TREATMENT OF LEPROSY BY OXYGEN UNDER HIGH PRESSURE ASSOCIATED WITH METHYLENE BLUE

By A. OZORIO DE ALMEIDA and
HENRIQUE MOURA COSTA

In a communication read to the National Academy of Medicine of Rio de Janeiro, on November 25th, 1937, A. OZORIO DE ALMEIDA and EDUARDO RABELLO reportd the positively favourable effect of oxygen under high pressure on Leprosy. At the request of the Dermatological Society, their paper was read before the Society on December 6th, with photographic documents in supoort of the results claimed.

For a clearer insight into what follows, a short summary will be made of the theoretical bases which led up to our experiments in the application of oxygen under pressure in leprosy. PAUL BERT'S experiments, which date back to last century, proved that oxygen under pressure is extremely toxic to all living beings, from mammals down to plants and micro-organisms. His results were confirmed and subsequently developed by other research-workers.

Though oxygen under pressure is always toxic to all living beings, or to living matter in general, sensibility varies from species to species. It is now possible to range living beings on a scale from the most sensitive, like the strict or obligatory anaerobes, which no longer develop when oxygen is present at a pressure of 4 to 5 mm. of mercury, such as Bacteridium butyiicum, Bacillus tetani, Bacterium oedematis malign, etc to those of the greatest resistance, such as Bacterium coil commune, Bacillus prodigiosus and Micrococcus laevulans, which can support more than 4 atmospheres of oxygen, the last-named supporting even over 9 atmospheres of pressure of oxygen (A. P&TTER, "Allgemeine Lebensbedingungen" in BETHE'S

Handbuch der normalen and pathologischen Physiologie, Vol. 1, p.1345).

The human being occupies an intermediate post in the scale of resistance of living beings: in his researches on cancer, A. OZORIO DE ALMEIDA proved that oxygen, under pressures of 25 2.5 to 4 athmospheres can be applied currently to man, during certain lapses of time, without any danger to life, provided certain precautions be taken, such as that of submitting th patient to an established previous diet, etc.

Such being the case, the next step was to find out which offered greater resistance to oxygen, the leprosy-bacillus or its human host. All that was needed was to submit lepers to pressures of oxygen, which could be used without danger, and to ascertain the mode of reaction of the leprosy bacilli as well as the course taken by the disease, under these circumstances.

In A. OZORIO' and E. RABELLO'S experimental treatment of leprosy, the disease shows regression and the bacilli appear under forms that are considered degenerative, besides becoming scarcer and scarcer in the patients undergoing treatment. This proves that the leprosy germ is compartively more fragile to oxygen under pressure than the human body. Oxygen is, therefore, a favorable agent for the treatment of leprosy, though the differential margin of resistance is a small one between the human body and the leprosy agent, so that numerous applications of oxygen and an extended period of treatment would be required.

The encouraging and undeniable results achieved in leprosy with the use of oxygen under pressure alone, made it imperative to perfect the method. In other words, a new and difficult problem had presented itself, i.e. how could the effects of oxygen upon the leprosy bacilli be increased, without at the same time raising its toxic effects upon the patient ?

Fortunately a series of circumstances permitted a solution of this problem to be found in the association of methylene blue to the treatment by oxygen.

Methylene blue was first used in the treament of leprosy by GALLAY (74) in 1894, and its use was subsequently taken up again by MONTEL in 1934.

However, it did not live up to the expectations of its patrons, as observation, both clinical and microbiological soon showed, even after large intravenous doses nowadays its use has ,been discontinued. Nevertheless, one fact remained on record and was later the subject of considerable study, Le. that, whereas the human organism eliminates all methylene blue a few days after its injection, the lepra-bacilli in the tissues are strongly impregnated by the dye, and keep it fixed for months, so much so, that leprous lesions rich in bacilli are stained blue and show a blue tinge for a long time. In short, methylene blue has a far greater affinity for, and fixation power over, the leprosy germ than over the normal tissues of the body (39 to 73).

Now, quite aside from this fact, A. OZORIO DE ALMEIDA, in his researches upon the action of oxygen on cancer, had tried out numerous substances, intended to intensify the effects of oxygen, among others methylene blue. He was able to find out that this was the substance that made oxygen under pressure most poisonous for animals. This action is shown by precocious and violent phenomena of intoxication exhibited during the application of oxygen, as well as by the ensuing general disorder leading up to death after the application as was seen in animals impregnated with methylene blue and subjected to the action of oxygen. Since cancer tissue has no more affinity for methylene blue than normal tissue, no advantage could be gained from its use in connection with oxygen in that disease. In leprosy, on the contrary, the fixation of the dye upon the bacilli, so as to be present at a time when the tissues of the host had already got rid of the methylene blue injected into him, made it possible to obtain a greatly reinforced action of oxygen on the germs, without raising the susceptibility of the patient. A few days after injection, the patient has already eliminated the methylene blue not fixed into the germs.

We must make it quite clear that the association of methylene blue and oxygen is not intended to sum the effect of one to the other This would be of small avail, in our opinion. As has been made sufficiently clear, the gist of the question is the increased toxicity of oxygen, brought about by the presence of methylene blue, so that, when it is fixed upon the bacilli, these become very much more sen-

sitive to oxygen. Methylene blue enables us to obtain a great selectivity of the action of oxygen upon the bacillus of leprosy.

Methylene blue is, by itself, very slightly toxic. By the mouth, animals can stand for days up to 60 centigrams a day, per kilo ; in the case of dogs, a fatal dose exceeds 1 gram per kilo (76).

The increased toxicity brought about by the association of oxygen and methylene blue is a consequence of the chemical action of this dye. Biochemical studies have shown that methylene blue acts as an acceptor of hydrogen, which it withdraws from numerous organic compounds. It thereby changes itself into a more hydrogenated compound, which is colourless. This is the leuco-form of methylene blue ; this form is a stable one, in the absence of oxygen. In the presence of oxygen, however, the hydrogen that had been fixed by methylene blue is given up to the oxygen and the dehydrogenated blue form is regenerated. This cycle, in which methylene blue alternately takes up and gives back hydrogen, repeats itself indefinitely. Methylene blue, in the presence of oxygen, is therefore a catalysar of indirect oxidation, acting by dehydrogenation, and is, consequently a reinforcer of the action of oxygen. The action of methylene blue reinforcing that of oxygen can be proved experimentally. Suffice it to say that the system "hemoglobin+glucose+methylene blued+oxygen" develops an activity which is 30 times greater than the system "hemoglobin+glucose+oxygen" (77). To obtain a general idea on the action of methylene blue, reference should be made to the article by W. LIPSCHITZ (78).

TECHNIQUE FOR THE EMPLOYMENT OF METHYLENE BLUE AND OXYGEN IN LEPROSY.

Patients are given intravenous injections of methylene blue. A number of days then elapse sufficient to ensure no further elimination of this substance in the urine. After that the patient is submitted to oxygen treatment. This is applied, as in the case of cancer, in a metallic compartment specialy built for the purpose (for further details, see the corresponding publications on the subject by A. OZORIO DE ALMEIDA (79 — 80 — 81 — 82).

TOLERANCE TO TREATMENT.

Tolerance to Methylene Blue. Tolerance to intravenous injections of methylene blue has been variable, despite the comparatively small doses given, which we attribute to impurities in the product. As a rule, the presence of zinc or hydrocyanic acid has been responsible. Symptoms observed in almost all patients consisted in a condition of nausea, oppression, anxiety and giddiness.

It should be pointed out. by the way that no less than four different products, differing in formula, are on the market under the same name of methylene blue : New Methylene Blue 2G : (C_{20} — H_{20} — N_3 — OCI), Methylene Blue B, 4B, 4BE-BG, NSZI : (C_{16} — N_{18} — N_3 — SCI), Methylene Blue in Powder extra BB : (2(C_{16} — H_2 — N_2 — SCI) 2n — CI; — H;O) and New Methylene Blue N : (C_{18} — H_{22} — N_3 — SCI).

Tolerance to Oxygen. In our experiments, we only made use of medium pressure, much lower than those employed by one of us in the treatment of cancer, as a lesser resistance to oxygen on the part of lepers had been noted previously.

In all the patients the tolerance was perfect, except in two, who showed a few unimportant effects : a slight sensation of discomfort and oppression, sickness, a burning sensation at the leprous lesions.

CLINICAL RESULTS.

Immediate Effects. Immediately after the applications, on coming out of the apparatus, all the lesions appeared congested and slightly edematous ; around the reddish tubercles, a very dark halo could be seen.

On the days following, in some cases one could ascertain a decrease in the elevation of the infiltrated areas, increased softness of the lesions, a greater suppleness of the infiltrated ear, decrease in size of some tubercles with a small scab in the centre, white others were in full suppuration period with partial elimination.

Reactional Effects — After a lapse of time varying from 3 to 11 days, on an average after one week, on all patients, the apperance of small, red pruriginous and short-lived patches was noted, located

on the lesions or on apparently healthy skin. When these patches occur over a tubercle, this tubercle immediately shrivels or suppurates as a result.

These patches appeared successively, at times overlapping, at others spaced at long intervals, and in some cases took the form of rashes.

In the case of one patient, three months after the last application, a renewal of this eruption occured, with the successive appearance of patches merging into one another and forming large scarlatiniform rashes. After these lesions had subsided, a special sepia hue of the skin was noticed, not only where the patches had been, but on the skin as a whole.

In some cases, there appeared, contemporaneously with the red patches, cutaneous nodules looking like boils, which suppurated in a day or two, and left atrophic scars (the pus, on examination, proved to be sterile).

Finally in various cases we noticed the appearance of large, deep-seated, hard and painful nodules of a nodular-erythematous type. These reactive effects were less pronounced in some cases, without any disturbance of the general condition of the patients, while in others they were more pronounced and then coincided with general symptoms such as muscular pains and swelling of the lymphnodes.

In one of the patients, more marked general effects were noticed: considerable weakness, vesperal fever, anorexia, intence muscular pains, pronounced and painful swelling of the lymphnodes and the presence of a large amount of albumen in the urine, three months after the applications.

Clinical Improvements. Clinical improvements occur in stages, with quiescent intervals, showing up suddenly and conspicuously, in the shape of : a marked decrease of the infiltrations, with consequent wrinkling of the skin, a softening of the lesions, a decrease or disapperence of the tubercles through shrinkage or atrophy of the skin, leaving a crater-like aspect (fig. 51, 52 53), or by inflammation and elimination leaving dark scars, which remind one of treated impetiginous or ecthymatous lesions (fig. 42.ª, 43a, 44a, 47, 48, 49). These improvements are characteristically not continuous and progression of the disease, alternating with stationary periods.

They extend over several months, quite independently of any treatment or any further interference.

The more accentuated improvements were seen in cases with little or no previous treatment. In cases having numerous previous intradermic infiltrations, the lesser efficiency of the method can be readily understood, by taking into account the prolonged permanence of chaulmoogra at the sites of infiltrations as was shown by NOLASCO (18), in his papers. By hindering the staining with methylene, blue it would consequently prevent the reinforcement of the effects of oxygen by the dye.

The observation of all cases, considered as a whole, shows the almost complete disappearance of the lesions in some of the patients, while the others show evident improvement, which is more conspicuous in some cases and less so in others. All of them, however, show beneficial modifications after the treatment.

Although naturally incomplete, owing to the insufficient lapse of time during which the cases have been observed, these results are, in many instances of great interest, since they would have been difficult to achieve by any other means of treatment, the more so if the severity and extension of the disease in our cases is taken into account.

Our studies are being continued, and, since observations has shown that the effects of regression are still in continuous progress, it may not be too much to hope for and even to assert that there will be a complete regression of all the lesions, in the near future.

BACTERIOLOGICAL RESULTS.

The effects of the treatment on the bacili is very prompt and intense. Remarkable alterations in the appearance of the bacilli take place immediately after the applications.

Before passing on to describe these alterations, a brief summary must be made of the normal morphology of the bacilli and of their granular forms, which are not interpreted in the same way by the different writers.

Normal Morphology. After staining according to the classical Ziehl Nielssen method, the typical Hansen bacillus is seen in the shape of a red rod, measuring 3 to 5 micra in length by 0.2 to 0.45

in width. These rods are straight or slightly bent, sometimes with tapering extremeties at other times not, They stain uniformaly throughout the body.

Using a special staining method, LUTZ (1), in 1886, showed the bacillus of leprosy as a rod full of granulations. He proved that these granulations are normal and characteristic of the bacillus, which he placed in the genus Coccothrix.

UNNA (2) is also of the opinion that the bacillary form of the lepra organism is merely a one-sided aspect of the same, its multiplicity of form being so great that it cannot be fully encompassed by any single method of staining.

PALDROCK (3), using the classical method of staining but devising a special process for the taking and preparation of the smears (4), described, at the Third International Leprosy Conference, some special forms. Adhering to the op inion of Meirowsky (5), he considers these forms to be phases of a cycle of the leprosy organism like those of fungi. MARCHOUX (6), however, disputed some of these, at the same leprosy Conference. He attributed them to a faulty interpretation of the morphological figures.

Granular Forms. — In old lesions undergoing spontaneous regression, in lesions submitted to much treatment and in phases of leprotic reaction, even the classical staining method shows atypic forms of the Hansen bacilli. The forms studied by different writers are : slender forms, long and bent forms, chains of fragmentated bacilli (strepto-bacilli), dumb-bell-shaped bacilli with granulations at both ends, bacilli in the shape of a note of music, rodlets with granular body, bacilli with clusters of granulations at one end (umbelliform bacilli), chains of granulations having no bacillary body (strepto-coccoid), as well as free granulations. (UNNA and other writers make reserves as regards these free granulations, which they consider to be simply albuminoid granulations of an origin other than the bacilli, especially as disintegrated cells from sweat glands).

NEISSER was the first to describe clear spaces or vacuoles in the bacillary body. He atributed them to the interstitial formation of spores.

JOHNSTON (7) divides the forms of leprosy bacilli into :

1. Clacaical Type

(a) coarse granules

2. Fragmentary or degenerative type. (b) fine granules.

3. Solid type ..... (a) long forms

(b) short forms.

4. Streptococcoid or nocardial type.

ROGERS and MUIR (8) describe :

a Diphtheroid rods with bipolar coloration

b Rods containing a series of dots, giving the appearance of a string of beads.

c Large round sporelike forms with an attenuated rod projecting either from one side or from both sides, at opposite poles.

d Spore-like forms with no projecting rod.

MUIR and CHATTERJI (9) describe two types of granulations :

a MUCH's granules.

b LUTZ'S particles.

MANALANG (10) considers three types :

1 Solid type

2 Segmented type.

3 Granular type.

The interpretation of these forms is still a moot point. The general consensus of opinion is to consider them as degenerative and to look on them as signs of extincion of the germ. Nevertheless, some writers have interpreted them as forms of resistence and propagation (UNNA, DENNY, PALDROCK).

Others again, like MARCHOUX (11), MARKIANOS (12), MANALANG (13), consider that the granular state forms a part fo the evolution of the ultra-virus into the bacillary state and that the post-virus granular phase is young and resistant, whilst the pre-virus phase is of age and degeneration.

HOFFMANN (36) is also of the opinion that the granular forms may be constituted by elements of resistance and elements of degeneration.

In any case, the granulations which we shall describe in the cases submitted to treatment are not granulations shown by normal

bacilli under special methods of staining, but granulations seen with the ordinary stain for acid-fast bacilli, in which they are stained by fuchsia.

Action of the Treatment on the Hansen Bacillus. Almost immediately after the first applications of oxygen, marked alterations are seen in the appearance of the leprosy-bacilli from the patients.

Smears from patients, who had previously shown an abundance of absolutely typical bacilli, were found, upon examination, immediately after, as well as in subsequent examinations, to contain great alterations in the morphology of germs, Some of these alter-rations were transitory, others persistent. They represented a complete revolution in the appearance of the bacilli.

In the firts place a description will be given of the principal types of anomalous forms occurring most frequently :

1.° Slender bacilli (fig. 1). Very slender bacilli, staining very lightly and showing up like "shadows" of the bacilli. Found free or in broken-up globi, granular at times.

2.° Elongate bacilli and elongate granular bacilli (figs. 2 and3)

Elements with a length almost three times the normal, many of them with a granular appearance. These forms were rarely seen. When found, however, they took up the preparation in great masses. Their appearance was observed in one patient, 15 days, and in another 25 days after the last application.

3° Short bacilli (figs. 4 and 5). The appearance is that of a fragment of bacillus, at times almost of a granulation, generally dense and strongly stained.

They occur free and in disrupted globi, together with fragments of bacilli and free granulations. Sometimes they show chain-formation (like strepto-bacilli).

Very frequent in almost all preparations and appearing very soon after the treatment.

4.° Short granular bacilli (fig. 6). Diminutive bacilli, commonly found, showing two granulations which make up almost the whole bacillary body. Frequently free and in disrupted globi.

Also, as with the previous form, very frequently found in the patients, treated and appearing with great precocity, as also in various later phases.

5.° Bacilli with a granular body (figs. 7, 8, 9 and 10). The bacillary body may be normally stained (fig. 8) or almost invisible, the granulations then appearing like a string of pearls (fig. 9). The granular bacilli appear free or in globi, which then take on a special dotted appearance (fig. 10). Generally found in preparations together with free granulations and short bacilli. They occur at various periods, more often in the early phases immediately after the applications, or else, later on, after an outbreak of typical bacilli or of anomalous forms. At times the granulations appear detached from the bacillary body, as can be seen in fig. 11.

6.° Bacilli in dumb-bell or diphtheroid form (fig. 12). In these forms the bacillary body is sometimes barely visible (as described by ROGERS and MUIR (8) or else it maintains its normal staining affinity, with more deeply stained granules at the extremities. Found at various periods after the applications, generally in association with other forms.

7.° Bacilli shaped like notes of music (fig. 13). Very similar in shape to the tetanus bacillus. The bacillary body is attenuated and reduced, with an enlargement at one end occupied by a granulation. Of fairly frequent occurence in various periods subsequent to the treatment and associated with other granular formations.

8.° Bacilli with granulations in clusters (fig. 14). These are bacilli with a slightly bent and granular body showing at one end an agglomeration of granulations and clear spaces. These formations, described under the name of umbellae by Paldrock and Meirowsky (3-5), were attributed by these authors to an evolutive phase of a life cycle of the germ of leprosy akin to those of fungi. They are very rarely found.

9.° Bacilli with a large granulation or nodular bacilli (figs. 15, 16 and 17). — These forms consist of a perfectly coloured bacillus, slightly bent, with tapering ends and showing, generally in its centre and more rarely near its ends, a large spherical granule of a far darker colour than that of the bacillary body, almost black and widely exceeding the bounds of the rod on both sides. This granule is as a rule a single one ; rarely has more than one been found for one barilhs.

The almost black colour of this granulation is not caused by the methylene blue, for it also appears in the same way in the pre-

partitions tinted exclusively with fuchsine, being due to a concentration of the colouring matter at that point.

In checking up with all the descriptions of the Hansen bacillus, we find no reference to forms like these, either because they have escaped observation owing to being extremely rare, or because they are due to the applicztions of oxygen.

In tuberculosis, entirely similar figures were found on one solitary occasion by Dr. Guilherme Lacorte who published them under the title "A curious morphological aspect of the Koch bacillus", without, however, putting forward any explanation for this phenomenon (14). At first sight one would immediately imagine it to be a case of a spore. Dr. Lacorte howver in the similar forms which he found in the Koch bacillus eliminated this hypothesis by showing that, whilst the unheated material, inoculated into a guine a pig, reproduced tuberculosis, samples heated to 65° gave negative inoculations.

These forms showing a large granulation may be found either in bacilli of normal size or in very long or in very short ones. They are generall commoner and more numerous in smears from the skin.

They may occur free or in globi, associated in the same preparation with typical or granular bacilli.

They appear systematically in all patients given oxygen treatment, in lapses of time varying between 23 and 56 days after the the application, in practically all cases after 39 days.

In many cases there is the appearance of a mass-invasion, completely filling the whole preparation.

As a rule they put in their appearance after periods of absolute negativity lasting days and days, or else they occur after days with only rare atypic formations. These days of rare atypic formations can then be followed by a new phase of negativity or of great scarcity of germs, among which the more accentuatedly granular forms predominate.

Their appearance generally coincides with a clinical phase of reddish reactional patches to be noted on the lesions or on apparently healty skin.

10.° Free granular forms. Marchoux's pulveriform Mycobacterium (figs. 17 and 18). These are minute granulations, a kind of granular powder, inside globi, together with tiny bacilli and ba-

ciliary fragments, or else free in the neighbourhood of a disaggregated globes from which they have come.

At times, an immense quantity of conglomerated granulations will be found in all the fields of a preparation. These masses are made up of very granular and imperfectly stained bacilli, of bacilli in fragments and of enormous globi full of nothing but granulations, just as if a violently destructive process had reduced all the bacillary bodies to powder.

These forms appear, in their milder aspect, at various perids, either precociously, following immediately the applications or at later periods, following outbreaks of typical bacilli or of similar forms.

When they occur alone, making up enormous masses, their appearance is not so prompt.

It should be noted that all these forms were seen using the usual Ziehl Nielssen staining method for acid-fast bacilli. The fact that these granulations and other anomalous forms were seen with the aid of this method, when normally special methods are required for granulations, is in itself an argument in favour of the changes undergone by the germ, under treatment.

This confusing multiplicity of forms seen after applications reminds one of what is seen in preparations of acid-fast bacilli grown on culture-media (fig. 1, 2, 4, and 5).

Signification of the Anomalous Forms. As already stated above, considerable divergencies exist among the various writers in their interpretations of these granular forms.

The few who elect to interpret them as forms of resistance, of evolution or of reproduction of the germ, base their contention on the following arguments :

1. On the fact that at times they may appear in recent lesions, still in evolution. Nevertheless their appearance in old and treated cases is the rule. VELASCO's observations of the typical forms in relapsed cases is conclusive, in this respect.

2. On the co-existence in the same preparation of typical and of granular forms.

This fact is easily explained by the possibility of the occurrence, in the skin, of bacilli of different ages and resistances, some of them lying more sheltered inside cells and others exposed for a longer time to the action of the defensive forces of the body.

3. On the appearance, at the same time but in different spots, of normal and of granular forms.

   This is easily explained by a local defensive reaction, which may be newer here and older there, greater in one place smaller in another. Local immunity allows for the existance of more protected or more exposed zones, even in the course of the disease.

In opposition to these, the arguments put forward in favour of interpreting these forms as a degeneration and death of the bacillus are far more logical and convincing :

1. Their almost constant appearance in old lesions, undergoing regression, and prior to the complete disappearance of the bacilli.

2. The often observed fact of finding them in treated cases evolving towards improvement (MANALANG (10))

3. VELASCO'S observations do not bear out those of MANALANG, regarding the forms appearing in relapsed lesions.

4. In old cultures of acid-fast germs, granular forms predominate, whereas, in young ones, typical bacilli prevail SALLE (15).

5. In the leprotic reaction, in which there is a considerable increase of the allergic phenomena, the predominant forms are the granular (FERNANDEZ (16), BECHELLI and CAMPOS SAMPAIO (17).

Finally the systematic and almost immediate appearance of these forms, after the applications, would seem difficult to explain otherwise than as a suffering and degeneration of the bacillus.

The appearance of certain bacillary forms seen in the course of the treatment show an arresting resemblance to the figures of

degeneration occurring in the myelin strands of nerves. The comparison between the two is all more tempting if we bear in mind the similarity of their lipoid composition.

Indeed, myelinic nerve fibre and the leprosy bacillus are both made up, roughly, of a filament of protoplasm enclosed in a sheath of lipoid substance.

When the nerve is cut and its peripheral extremity undergoes secondary or Wallerian degeneration, the phenomena which occur in it remind one of the alterations frequently seen in lepra bacilli and might well be used in interpreting these alterations, as we shall next try to show, after summarizing the detailed description of the phenomena of nerve-degeneration, given by SPIELMEYER (in the 9th. volume of BETHE'S Handbuch der normalen and pathologischen Physiologic).

In the myelinated nerve fibre, degeneration begins at the axon, followed later by the transformation of the lipoids of the myelin sheath.

At the first signs of degeneration, the axon presents parts swollen by imbition side by side with shrivelled parts, which cause it to break up into minute irregular particles. The axon often shows cork-screw formations, granulation, loop-formation, or enovelated formations : it subsequently is transformed into irregular fragments or into granular masses.

The myelin sheath breaks up progressively, giving rise to the formation of ellipsoids, balls and even small spheres. With the alterations of their microscopic appearance, comes a change in affinity for dyes, indicating the regression of lipoid substances of different composition into substances with the simple characteristics of neutral fats.

The series of alterations of the axon and the myelin sheath, combined one with the other, produce aspects which recall to mind those, seen with the ordinary Ziehl-Nielssen method of staining acid-fast bacilli, under the shape of lepra-bacillus granulations and globi filled with these granulations.

If the alterations observed in the nerves are of the same nature as those occurring in lepra-bacilli, these become easier to undertand. Imbibition and swelling of the nerve-protoplasm at one point,

accompanied by shrivelling at other points, formations of globes and fragmentation, occurring together with the rupture of the waxy sheath and the formation of granulations, can be compared with the variation in length and in thickness of the bacilli, with the appearance of large granules or lumps between the bacilli, with the bent shapes and the shapes looking like a note of music, and with the free granulations seen in our preparations after the action of oxygen. By careful observation, one may even ascertain a condensation of chromatin in one half of the rod or at its extremities, as a primitive phenomenon, giving rise, probably, to the nodule formation and the dumbbell formations, together with a later appearance of comma-shaped aspects, bacillary segments and granular powder.

Bacteriological examinations were numerous in all the patients treated. Examination was made before treatment, immediately after and then periodically, throughout a period of 83 to 96 days.

A uniform technique of collection of material, fixation, staining and examination was always used.

In patients with abundant and typical bacilli at the time the treatment was begun, great alterations could be seen in the bacilli, in some patients two days after the treatment, in others later.

In all instances, as an almost instantaneous effect of the applications, the normally shaped bacilli either disappeared or diminished in number.

Granular bacilli, shapeless bacilli and free granulations with the aspect of disaggregation made their appearance at the same time. At times, these changes took a few days longer.

After the lapse of a varying period of poverty or absence of typical forms. there occured the appearance of bacillary outbreaks, at times intense, always mingled with a large number of degenerate forms predominating in the preparations. These periods of crisis were usually short-lived. They lasted one or two days and gave way to an absence or rarity of bacillary formations.

The granular formations also increased or decreased in crises, in connection with bacillary crises or otherwise.

Upon the repetition of the applications, the same effects took place.

Consequently some alternation exists, without apparent regularity, between enduring periods of scarcity or even of absence of bacilli, either normal or anomalous, with other short-lived periods of a mild invasion of normal bacilli or of an appearance in mass of anomalous forms, including a mass pulverization of the bacilli.

From an examination of the bacteriological graphs (fig. 33) the following constant observation may be derived:

a) Decrease or disapperance of the typical forms almost immediately after each application ;

b) After the first application, an immediate appearance of granular forms (if they had been absent before) and an increase in their number (if they had existed, at all, before). This action is reinforced by each subsequent application :

c) Typical bacilli become scarce or absent in preparations during periods, separated by short-lived outbreaks, during which they were seen ;

d) The granular forms, ever increasingly conspicuous, showed also short-lived crises, during which they were extremely numerous ;

e) Examinations made on nasal mucus show greater variation than the smears obtained from the skin ;

f) Complete negativity (absence of bacilli etc). in the preparations in several cases and, in all, a drop in the bacteriological graphs. with a clearly defined trend towards the complete extermination of all germs.

Histo-pathological examination of sections of the skin in some patients were negative in regard to germs after treatment, including in the less superficial strata of the sections, and only revealed acid-fast powder in the case of the remaining patients.

In an almost complete ignorance of the manner in which the leprosy bacillus actually behaves in the human body, an exact explanation of these phenomena is a matter of difficulty. Basing ourselves, however, on certain clinical facts, we feel entitled to attribute these crises described, to a mobilisation, from visceral foci, of germs already weakened or killed by the action of oxygen.

As a first effect of the treatment, a more extensive or complete destruction would take place at the body surface. Here they would

already be naturally weakened by the defensive action of the skin. The skin is normally considered to be endowed with greater resistance and to produce anti-bodies, the defensive powers of which would be heightened and stimulated by such destruction of germs.

The bacilli, already killed or weakened by the action of oxygen, would be set free from the deep-seated foci and brought, in great masses to the skin, by the circulation. Here they would undergo rapid destruction by the defensive action of the skin, alredy stimulated and increased. This is the only way that the rapid disappearance, at times within a few hours, of enormous outbreaks of bacilli or granulations can be understood. It is obviously not possible to conceive that enormous amounts of non-motile bacilli spread among great quantities of cells could disappear by returning to the viscera. In support of these ideas, we observed in all these patients cutaneous phenomena of allergic type. In various cases the lepromin test became positive. Our interpretation also agrees with the description, by several writers, of cases of leprotic reaction forms (FERNANIEZ (16), BECHELLI and CAMPOS SAMPAIO (17). PHILLIPSON (19) made remarkable publications in this respect, showing histo-pathological sections of leprotic reaction, with bacilli entirely normal in appearance protected inside the capillaries, while those dispersed outside these shelters and in contact with the reaction of the tissues, showed themselves profoundly modified.

We may, therefore, infer that there is a direct an immediate action on the bacillus ; this action would disturb or annul its metabolism and affect or destroy a large number of germs. This would allow the human organism to increase its defensive capacity, reacting more efficiently, completing the destruction of the residues. In our opinion, this is the essence of allergic cutaneous processes.

One way or another, oxygen under pressure in patients prepared with methylene blue, has shown itself capable not only of clinical improvement of leprosy patients but also of modifying profoundly or destroying the lepra-bacillus.

We shall next proceed to the results of Lepronime-Tests and to the histo-pathological sections, which were diagnosed by Dr. H. PORTUGAL.

RESULTS OF IEPROMINE TEST

CASES	BEFORE TREATMENT	AFTER TREATMENT
Case I	negative	+
Case II	negative	+ +
Case III	negative	+ +
Case IV	negative	+
Case V	negative	+ +
Case VI	not made	negative
Case VII	negative	negative
Case VIII	negative	+
Case IX	not made	negative

HISTO-PATHOLOGICAL EXAMINATIONS AFTER TREATMENT
SUMMARY OF CASES HISTORIES

All material for these examinations was taken on Jan. 1938

Case I. *Material:* Fragment of tubercle in regression on forearm.

Description: Large foci of cellular infiltration round dilated vessels. Infiltration cells are histiocytes, plasma-cells and neutrophils. Absence of germs.

Diagnosis : Non-specific infiltration.

Case II. *Material:* Zone of leprotic infiltration on forearm.

Description: Diffuse infiltration of the corium, only the papillary body being free.« Epidermis diminished. Infiltration composed of vacuolized histiocytes and giant-cells with a large central vacuole, some of them containing hemateiphil masses. Absence of bacilli and granulations. This patient had never undergone any local treatment of any kind whatsoever.

Case III. *Material:* Reddish reactional patch on forearm.

Description: Marked hyperkeratosis. Telangiectasias of the papillary body. The remainder of the thorium occupied by a proliferation of a neoplastic aspect, composed of histiocytes and numerous vessels. Absence of bacilli and granulations.

Diagnosis : Histologic appearance like that of a histiocytoma.

Case IV. *Material:* Tubercle undergoing regression on forearm.

Description: Numerous vacuolized cells. Rare giant-cells with large central vacuole. Some plasma-cells and lymphocytes to be noticed in the infiltration. Acid-fast granulations in powder. Absence of bacilli.

Diagnosis: Leprotic Granulomsa in regression with signs of oily infiltration.

(Before treatment the histo-pathologic diagnosis was "Leprotic Granuloma").

Case V. *Material:* Leprotic nodule undergoing regression on wrist.

Description: Diffuse superficial infiltration of the corium, with dilated vessels. infiltration-cells are histiocytes and plasmacells. Deep nodular infiltration round the hair-follicles, sebaceous and sweat glands. Absence of bacilli and granulations.

Diagnosis: Non-specific infiltration.

Case VIII. *Material:* Tubercle undergoing regression on forearm.

Description: Nodular infiltration of the deep part of the corium, with numerous vacuolized cells, plasma-cells and giant-cells. Presence of exceedingly rare acid-fast elements.

Diagnosis: Leprotic Granuloma in regression.

SUMMARY OF CASE HISTORIES.

Observation of Cases was made up to Jan. 27, 1938, when this report was closed. Further reports will be published later.

Care I. A. T. C. One year infection. Previous treatment nil. Coryza. Epistiris. Tubercles on ears, arms, forearms, buttocks, thighs and legs. Slight infiltration on malar regions. Signs of atrophy of muscles of the hand. Perturbations of sensibility to heat.

C2N1 Nasal Mucus : frequent bacilli and globi. Skin : frequent bacilli and globi. Mitsuda : negative. Sedimentation : 67. Weight : 60 kilos.

9 intravenous injections of Methylene Blue at 1% (6 of 6 c.c., 4 of 10 c. c.), 3 series of oxygen, at 3.0 to 3.5 atmospheres, totalling 11 application or 15 hours at 3.0 or 3.5 atmospheres.

Reactional patches 11 days after the second application. Great outbreak of patches three days after the third application, with fever, anorexia, pains in the joints, lymph-node enlargement. Disappearance by shrivelling or suppuration of all tubercles, except

a few on the right ear, which, however, were greatly reduced in size. Pigmented scars in the place of the tubercles. Beginning of treatment : Aug. 24, 1937 ; end : Oct. 23, 1937.

On Jan. 27, 1938: Nasal mucus : absence of bacilli ; very rare granular forms. Skin : absence of bacilli ; very rare granular forms.

Mitsuda : +. Sedimentation : 108. Weight : 45.5 kilos.

Case II — A. A. S. — 6 years infection. Chaulmoogra treatment 3 months ago without result.

Total fall of eyebrows. Great infiltration of forehead, regions of eyebrows, cheeks, jaw, neck and ears. Mass infiltration of arms and forearms. Large patches of infiltration on back, buttocks, thighs and legs. Deepseated nodules in forearms and thighs.

C3 — Nasal mucus — presence of bacilli and globi. Skin — presence of bacilli and globi. Mitsuda — negative. Sedimentation — 95. Weight 66 kilos.

9 intravenous injections of Methylene Blue at 1% (5 of 5c. c., 3 of 10 c. c. — 1 of 15 c. c.), 3 series of oxygen or a total of 11 applications. (13 hours at 3 atmospheres).

Appearance of reactional red patches 6 days after the 2nd. application; 18 days after the 3rd. application, appearance of nodules of furunculoid aspect, with suppuration in 2 days'time. Deep-seated and painful nodules. Pains in the joints.

Considerable decrease of all zones of infiltration, especially in the regions of the eyebrows, ears and forearms. On the forearms upon the rapid disappearance of the infiltration, the skin became loose and wrinkly. Disappearance of deep-seated nodules. Nasal mucus — negative. Skin — negative.

Mitsuda++ Sedimentation — 62. Weight — 71, 1 kilos. Commencement of treatment : Aug. 24, 1937 ; end : Oct. 23, 1937.

Case III — G. N. — 5 years'infection. Chulmoogra treatment more than 1 year without appreciable results.

Coryza. Epistaxis. Eyebrows completely fallen away Infiltration of the regions of eyebrows and jaw. Tubercles on face, jaw nose and ears, elbows, legs and chest. Patch of tubercles on the abdomen.

G3 — Nasal mucus — bacilli and granular forms. Skin — negative. Mitsuda — negative. Sedimentation — 47. Weight 77,5 kilos. Nine intravenous injections of methylene blue at 1% (5 of 5 c. c., 3 of 10 c. c. , 1 of 16 c. c.). 3 series of oxygen treatment or a total of 11 applications, summing up 13 hours at 3 or 3.5 atmospheres.

3 days after the 2nd. series of applications, a limited appearence of reactional red patches.

Disappearance or decrease of tubercles by inflammation. Natal mucus — Very rare bacilli and very rare degenerated forms. Skin — negative. Mitsuda — ++. Sedimentation — 68. Weight 75, 8 kilos. Beginning of treatment : Aug. 24, 1937 ; end : Oct. 23, 1937.

Care IV — M. J. B. — 4 Years'infection. Chauhmmogra treatment 2 months ago, with small result.

Coryza. Fall of eyebrows. Erythematous patches on cheek and ear. Pigmented patches on arms and forearms. Leprous infiltration on forehead, regions of cheeks, ears, back and buttocks. Isolated tubercles on forearms. thighs and legs. Anesthesia in forearms, backs of hands and feet.

C2N1 — Nasal mucus — presence of globi. Skin — presence of bacilli and globi. Mitsuda — negative. Sedimentation 118. Weight 58,8 kilos.

Six intravenous injections of 1% Methylene Blue (3 of 5 c. c., 3 of 10 c. c.) 2 series of oxygen forming a total of 9 applications (10 hours at 3.0 or 3.5 atmospheres).

Appearance of red patches, 7 days after the 1st. application. Decrease of infiltrations of the tubercles with disappearance of a large number.

Nasal mucus — negative. Skin — negative. Mitsuda + Sedimentation — 86. Weight 65.6 kilos.

Beginning of treatment : Aug. 24, 1937 ; end : Oct. 23, 1937.

Case V.— B. M. C. — 8 year's infection. 15 chaulmoogra injections more than 2 years ago, with constant reactions.

Total fall of eyebrows. Extensive infiltration of the regions of the eyebrows, cheeks, ears, arms and forearms. Isolated tubercles on forearms and knees. Voluminous nodules on wrists and hands. Dry ichthyosiform eczema on legs.

C3 — Nasal Mucus — abundance of bacilli and globi. Skin — negative. Mitsuda — negative. Sedimentation 97. Weight 55.6 kilos.

6 intravenous injections of Methylene Blue at 1% (2 of 5 c. c., 4 of 10 c. c.).

2 series of oxygen treatment forming a total of 9 applications. (11 hours and 15 minutes at 3 atmospheres).

Small red patches on arms and malar regions, 5 days after the 2nd. series of applications. Appearance of deep-seated nodules on thighs. Accentuated decrease of infiltrations especially in malar regions and ears. Desapperance of tubercles on forearms and knees. Decrease of nodules on forearms and hands.

Nasal mucus — frequency of bacilli, rare granular forms. Skin — negative. Mitsuda+ + Sedimentation — 86. Weight 53,8 kilos.

Beginning of treatment : Sept. 25, 1937 ; end : Oct. 30, 1937.

Case VI — A. S. — 13 years'infection. Chaubnoogra treatment several years ago, without result, constant reactions and new lesions. Coryza. Epistaxis. Collapse of nose. Total fall of eyebrows. Leonine features. Enormous infiltration of the whole face, arms, forearms, hands, buttocks, thighs and legs. Isolated tubercles on lips and jaw.

Voluminous masses of lepromas on forearms and backs of hands. Atrophy of the muscles of the hand, with signs of claw deformation. Atrophy of the muscles of the soles of the feet. Neuritis in feet. Anesthesia of forearms, backs of hands and of feet.

C3N2 — Nasal mucus — abundant bacilli and globi. Skin — many bacili and globi. Mitsuda — not effected. Sedimentation —127 Weight 53 kilos.

7 intravenous injections of 1% Methylene Blue (4 of 5 c. c., 3 of 10 c. c.) 2 series of oxygen treatment, totalling 9 applications or 13 hours at 3.0 or at 3.5 atmospheres.

Appearance of some reactionary patches 10 days after the first applications. 30 days after the 2nd. series of applications, swelling of lymph-nodes, muscular pains, fever.

Decrease in size of tubercles on lips. Decrease in size of the voluminous nodules on forearms and hands. Improvement of the neuritis.

Nasal mucus — bacilli exceedingly rare, very rare granular forms.

Skin — bacilli very rare, granular forms very rare.

Mitsuda — negative. Sedimentation — 60 Weight 59 kilos.

Beginning of treatment : Sep. 25, 1937 ; end : Oct. 30, 1937.

Case VII — A. E. N. — 7 years'infection. Previous chauhnoogra treatment with small results.

Some infiltration of the malar regions. Small tubercles in the region of the eyebrows, face, neck, forearm and legs. Limited zone of anesthesia in forearms. Slight atrophy of the muscles of the hand.

C2N1 — Nasal mucus — frequent bacilli and globi. Skin — frequent bacilli and globi. Mitsuda — negative. Sedimentation 75. Weight 60,5 kilos.

6 intravenous injections of Methylene Blue at 1% (5 of 5 c. c. , 1 of 10 a c.) 2 series of oxygen treatment forming a total of 6 applications (8 hours and 15 minutes at 3 atmospheres).

Appearance of a few reactionary red patches 30 days after application. Disappearance of tubercles.

Nasal mucus — negative. Skin — negative. Mitsuda — negative Sedimentation — 58. Weight — 67 kilos.

Beginning of treatment : Oct. 6, 1937 ; end : Nov. 6, 1937.

Case VIII — D. G. - 2 years infection. Previous treatment — nil. Coryza. Epistasis. Fall of eyebrows. Conglomerated tubercles on forehead and knees. Isolated tubercles on nose, face, malar regions jaw, ears, forearms, hands, buttocks, back, thighs and legs. Lepromatous infiltration on forearms, legs and buttocks, Dry eczema on legs.

Anesthesia in forearms, left leg and feet.

C3NT — Nasal mucus — frequent bacilli and globi. Skin — frequent bacilli and globi. Mitsuda — negative. Sedimentation —60. Weight 52,7 kilos.

6 intravenous injections of 1% Methylene Blç (5 of 6 c. e., 1 of 10 c. c.) 2 series of oxygen treatment totalling 6 applications or 8 hours and 15 minutes at 3 atmospheres).

7 days after the 2nd. series of applications, appearance, of rashy patches, 40 days after the 2nd. series, of reactionary nodules.

Disappearance of infiltrations of forearms and decrease of infiltrations of legs. Decrease of tubercles on ears and jaw. Disappearance of a large number of tubercles on forehead, forearms and legs through shrivelling. Many of the tubercles show complete central depression, with atrophied skin, presenting an appearance of a lunar crater. In the place of the vanished tubercle a clear atrophy of the skin is to be noted.

Nasal mucus — absence of bacilli, very rare degenerated forms. skin — negative. Mitsuda + Sedimentation — 53. Weight — 55.3 Has.

Beginning of treatment : Oct. 6, 1937 ; end : Nov. 6, 1937.

Care IX — I. M. — Period of infection unknown. Previous chauhnoogra treatment with fair results.

Coryza. Rarefaction of eyebrows. Tubercles and infiltrations on face and forearms.

C2 — Nasal mucus — Bacilli and globi most abundant. Skin — negative. Mitsuda — not effected.

Three intravenous injections of 5 c. c. of 1% Methylene Blue. One series of oxygen treatment, 3 applications all told (3 hours and 45 minutes at 3 atmospheres.)

Upon patient's return to the hospital, a slight decrease in the lesions was ascertained.

Nasal mucus — exceedingly rare bacilli, infrequent granular forms. Skin — negative. Mitsuda — negative.

Beginning of treatment: Nov. 3, 1937 ; end : Nov. 6, 1937.

SUMMARY AND CONCLUSIONS.

The work reported in this paper rests theoretically on the following facts : a) Oxygen under high pressure has a harmful effect on all living beings ; the sensitiveness varies, however, from species to species ;

b) Methylene Blue, which acts as a catalyzer of indirect oxidations, by dehydrogenation, increases the toxic action of oxygen under pressure ;

c) Methylene Blue has a special and elective affinity for bacilliferous leprous lesions, in which it is retained for months, whereas in the body, as a whole, it is eliminated in a few days ; d) the beneficial effects of oxygen under pressure on leprosy were previously ascertained. In the treatment of leprosy by oxygen under high pressure, the authors conceived the idea, and put into practice, the previous administrations to patients of injections of methylene blue, a lapse of time being allowed for the patients fo get rid of the dye through the urine, although still retaining methylene blue in their leprous lesions.

Experimental treatment here reported started about five months ago, on Aug. 23, 1937. Nine patients in all were treated. The period of treatment by oxygen, which varied individually, was commenced on Aug. 23, 1937 and was finished for all patients by Nov. 6, 1937. After this the patients were merely kept in observation. The total amount of methylene blue injected into one patient, during the whole course of treatment, varied from 15 to 70 centigrams, leaving an average of 48 centigrams for a single patient. Oxygen was used at a pressure varying from 2.5 to 3.5 atmospheres. The mumber of applications of oxygen varied from 3 to 11, with a mean of 8 sittings. The length of each application was variable and the sum total of the number of hours each patient was exposed to oxygen varied from 3 hours and 45 minutes (minimum duration) to 15 hours (maximum duration), allowing a mean of 10 hours and 36 minutes for the total duration of oxygen treatment in one patient.

The results are the following :

a) Clinical results consisting of marked decrease of the infiltrations, with folding of the skin, disappearance of tubercles by acute processes of reabsorption with shrivelling and atrophy of the

skin, improvement in general condition with a considerable gain in weight of the patients.

b) Bacteriological results consisting in prompt and intense alterations in the morphological appearance of the germs, reduction in number of germs right upto the disappearance of typical bacillus 6 cases out of 9.

c) Histopathological results, in the six examinations, showing the regresion of the leprotic granuloma in 2 and no specific infiltration in 3. of them Absence of any acid-fast elements in the strata of the skin, in 4 cases.

d) Immunity-test results showing changes of the Mitsuda test. Of the 9 cases, 7 were negative and 2 unexamined prior to treatment. Of the 7 former, 5 became positive, one doubtful and one continued negative after treatment. The two unexamined before treatment, were negative after treatment.

These results leave no room for doubt as to the existance of an intense and rapid action of oxygen-methylene:blue upon leprosy. The efficacy of this action is considerably greater than that seen previously by A. OZORIO DE ALMEIDA and E. RABELLO, when oxygen under pressure was used alone.

If the severity of most of the cases treated is taken into account together with the few applications of Oxygen-Methylene-Blue, the obviously insufficient lapse of the and the magnifitude of the results obtained, we feel entitled to claim that oxygen udenr pressure associated with Methylene Blue, according to the technique described, exceeds in efficacy all other treatments used up to now in the treatment of leprosy, not excepting the modern treatments by esters of chaulmoogra. In other words leprosy appears fragile in the face of oxygen under pressure associated with methylene blue.

The work here reported was carried out as an extension of work of the ward for the experimental study of oxygen-therapy supported by the generosity of MR. GUILHERME GUINLE, to whom all thanks are due. MR. GUILHERME GUINLE's keen interest in the question was also shown by Ms having the experimental study of oxygen under pressure in leprosy, included in the list of recommendations made by the "Centre Internacional d'Etudes sur la lepre" of the League of Nations. We are grateful to Dr. Theophilo de

Almeida for his kindness in permitting ours experiments in the pacients of the Curupaity Leprosy Hospital

REFERENCES

1) — ADOLPHO LUTZ — Zur Morphologic des Mikroorganismus der Lepra-Dermatologische Studien — 1886 — Heft 1,S.77.
2) — P. G. UNNA — Histotechni der leprosen Haut — 1910.
3) A. PALDROK — Contribution á la morphologic du microbe de la lepre — 1924.
Bème Conférence Internationale de la Lèpre — 1924.
4) — A. PALDROCK — Eine einfache Methode Leprabazillen in der zu untersuchenden 7aut nachzuweisen — Dermatologisches Centrelblatt n.° 4 — Jan. 1913.
5) — E. MEIROWSRY — Protozoischer oder pfanzlicher Entwicklungskreis der Spirochaeten ? — Dermatologische Wochnschrift n.° 8. Feb. 1914.
6) — Marchoux — 3éme Conférence Internationale de la Lépre. 1924. p. 139.
7) — Johnston — The varying morphology of B. leprae and the routine microscopic examination of nasal mucus in lepers — Philippine Journal of Sciences. n.° 9-1917.
8) — ROGERS AND MUIR — Leprosy — New York — 1925, p. 141.
9) — MUIR AND CHATTERJI — Leprous nerve lesions of the cutis and subcutis — International Journal of Leprosy, vol. 1-1933.
10) — J. MANALANG — The morphology of Myc. Leprae during the course of treatment. Monthly Bull. Bur. of Health. Vol. 18 n.° 1-1937.
11) — MARCHOUX — La lepre des rats. 2ème Conférence Internationale de la Lèpre — 1924.
12) — MARKIANOS — Lépre et virus filtrable — Annales de l'Institut Pasteur — Paris — 1931.
13) — J. MANALANG — The morphology of Myc. Leprae before treatment in readmitted relapse cases. Monthly Bull. Bur. of Health. Vol. 18 n.° 1-1937.
14) — GUILHERME LACORTE — Curioso aspecto morphologico do Basílio de Koch. — O Hospital — Jan. 1936.
15) — SALLE — On the cultivation of an acid-fast organism from leprous lesion in tissue culture and other media — International Journal of Leprosy. Vol. 2 n.° 1-1934.
16) — JOSÉ M. M. FERNANDEZ — Bacteriologia de la reacción leprosa — Revista Brazileira de Leprologia — Vol. 4 n.° 1-1936.
17) — LUIZ MARIANO BECHELLI AND JOSÉ CAMPOS SAMPAIO — A pesquiza do bacillo de Hansen nas lesões cutaneas da reacção leprotica — Revista Brazileira de Leprologia — Vol. 4 — n.° especial — 1936.
18) — NOLASCO — Histopathology of leprosy under local infiltration ; the nature of the yellowish globules in treated lesions. The Journal of the Philippine Islands Medical Association — 1930.
19) — PHILIPPSON — Histopathology of the acute patches and erythymatose spots of tuberous leprosy — Virchowschrift — Vol. 32. Booklet 2.
20) — Josef Kyrie — Vorlesungen uber Histo-Biologie der mensrhlichen Haut and ihrer Rrkrankungen — 1927.
21) — Cerqueira — Sobre a coloração do bacillo de Hansen — Rio de Janeiro — 1925.
22) — Gay and associates — The leprosy bacillus — Agents of Dis. and host resistance — L. 968 — 1935.
23) — A summary of our present day knowledge of the pathology and bacteriology of human leprosy — The Indian Medical Gazette n.° 2-1930.
24) — Terebinsky — Qu'est ce que les bacilles jaunes (Unna) de la lepre — Anneles de Dermatologie et Syphiligraphie — n ° 2-1911.

25) — MAKIANOS — La bacillémie et la fiévre lépreuse — Annales de Dermatologic et Syphiligraphie — 1933.
26) — PALDROCK — Treatment of leprosy by Solganol and carbonic snow — Archiv. fur Schiffs — and Tropenhygiene. Vols. 31 and 32.
27) — P. PEREIRA CERQUEIRA — Etiologia da leprose — Revista Medica de Minas (Brasil) n.° 47-1937.
28) — J. RODRIGUEZ, E. MALABAY AND J. C. TOLENTINO — Gram positive forms of M. leprae from leprotic lesions bacteriologically negative for acid fast organism — Philippine Journal of Sciences — Vol. 6 n ° 4.
29) — A. J. Salle and J. R. Moser. — Bacteriology of leprosy. International Journal of Leprosy — n.° 5 — 1937.
30) — BERNICE E. EDDY — Attempt at cultivation of M. Leprae — International Journal of Leprosy n° 5-1937.
31) — VAUDREMER AND MLLE BRUN — Culture et cycle évolutif do bacille de Hansen — Presse Medicate. n.° 92 — 1936.
32) — W. I. KEDROWSKY — Modern aspects of the epidemiology of leprosy — Internation Journal of Leprosy. n° 3-1935.
33) — D. DE LOIZACA — Bacilos de Hansen y otros acidoresistentes aislados de lesiones leprosas cobreados por el método de Fontes. Revista Médica Argentina de Dermatosifi1ologia n.° 6 — 1936.
34) — GIL DE CASTRO CERQUEIRA — Eliminação do bacilo de Hansen pela via cutanea. Revista Brasileira de Leprologia. Vol. 2 n.° 2-1934.
35) — MCKINLEY — LEON — Cultivation of lepra bacillus. International Journal of Leprosy — n.° 3-1937.
36) — H. W. HOFFMAN — The granular forms of the leprosy bacillus. International Journal of Leprosy n.º 1-1933.
37) — AEGEMIRO RODRIGUES DE SOUZA — Destino do bacillo de Hansen fóra do organismo. Revista Brasileira de Leprologia. Vol. 4 n.° especial — 1936.
38) — ERNESTO MENDES AND VICENTE GRIECO. — Interpretado da reacção leprotica, suas relações com a parallergia. Revista Brasileira de Leprologia. Vol. 4 n° especial — 1936.
39) — R. MONTEL — Résultats obtens dans le traitement de la lepre par le blea de methylene. BulL Soc. Path. Exotique. n.° 291936.
40) — R. MONTEL. AND LE VAN PHUNG — Un cus de lepre tubereuse generalise blanchi cliniquement et bactériologiquement en 10 moil. Traitement micte par le bleu de methylene et le chaulmoogra. BuL Soc. Path. Exotique. n.° 29-1936.
41) — R. MONTEL — Poussées de lépromes furonculoides au cours du traitement par le bleu de methylene. Bull. Soc. Franc. Dermat. Syphiligraphie n° 2-1935.
42) — R. MONTEL — Conference ser le bleu de methylene. Bull. Soc. Paul, Exotique. 1936.
43) — R. MONTEL, J. BABLET, NGUYEN, NHUA AND DO VAN HOANH — Deux cus de lépre traités par le bleu de methylene sent, d'abord, et par l'association : bleu de methylene-chaulmoogra ensuite. Action du traitement snr lee symptomes cliniques, sur lee tissue et sur le M. leprae — Bull. Soc. Path. Exotique n.° 29-1936.
44) — L. VIALLETON — Colorations vitales — Précis de technique histologique et embryologique.
45) — A. AFANADOR — Traitement de la lépre par lea injections intravcineuses de bleu de methylene. Bull. Soc. Path. Esotique n° 9-1934.
46) — P. LÉPINE AND J. MARKIANOS — Action directe do bleu de methylene sur le bacille de Hansen dans forganisme humaine. Comptes rendas de la Soc. de Biologie. — 1935.
47) — P. LÉPINE E J. MARKIANOS — Résultats obtens dens le traitement de la lépre par le bleu de methylene. Bull. Soc. Path. Exotique. n.° 29-1936.

48) MERCHEN — Essai de traitement de la lepre par les injections intra-veineuses de bleu de methylene. Ann. Soc. Beige Med. Tropicale. n.° 16-1936.
49) A. OCCHINO AND Y KERNHAMP — Le bleu de methylene dans le traitement de la lepre. Ann. Soc. Belge MM. Tropicale n° 16-1936.
50) — G. BERTACCINI — Sulfa colorazione vitale dei bacilli della lebbra. Bol. Sez. Regionale n.° 14-1936.
51) G. BERTACCINI — Sulla cura della lebbra col bleu di metilene, Giorn. Ital. Dermatologia e Sifilologia n.° 77-1936.
52) BENETAZZO — Il bleu di metilene nella terapia della. lebbra. Giorn. Ital. Derm. Sifil. n.° 76-1935.
53) — C. LOMBARDO — Ulteriori ricerche sulla colorazione in vivo dei bacilli della lebbra. Boll. Sez. Regionale n.° 14-1936.
54) — C. LOMBARDO — Osservazioni sulla terapia della lebbra col bleu di metilene. Arch. Ital. Dermot. Sifil. Vener. n.° 4-1937.
55) — C. LOMBARDO — Nuove Indagini sulla colorabilitá in vivo dei bacilli della lebbra e della tuberculosa. Boll. delle Sezioni Regionals. n.° 15-1937.
56) M. LA SCALA — Il bleu di metilene nella terapia della lebbra. Boll. delle Sez. Regionali, n.° 15-1937.
57) J. C. URUENA AND L. J. LOZANO — El tratamiento de lepra por el azul de metileno. Giornale Ital. Derm. Sifil. n° 78-1937.
58) P. Benny — Action du bleu de methylene adminiatré par voie buccale sur les douleurs des lépreux. Bull. Soc. Path. Exot. n.° 29-36.
59) MME. DELANOE. — Le bleu de methylene dans le traitment micte de la lepre. Bull. Soc. Path. Exotique n.° 5-1935.
60) Dubois and Degotte. Essai therapeutique dans la lepre, le bleu de methylene. Bull. Soc. Soc. Path. Exotique. n° 2-1935.
61) MME. MILIAN AND GEORGES GARNIER — Le traitement de Ia Lepre par le bleu de methylene. Bull. Soc. Franç. Derm. Syphil. n.° 2-1935.
62) J. FERON — Une indication du bleu de methylene dans le traitement de la lepre. Bull. Soc. Path. Exotique. n.° 29-1936.
63) M. LANGALEZ — Le bleeu de methylene dans l'intoxication par l'oxyde de Carbone. Bruxelles Médical — 1937.
64) J. M. FERNANDEZ AND SALOMON SCHUJMAN — El empleo de las anilinas en el tratamiento de la reacción leprosa. Revista Brasileira de Leprologia.
65) J. M. FERNANDEZ AND SALOMON SCHUJMAN — Nuestra experiencia acerca del valor de algumas analisas en el tratamiento de la lepra. Rev. Brasileira de Leprologia. Vol. 2 n.° 3-1935.
66) — NELSON DE SOUZA CAMPOS — Coloração vital pelo azul de methyleno. Rev. Braz. Leprologia. n.° especial — 1935.
67) — Renato Braga — Tratamento da lepra pelo azul de methyleno. Rev. Braz. Leprologia. n.° especial — 1935.
68) — FLAVIO MAURANO — Os resultados do tratamento da lepra pelo azul de methyleno endovenoso. Rev. Braz. Leprologia. n.° especial — 1935.
69) — RENATO BRAGA — Considerações sobre os resultados do tratamento da lepra pelo azul de methyleno. Rev. Braz. Leprologia. Vol. 2, n.° 2-1935.
70) — J. COSTA DE CARVALHO — Tratamento da lepra pelo azul de methyleno no Asylo Colona de Aymorés. Rev. Braz. Leprologia. n.° especial 1935.
71) — F. RIBEIRO ARANTES — (Accidentes observados no tratamento da lepra pelo azul de methyleno. Rev. Braz. Leprologia. n.° especial 1935.
72) — E. COSTA VALENTE AND LUIZ M. BECHELLI. O azul de methyleno no tratamento das reacções leprosas. Rev. Braz. Leprologia n.° especial — 1935.
73) — LUIZ M. BECHELLI — O azul de methyleno no tratamento das algias leprosas. Rev. Braz. Leprologia n° especial — 1935.
74) GALLY — Arch. medic. naval. Colon — 1894.
75) A. OZORIO DE ALMEIDA. — Researchon the treatment of experimental and human cancer by oxygen under pressure. 6th. Congress of the Pan-American Association in Rio de Janeiro. July 16th. 1935.

76) — SOLLMANN — A manual of Pharmacology, 3rd. edition.
77) — O WARBURG, F. KUBOWITZ UND W. CHRISTIAN — Uber die katalytisch Wirkung von Methylenblau in lebenden Zellen. Biochem. S. Vol. 227-1930.
78) — W. LIPSCHITZ — Ubersicht uber die chemischen Systeme des Organismus in Bethe's Handbuch der Physiologie.
79) — A. OZORIO DE ALMEIDA — Recherches sur l'action tosque des hautes pressiona d'oxygène. C. R. *Soc.* Biologie. V. CXVI — 1934.
80) — A. OZORIO DE ALMEIDA — Le traitement et guérison par l'oxygène du cancer expérimental des rats. C. R. Soc. Biologie — V. CXVI — 1934.
81) — A. OZORIO DE ALMEIDA — Essai de traitement du cancer humainpar l'oxygéne sous pressiona. Ac. Braz. Sciencias. Mai 1935.
82) — A. OZORIO DE ALMEIDA — Recherches sur l'action de l'oxygcne sous pression. Acad. Braz. de Sciencias. Mai 1935.

PICTURE OF THE GRANULOUS FORMES

All microphotographies were increased 4 times. Objective 100 ×, Ocular 8 ×.
Photographs amplified 2 times. Drawings increased of half.

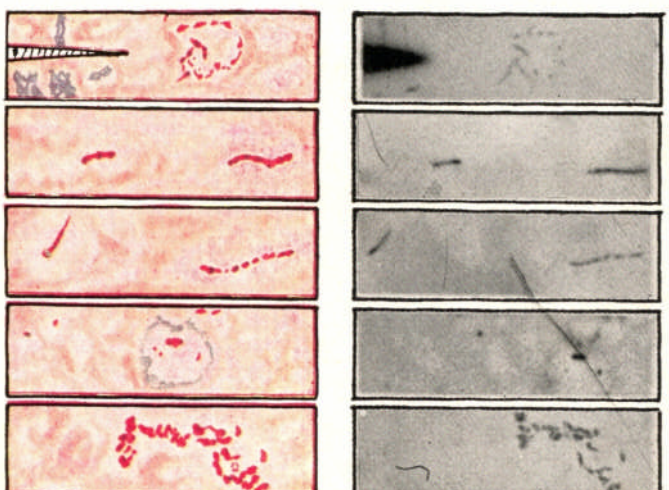

Fig. 1.	*Thin bacilli.*
Fig. 2.	*Long bacilli and bacilli of normal sige.*
Fig. 3.	*Long and granuloses bacilli and bacilli of normal size.*
Fig. 4.	*Short bacilli.*
Fig. 5.	*Short bacilli (globi).*

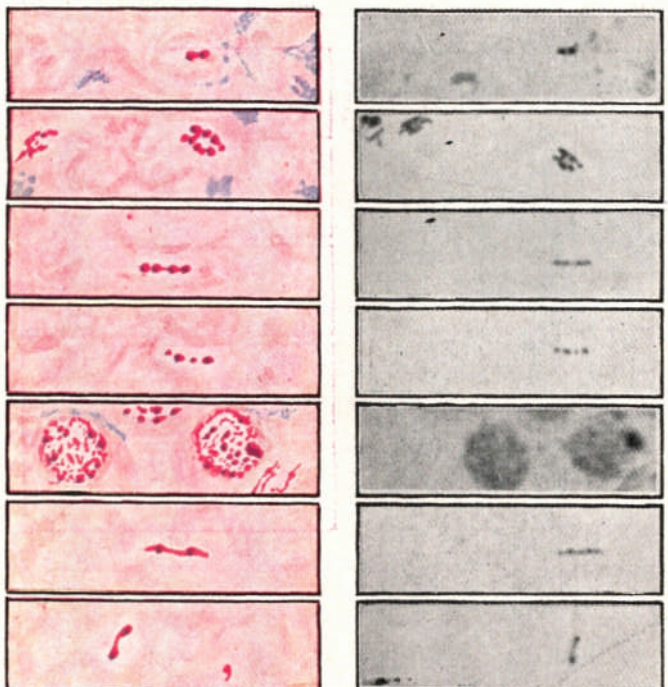

Fig. 6.	*Granulous short bacillus.*
Fig. 7.	*Bundle of granulous bacilli.*
Fig. 8.	*Granulous bacillus.*
Fig. 9.	*Granulous bacillus.*
Fig. 10.	*Globi of granulous bacilli.*
Fig. 11.	*Bacillus with excentric granule.*
Fig. 12.	*Bacillus showing the form of dumbbels.*

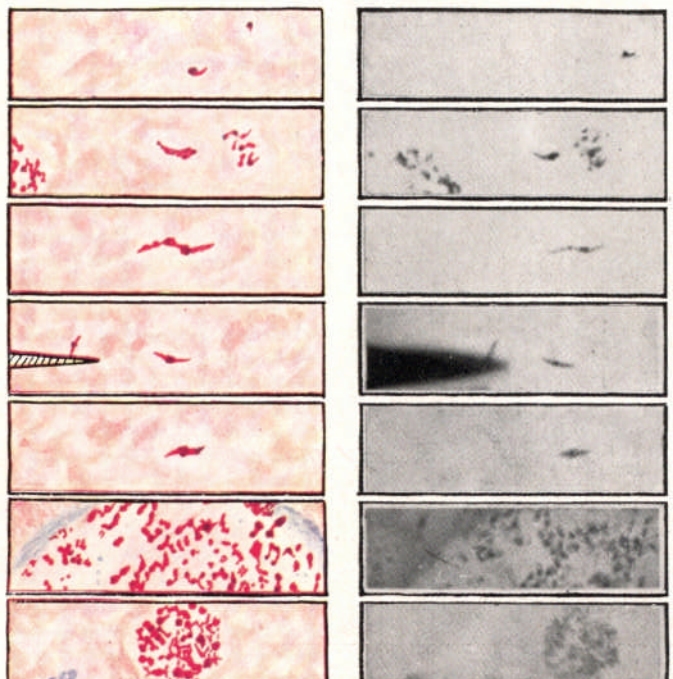

Fig. 13.	*Bacillus showing the form of musical note.*
Fig. 14.	*Nodular bacillus.*
Fig. 15.	*Nodular bacillus.*
Fig. 16.	*Nodular bacillus.*
Fig. 17.	*Fragment of a globi with granules.*
Fig. 18.	*Globi of bacilli-fragments.*
Fig. 19.	*Globi of bacilli-fragments.*

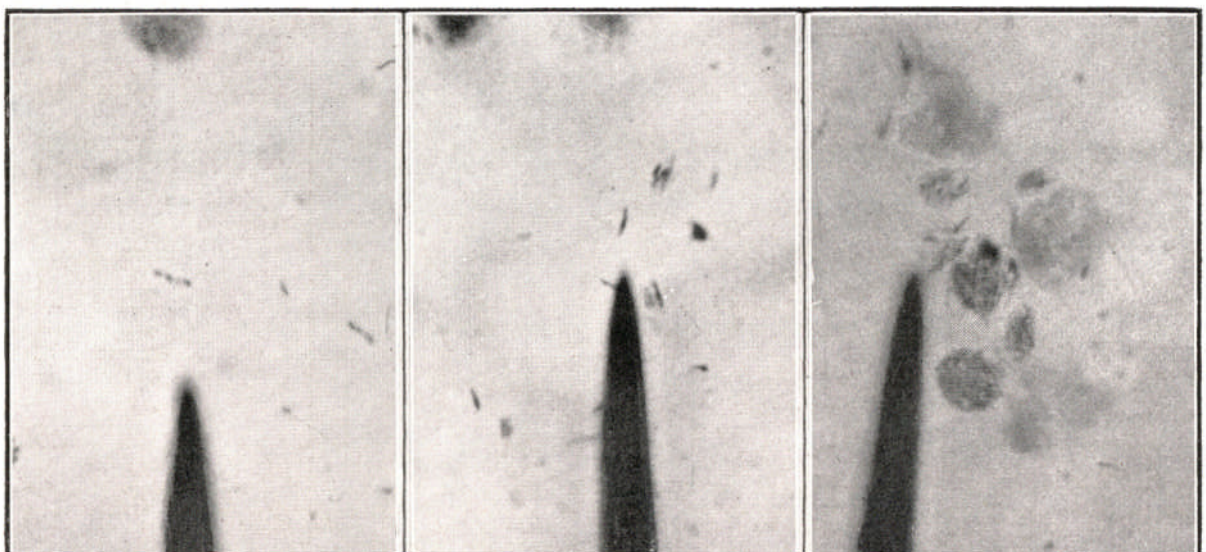

Fig. 20 — *Mucus.*

40 days after the first application. Diphteroid aspect.

Fig. 21 — *Skin.*

23 days after the first application. Nodular bacilli.

Fig. 22 — *Skin.*

23 days after the first application. Granulous globi.

Fig. 23 — *Skin.*

23 days after the first application. Nodular bacilli. Bacilli showing condensation of the protopleim.

Fig. 24 — *Mucus.*

40 days after the first application. Bacilli showing umbel disposition.

Fig. 25 — *Mucus.*
51 days after the first application. Small bacilli.

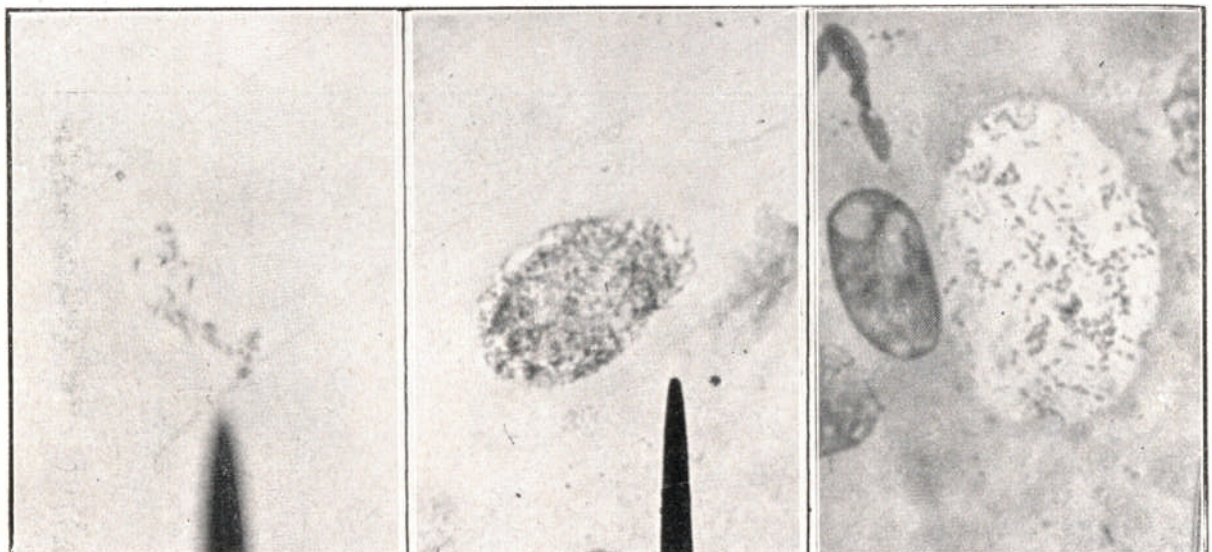

Fig. 26 — *Skin*

26 days after the first application. Group of small bacilli.

Fig. 27 — *Mucus.*

30 days after the first application. Globi of granules.

Fig. 28 — *Mucus.*

37 days after the first application. Globi of granules.

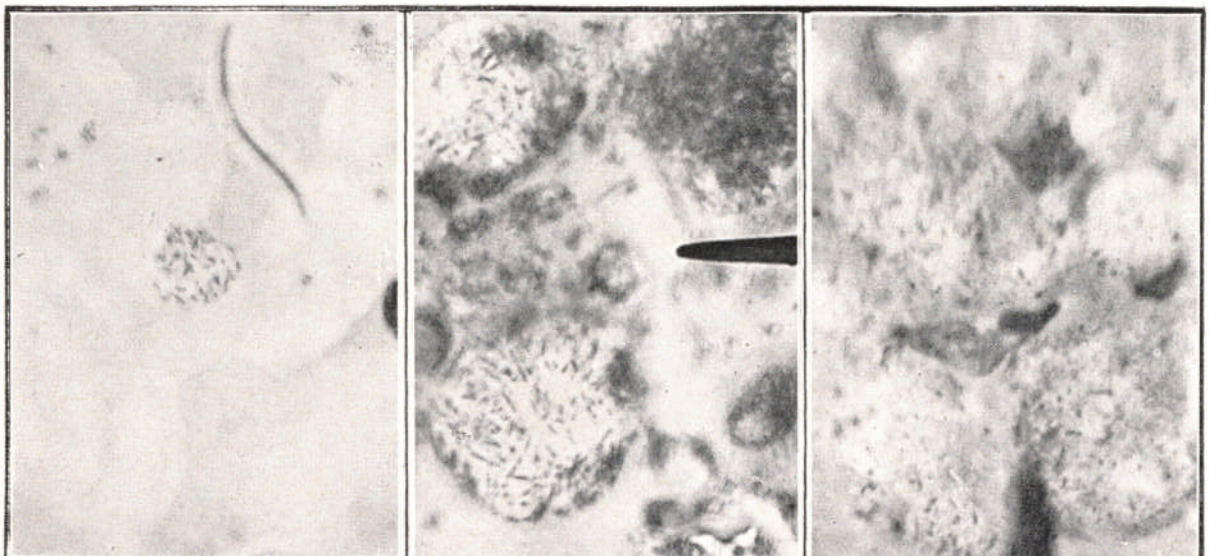

Fig. 29 — *Skin.*
5 days after the first application. Globi of fragments of bacilli.

Fig. 30
98 days after the first application. Globi of granules. Globi of granulous bacilli.

Fig. 31
25 days after the first application. Granular dust.

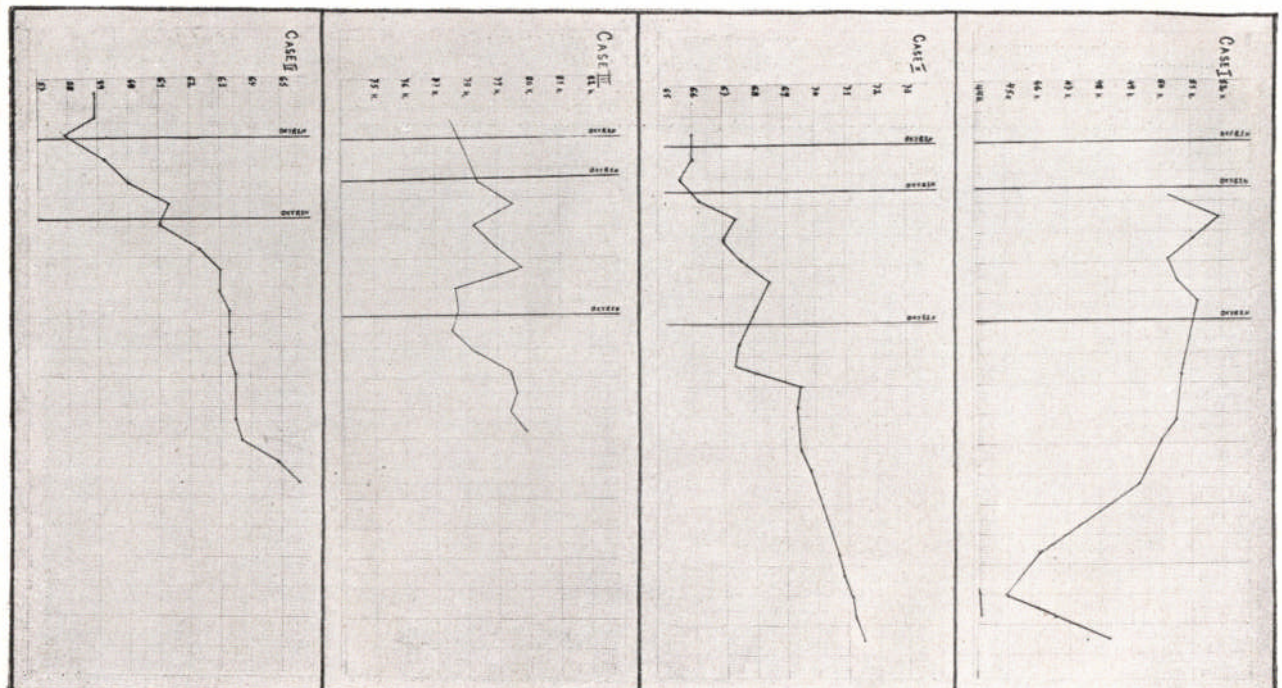

Fig. 32 — *Chart of weight.*

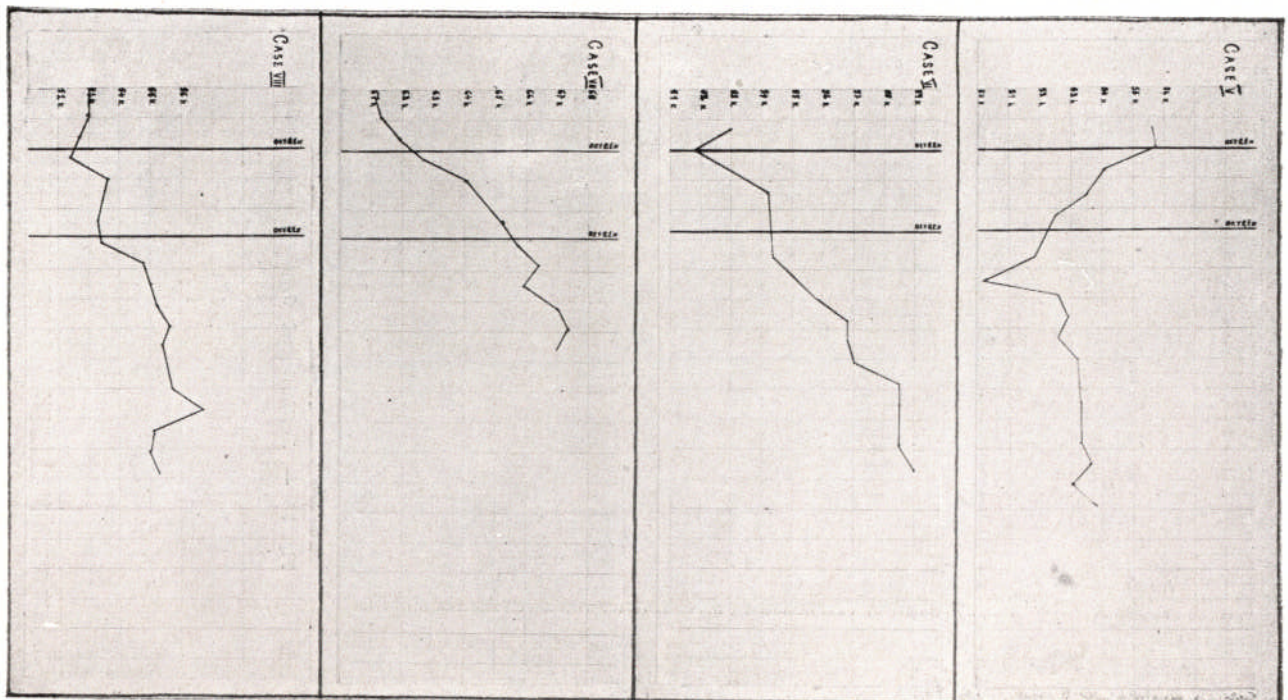

Fig. 32a — *Chast of weight.*

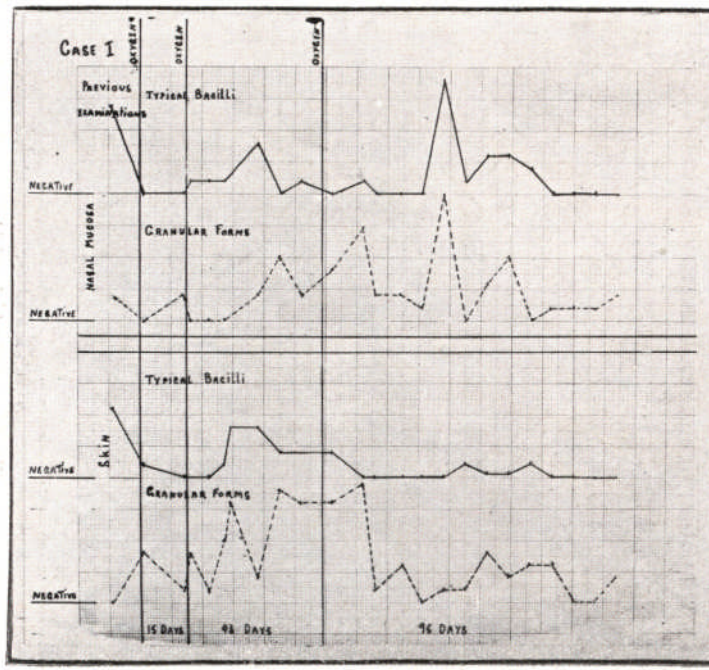

Fig. 33 — *Bacteriological Graph.*

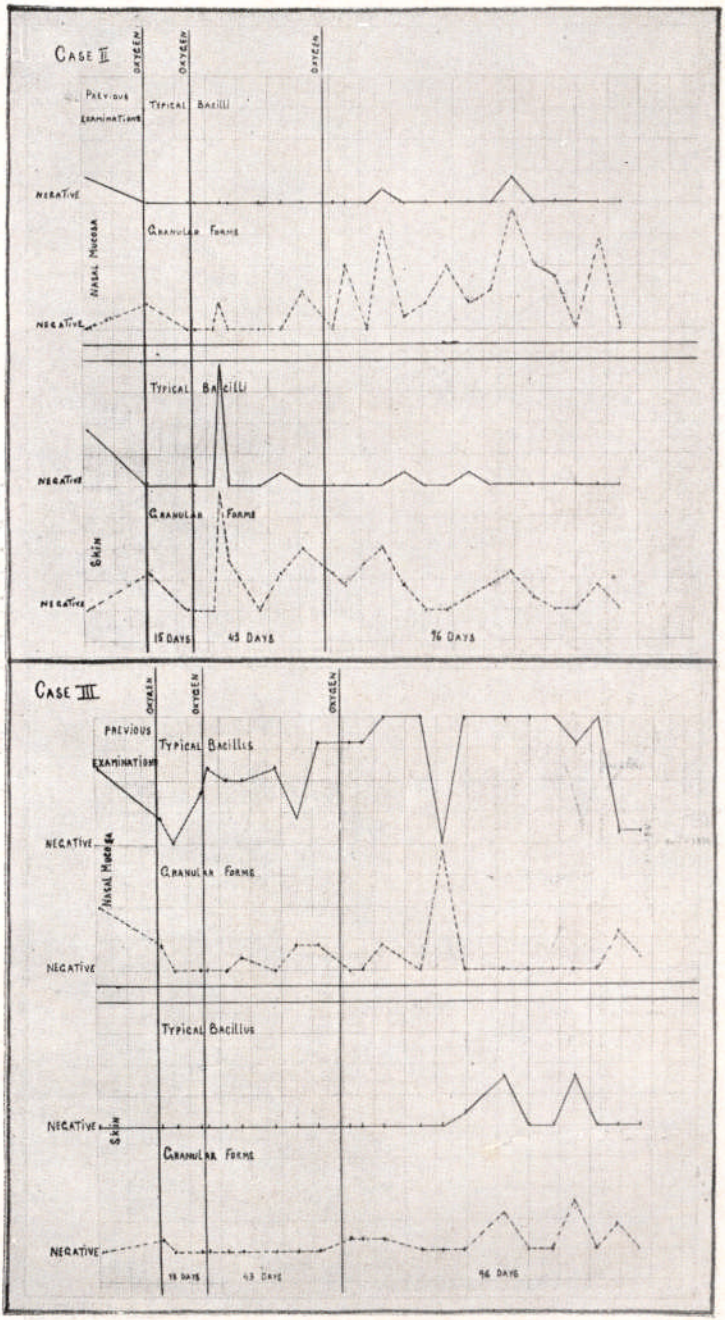

Fig. 33a — *Bacteriological Graph.*

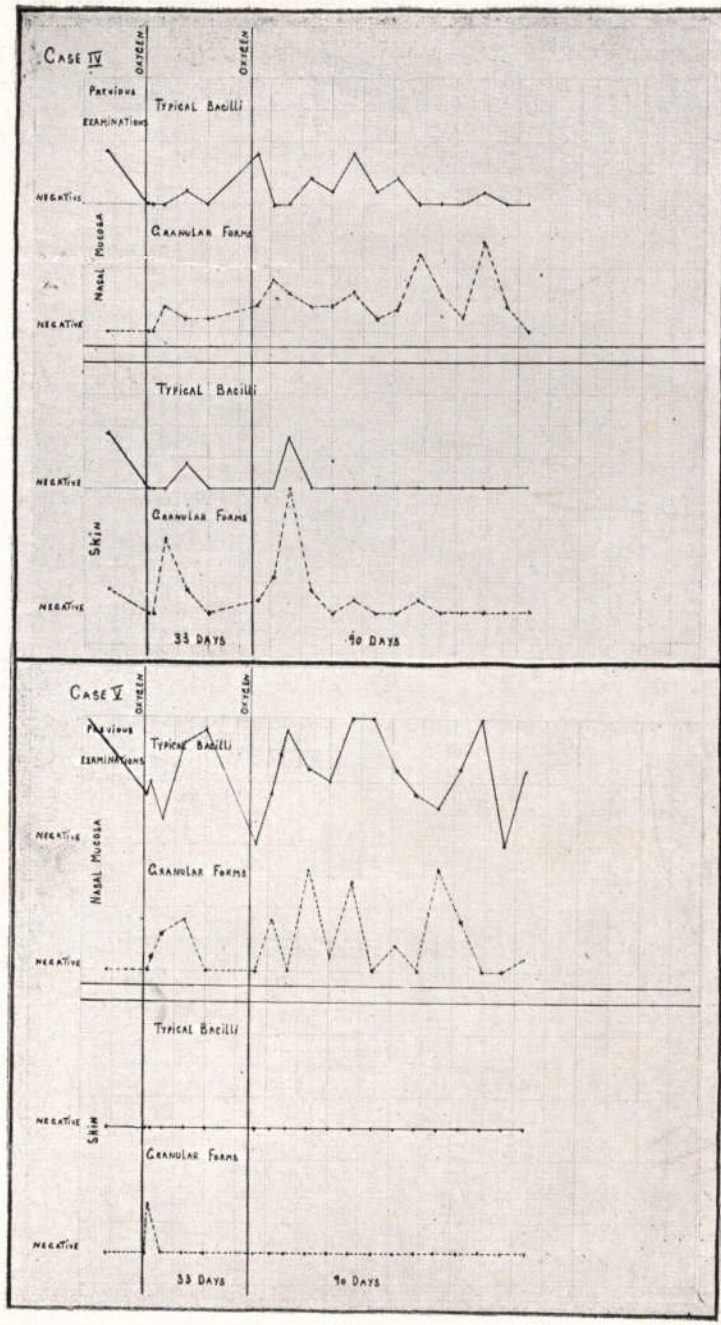

Fig. 33b — *Bacteriological Graph.*

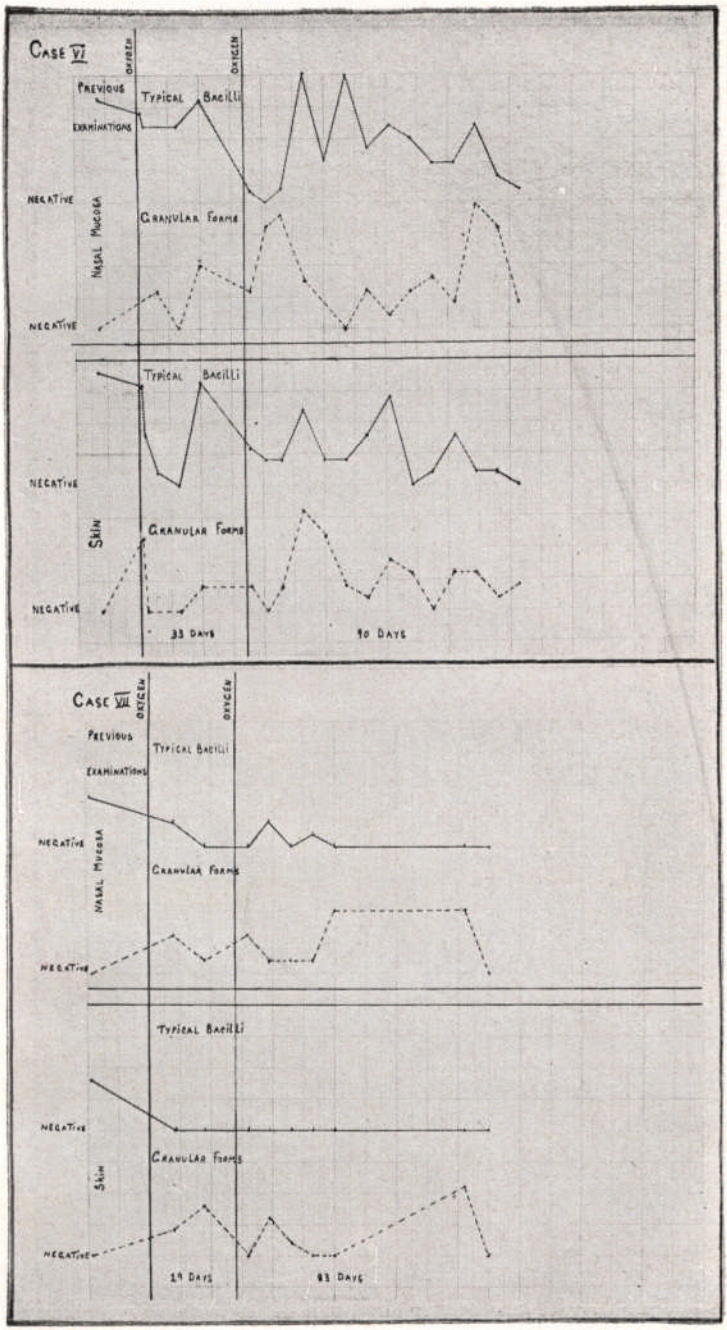

Fig. 33c — *Bacteriological Graph.*

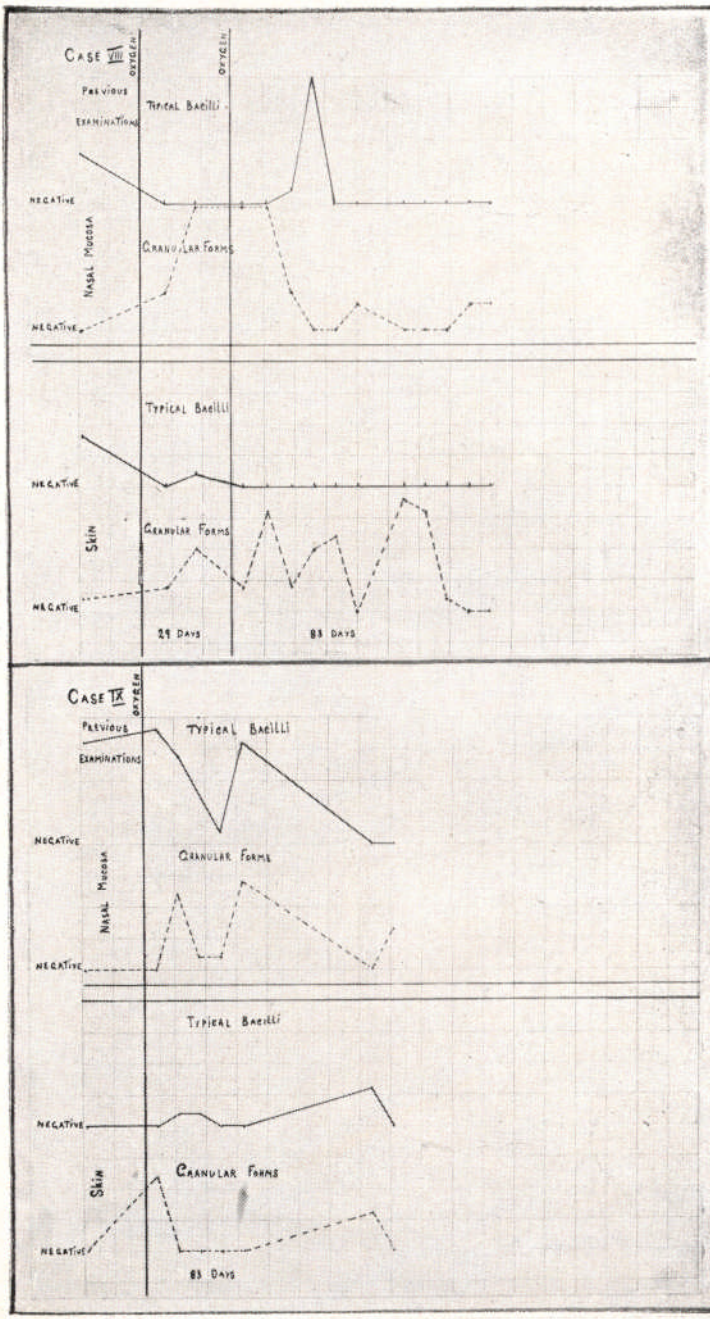

Fig. 33d — *Bacteriological Graph.*

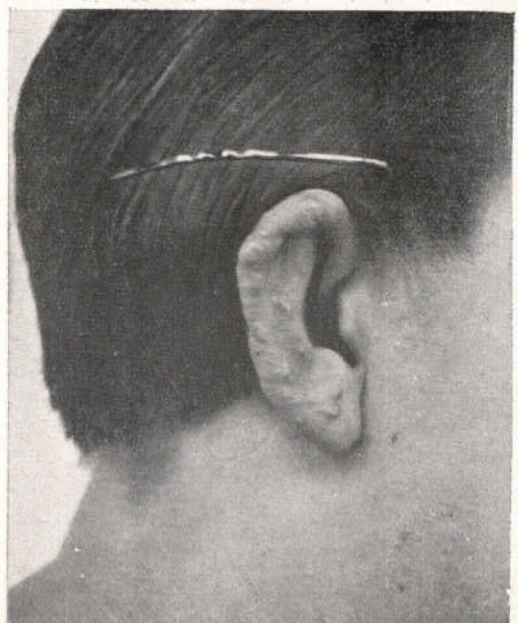

Fig. 34 — Case I — *Before treatment.*

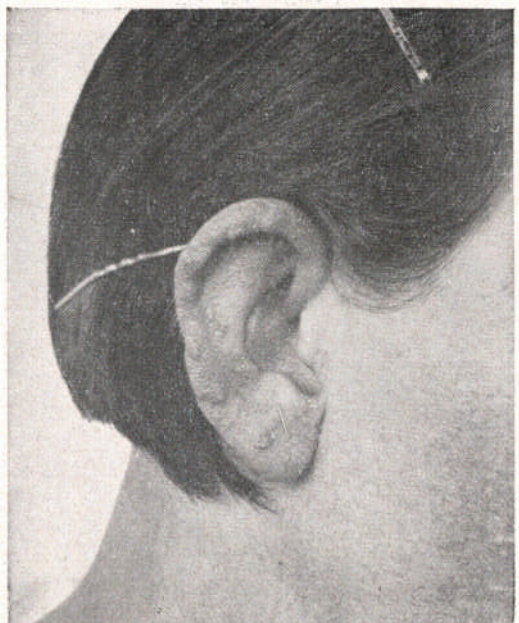

Fig. 34a — Case I
90 days after the last application.

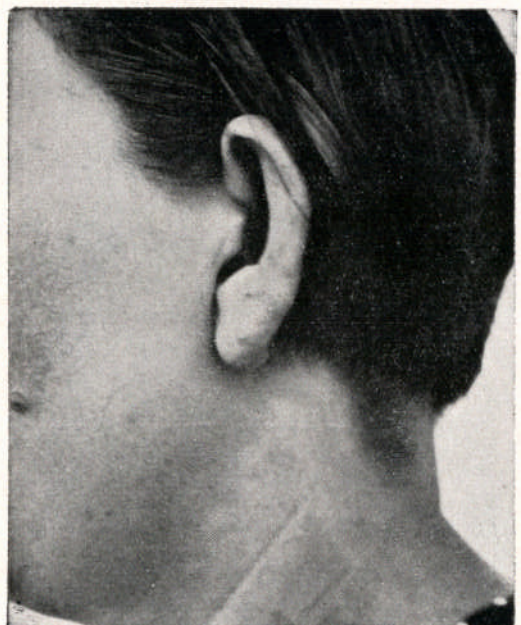

Fig. 35 — Case I — *Before treatment.*

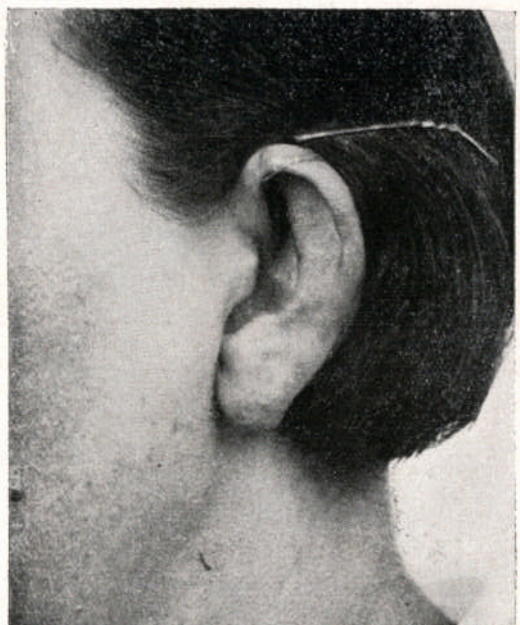

Fig. 35a — Case I
96 days after the last application.

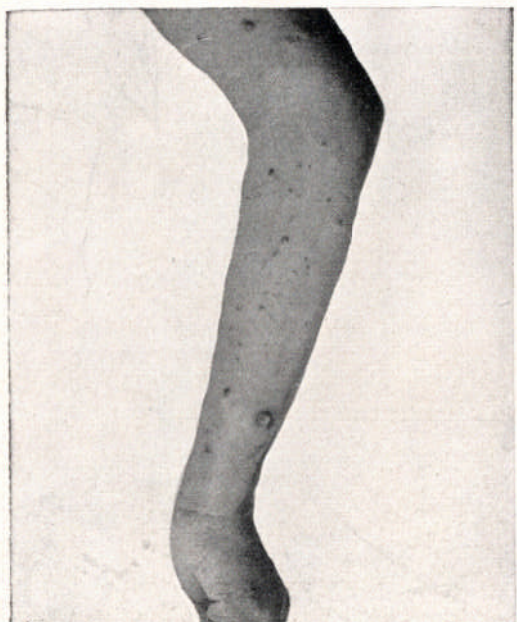

FIG. 36 case I — *Before treatment.*

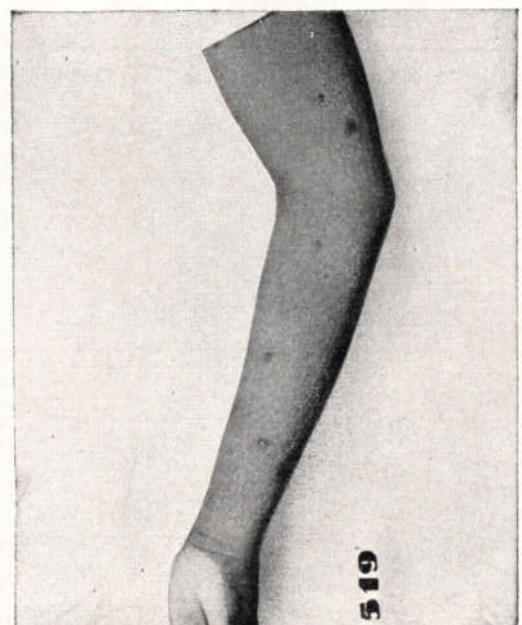

FIG. 36a case I
96 *days after the last application.*

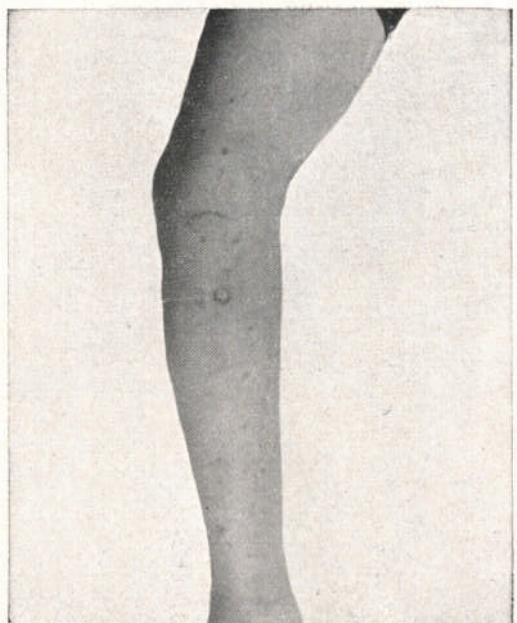

FIG. 37 case I — *Before treatment.*

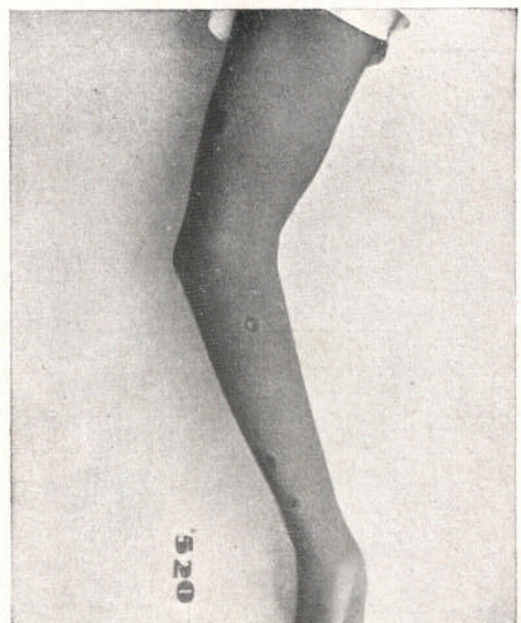

FIG. 37a case I
96 *days after the last application.*

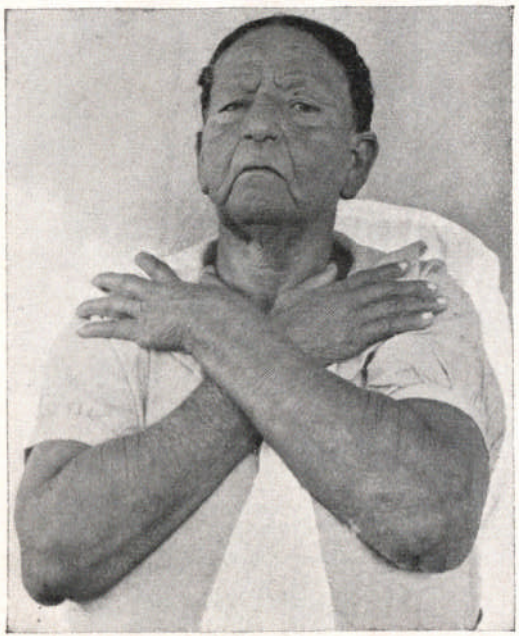

FIG. 38 case II — *Before treatment.*

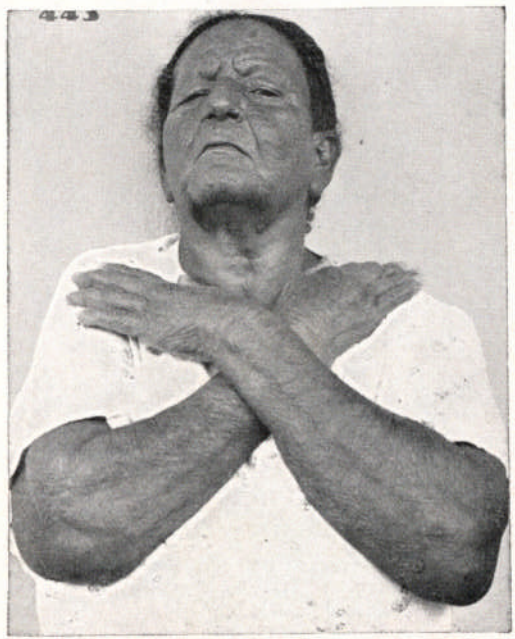

FIG. 38a case II
96 days after the last application.

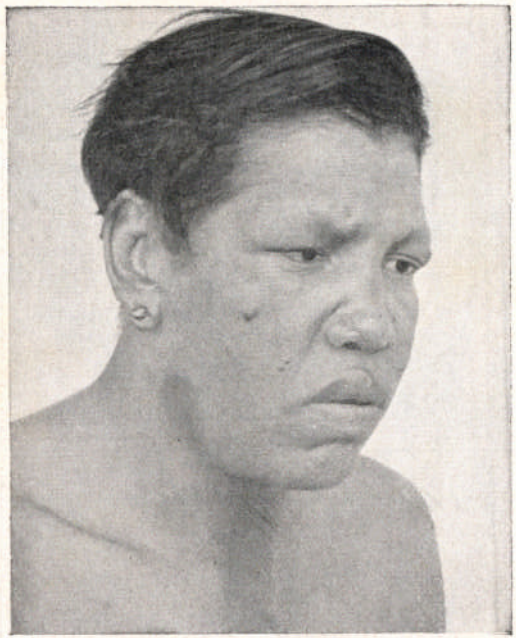

Fig. 39 — Case III — *Before treatment.*

Fig. 39a — Case III
96 days after the last application.

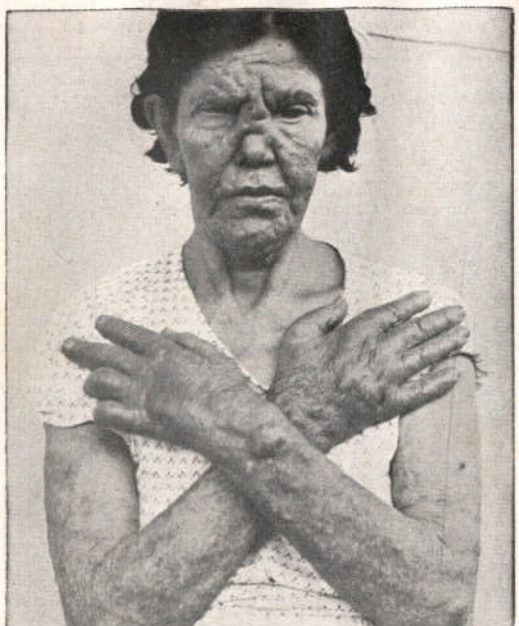

Fig. 40 — Case IV — *Before treatment.*

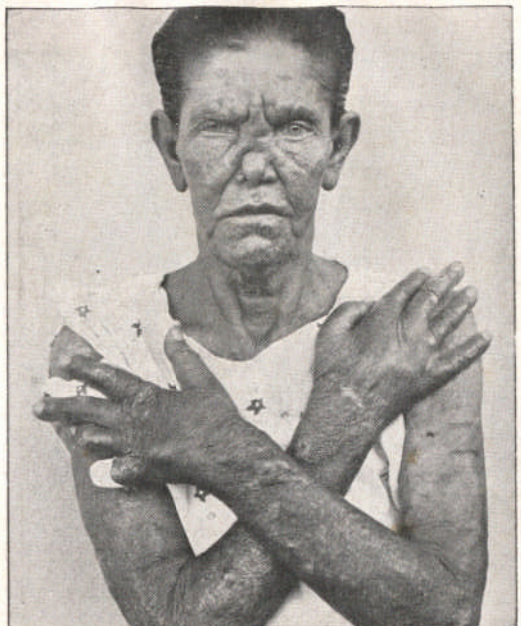

Fig. 40a — Case IV
90 days after the last application.

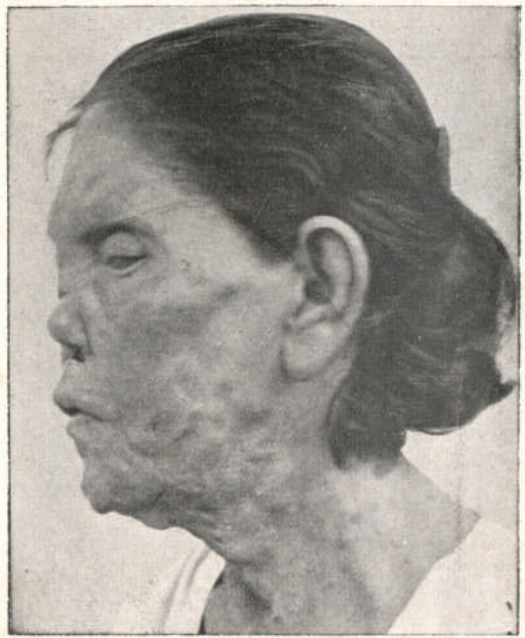

Fig. 41 — Case VI — *Before treatment.*

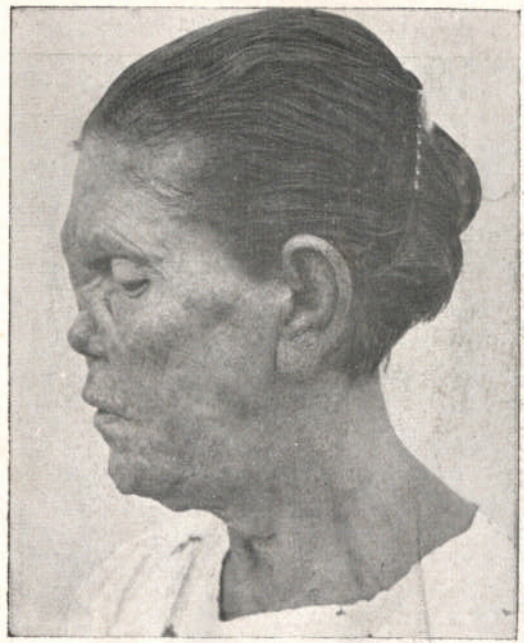

Fig. 41a — Case VI
90 days after the last application.

Fig. 42 — Case IV — *Before treatment.*

Fig. 42a — Case IV
90 days after the last application.

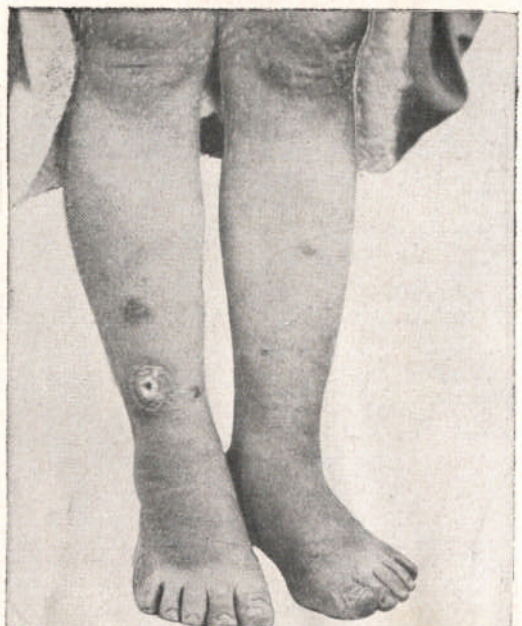

Fig. 43 — Case IV
During treatment. 33 days after the last application.

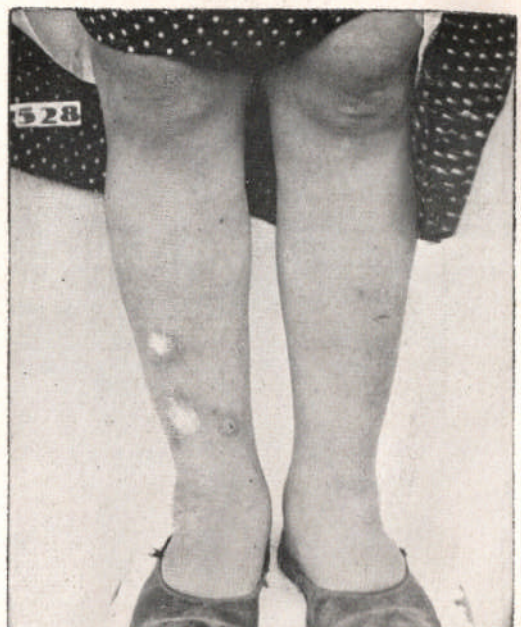

Fig. 43a — Case IV
90 days after the last application.

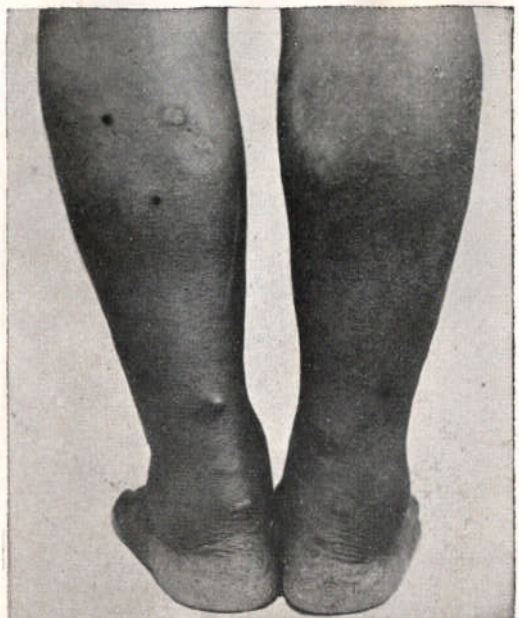

Fig. 44 — Case IV
During treatment. 33 days after the last application.

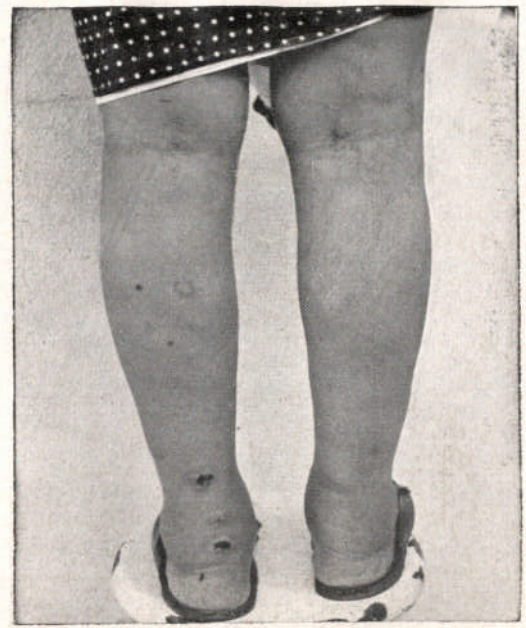

Fig. 44a — Case IV
90 days after the last application.

Fig. 45 VIII — *Before treatment.*

Fig. 45a — Case VIII
82 days after the last application.

Fig. 46 — Case V — *Before treatment.*

Fig. 46a — Case V
90 days after the last application

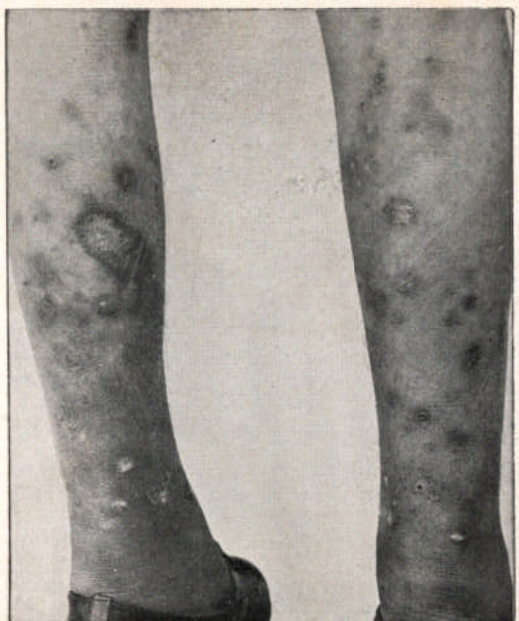

Fig. 47 — Case I
Aspects of healed lesions. 96 days after the last application.

Fig. 48 — Case I
Aspects of healed lesions. 96 days after the last application.

Fig. 49 — Case I
Aspects of healed lesions, 96 days after the last application.

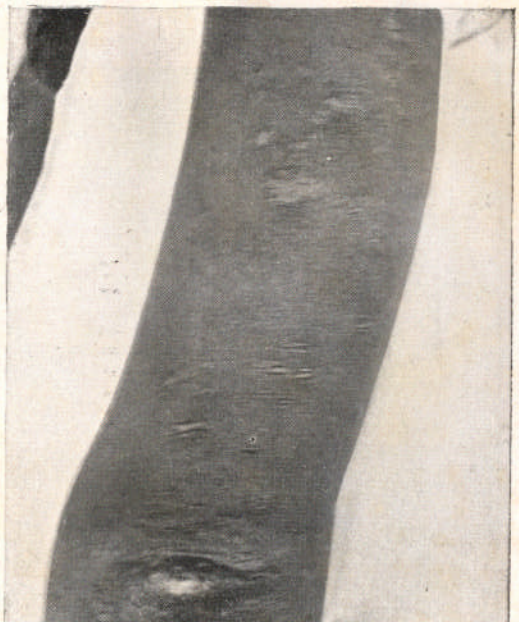

Fig. 50 — Case III
Aspects of pealed lesions with atrophic skin. 96 days after the last application.

Fig. 51 — Case VIII

Aspects of regrenive lesions, with atrophy of the skin. 82 days after the last application.

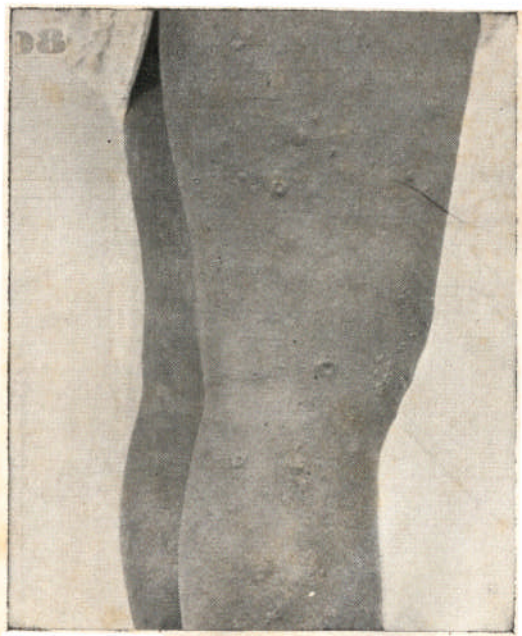

Fig. 52 — Case III

Aspects of regrenive lesions, with atrophy of the skin. 82 days after the last application.

Fig. 46 — Case V — *Before treatment.*