transversally elongated nodules, about 2 and 3 cm long, located in the space between the primary alopecial spot and the knee; also, on the posterior view of the right thigh, two lepromatous patches, of which one, on the popliteous area, is circular with a depressed center, while the other, is larger and seen above the former, on the postero-external surface of the thigh; a few isolated lepromata are also seen in that part of the same limb; a lepromatous patch, larger on the left side, is seen just below each knee; small areas of alopecia and small erythematous maculae are found on the legs, three of them, on the calf of the right leg, being already infiltrated; a prominent, hard, lepromatous patch, about 4 cm broad and 8 to 10 cm high, is now seen in the place where the patient says he had the tetter — like spot, between the malleolar region and the Achillean tendon of the left leg; marked cyanosis and anaesthesia on the 2nd and 5th toes on the left foot (see graphic n. 1).

We classified this case as of L2-N1.

Nasal mucus collected on both nostrils * gave positive results for leprosy bacillus. Besides we excised the larger nodule found on the upper part of the left ear, made two smears from it and stained them by the Ziehl-Neelsen’s method. Both smears displayed large and impressive numbers of acid-alcohol fast, granular and little homogeneous bacilli. One of these smears was sent to Doctor Tostes and the other kept as a most interesting specimen. Both patient and nurse were shown this material under the microscope and, as ourselves, were

* For many years we have been using to collect nasal mucus with a curette from both sides of the nasal septum, making, on the same slide, a circular smear with the material from the right and a square smear with that of the left side. The technician in charge of the microscopical examination is required to state which one of the smears has been positive or, if both of them have been positive. This information is used in order to prescribe local treatment by fulguration or electrocoagulation on the baciliferous lesions.
astonished with the large number of bacilli. This microscopical features
have been very well represented by A. Pugas' drawings on figure 1,
plate 1 of this work. Both Processor Lamere, President of the New
York Academy of Medicine, and Professor Otto Bier, of Sao Paulo,
having had the opportunity of comparing the drawing and the smear,
stated that they did not find anything schematical in the former.

One half of the excised nodule was immediately taken to the late
Professor Penha de Azevedo, head of the Section of Pathological Ana-
tomy of the Instituto Oswaldo Cruz, for histopathological investiga-
tion. We trititated the other half, treated it with sodium hydrate (Petroff's
technique), and after having three times centrifugated it, the sediment
has been smeared into ten tubes of Loewenstein's medium and one
tube of 5 per cent glycerin broth. The blood obtained from the bleeding
of the excision wound has been collected with a pipette, and, without
any special treatment, was transferred into three tubes of Loewenstein's
medium. All these 14 cultures, kept for 2 months at 37°C., in the
incubator, remained sterile and finally have been discarded.

We prescribed daily injections of Promin at the increasing dosis
of from 5 to 12.5 cm², associated with physiotherapy.

Before leaving our office, the patient showed us the results of
two previous examinations, one of them being a negative nasal mucus
examination and the other an histopathological report which reads as
follows:

"N.º 132.296, June 13.1949. Histopathological findings of the
nodular lesion on the left thigh and of the lymph collected on the ear
lobule:

"Large nodules formed by epithelioid, vacuolated cells and a few
lymphocytes and plasma cells, located in the deep, subpapillar layer
of the derma and, in part, in the epidermis. Oedema of the papilar
region, hyperaemia of the superficial and deeply seated blood-vessels.
Very numerous acid fast bacilli were found. Tuberculoid rectional
leprosy. Search for M. leprae on the ear lobule: positive.

(s) Hildebrando Portugal

(see photomicrograph 7, plate 3).

June 25. With part of the sediment which remained in the mortar,
in the bacteriological chamber, we made still another smear, which,
stained by the Ziehl-Neelsen method, displayed an extraordinary
amount of acid-alcohol fast bacilli, most of them with a granular
structure, as were seen in the smears made the day before with fresh
materials. Also compact masses of bacilli were found, which are illus-
trated on photomicrographs 2 and 3, plate 2 (It is impossible to concieve
such a richness of bacilli in a case of tuberculoid leprosy).

Sodium hydrate, after 24 hours action, had introduced no changes
in the morphological appearance or in the acid-alcohol fastness of the
bacilli, a fact which has not as yet been reported in previous publications.

July 7. The report on the histopathological findings on the skin fragment excised from the ear of the patient on June 24, reads as follows:


Result of the histopathological study: Lepromatous leprosy; large numbers of acid-alcohol fast bacilli in sections stained by the Ziehl-Neelsen's method.

Manguinhos, July 7. 1949.

(8) Dr. A. PENNA DE AZEVEDO.

(See drawing 2, plate 1 and photomicrographs 11 and 12, plate 4).

Sections from this material, as well as smear I, taken on June 24, have been used by Dr. PENNA DE AZEVEDO for demonstration in the Ipase Hospital, Rio de Janeiro, and for the preparation of lantern-slides.

July 13. Due to the disagreement between the two above-mentioned histopathological reports, we decided to have a new biopsy performed, and made it to-day by excising a large skin fragment from the patch located on the left malleolar region, which had a lepromatous appearance. The fragment excised was divided in three portions. We triturated one of them, treated it, with sodium hydrate, and smeared the sediment, which had many bacilli into Loewenstein's medium. The other two portions, both of them labeled XX, in order to avoid any preconceived opinion, were sent, for histopathological diagnosis, one to Doctor PENNA DE AZEVEDO and the other to Doctor H. PORTUGAL.

Report from Doctor PORTUGAL:

"No. 133.506, July 18. 1949.

Patient XX. Doctor: Prof. SOUZA-ARAUJO.

Histopathological findings on a fragment of skin recieved:

In the dermis and epidermis, there are large nodules of cellular infiltration, formed by groups of epithelioid, partly vacuolated cells, surrounded by lymphocytes and plasma cells. In the dermal infiltrates, the epithelioid cells, are in direct contact with the collagen. No acid-fast bacilli. Tuberculoid granuloma (sarcoid type).

One slide accompanies this report.

(8) H. PORTUGAL."
Report from the Section of Pathological Anatomy, Instituto Oswaldo Cruz:


(s) Dr. Jorge P. Guimarães."

(See drawing 4, plate 1 and photomicrographs 14 and 15 plate 5).

July 20. We made a 4th. biopsy for bacteriological study, excising two nodules on the right ear, the smears of which displayed a large amount of bacilli, the ones having an homogeneous appearance predominating over those displaying a granular structure. This was something different from what had been seen in the material obtained by biopsy from the left ear on June 24. 1949. One of the nodules, labeled with the real name of the patient, J. C., was taken by himself to Doctor H. Portugal, for histopathological examination. The following result came in and was confirmative, both of our clinical diagnosis and of the histopathological diagnosis made in the Instituto Oswaldo Cruz:

"No. 133.760, Rio, July 22. 1949.

Patient J. C. Doctor: Prof. Souza-Araujo.

Histopathological findings on a skin fragment from the ear.

Massive cellular infiltration of the dermis and the hypodermis, isolated from the epidermis by a band of oedematous collagen. The infiltration is formed by lymphocytes, plasma cells, mastcells and vaculated cells, the latter being grouped in more or less limited areas. Many acid-fast bacilli were found, either homogeneous or granular, isolated or in bundles, intra — or extracellular in position. *Lepromatous lesion*. A slide, stained by haematoxylin-eosin, accompanies this report.

(s) H. Portugal."

(See drawing 3, plate 1 and photomicrograph 13, plate 4).

In the short period of 40 days, Doctor Hildebrando Portugal, who is justly considered the most authorized specialist in the histopathology of leprosy in Brazil, made three different histopathological diagnosis of this patient: 1) tuberculoid reactional leprosy, with large numbers of acid-alcohol fast bacilli; 2) tuberculoid granuloma (sarcoid type), with no acid-alcohol bacilli; 3) lepromatous leprosy, with very many bacilli.

When the patient was acquainted with these results, he parted with his wife, leaving her under the care of an aunt, who interned her in a nun's school in the district of Laranjeiras, Rio. On this occasion, her nasal mucus was negative and she seemed to be free of leprosy.
July 22. **Blood picture:** red blood corpuscles 4.5 million; haemoglobin 14.0%; white blood corpuscles 6.500; everything else was normal.

(a) **Dr. Alvaro Lobo**.

July 26. Both the general health conditions and the cutaneous lesions were improved. A biopsy was performed from various nodules in the left ear, for bacteriological study and experimental inoculations. The 5 black American mice inoculated with an emulsion of this material died during my absence from the laboratory, on a trip to Bahia, and a *post-mortem* examination has not been performed by our laboratory assistant on the ground that they did not show any cutaneous tumors.

August 1. Clinical check-up: After 34 injections of Promin, 25 of which of 5 cm$^3$ and 9 of 12.5 cm$^3$, amounting to a total 237.5 cm$^3$, the patient displays an acute eruption, with no fever and without the old lesions becoming any worse. The patient becomes pessimistic and talks of committing suicide.

August 9. New biopsies are performed. A few nodules of the right ear are excised for therapeutical and bacteriological purposes. On the left thigh, in a place above the location where the 1st. biopsy made by Dr. Portugal has been performed, a skin fragment is taken for histopathological study by Doctor Penna de Azevedo who, unfortunately died before he could do it. The Department of Pathological Anatomy of the Instituto Oswaldo Cruz furnished us with the following report:


Nature and site of the tissue: Skin fragment from the left thigh.

Result of the pathological study: **Lepromatous leprosy**. The Ziehl-Neelsen's stain showed acid-alcohol fast bacilli.

Manguinhos, August 9. 1949.

(s) **Dr. Jorge P. Guimarães**.

September 5. After 60 injections of Promin, we performed a biopsy of the lesion on the posterior view of the right thigh, taking a fairly large coinshaped fragment, of which one portion was sent to Dr. Magarinos Torres, another to Dr. H. Portugal, a third one being used by us for bacteriological cultures, after treatment by Petroff's technique. The smears then made, after 30 days, furnished us with a chromogenic culture which will be used for a number of studies.

Once more the histopathological reports from the Instituto Oswaldo Cruz, on one side and from Dr. H. Portugal on the other, disagreed. They read:
Patient: J. C., Doctor: Prof. SOUZA-ARAUJO.

Histopathological study of a skin fragment from the right thigh: Infiltrative nodules formed by epithelioid cells and rare lymphocytes, located in the median and deep layers of the chorium. No acid-fast bacilli were found. Tuberculoid granuloma (sarcoid type).

One slide accompanies this report. 

(See photomicrograph 16, plate 5).

"Department of Pathological Anatomy, Instituto Oswaldo Cruz.
Patient: J. C., Doctor: Prof. SOUZA-ARAUJO.

Nature and site of the tissue: Skin fragment from the right thigh.

Anatomo-pathological findings: the microscopic sections show a granuloma with the characteristic features of the one of leprosy. Search for acid-alcohol fast bacilli positive. Leproma.

Manguinhos, September 15, 1949. 

(s) JORGE P. GUIMARÃES”.

(See photomicrograph 16, plate 5).

September 8. After 62 injections of Promin, the patient is becoming cyanotic, feeling ill and displaying other symptoms of poisoning. We advised him to discontinue the injections for a period of two weeks. The examination of the nasal mucus, collected this very day and stained by us, showed on the right side many acid-alcohol fast bacilli (++++) and on the left side groups of bacilli looking like the ones seen in culture emulsions. This result makes a contrast with the one obtained on June 24, 1949, when only a few bacilli could be found. Is it possible that the sulfone has produced an exacerbation of the disease?

September 14. The skin lymph from the cutaneous lesion on the right knee (Lleras Acosta technique) was smeared in natura, into 12 tubes of Loewenstein’s medium. The smears from this lymph were highly positive for acid-alcohol fast bacilli, the ones with a granular structure predominating over the homogeneous ones the later sometimes being larger than the ones previously seen or displaying a form of binary division. A few globies were also found.

September 19. The skin lymph collected on the incipient lesion of the left forearm, with the aid of Pean forceps and a vaccin stylet, was transferred into 6 tubes of Loewenstein’s medium, without any previous treatment. Smears from this material had been scarcely positive for acid-alcohol fast bacilli, and the cultures did not grow.
The patient moved for S. Gonçalo, State of Rio, where he went to live in an isolated country house.

September 26. Clinical check up: After having had 665 cm³ or 266.0 g. of Promin, the patient has all the old lesions exacerbated, new lesions also appearing which do not have the characteristics of *erythema polymorphum*. The infiltration on the face corresponds to L2, on the ears to L3. The patient complains of nasal obstruction. The skin on the neck and the breast, which had been previously free from lesions, shows now an acute moderate exanthema; on the skin of the abdomen, besides the old lepromatous patch, no exacerbated, there appear many papulae of different sizes; nothing new on the skin of the back; in the lombar region an extensive, squamous patch; the nodules on the arms, having been treated by physiotherapy, did not change for the worse; on the buttocks, a few sparse nodules appear, together with many, small, erythematous, slightly elevated maculae of various sizes; inguino-crural glands still not palpable, a token of recently acquired leprosy; red spots on the preputium and on the glans penis; on the inferior limbs the exacerbation has been more marked, specially, on the feet, which display several patches of the type L2.

October 3. Three nodules on the right ear were excised for bacteriological investigation and inoculation to two groups of brown and of black mice, as well as of white rats. The smears from these nodules showed many bacilli and a few globias.

October 5. A growth appeared on six tubes of Loewenstein's medium to which an emulsion of the skin lesion on the right thigh, had been smeared. There were a few disk-shaped, elevated, leadenbrown, shining colonies, with a golden-yellowish tone at the top. On September 29., one of these six tubes had already three of such brownish colonies, besides another one, also disk shaped and elevated, but of a golden-yellow colour. The smears from this colony, made to-day, shows only acid-alcohol fast bacilli of homogeneous structure. This colony has been transferred into 4 tubes of Loewenstein's medium which did grow normally. The smear from one of the brownish colonies, stained by the Ziehl-Neelsen's method, has shown cyanophil bacteria intermixed with roundish groups of acid-alcohol fast bacilli looking like globias. With a red-heaten platinum spatula, we destroyed the three brownish colonies of this tube, leaving the yellow one which being bathed every day by the condensation water of the culture tube, developed and spread, as illustrated by drawing 6, plate 1. The study of this culture will be the subject of a paper presented at 5th. International Congress for Microbiology, to be held at Rio de Janeiro on August 17 to 24. 1950.

We deem it a great success to have obtained such a culture after several series of sowings. Having always used for these smears materials which were very rich in bacilli, these being probably all alive for they came from florid lesions, one could not explain this lack of success. It could not possibly be ascribed to a deficient technique
since the contaminations have been extremely rare and when they occurred the cause was *Aspergillus niger* whose spores came with the cotton used for the plugs of tubes, which were always buried and then sealed.

In our opinion the conditions which regulate the growth of the *Hansen* bacillus have not yet been discovered and we must reach the conclusion that, if on one side we do not venture to state that the bacillus we isolated is the true *Hansen* bacillus, on the other side we cannot either be sure that it is not really this one. Since it is extremely difficult to obtain, directly from the leprous skin, pure cultures of an acid-alcohol fast bacillus pathogenic for laboratory animals, we feel happy to have gotten in the same year (1949) two of such strains, the strains "Hecke" and "Chaves". The future will tell if they deserve to be included among the *Mycobacteriaceae* suspected to be or considered as being the leprosy bacillus.

After more than twenty years of research on the bacteriology of leprosy (1928 to 1950) we feel authorized to admit as true Marchoux' hypothesis "that no doubt there are several leprosy bacilli, as there are several tubercle bacilli, and that the virulence of one strain may vary within very wide limits," an idea he expressed in his speech in the opening session of the 4th. International Congress for Leprology, held in Cairo, in March 1938.

October 10. A new biopsy (the 9th.) was made, this time on the anterior view of the left thigh, in the same place where the first biopsy had been made on June 10, by Dr. Portugal. The smears prepared with the section surface of the skin fragment taken on this biopsy, were stained by Ziehl-Neelsen's technique, were strongly positive for acid-alcohol fast bacilli. This material was divided in three portions, of which one was sent to Dr. Portugal, one for Dr. M. Torres, while the third was used for a new series of cultures. Once more Doctor Portugal's results disagreed from those of the Department of Pathological Anatomy of the Instituto Oswaldo Cruz. Those reports, read as follows:


Patient: J. C., Doctor: Prof. Souza-Araujo.

Histopathological examination of a fragment from a lesion on the thigh:


Two slides accompany the report.

(s) H. Portugal"
Souza-Araujo: Acute malignant leprosy

(See microphotographs 8 and 9, plate 3).

"P. C. 15.990, October 12, 1949.

Patient: J. C., Doctor: Prof. SOUZA-ARAUJO.

Nature and site of the tissue: Skin fragment from the left thigh.

Histopathological findings: Under the microscope, an inflammatory granuloma, with the common characteristics of lepromata. In other fields, the predominating cells display the same morphology as epithelioid cells. The large number of lymphocytes and plasma cells in the immediate neighbourhood of the granuloma calls the attention. Search for acid-alcohol fast bacilli, positive.

Diagnosis: Leproma, containing in some places, many epithelioid cells. In the neighbourhood, an inflammatory cellular infiltration with many plasma cells.

Manguinhos, October 24, 1949.

(s) JORGE P. GUIMARÃES”.

October 10. Three smears prepared by excoriating and scraping with a vaccin needle the three sides of a small lesion, tuberculoid in appearance, of the anterior surface of the right leg of Maria Domingas, the wife of Chaves, were stained and examined by us, being positive for HANSEN bacillus, which were found in small nubers.

The pessimistic state of mind of the patient became more marked, until it changed a few days later into a plain satisfaction when we told him that his wife should come back to his company and submit to treatment with Diasone associated to physiotherapy.

October 17. In the morning, sputum was collected from CHAVES, the smears prepared by the Ziehl-Neelsen staining technique with no other special treatment, being negative for acid-alcohol fast bacilli. However, after treatment by sodium hydrate and centrifugation the result was reversed to positive. The sediment obtained by centrifugation was transferred to Loewenstein’s medium, in one of the tubes three small, white colonies appearing, whose growth was extremely slow. These colonies have not yet been studied and will be subject to further research.

October 24. A lepromin-test was weakly positive a week later (October 31.), to decline in the next week and become entirely negative on December 5. On the same day, October 24, we sent the patient to the Dermatological Clinic of the National School of Medicine to be examined by Doctor HILDEBRANDO PORTUGAL to whom we wrote the
letter we transcribe in the footnote *, together with Doctor PORTUGAL’S answer which contributes a great deal to clarify this case.

October 31. Blood-picture (2nd., after having had 1.700 cm³ of Promin): red blood corpuscles 5.2 million; haemoglobin 15.0g%; white blood corpuscles 8.700; basophils 1%; eosinophils 8%. At it is easily seen, the only abnormality is the eosinophilia (Doctor Lobo).

November 21. We had previously made it sure that rats and mice resisted to subcutaneous inoculations, for a period of several days, of 0.4 cm³ of the emulsions of strains “Chaves” and “Hecke”. Then, having obtained the patient’s and his wife’s consent, at first verbally then by writing, we proceeded to inoculate the patient. We injected intradermally 0.2 cm³ of the emulsion of a culture on glycerin agar of the “Chaves” strain in the lower third of his right thigh and 0.2 cm³ of the emulsion of a culture on Loewenstein’s medium of the “Hecke” strain in his left thigh. To his wife we injected equal amounts of the same emulsions in the same regions.

The patient reacted strongly to the inoculation of his own strain of bacillus, which produced in loco, in the first few days, an erythematous, warm and painful patch, about 15 cm in diameter. On the opposite side, a similar but less marked reaction was produced, the erythematous patch being 6 cm. in diameter. The patient complained also of fever, cephalaea and depression. The patient’s wife had equally strong reactions both to the “Chaves” and to the “Hecke” strains.

During the next two weeks the erythematous patches became infiltrated, hard, painful at the palpation, these symptoms being more

* Manguinhos, October 22. 1949.

Dear Doctor Portugal

Tis will introduce you of my patient, Mr. J. C., whose case deserves your inspection on the lesion on which biopsies have been performed.

On June 13. 1949, based on biopsy material from a lesion in the left thigh of this patient, you established the diagnosis of tuberculoid reactional leprosy, with many acid-alcohol fast bacilli in the sections (P.C. 132.286).

On June 24. our colleague, the late Penna de Azevedo, established the diagnosis of lepromatous leprosy, based on material from a biopsy of the skin on the left ear. On July 13, we performed a biopsy of a lesion on the left leg (malleolar region) which is still worth seeing since it has undergone little retrogression. You supplied me with the following information on this material: tuberculoid granuloma (sarcoid type) with no acid-alcohol fast bacilli. P.C. 133.506. I would like to have a slide stained by the Ziehl-Neelsen’s technique.

On July 20. (40 days after your 1st. examination) I excised several nodules from the right ear of the patient for bacteriological investigation and sent you one of them for histopathological study. Your result (P. C. 133.760) reads: Lepromatous lesion.

On October 10. a new biopsy was performed on the lesion on the left thigh, on the place adjoining the scar of the 1st. biopsy by you. With the skin fragment I sent you, I made three smears, of which I forwarded two to you, one unstained and one stained by the Ziehl-Neelsen’s method. You reported on this material (P.C. 137.011, of October 12.): tuberculoid granuloma
marked on the right thigh of the patient and equally accentuated on both thighs of his wife.

December 14. Three weeks elapsed with no alarming symptoms; the oedema was reduced and craters appeared on the inoculated points. At this time we decided to repeat the inoculation of both volunteers, which was made with the remaining of the emulsions previously used, the injections being made 10 cm. above the first ones.

From December 19 to January 9, 1950, we collected the serous fluid from the experimental lesions, for bacteriological study and every time we succeed to obtain pure retrocultures of both strains inoculated, without any contamination and using either the serosity in natura or the serosity treated with sodium hydrate. Retrocultures were also obtained from the emulsions of biopsy materials from the craters of the experimental lesions of both patients.

Biopsy material from these experimental lesions is being studied in collaboration with the Department of Pathological Anatomy of the Instituto Oswaldo Cruz.

January 23. A new biopsy was performed on the border of lesion 2 “Chaves” on the right thigh. The lesions produced by the “Hecke” strain seem to be healed.

(sarcoid type) with no acid-alcohol fast bacilli. Both slides which accompanied your report were stained by haematoxylin-eosin. I beg you the favor of sending me a slide stained by the Ziehl-Neelsen’s technique. From your section of the ear, I obtained excellent photomicrographs and drawing. The bacilli with granular structure have the same characteristics as those found in the smears taken from the left ear on June 24, 1949.

With best regards,

Yours sincerely,

(s) H. C. de SOUZA-ARAUJO”.


Dear Doctor SOUZA-ARAUJO,

I acknowledge receipt of your letter October 22 and take due note of what you say, entirely in accordance to what is found on our laboratory files. According to your desire, I examined the patient and have nothing to add to what you say. I will take the necessary steps to send you the two slides you ask me; however, I will not be able to do it before next week, for I will be absent from Rio from the 27. to the 30. of this month. I wish to call your attention to the last examination (P.C. 137.011 of October 12.), whose report mentions the finding of Gram-positive bacilli in the sections. This technique is more accurate for the sections, because the different steps in the inclusion procedure, weaken the acid-fastness of the bacteria. One of the slides I sent you of the material of the last biopsy, marked H. Z., was stained by Ziehl’s technique, only the contrast staining by the methylene blue having been replaced by haematoxylin. This is a technique commended by Schmorl and which has the advantage of providing at the same time the staining of the bacteria and of the tissues.

Very cordially yours,

(s) PORTUGAL”.
January 28. Hemoculture: 5 cm$^3$ of blood taken from the right elbow veins of the patient were transferred in natura to 100 cm$^3$ of glycerin broth.

February 25. Clinical check-up: The figure is round, fat and with a good appearance. Erosions on the left side of the nasal septum. Ears almost normal. The experimental lesions of the left thigh, produced by the cultures of the “Hecke” strain, disappeared; the ones on the right thigh produced by the patient’s own strain, display half-smooth scars and cyanosis in an area of 2 by 2 cm., both having the same appearance. The blood-culture of January 28. shows no growth but a sediment which will be examined. The improvement is remarkable, both of the general health conditions and of the cutaneous lesions.

February 27. Examination of the nasal mucus: very rare acid-alcohol fast elements. Smears from a skin fragment taken from the lesion on the left malleolar region also show only very rare, atypical, acid-alcohol fast elements. With the emulsion prepared with this skin fragment we made transfers, besides sending a portion of the tissue to Dr. Torres for histopathological investigation. The lesion shows marked regression; the skin is smooth and only slightly cyanotic. There remains only one lesion with lepromatous appearance, located below the left knee and on which a biopsy will be perfomed next week.

In spite of the fact that the lesion on the left leg is already regressive one, the histopathological diagnosis, has still been of lepromatous leprosy, as may be seen in the following report:

“Instituto Oswaldo Cruz. Department of Pathological Anatomy.


Patient L. N. (name used for J. C., in order to avoid preconceived opinions). Doctor: Prof. Souza-Araujo.

Nature and site of tissue: Skin fragment from the left leg.

Anatomo-pathological findings:

The skin fragment is covered with stratified pavement epithelium (epidermis) with no particular characteristics. In the dermis and the subcutaneous tissues small cellular infiltrates can be seen formed by lymphocytes, fibroblasts and mononuclear cells with vacuolized cytoplasm and with intermingled normal collagen. There are very rare acid-alcohol fast bacilli. Diagnosis: Lepromatous leprosy.

Manguinhos, March 15. 1950.

(s) Dr. Jorge P. Guimarães"
On March 6 we performed a new biopsy on the patient, this time on the only lesion that still seemed to be lepromatous, which was located below the left knee. We stained and examined the smears from this skin fragment and found very rare acid-alcohol fast bacilli in marked degenerative condition. In spite of this, we made sowings from part of this material and sent part to the Department of Pathological Anatomy of the Instituto Oswaldo Cruz, from which we received the following report which we transcribe on account of its great scientific interest:


Patient: J. C., Doctor: Prof. SOUZA-ARAUJO.

Nature and site of the tissue: Skin fragment from the left knee.

Note: Patient on the 8th month of treatment by sulfones and physiotherapy.

Anatomo-pathological findings: The fragment is covered by stratified pavement epithelium (epidermes) with no particular characteristics. In the derm and subcutaneous tissues small infiltrates are seen, located specially in the neighbourhood of blood-vessels, glandulae and hair follicles and formed by mononuclear cells with vacuolized cytoplasm, lymphocytes, plasma cells and fibroblasts. Very rare acid-alcohol fast bacilli have been seen. Diagnosis: Lepromatous leprosy.

(s) JORGE P. GUIMARÃES”.

All Doctor GUIMARÃES’ slides and reports are submitted by him to the examination and criticism of the head of the Department, Doctor MAGARINOS TORRES.

March 13. As the patient continues to improve, we decided to perform a last biopsy (the 13th.), excising a skin fragment from each ear, on the sites where there was still a nodular appearance. It interested us a great deal to know the conditions on this part of the skin after eight months had elapsed since the first biopsy on the left ear which had furnished us with smears so rich in acid-alcohol fast bacilli. To our great surprise and still greater satisfaction, we verified that the different smears prepared with the excised skin fragment were practically negative, for only with a great difficulty could we detect two or three acid-alcohol fast bacilli and even these entirely broken into fragments. In spite of this, we prepared transfers on Loewenstein’s medium with blood in natura, as well as with a skin emulsion. The remaining material was sent to the Department of Pathological Anatomy, with the following note:
"Dear Doctor Guimarães.

I am sending you two skin fragments from the ears of J. C. (13th. and perhaps last biopsy to be performed on this patient). I ask you a prompt report on this material.

It has been for us a pleasant surprise to ear from this colleague that the tissue structure was that of a leprosy in marked regression, a statement which confirmed our clinical opinion. Here is the report:


Patient: J. C.

Nature and site of the tissue: Skin fragment from the ear.

Anatomo-pathological findings: The fragment is covered by strati-fied pavement epithel (epidermis) with no special characteristics. In the dermis small infiltrates can be seen formed by mononuclear cells in some places associated to cells with vacuolized cytoplasm, the total recalling the structure of a leprosy. Search for acid-alcohol fast bacilli negative.

(S) Dr. Jorge P. Guimarães".

A synoptic table showing the biopsies performed and the respective findings, will be seen below. See Graphic n.º 2.

March 13 — 3rd. Blood-count: red blood corpuscles 4,5 millions; hemoglobin 13,0 gr; leucocytes 6.000; basophils 1,0%; eosinophils 3,0% neutrophils: myelocytes 0,0; young 0,0; rod-like nuclei 2,0; seg-mented nuclei 58,0; lymphocytes 29,0; mononuclear 7,0%. (Dr. Al-varo Lobo).

March 27 — General conditions of the patient continue to improve. He says that: "he feels as well as if he was not ill, taking Promin". The cicatrical lesions resulting form experimental work, "H"1 and "H"2; "C"1 and "C"2, remain unaltered. The lesion on the left heel is still painful under pressure.

Microscopic examination: The smears of nasal mucus, on both sides of the septum are still positive (+). In the 4 smears of lymph and blood from the ears I found very rare and degenerated acid-alcohol fast bacilli.

Rhesus monkey: On the 22. March I inoculated the "Chaves" culture in the frontal and malar regions of a female rhesus monkey, 5 months old, weighing 2.5 Kg. In the first 3 days an intense inflammation resulted in the inoculated areas, which became attenuated and almost disappeared between the 5th and the 10th days, when nodules appeared on the three inoculated regions. On the 19th day after the
### TABLE I
SYNOPSIS OF THE ANATOMIC-PATHOLOGIC DIAGNOSTIC OF TEN SAMPLES OF LEPROUS SKIN FROM "CHAVES"

<table>
<thead>
<tr>
<th>N.º</th>
<th>Date</th>
<th>Sites</th>
<th>Dr. Hildebrando Portugal Laboratory</th>
<th>Division of Pathology Instituto Oswaldo Cruz</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>13–6-49</td>
<td>Left thigh anterior face</td>
<td>N.º 132,296: Tuberculoid leprosy (reactional) Innumeros a. f. bacilli</td>
<td></td>
</tr>
<tr>
<td>2nd</td>
<td>24–6-49</td>
<td>Left ear: big nodule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd</td>
<td>13–7-49</td>
<td>Intern malleolous, left leg.</td>
<td>N.º 133,506: Tuberculoid granuloma (Sarcoïd Type). No bacilli.</td>
<td></td>
</tr>
<tr>
<td>4th</td>
<td>20–7-49</td>
<td>Nodules: right ear</td>
<td>N.º 133,760: Lepromatous lesion.</td>
<td></td>
</tr>
<tr>
<td>5th</td>
<td>26–7-49</td>
<td>Nodules: left ear.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6th</td>
<td>9–8-49</td>
<td>Left thigh and right ear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7th</td>
<td>5–9-49</td>
<td>Right thigh: posterior face.</td>
<td>N.º 135,527: Tuberculoid granuloma (Sarcoïd type). No bacilli.</td>
<td></td>
</tr>
<tr>
<td>8th</td>
<td>3–10-49</td>
<td>Nodules from the right ear.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10th</td>
<td>2–1-50</td>
<td>Right thigh: 1st experimental lesion.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11th</td>
<td>27–2-50</td>
<td>Intern malleolous, left leg.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12th</td>
<td>6–3-50</td>
<td>Right knee.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13th</td>
<td>13–3-50</td>
<td>Skin of both ears.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For bacteriology.

N.º 15.889: Lepromatous leprosy. Dr. Jorge P. Guimarães.

N.º 15.945: Lepromatous leprosy. Dr. Jorge P. Guimarães.

For bacteriology and inoculations.

N.º 15.990: Lepromatous leprosy. Dr. Jorge P. Guimarães.


N.º 16.336: Lepromatous leprosy. (No bacilli for 1st time).
inoculation (April 10). I made a biopsy of the nodule on the right malar region and on April 14. Doctor MURILLO FONTEs excised the nodules of the frontal region and took also one half of that on the left malar region. (See Photos 20 e 21).

The histopathological examination of the material of the 1st biopsy (19th day after the inoculation), gave the following results:

"Section of Pathological Anatomy, Instituto Oswaldo Cruz": P.C. 16.398, April 10.1950 — Skin from the malar region of rhesus monkey, inoculated with the "Chaves" culture, 19 days ago.

Anatomo-pathological findings: There is an extensive inflammatory reaction on the dermis, with predominating great mononuclear sometimes vacuolated cells (macrophages). In several areas there are also, heterophile leucocytes (purulent inflammation). In the points where the exudate is formed almost exclusively of macrophages, there appear acid-alcohol fast bacilli, however in small numbers. One characteristic which is found to be similar to that of human lepromata is that the lesion is well limited, to the typically granulomatous area, another being following in which the dermis keeps its normal structure.

The above-mentioned description is valid for both fragments examined.

Manguinhos, April 12, 1950. (s) Dr. C. B. MAGARINOS TORRES, chief of the Division of Pathology".

On April 14 — I inoculated another rhesus monkey with an emulsion of nodules from the frontal and left malar regions of this monkey which is still kept under observation.

Everything related to the experiments made with the "Chaves" strain, will summarized in a paper to be read before the III Panamerican Conference of Leprology.

April 17 — New microscopic studies made on four different points of both patient's ears, after a very thorough examination, did show only the remaining of completely desintegrated bacilli. Five smears from the skin, from places on the patient's trunk, where no leprous lesions had ever been observed, did not show any acid-alcohol fast bacilli.

Treatment: From June 25, 1949 to April 20, 1950 (about 10 months) Chaves was given 251 intravenous injections of Promin, of which 25 of 5cm³, 6 of 7.5cm³ and 220 of 12.5cm³, amounting to a total of 2,920cm³, or 1,168 grams of the active substance.

During the first 6 months of treatment this had to be interrupted once due to toxic symptoms developed and twice on account of a severe leprotic reaction. Toxic symptoms never appeared again since the patient has been making constant use of liver extracts and anti-anemic products.

During this period, we submitted the patient to an electrocoagulation session for the ears and thirty two sessions of galvanocau-
terisation, followed by local use of a 33% solution of trichloracetic acid on the cheeks, buttocks, abdomen, arms and legs. On the heel, malleolar regions and calf of the left leg, we made 22 subcutaneous injections of Neovaleol added with strong Betaxin, at least on three different points of the skin each time, with excellent results on the point of view of relief of the pain, which, from the begining of the disease was felt on the left heel. On the clinical point of view the conditions of the patient had improved greatly. I should say, even that never have I had in my practice a so severe case of leprosy which responded so fast to the treatment.

The bacterioscopic and histopathological examination which lately have been performed, confirm the remarkable improvement of the condition of the patient who is now fat, with a clean skin on the cheeks, and with an excellent morale, being only worried by his wife's disease which has been progressing slowly but steadily.

The cooperation given me by Doctor João Fonte, in the study and treatment of this patient, has been most valuable, and it is a pleasure for me to acknowledge it with my best thanks, as also to Drs. Magarinos Torres, H. Portugal and Jorge P. Guimarães.

As told above, before submitting Chaves and his wife to experimental inoculations, I obtained their verbal consent which has been confirmed by the following certificate which may have, in the future, an historical value:

Certificate

"With experimental purposes, sure that we are contributing to scientific progress, we allow Doctor H. C. de Souza-Araújo, twice inoculate us with small amounts (0.2cm³ of emulsion) of cultures of acid-alcohol fast bacilli which he had isolated from lepers.


(ss) José Leonel Chaves Nava and Maria Domingas Chaves Nava"

CONCLUSIONS

1. In the present case there has been a disagreement about the histopathological diagnosis. When this occurs in the two most specialized centers of the capital city of the country, what cannot be expected to happen in the different states?

2. This disagreement proves that it is impossible to recognize the clinical type of leprosy through the histopathological examination of a skin fragment. As a matter of fact, Professor Francisco E. Rabello says: "... that he always protested against stabilishing the diagnosis of the form of leprosy through the examination of a skin fragment". (Brazilian Society of Dermatology, session of July 28, 1948).
3. To accept the above studied case as being of tuberculoid leprosy, would be contrary to the statements of several authors who profess that such a clinical form does not produce secondary cases, contrary to which the patient we studied after three months of cohabitation transmitted the disease to his wife who displayed the lepromatous form.

4. If we accept that this patient has both the lepromatous and the tuberculoid forms, accepted by as Stein, Wade and Rodriguez, John Lowe and others, would mean to refute the classification adopted by the 5th International Congress for Leprology (Habana, 1948).

5. It is important to register that, after showing large numbers of bacilli during 4 to 6 months, suddenly in the last three months, after being treated with Promin associated to physiotherapy, the patient showed spectacular improvement the clinical symptoms in evident regression and the number of bacilli being reduced to the point that the microscopical examinations were almost negative.

6. A long and steady observation of this couple of lepers might elucidate several problems, particularly the ones related to experimental leprosy.

Manguinhos, April 20th, 1950.

ADDENDUM

May 22nd — Clinical re-examination: After the patient having taken 3.220 cm³ of Promin (or 1.288 (gm.) of its active principle), by veins, and 36 sessions of galvanocautedisation, shows his cutaneous lesions much better. Search of Hansen bacillus in his nasal mucus was positive and feable positive in his skin. His general health is excellent. The experimental lesions are reduced to smooth scars with erythematous margins of 1 to 1 cm. His lesion on left knee is the unic showing lepromatous aspect: its biopsy (14th biopsy in this patient), gave me a fragment of skin, which was divided into two pieces, the 1st I gave to Dr. J. P. Guimarães in which smear he found a microscopic field with many a.f. bacilli and others poor. His histopathological report (P.C. 16.497, June 5,50) showed no structure of granuloma similar to leproma.

Bacteriology: May 23rd I sowed on 4 tubes of Loewenstein medium emulsion of the second piece of the skin, after being treated with soda.

June 12th, 20th day of incubation at 37°C: 3 of the 4 tubes showed many very small yellow colonies.

June 16th — Microscopical examination of one of these colonies revealed a pure culture of a.f. bacilli similar to those of the 1st strain "Chaves". Such finding was an agreeable surprise. Into the 4th tube smeared on May 23rd I added 1 cc. of fresh Loewenstein water.
June 25th: The patient completes to-day one year of treatment, having taken 3,495 cc. or 1,398 gm of Promin.

July 10th — The 4th tube sowed on May 23rd shows very small yellow colonies similar to the others. Success 100% of the sowings without any contamination. This new culture is Strain “Chaves-II”. Promin did not avoid, or perhaps has favoured, the germination of many Hansen’s bacilli, because in each tube there are many isolated colonies. The transplants of June 16th are germinating well.

Dr. Laerte Andrade proved that this strain “Chaves-II” is fluorescent as strong as the 1st Chaves strain and positive for cyto-chemical test of Dubos for virulence, but he is not quite sure in this point because the culture is too chromogenic.

This new culture will be inoculated into rhesus monkey as soon as new specimens be available. There is evidence that the Strain “Chaves” in the true Hansen’s bacillus.

Clinical history of the contaminated consort.

M.D.N., 18 years old white girl, born in Pedreiras, State of Maranhão, 180 Km. from Barra do Corda, where her husband was born. M.D. lost her mother many years ago. Her father and her 5 brothers are reported as healthy. She says that Pedreiras is no leprosy focus, instead of her husband’s native land. She was betrothed to the patient for four months, during which, she says, she had only 8 days of intimate contact with her bridegroom-to-be. She married him in São Luiz, State of Maranhão, on April 9, 1949, and travelled to Rio, where they settled in the district of Ipanema, in the same house in which her husband had lived since February 1947.

On July 11, on the first dermatological examination we could not see anything abnormal and we did not proceed to collect nasal mucus because she wept copiously after knowing about her husband’s disease.

July 25. She came back to our office, with her aunt, in order that we could collect nasal mucus. We stained and examined this material and found it negative for Hansen bacillus. Submitted the same day to a second dermatological examination, we discovered on the middle of the right leg, over the tibia, a small erythematosous spot, 2 by 1 cm. in diameter, which was left under observation. Her aunt took her away from her husband and had her enter a nun’s school in the district of Laranjeiras, Rio.

October 10. The lesion discovered on July 25. — a little more than three months after her marriage — located on the anterior tibial nerve, which is a branch of the external sciatic popliteum (see graphic n.º 4), increased a little and became tuberculoid. It is triangular in shape, 2 cm. high and its base has 1.5 cm. With a vaccin needle we collected serosity from the three sides of the lesion and prepared three different smears which we stained by the Ziehl-Neelsen’s technique and exam-
ined. A few typical acid-alcohol fast bacilli were found in each one of the smears.

The patient reported that since the end of September she has acute pricking sensations in the plants near the heels and a numbed skin on the same region (innervation territory of the common branch of the palmar cutaneous, of the calcanean branch of the posterior tibial nerve).

October 18. We destroyed by electrocoagulation (with Gaiffe's apparatus) one portion of the lesion, the destruction being incomplete on account of the resistance offered by the patient. The same day, a lepromin — test was made on the anterior view of the left forearm, this test being positive + on the 4th day, ++ on the 10th day and +++ from December 5 on, leaving a nodule 0.5 cm. in diameter which did not ulcerate. A new nasal mucus examination performed the same day was negative.

We prescribed Diasone at the dosage of two tablets a day, during the meals, and vitamin B.

October 31. The patient went back to her husband's company in an isolated county house in São Gonçalo, State of Rio.

November 21. First galvanocautery-trichloroacetic treatment of the lesion on the right leg. An acute nodule, 1.5 cm. broad and 1 cm. high, was discovered on the left leg, over the tibia, in a position similar to that of the lesion of the right leg. This lesion was kept under observation. On this occasion we discovered and called to it the attention of Doctor Joir Fonte, also an incipient ichthyosis simulating Chiyuto's sign on both legs of the patient, above the malleolar region.

After having her consent and that of her husband, we inoculated the patient with 0.2 cm³ of an emulsion of a culture of the "Chaves" strain. The site of the inoculation was the lower third of the right thigh. The symmetrical region on the left thigh was inoculated with 0.2 cm³ of a culture of the "Hecke" strain. The inoculation was intradermal and the dose was one half of the infective dose used for mice and rats.

November 28. The patient has a moderate fever and a marked local reaction on both inoculated points. This reaction is under the form of an infiltrated painful and warm area of hard oedema about 15 cm. in diameter. The results of this experiment will be reported later in a paper prepared in collaboration with the Department of Pathological Anatomy of the Instituto Oswaldo Cruz and to be read at the 3rd Panamerican Conference for Leprology to be held in Buenos Ayres on October 1950.

Doctor Joir Fonte punctured an inguino-crural lymph-node of the patient, the sero-bloodish fluid obtained being used to prepare smears which we stained and examined, finding a few isolated acid-alcohol fast bacilli and a group of forms looking like a colony (see drawing 10), Plate 1. The patient looks pale and depressed.
December 5. The lepromin-test became more clearly positive (++++), under the form of a well circumscribed, non-ulcerated nodule. The lesions produced by the experimental inoculations were reduced to a marked erythema and an indurated patch 5 cm. in diameter, with a nodular center, and painful on palpation.

December 14. In spite of the fact that the nodule on the left leg is now regressive, we punctured it with a vaccin needle, using the serosity collected for microscopical examination which has been negative for acid-alcohol fast bacilli. The infiltrated areas on the thighs being reduced, we collected with a pipette some serosity from the two “Chaves” and “Hecke” nodules and transferred it into Loewenstein’s medium, obtaining in from one to two weeks pure retrocultures of the inoculated cultures.

On December 14, we made the 2nd. inoculation with the same emulsions previously used, of strains “Chaves” and “Hecke”, at the same dose of 0.2 cm³, on a site 15 cm. above the 1st. ones, respectively on the right and left thighs.

December 19. For the second time we collected serosity from the craters formed on both lesions, on the right thigh, by the inoculation of the “Chaves” strain. The retrocultures were positive both with non-treated material and with the serosity previously treated with sodium hydrate. The lesions were still painful on palpation. The lesions on the right thigh showed marked regression.

January 2, 1950. A biopsy was performed on the borders of the crater formed on the site of the 2nd. inoculation of strain “C”. The retroculture was positive. New sowings were made on January 9.

January 16. A large intradermal nodule appears, with the consistency of an abscess, in the point where the Mitsuda lepromin was injected. The appearance is abnormal and alarming.

January 23. A large biopsy of this nodule was performed, the emulsion prepared being positive for acid-alcohol fast bacilli. A portion of excised fragment was sent to the Department of Pathological Anatomy of the Instituto Oswaldo Cruz and another portion was triturated and treated by sodium hydrate the sediment being transferred to Loewenstein’s medium. At the same time, the blood from the biopsy wound, was also transferred to six tubes of Loewenstein’s medium. A small biopsy intended just to clean the borders of lesion 2 “Chaves” was also performed, the smears showing rare acid-alcohol fast bacilli.

January 28. An inguino-crural ganglion on the left side was punctured and the material obtained transferred in natura to Loewenstein’s medium. 5 cm³ of blood, taken from a vein on the right arm, were transferred with no special treatment to 100 cm³ of glycerin broth.
Blood picture: Red blood corpuscles 4.5 millions; haemoglobin 12.0%; leucocytes 9,500 (eosinophils, myelocytes and young forms 0%); basophils and rod-shaped nuclear forms 2%, each; segmented nuclei 57%; lymphocytes 33%; monocytes 6% (Doctor Alvaro Lobo).

February 25. Gastralgia, inappetence and diarrhoea appeared, the use of diasone being discontinued for two weeks. Nasal mucus examination positive +, rare acid-alcohol fast granules.

The experimental lesions on the left thigh ("H") disappeared; those on the right thigh ("Chaves") were reduced to a cyanotic area, 2 cm. in diameter. The nodule due to the Mitsuda's test remains. Three of the six tubes to which transfers were made on January 23. show a growth of the "Chaves" strain.

March 3 — A biopsy having been performed on January 23. of the subcutaneous tumor produced under the nodule resulting from the Mitsuda test, the Section of Pathological Anatomy, furnished us with the following statement:

"P.C. 16.223, January 24. 1950 — Patient: Maria D. Chaves, Doctor Souza-Araujo. Nature and origin of the tissue: Tumour of the left forearm. Anatomo-pathological findings: In the a great many formations of a follicular type with epithelioid cells and rare giant cells of the Langhans type. The histological picture is the same which has been described in the lesions produced by a positive Mitsuda test.


(s) Doctor Jorge P. Guimarães".

March 10 — Retrocultures from the "Chaves" strain obtained by biopsy of the tumour of the Mitsuda test, as well as retrocultures of the "Hecke" strain obtained from the secretions of the experimental lesions on the left thigh, are similar to the original cultures, both macro-and microscopically, not the slightest sign of association of these strains being found. The facts show that the lesion produced by the Mitsuda-Hayashi lepromin acted as a locus minoris resistantiae. However, there is no explanation to the fact that such lesion was attained by the bacilli of the "Chaves" strain inoculated in the right thigh of the patient, while the "Hecke" strain organisms were inoculated at the same time in the left thigh and were nearer to the point of attraction. Could not the identity or near relation of the "C" strain and of the organism responsible for the natural infection explain this secondary point of infection?

March 20 — On the point of the Mitsuda test there is still an intradermic, painless nodule, 2 x 2 cm. To this nodule, I applied 9 galvanopunctures. The experimental lesions are in the following state: Left thigh: "H"1, cyanotic a little depressed scar, 0.5 x 0.5 cm.; "H"2, a 1 x 0.6 cm. scar covered by a thein scab. Right thigh: lesion
“C”1, cyanotic area, 3 x 1.5 cm., with a central, plain, depressed scar;
“C”2, cyanotic area, 3 x 2 cm., with a central, depressed scar, covered
by a dry scab.

The nasal mucus, at this time, showed under the microscope:
right side +, left side ++ (bicilli granular as found on the first
examination of the skin of her husband). Blood was taken, the serum
being sent by airmail to Doctor FREDERICK EBENSON, chief of service
in the Kennedy VAMTG Hospital, Memphis, Tennessee, for study of the
classification of strain “Chaves” and “Maria Domingas”.

April 10 — Six months ago (October 10.) the initial lesion of this
patient had a tuberculoid appearance, but three smears with the
material obtained by scratching three sides of the lesion showed a
few typical acid-alcohol fast bacilli. Galvanopuncture, twice applied
to this lesion produced a small change in its appearance. After 4
months rest, a biopsy was performed on this lesion, the fragment taken
being sent to Doctor MAGARINOS TORRES.

The following statement was made:

old — Doctor SOUZA-ARAUJO. Nature and origin of the tissue: skin
lesion on the right leg. Clinical diagnosis: Tuberculoid leprosy?
“Anatomo-pathological findings: In the dermis there are nodules with
the structure displayed by lepromata, the histopathological picture
being that of lepromatous leprosy. Acid-alcohol fast bacilli are not
found.

Manguinhos, April 12. 1950.

(s) Dr. C. B. MAGARINOS TORRES, chief of the Division of Pathology”.

April 10 — As a subcutaneous nodule was still found to the left
of the scar produced by the Mitsuda test, it was incised with the
galvanocauterium. Under pressure, a regular amount of pus was
obtained, smears stained by Z.N. not showing any more a.a.r.
bacilli. The nasal mucus, examined at the same time, was ++. The
pus, treated by soda and transplanted to Loewenstein medium, is
still under observation.

Treatment. From November 6. 1949 to April 20. 1950, the patient
was given 383 tablets of Diasone Abbott, 2 or 3 a day, at meal time,
the total amounting to 115.0 gr. of the active drug. As no new skin
lesions appeared and as the ichthyosis of the legs improved, it look
as having been beneficial. Twice a month I examine this patient again,
repeating the microscopical examinations. As yet it is not possible to
have a prognosis.

I am grateful to Doctor JOIR FONTE for his cooperation in the
study and treatment of this patient, whose fate greatly interested us
not only on the humanitarian but also on the scientific point of view.