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A FOURTH ORIENTATION ON THE THERAPEUTIC VALUE OF AN ANTI-LEPROSY SERUM*

IN COLOMBIA AND VENEZUELA[1]

By John Reenstierna, M.D.

Professor of Hygiene and Bacteriology at the University of Upsala, Sweden

Researches on the microorganism of leprosy, especially regarding its nonacid-fast life forms and mycotic nature, which I started in 1912 at The State Bacteriological Institute of Stockholm (Director at that time Professor A. Pettersson) and which have been in progress since then, had in the summer of 1933 arrived at the following stage: by a method of immunization, described below, I had succeeded in preparing a small quantity of Anti-Leprosy Serum.[2]

With this serum preliminary therapeutic tests were performed at Järvsö Leprosarium, Sweden (Director, Dr. R. Zacchrisson) on two patients suffering from very advanced ulcerating nodular leprosy. The results were stated by Charles Nicolle at the session of the French Academy of Science on Sept. 25th, 1933.[3]

In 1934 I had the opportunity of testing another quantity of the serum at Järvsö, this time also on two patients with nodular leprosy.[4]

*Reprinted, with minor changes by the author and without the supplement containing the case reports, from *Acta Medica Scandinavica*, Supplementum LXXXV, 1937. Plates supplied by the author.

[1] In this article I had hoped to be able to include some tests on a smaller scale, carried out in other countries, but as yet I have not received the promised reports.

[2] This first quantity had been prepared in a small laboratory which Count I. Andrássy had put up for me on his estate Letenye in Hungary. Since 1933, when I was appointed Director of the Institute of Hygiene and Bacteriology at the University of Upsala, the serum preparation has taken place there. Regarding previous attempts to prepare an Anti-Leprosy Serum I refer to the handbooks of Jadasshon and Klingmüller, and Jeanselme's work "La Lèpre".

[3] C. R. Acad. Sc. T. 197, 2 October 1933.

[4] Svenska Läkartidningen, 1935, No. 41.

The third orientation—the first one on a larger material—was carried out at Addis Abeba in Ethiopia, where I stayed from May 7th to June 21st, 1935. All in all 65 cases of leprosy were treated, 31 (beggarmen from the streets) as outpatients at the hospital of the Emperor, Betsaida (Director, Dr. K. Hanner), 33 at "Haile Selassie Leprosarium" (Director, Dr. R. Hooper), and finally 1 case at the Ethiopian Army Hospital (Director, Dr. H. Nyström).

On account of the prevailing primitive and, at that time, difficult conditions at Addis Abeba it became evident to me from the first that only quite crude, orientating tests could be carried out. Our object was thus to give as many patients as possible, before the outbreak of the war, at least a first series of injections of Anti-Leprosy Serum, so as to obtain a conception of what type of leprous manifestations the serum acted on (if it really did act), how rapid this action was, and if the treatment incurred any risks.

As is seen from a short paper of mine (published last year[5]) the physicians at Addis Abeba established the fact that the serum, even if only temporarily, was able to bring about a very rapid healing of cutaneous ulcers, even large ones, and of periosteal fistulas, diminution in size (although not so rapidly) of nodules, return (partially) of lost sensibility (in more than 65% of the cases), renewal of mobility in paralyzed fingers[6] and disappearance of swellings of the feet to such an extent that shoes and sandals became too large. No real contraindications were met with.

Thanks to support and subventions from the governments of Sweden, Colombia, and Venezuela, I was enabled, in 1936 and 1937, to continue my investigations on the therapeutic value of the Anti-Leprosy Serum in these two South American countries where lepers are so numerous.[7] This constitutes my fourth orientation.

Before stating the results of the tests in Colombia and Venezuela I wish to give a description of the method for the preparation of the Anti-Leprosy Serum, as well as of the bacteriological properties of the strains used.

The principle of the serum preparation is based on the opinion I have formed during my research work on the leprosy and

[5] Reenstierna, *Acta Med. Scandinavica*, 1936, LXXXVIII, fasc. II-IV.
[6] In fact, two patients were able to pick up needles and start sewing within three weeks of the commencement of the serum treatment.
[7] In Sweden there are at present no more than 9 cases of leprosy.

tubercle bacillus, in parallel. The purport of this opinion is that the acid-fast bacilli of both Hansen and Koch are merely fragments or evolutionary forms of lower fungi, probably quite commonplace, that occur in nature and which at one time, far back in the past, entered and at times still enter the human organism. In their struggle against the natural defensive forces of the body they then adopted and are still able to adopt pathogenic qualities, after which, while retaining more or less these qualities, they are passed on from man to man. We think that, under certain conditions of which we know nothing, they may be able to return to their saprophytic mode of life, and later to acquire pathogenic qualities once more.

The method for the serum preparation, which in 1932-33 was comparatively complicated, has now been considerably simplified. Briefly, it consists of repeated injections into sheep of increasing doses of toluol-treated, fresh and older glycerin bouillon cultures of Kedrowski's and Reenstierna's leprosy strains, which contain both acid-fast and nonacid-fast life-forms of the mycotic microorganism of leprosy together with their disintegration products and toxins.

Kedrowski's well-known strain, isolated in 1900 from a leproma in a Russian patient, was at first nonacid-fast and of diphtheroid type (Pl. 1, fig. 1), but later on, after having been passed through rabbits, it changed almost entirely into acid-fast rods.[8] It still exists on different solid media in laboratories all over the world. In 1927, after repeated failures I succeeded, by the aid of small floating pieces of cork, to make it grow on the surface of common glycerin bouillon. On this medium it forms a light lead-grey, creamy, wrinkled pellicle (Pl. 1, fig. 2). Ziehl-Neelsen stained smears of the growth show both acid-fast and nonacid-fast elements, varying in different parts of the pellicle (Pl. 1, figs. 3 and 4). As a rule, the acid-fast forms predominate.

Reenstierna's strain was isolated in 1912 from the blood of a patient suffering from nodular leprosy in the acute stage, who had been admitted to Järvsö Leprosarium, Sweden.[9] In a medium consisting of glycerin bouillon with ascitic fluid, glucose, and lumps of human brain I succeeded in obtaining from this blood, first a culture of a nonacid-fast microorganism of rather a queer appearance (Pl. 1, fig. 5) which formed long chains of up to

[8] Kedrowski, *Zeitschr. f. Hygiene*, 1901, Vol. XXXVII, p. 52 and 1910, Vol. LXVI, p. 1.

[9] Reenstierna. *Arch. f. Dermat. u. Syphil.*, 1913, Vol. CXVI, No. 3.

500 links or more, and the mycotic nature of which immediately became evident to me, and later on a culture of an acid-fast microorganism of the classical Hansen type (Pl. 1, fig. 8). A closer study of these two forms, the nonacid-fast and the acid-fast, convinced me that they were merely different stages of a fungus which causes leprosy.

Walker, who worked at Honolulu, states in an article published 1929[10] that he has succeeded in cultivating an exactly similar microorganism from the blood of lepers during acute reactions.

My own nonacid-fast strain, which as early as in 1912, at the State Bacteriological Institute of Stockholm, had shown its ability to change, partially, into acid-fast forms—both in cultures and after inoculation into a monkey (Pl. 1, figs. 6 and 7. Pl. 3, figs. 2-5)—was sent, in 1913, to diverse foreign laboratories, in several of which it still exists. In some places the nonacid-fast stage predominates, in others the acid-fast stage. In a letter dated Moscow, Sept. 17th, 1931, Kedrowski informs me *inter alia* that my strain is practically identical with his. Paldrock has found that it has the same chemical properties as the bacillus of Hansen which occurs in lepromas.[11]

This strain of mine is now growing on glycerin agar and other solid media, e. g., those of Hohn and Petragnani, on which it forms a greasy layer of greyish colour. Last winter, when there occurred an accidental decrease of temperature at the Bacteriological Institute in Upsala, it was observed that the colour at times showed a light yellowish tint. On smears stained by the Ziehl-Neelsen method the microorganism appears pleomorphic, in the shape of short rods, pears, sausages, streptococcus-like elements with or without a connective substance, groups of small cocci, filaments, etc. The majority of these types are quite nonacid-fast, but here and there, sometimes within the bodies of the microorganisms, sometimes detached from them, there are to be found small, red, round or eggshaped corpuscles (Pl. 2, figs. 1 to 4). The forms now mentioned correspond entirely with those reproduced in the excellent papers of Kedrowski, Bayon,[12] and Walker. (*Vide* the explanation of plates 1, 2, and 3 of this article.)

[10] Walker, *Jour. Prev. Med.* 1929, Vol. 3, No. 3.
[11] *Dermat. Wochenschr.*, 1927, Bd. 84, No. 9.
[12] Bayon, *Ann. Trop. Med. & Parasitol.*, 1915, Vol. IX, No. 1.

In 1927, after having failed several times, I succeeded in transfering my greyish culture from a tube containing nutrose glycerin agar, which Paldrock had sent me, to common glycerin bouillon (by the aid of small floating pieces of cork). On the surface of this medium the strain forms a wrinkled pellicle, the colour of which is yellow (Pl. 2, fig. 5) though of different shades, at times, however, changing more into grey.[13] On smears stained by the Ziehl-Neelsen method both acid-fast and nonacid-fast elements are to be seen, the acid-fast type predominating in some parts of the pellicle, the nonacid-fast type in others (Pl. 2, figs. 6 and 7).

To such glycerin bouillon cultures (in 1-litre flasks) of Kedrowski's and Reenstierna's strains, aged from one to several months, sufficient toluol is added to form a layer about 1 cm. in height on the surface of the bouillon. The flasks are then shaken up thoroughly and the contents mixed in large bottles with rubber stoppers, about equal quantities of the two different strains being brought together. The bottles are shaken well from time to time. In the Kedrowski and Reenstierna cultures, which before the treatment with toluol contain both nonacid-fast and acid-fast elements, the latter, through the ability which toluol has of dissolving waxy-fatty substances, are partly transformed into nonacid-fast ones, the amount of which in this manner becomes increased. Naturally, the mixtures also contain disintegration products and toxins.[14]

It is not possible to prove that all the microorganisms (which no doubt are resistant) in the toluol treated cultures really are killed. Personally, I think that in the newer cultures, besides the dead microorganisms, there also exist those which, though weakened, are still alive. Those are just the kind I primarily aim at using in order to obtain the best results of the immunization.

As mentioned above, sheep, both rams and ewes, of different ages are used for the immunization. During a period of 3 to 4 months increasing doses of new and older, well shaken culture mixtures are injected subcuta-

[13] Such changes of colour have been observed in other strains of leprosy. Ota and Sato, among others, found that "whitish strains change to ochre colour during subculture" (*Internat. Jour. Lep.* 1934, Vol. 2, No. 2). This change in colour probably depends on the composition of the media, the temperature, etc. In this connection I further wish to mention that, during my stay at Bogotá, Professor Lleras Acosta in his laboratory had a culture signed "Kedrowski" (from the collection of Souza Araujo, in Rio de Janeiro), which on a modified Petragnani medium grew with a bright ochre colour.

[14] It is a well-known fact that there are still scientists who declare that the microorganism of leprosy, contrary to its twin microorganism, i.e., that of tuberculosis, is unable to produce toxins. I do not consider it worth while to discuss such an hypothesis here. Several clinical symptoms in leprosy, e.g., the leprous fever and the general reaction of the animals during the period of immunization (*vide infra*), are conclusive evidence to me of the ability of Hansen's microorganism to produce toxins, although much weaker than those of the microorganism of Koch, and regarding this subject I place myself unreservedly on the side of Babes, Marchoux, Jadassohn and others.

neously into the sheep every 9th or 10th day, approximately according to the following schedule:

1st injection ..... 25 c.c.
2nd injection ..... 50 c.c.
3rd injection ..... 50 c.c.
4th injection ..... 50 c.c.
5th injection .....100 c.c.
6th injection .....100 c.c.
7th injection .....100 c.c.
8th injection .....200 c.c.
9th injection .....200 c.c.
10th injection .....300 c.c. (possibly less)
11th injection .....300 c.c.
12th injection .....400 c.c. (in some of the largest animals)

On the same day as a larger dose, from 100 c.c. upwards, is administered, and on the following day an injection of 3 to 5 c.c. of camphor is given intramuscularly, as the animals are drowsy and off their food on these days. At times subcutaneous abscesses of no importance may appear in some of the places where culture mixture has been injected. At the end of the period of immunization several of the animals become lean, and the risk of losing some of them in general intoxication is then very great. In spite of our large experience regarding the maximum of doses which the animals will stand, we always lose about 10% of our sheep.

The animals are divided into two groups. On the 8th (1st group) and 9th (2nd group) day respectively after the last injection the animals are chloroformed, the carotid artery is laid bare and cut through, and the blood is allowed to flow into newly sterilized[15] bottles. Two days later the serum is drawn off and to this is added acid. phenylicum so as to make 0.5%, after which it is stored in a refrigerator, at a temperature of 4°C. for about two months. After that time serum from different animals of the 8th and 9th day-groups is mixed and the bottles are then kept in the refrigerator for another week or two. After that the unfiltered serum is poured into flasks of 10 c.c., which are stored in the refrigerator for at least two weeks before they are sent to warmer countries.[16] Every lot of serum is tested as to sterility, as to the absence of tetanus toxins (on guinea-pigs), and regarding the amount of acid. phenylicum (on mice). That some sera acquire a little colouring of haemoglobine is unavoidable but of no importance.

This is a brief description of the preparation of Anti-Leprosy Serum. It is very probable, however, that further modifications of the method will be made.

In Colombia I first worked for about a fortnight in the capital, Bogotá, especially in the laboratory of the leprosy investigator, Professor F. Lleras Acosta, who had the opportunity of testing the serum on two patients. One of these suffered from anaesthetic leprosy and had a considerable diminution of sensibil-

[15] The whole procedure for obtaining the Anti-Leprosy Serum is naturally carried out under the strictest sterile conditions.

[16] As a rule, only serum which has been stored for about 3 months in a refrigerator should be used for treatment.

ity, while the other one had nodular leprosy with a large cutaneous ulcer. In both of these cases Lleras Acosta observed rapid improvement.

I then stayed from Oct. 4th to Dec. 10th at Agua de Dios. This leper colony, the largest in South America and the second largest in the world, had at that time about 5,000 inmates, and new patients arrived almost every week. The director of Agua de Dios is Dr. P. Velasco.

At Agua de Dios leprous manifestations of all conceivable kinds and ages were represented, both in untreated patients and in patients who had received treatment with chaulmoogra oil for shorter or longer periods of time.

During my stay at the colony 51 patients were treated, under the constant supervision of Dr. Velasco, with Anti-Leprosy Serum by the following physicians: L. Albarracin, M. Bernal Londoño, E. Buitrago Mantilla, B. Castañeda, B. Castro, E. Perilla, A. M. Riveros, and J. Tovar Daza. In addition, occasional observations were made by Drs. M. Arenas and M. Medina. The results of the serum treatment, up to Dec. 9th, 1936, will be found in the records kept by the above-mentioned physicians, which have been brought together in an act officially certified by Dr. Velasco. According to a report dated Feb. 4th, 1937, which Dr. Velasco, by request of the Minister for Foreign Affairs, Bogotá, sent to the Director of the Colombian Board of Health, another 8 patients have subsequently received serum treatment at Agua de Dios. The total number of patients which have been treated with the Anti-Leprosy Serum in Colombia is thus 60.[17]

My stay in Venezuela lasted from Dec. 21st, 1936, to Feb. 14th, 1937. At the leprosarium of Cabo Blanco, which had about 500 inmates, the serum was tested on 30 patients by the Director, Dr. M. Vegas, who was assisted by Dr. C. Gil Yepez and Dr. G. Negrette de Windt. Although the material at Cabo Blanco was not as good as at Agua de Dios, certain manifestations were, nevertheless, always found (*vide infra*). The records have been brought together in an act officially certified by Dr. Vegas.[18]

[17] One of Dr. Lleras Acosta's patients had continued his treatment at Agua de Dios.

[18] As the records from both Colombia and Venezuela, in spite of their only covering rather a short period of observation, are valuable for the estimation of several questions connected with this matter, they have been published in extenso in the original language in *Acta Medica Scandinavica*, Supplementum LXXXV, 1937. They were made by 13 physicians, all of whom are well experienced in leprosy.

On account of the great amount of work at such large leper colonies as Agua de Dios and Cabo Blanco, it was on several occasions necessary to include only some of the principal manifestations in the records. Furthermore, the examination of sensibility could, at Agua de Dios, only be made by testing with a pin and, at the same Leprosarium, the examination of obstructed and ulcerated noses was several times not so extensive as would have been desirable, especially as the favourable action of the serum on these leprous manifestations came as quite a surprise.

As far as possible the tests were carried out on the most discriminating patients in order to obtain the most exact statements, while eliminating the suggestive element. In nearly all cases photographs were taken some hours before the serum treatment started, but of course only a few of the most illustrative ones can be reproduced in this article. In those cases where the patients were undergoing treatment with chaulmoogra oil, this treatment was stopped and the serum injections were not administered until an interval of about a month had elapsed.[19] No additional treatment was used in any of the cases. On cutaneous ulcers only dry bandages (of sterile gauze) were used. These were changed morning and afternoon, after having been moistened with cold (never hot) water in order to prevent the tearing up of fresh granulations. The patients with ulcers were not kept in bed. Generally, the same conditions as before were maintained as far as possible.

The manifestations on which the serum treatment has shown a favourable action, and the changes observed, are as follows:[20]

1. *Nodules.*—Six cases were treated.[21] A slow diminution in the size of the nodules was observed. After 2 to 5 weeks it was possible to establish the fact that many of them were flatter and softer, the skin covering them being wrinkled as if too large, while some very small, fresh nodules had been resorbed. On larger conglomerates of lepromas, e.g., the leonine face, no effect or merely

[19] Dr. Hooper at Addis Abeba observed that patients with chaulmoogra oil in their bodies did not react so well to the serum treatment. It seemed to "interfere" in some manner with the action of the serum.

[20] If nothing to the contrary is expressly stated, the following observations refer to patients treated in Colombia up to Feb. 4th, 1937. At my departure from Venezuela on Feb. 14th, 1937, the time of observation in that country had been too short to admit of any more definite conclusions in more than certain manifestations. Regarding the details *vide* the records and the report of Dr. Velasco published in *Acta Medica Scandinavica.*

[21] In some cases with other manifestations, which also showed lepromas, no definite observations were made with regard to the effect of the serum treatment on these.

a very slight one could be seen. During the first days of the treatment it was observed several times that the skin covering or adjoining the leprous foci became redder (local reaction). In this connection it may be mentioned that in 5 cases with old, diffuse infiltrations of the face, the skin there had become manifestly softer about a month after the beginning of the serum treatment.

2. *Brownish leprous colouring* (of the skin of the face).—After 4 to 5 weeks it could be observed, in 4 cases, that the colour of the face had changed towards the normal.[22] In several cases reddish-violet maculae turned partially or entirely pale during the first weeks.

3. *Cutaneous ulcers* (of the face and extremities).—In Colombia 14 cases were treated. Rapid healing occurred in 8 cases and improvement in 5 (Pl. 3, 4 and 5), while in 1 case no effect was observed. The serum thus had a favourable effect in about 93% of the cases. In 5 of the cases treated in Venezuela some of the smallest ulcers healed up, and the majority of the larger ones diminished considerably in size in the course of 2 to 3 weeks.[23] In nearly all cases, both in Colombia and Venezuela, the ulcers had after 2 to 3 days already become cleaner and drier, with incipient red granulations at the bottom. In addition, the pains caused by the ulcers had, as a rule, disappeared.

4. *Nose* (with all or some of the following manifestations present: obstruction, difficulty in nasal breathing, swollen and hyperaemic mucosa, ulcers—especially of the septum—haemorrhages, and pains).—In Colombia 23 cases were treated, in Venezuela 25. All patients, i.e., 100% of the cases, showed improvement of some kind after 2 or 3 days; e.g., nasal breathing had become less impeded, or haemorrhages (of up to a few years duration) or pains had diminished or disappeared entirely. In nearly all the cases where we had the opportunity to examine the mucosa, it was also observed that the ulcers had become cleaner and begun to heal.[22] In addition, the mucous membrane had often become less swollen and paler.[24]

[22] Such a change in colour after treatment with this Anti-Leprosy Serum has been observed in other countries also.

[23] The healing of the cutaneous ulcers—as probably also of those of the mucosa—is generally only temporary, but experience has shown that when ulcers begin to reappear a few more injections are able to close them again and that such a procedure can be repeated when necessary.

[24] The majority of the patients with nasal affections had previously, for shorter or longer period of time (up to several years), been treated with chaulmoogra oil, with little or no improvement at all. The same applies to several other cases also (anaesthesia, etc.).

5. *Anaesthesia* (total or partial).—Thirty-eight cases were treated.[25] In 33 cases, i.e., about 87%, it was established by testing with a pin that in different regions the sensibility had improved and in some quite returned to the normal. Also so-called "spots whiter than snow" regained partial sensibility. In most of the cases the improvement in sensibility began very rapidly, generally after only a few days. In 5 cases no effect at all could be observed.

6. *Neuralgia* (of the head, elbows, fore-arms, hands, knees, legs, and feet).—In Colombia 3 cases[26] were treated, in Venezuela 11. A rapid and complete disappearing of the pains occurred in 7 cases. In 5 of the cases the pains diminished, while 2 cases remained uninfluenced. Also in a case of "neuritis" the serum treatment was without effect.

7. *Paralysis, paresis, and loss of or reduction in different functions of the hands* (without regard to the predominance of nervous lesions or atrophic conditions).—Four cases of paralysis and paresis of the eyelids were treated. In 3 of the cases a rapid and considerable improvement was observed; in the remaining one the serum injections had no effect. Of 3 cases with paresis of the mimic musculature improvement occurred in 2—one patient even demonstrated, on the 6th day, that he was able to whistle and, a few days later, that he could blow up his cheeks—while no change was observed in the remaining one. Treatment was given to 16 cases with different disturbances in the functions of the hands, such as inability of or difficulty in closing the hands, writing with a pen, type-writing and playing the organ (especially on account of weakness of the extensors—the fingers doubled up), combing the hair, grasping a glass, picking up coins and needles, etc. In 14 cases a considerable and rapid improvement was noticed, which often set in before the end of a week. In the remaining two cases no change occurred. Eleven patients (in Colombia and Venezuela) stated that the general stiffness of the hands, particularly of the fingers, had decreased after the serum treatment. Nine of 15 patients with reduced general strength in the hands reported that the strength had increased.

8. *Oedema* (of the face, legs, and feet).—Nine cases were

[25] Including that one of Dr. Lleras Acosta's patients who suffered from anaesthetic leprosy.

[26] Among these patients was a young, intelligent man who for the last 7 years had had such intense pains that he had been obliged to take aspirin almost every day (!). On the 11th day after the commencement of the serum treatment all the pains had disappeared.

treated in Colombia, 3 in Venezuela. In 3 cases the swellings disappeared, improvement occurred in 5, while 4 cases were unaffected by the treatment.

9. *Leprous fever.*—Seven cases treated, 2 in Colombia and 5 in Venezuela. The 2 patients in Colombia had had an average temperature of 38°C. for 3 and 9 months, respectively, and lay in bed. After 6 and 16 days, respectively, the temperature had returned to normal and the patients could leave their beds. Among the patients treated in Venezuela there was one who showed an especially remarkable improvement. This case had been kept in bed for 3 months with an average temperature of 38.5°C. From the 9th day after the first serum injection and for the next fortnight (i.e., until I left) the morning temperature was always lower than 37°C., and the patient did not need to stay in bed any longer. Of the 4 remaining cases, which had merely a moderate rise of temperature, the temperature had, after the treatment, returned to about the normal in 3, while the last one showed no change.

10. *Other manifestations.—Epiphora:* 2 cases treated in Colombia, 1 in Venezuela. Rapid and complete disappearance in two cases, improvement in one. *Eyesight:* 2 patients reported that, after the serum treatment, it was easier for them to read newspapers, etc., and one who suffered from photophobia declared that he did not shun the light as before. *Hoarseness:* 3 cases treated. A slight improvement in case No. 5, no effect in the others. *Perspiration:* 3 patients suffering from anaesthetic leprosy reported that the skin of the hands and of other parts of the body, which previously had been dry, had become moist.

Finally, it may be mentioned that, both in Colombia and Venezuela, several of the discriminating patients reported an improvement in their general conditions (better appetite, sleep, etc.) after the serum treatment.

After the serum injections a general reaction appeared as a rule, manifesting itself principally in a rise of temperature. In 73 of the 90 cases treated in Colombia and Venezuela this rise of temperature was less than 1°C., on an average 0.5°C.; in 10 cases it was about 1°C.; in 4 cases about 1.5°C.; and in 3 cases from 2° to 2.5°C. One patient, who for some 9 months had been obliged to stay in bed on account of leprous fever, for some days showed a bad general condition and had traces of albumine in his urine, though of short duration. As mentioned above a local reaction in the nodules was several times observed. In the same manner as after injections of other sera an itching exanthema, as a rule local (on the gluteae) could be

seen in several cases. In one case a rapidly passing general urticaria appeared shortly after the first two serum injections. In the second week 3 patients showed a few pemphigus blisters, while another patient had swellings on the left glutea and the right side of the face (including the regional lymphatic glands). Both the swellings and the accompanying fever (up to 39°C.), however, disappeared in a couple of days. No other discomfort from the treatment was observed.

Most of the patients received only one series of three intragluteal injections, 10 c.c. each, in the course of one week (on the 1st, 3rd, and 6th days). In a few cases which reacted more strongly only two injections were given, while others received more than three. About one month after the first series a second one of two or three injections (10 c.c. each) was given to some of the patients.[27] As yet, however, no rules can be advanced either for the amount of serum to be injected or for the frequency of the injections, etc.

We have the impression that the activity of the serum is greatest, during the first year after its preparation and that the activity has decreased in a serum about 2 years old. We have therefore been anxious not to use a serum older than a year and a half. The question of the length of time that the serum can be maintained is, however, as yet far from worked out.

In this connection an interesting fact may be noted, viz., that serum which had been exposed to tropical heat for about 2 months seemed to be just as active as the same serum number stored in a refrigerator.[28]

The mechanism of the Anti-Leprosy Serum's healing effect has as yet in no way been explained. I do not think we need assume that it is merely a question of pyrotherapy, as on the one hand the rise of temperature in most of the cases was very moderate (on an average 0.5°C.), and on the other hand the effect of the serum was by no means better in the few cases with a greater rise of temperature than in those with a lesser rise. In this connection I wish to mention the almost negative results which Jeanselme,[29] and Nocht

[27] In order to prevent the occurrence of anaphylactic trouble *every* serum injection, after the end of the first week, is administered in the following manner: First a preliminary dose of 0.5 c.c. subcutaneously and then, about 15 minutes later, the remaining contents of the flask intramuscularly. When these precautions have been observed no suggestion of an anaphylactic shock has ever occurred.

[28] Should it, however, in warmer countries become necessary to store the serum for some time before using it, I think it advisable to keep it at least in a cool place.

[29] Jeanselme. *La Lèpre*, Paris, 1934.

and Velasco[30] obtained in spite of their using injections of preparations which produced a temperature of more than 40°C. So great a rise of temperature did not occur in any of our cases.

Most probably the serum—like all other bacterial anti-sera—contains specific as well as nonspecific components.[31] Granted that the action of the serum is partly nonspecific, it must, however, be assumed that the principal action is a specific one, presumably of an antitoxic nature. The rapid influence on different leprous manifestations indicates such an action. In all probability the mechanism is, in reality, much more complicated. One might also assume an effect of other antibodies as well, either directly or by means of the tissue cells which, when relieved of the toxins, become more active.

Finally, the question of the manner in which a serum acts must surely be considered of secondary importance, at least from an humanitarian point of view, when compared to the indisputable fact that the serum treatment is able, even if only *temporarily* (and this is also of value to the patients) to bring about return (as a rule only partial) of lost or decreased sensibility (in up to 87% of the cases); disappearance or relief of neuralgic pains; return of mobility in paralyzed eyelids and fingers (so that the patients are able to write, comb themselves, pick up coins and needles, etc.), disappearance of swellings of the feet, etc., healing or improvement of cutaneous ulcers (in about 93% of the cases) and of affections of the nose—obstruction, ulcerations, bleedings of the mucosa extending over a year or more—(in 100% of the cases), cessation of leprous fever of several month's duration.

Even if it is too early to give an opinion of the effect which a serum treatment carried on for several years could be able to bring about in certain cases, it should to an experienced bacteriologist be evident that a cure through serum treatment alone of such a chronic disease as leprosy, caused as it is by a resistant microorganism, is highly improbable. But the rapid and favourable action of the Anti-Leprosy Serum, observed by more than 20 physicians in different

[30] Nocht and Velasco, *Jour. Philippine Islands Med. Assoc.* 15 (1935).

[31] From practical reasons it has as yet only been possible to make crude, orientating tests with normal serum from sheep on 5 lepers (with cutaneous ulcers, multiple small nodules, anaesthesia, obstruction of the nose, and red-violet macule respectively). In these patients, each of whom received one series of 3 injections of 10 c.c., no objective improvement could be established. It should also be noted that previous tests on the activity of normal sera in comparison to anti-tuberculosis (especially on lupus), antigonococcic, and antistreptobacillary sera have never given me results indicating a nonspecific healing effect in these diseases.

countries, on leprous manifestations on which injections of chaulmoogra oil and its derivatives have had little or no effect, entitles me to the assumption that treatment with Anti-Leprosy Serum, carried out in the most advantageous manner, should become a good auxiliary to the standard therapy with chaulmoogra. And that is all I hoped for from my serum, as I also stated in my first article, in the French Academy of Science, in 1933.

EXPLANATION OF PLATES

(1, 2 and 3)

PLATE 1

FIG. 1. Reproduced from Kedrowski's paper in *Zeitschr. f. Hygiene* 1901, Vol. XXXVII, p. 52. A microorganism isolated by Kedrowski in 1900 from a leproma in a Russian patient. Nonacid-fast of diphtheroid type but showing, here and there within the body of the microorganism, small, red, round corpuscles. After passage through rabbits the microorganism changed into almost entirely acid-fast rods, and as such the culture still exists in laboratories all over the world, growing on different solid media on which it forms a greasy layer of greyish colour. Stain: Ziehl-Neelsen. Magnification, about 1000 diameters.

FIG. 2. The same strain in 1937, growing on the surface of common glycerin bouillon. It was brought over from glycerin agar onto this medium by Reenstierna in 1927 by means of floating pieces of cork. On common glycerin bouillon it forms a light lead-grey, creamy, wrinkled pellicle.

FIGS. 3 and 4. Smears from this bouillon culture showing the microorganism in an almost acid-fast (fig. 3) and a half nonacid-fast stage (fig. 4). Stain: Ziehl-Neelsen. Magnification, 1000 diameters.

FIG. 5. Reenstierna's mycotic microorganism in a nonacid-fast stage, isolated in 1912 from the blood of a Swedish patient during an acute period of nodular leprosy. Stain: Ziehl-Neelsen. Magnification, 1000 diameters.

FIGS. 6 and 7. Acid-fast elements appearing in cultures of this microorganism. Stain: Ziehl-Neelsen. Magnification, 1000 diameters.

FIG. 8. Acid-fast stage of the same microorganism. Stain: Ziehl-Neelsen. Magnification, 1000 diameters.

(FIGS. 5 to 8 are reproduced from Reenstierna's paper in *Arch. f. Dermat. u. Syphil.*, 1913, Vol. CXVI, No. 3.)

This culture of Reenstierna's was obtained in a special medium, consisting of glycerin bouillon with ascites, glucose, and lumps of human brain. From the nonacid-fast stage (fig. 5) of this microorganism a single cell culture (from one diplo-link) was prepared in 1912 according to Burri's method. Only subcultures of this single-cell culture are now in existence.

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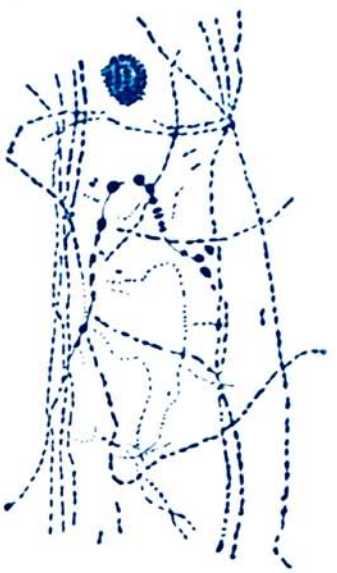

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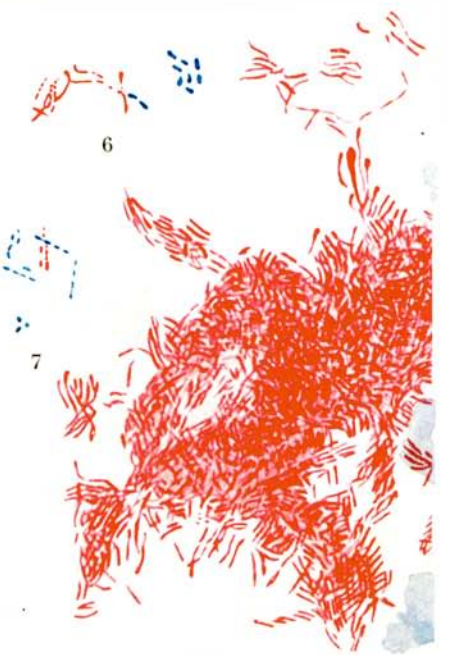

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PLATE 1 (7)

Plate 2

Figs. 1, 2, 3, and 4. Smears from the same mycotic microorganism in 1937, growing on Hohn's solid medium where it forms a greasy layer, the colour of which is greyish though sometimes with a light yellowish tint. The nonacid-fast stage is predominant. Note especially the round or egg-shaped red corpuscles of different sizes, some within the bodies of the microorganisms, others detached from them. (Cf. Pl. 1, fig. 1; Pl. 2, figs. 11 and 12; and Pl. 3, fig. 5). Stain: Ziehl-Neelsen. Magnification, 1000 diameters.

Fig. 5. Reenstierna's strain in 1937, growing on the surface of common glycerin bouillon onto which, by means of small floating pieces of cork, it was brought over by the author in 1927 from a tube containing solid medium (nutrose glycerin agar) which he had received from Paldrock. It forms a wrinkled pellicle the colour of which is yellow of different shades, at times, however, changing more into grey.

Figs. 6 and 7. Smears from this bouillon culture showing the microorganism in chiefly acid-fast (fig. 6) and chiefly nonacid-fast stages (fig. 7). Stain: Ziehl-Neelsen. Magnification, 1000 diameters.

Fig. 8. Bayon's strain. A diphtheroid, pleomorphic, filamentary and bacillary microorganism isolated at Robben Islands, South Africa, from nodules of a leper. These slightly acid-resistant bacteria regained acid-fast properties after injection into animals. Strain: Ziehl-Neelsen. Magnification, 1000 diameters. Reproduced from Bayon's paper in *Ann. Trop. Med. and Parasitol.*, 1915, Vol. IX, No. 1.

Figs. 9, 10, and 11. A mycotic microorganism isolated by Walker at Honolulu from different leprous foci. Obs. the egg-shaped red and blue corpuscles (fig. 11). Stain: Ziehl-Neelsen. Magnification, about 1380 diameters. Reproduced from his paper in *Jour. Prev. Med.*, 1929, Vol. 3, No. 3.

Fig. 12. A microorganism—the so called "Unterart D"—isolated from the bone-marrow of a rabbit inoculated in 1902 with Kedrowski's strain. Red and blue egg-shaped corpuscles. Stain: Ziehl-Neelsen. Magnification, about 1000 diameters. Reproduced from Kedrowski's paper in *Zeitschr. f. Hygiene*, 1910, Vol. LXVI, p. 1.

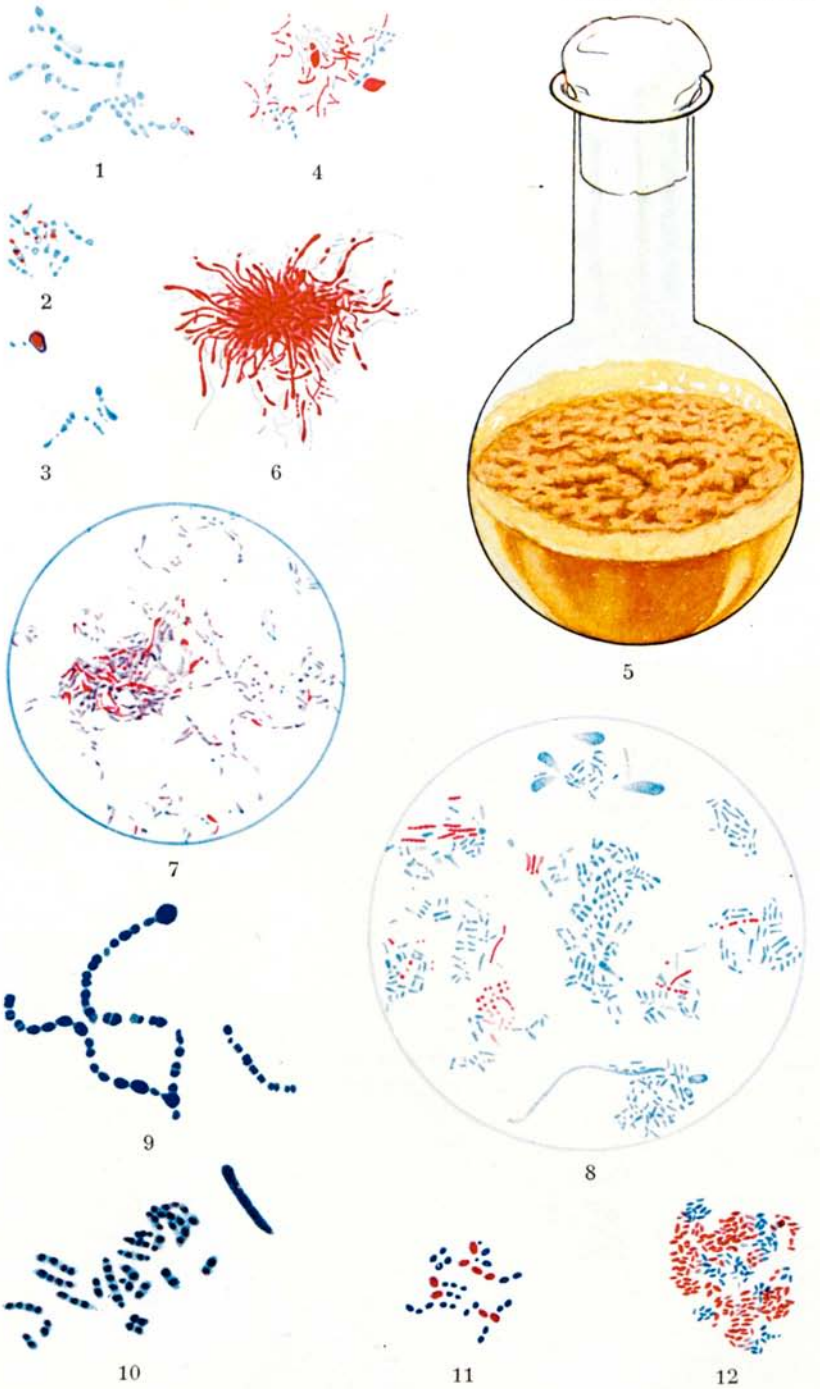

PLATE 2 (8)

Plate 3

Fig. 1. An intact purulent blister which appeared on the middle toe of the left foot of a *Macacus rhesus* 42 days after its inoculation (intraperitoneally, intraneurally, etc.) in 1912 with a culture of Reenstierna's microorganism in its nonacid-fast stage (*Vide* Pl. 1, fig. 5).

Figs. 2 to 5. Smears from the content of this blister. Both nonacid-fast and acid-fast microorganisms are to be seen. Note the egg-shaped red corpuscles (fig. 5). Stain: Ziehl-Neelsen. Magnification, 1000 diameters.

(Figs. 1 to 5 are reproduced from Reenstierna's paper mentioned above.)

Fig. 3. Smear from the contents of a bulla in a case of leprous pemphigus. Stain: Ziehl-Neelsen. Magnification, about 1380 diameters. Reproduced from Walker's paper mentioned above.

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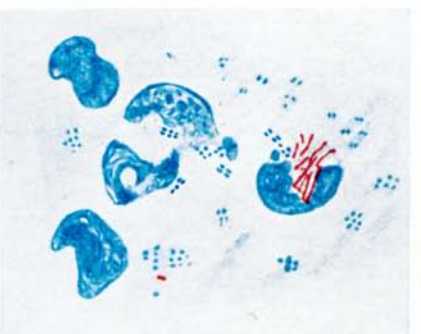

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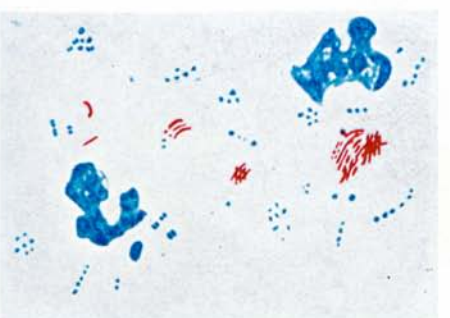

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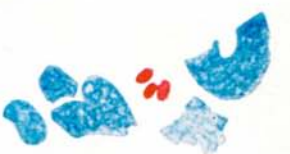

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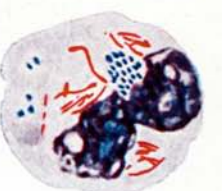

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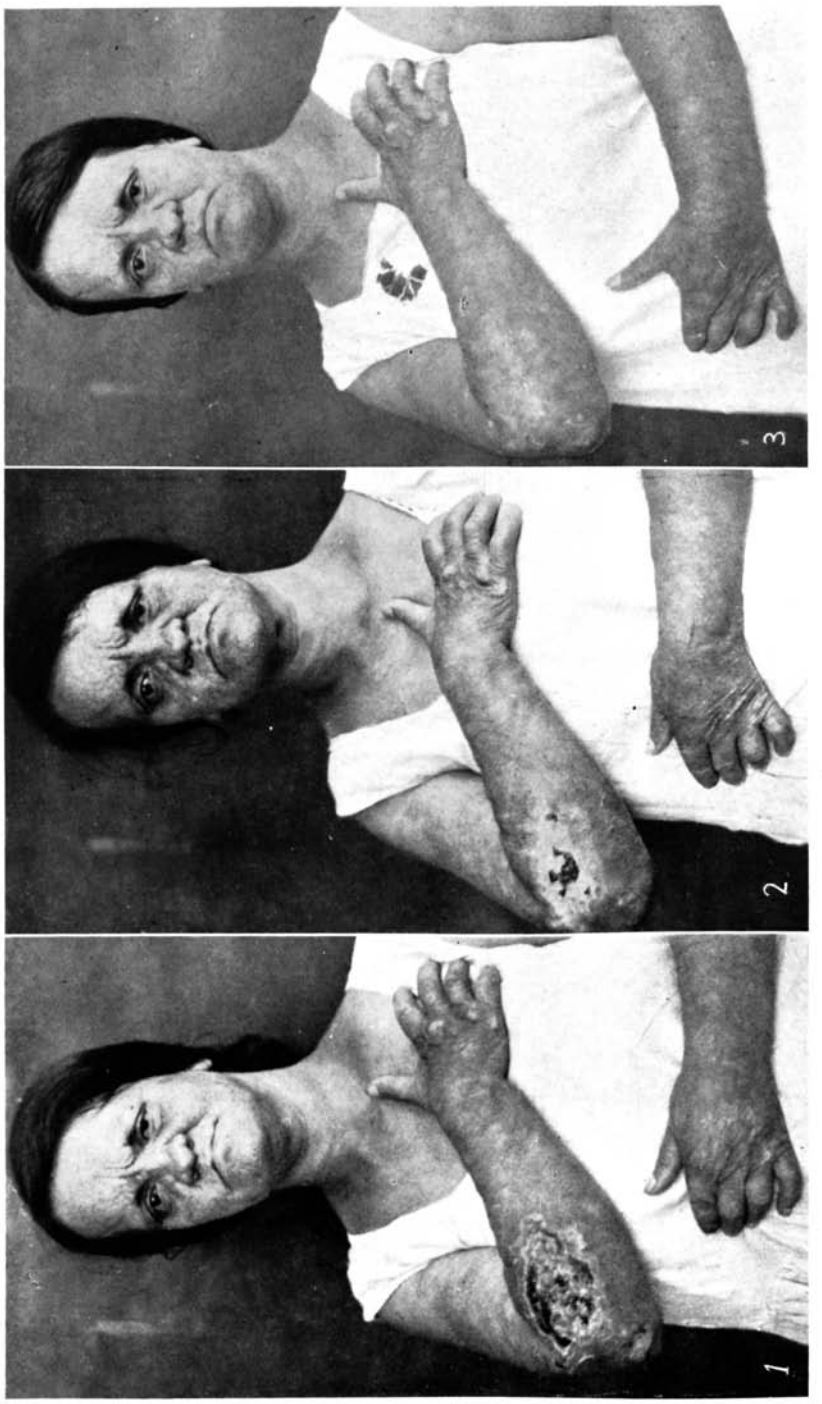
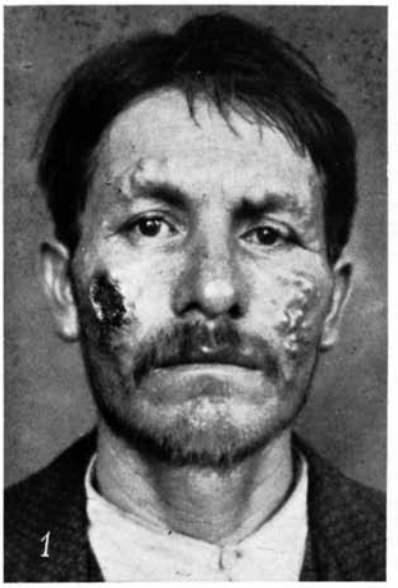
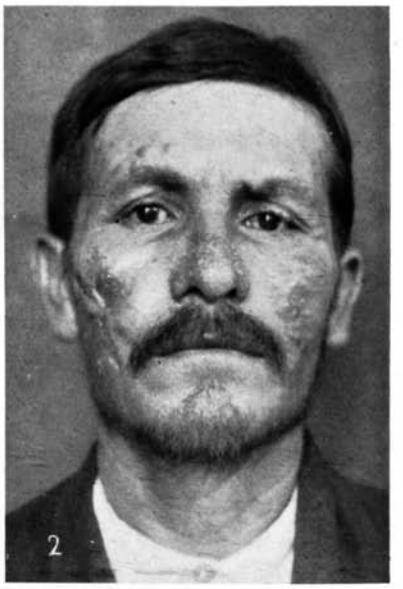
PLATE 3 (9)



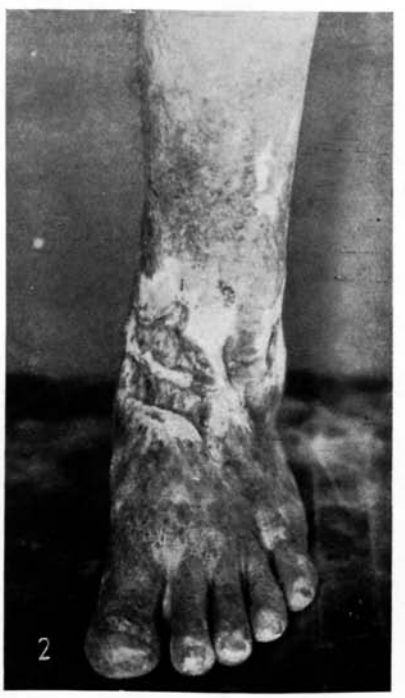
Case No 23, Agua de Dios (Colombia). November 3rd, 1936. November 16th, 1936. November 24th, 1936.

PLATE 4 (10)





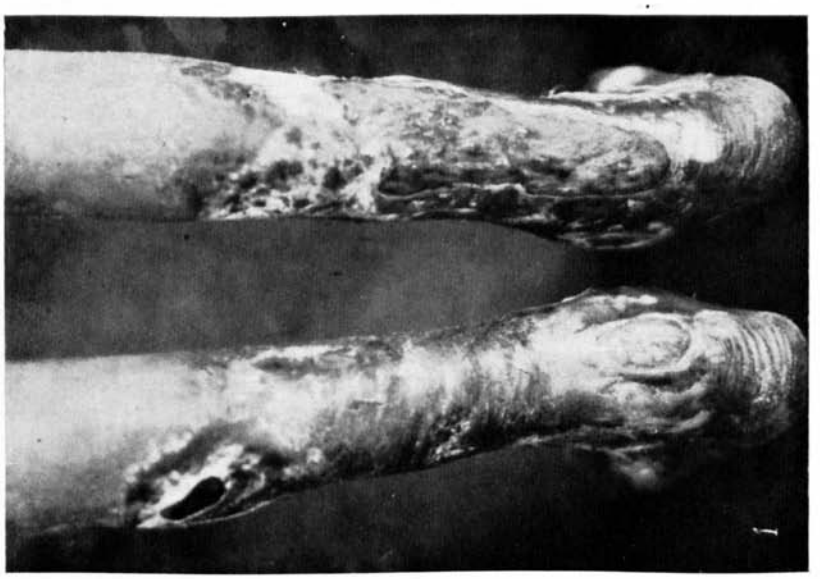
Case No 17, Agua de Dios (Colombia).
October 19th, 1936. November 26th, 1936.



Case No 7, Agua de Dios (Colombia).
October 8th, 1936. November 26th, 1936.

PLATE 5 (11)

November 26th, 1936.



October 14th, 1936.

Case No 10, Agua de Dios (Colombia).

PLATE 6 (12)