Tegumentary Leishmaniosis and its Transmission by Phlebotomi

BY

Dr. Henrique de Beaurepaire Aragão

(Chief of Section).

(With Plate 104)

The present paper is meant to give an account of observations and studies we were enabled to make by the occurrence of a remarkable focus of tegumentary leishmaniosis, arising in the heart of the city of Rio de Janeiro, in the course of 1921, as well as of the experiments made with the material obtained. These were specially directed towards ascertaining the transmission of the disease by blood-sucking Psychodids of the genus Phlebotomus. On the subject we published already a brief note (Brasil-Médico, March 18th., 1922), now to be compared with further details.

The site, on which arose the focus of leishmaniosis described, is about four or five kilometres from the centre of the city, and lies at Aguas Ferreiras, at the far end of the valley of Laranjeiras, and thence slopes up the Corcovado and up Santa-Thereza Hill, up to the place known as Lagoinha, in the vicinity of the Hotel Internacional. (Fig. 1).

This region is pretty densely populated, not only by the well to-do who have good residences with gardens and parks, as by the poorer classes who live in rough cottages and cabins on the slopes and more or less steep paths leading up to Sylvestre.

This section is inhabited not only by Brazilians, but also by a good many foreigners, who find it cooler than many other quarters of Rio, especially in the summer.

The whole region is well provided with trees and dampness and there is plenty of shrubbery and springs and small brooks.

These conditions are very favourable for phlebotomi, which are abundant. They belong to the species Phlebotomus intermedius LUTZ et NEIVA, the com-
monest species in Rio and plentiful almost everywhere in Brasil. (1)

At certain times of the year, from March to June, these insects are particularly abundant and become quite a nuisance, so much so that the dwellers have come to know them quite well and to distinguish them from other blood-sucking insects. These other blood-uckers are not very varied: they are mosquitoes of the species *Culex confirmatus*, *Culex taeniornynchus*, *Culex fatigans*, *Cellia argyrotarsis* *Aedes calopus* and other less important ones as well as of a Simulium-species, *Simulium pertinax*. No blood-sucking tabanid and no tick or other blood-sucking mite is to be found on the spot. The lack of ticks and of blood-sucking tabanids (for *Erophopsis ulicorinis* which is found there is not a bloodsucker) practically excludes their being taken into account, as possible transmitters of leishmaniosis, as was suggested by some writers. Transmission by bugs must also be excluded as extremely unlikely: the facts seen in this focus in Rio de Janeiro leave no opportunity for incriminating them, seeing that the disease spread pretty rapidly in a large zone and attacked people living in places entirely free from such vermin.

Thus only mosquitoes, simulidía and phlebotomi are left in the field, but all that is known regarding the transmission of leishmaniosis of the teguments tends to prove that the last-named are the real and perhaps the only transmitters.

The hypothesis of the transmission of leishmaniosis by phlebotomi was first brought forward in 1901, by PRESSAT. Since then other writers, in several countries, have accepted this theory and have contributed to its demonstration with varied arguments. Amongst others may be cited the names of the brothers SERGENT, WENYON. PIRAJA' DA SILVA, LUTZ, NEIVA. DUTRA E SILVA, CASTRO CERQUEIRA and others. The constant presence of phlebotomi in the foci of leishmaniosis and the observation of flagellate forms, similar to leishmania forms, in the digestive tube of phlebotomi has concurred to give weight to the theory.

A very close connection between the sting of a phlebotomus and the appearance of a leishmania-ulcer at the same spot had also been noticed.

The fact of a focus of leishmaniosis arising just in a part of Rio de Janeiro, in which phlebotomi are particularly abundant, is another very convincing argument in favour of their being the only transmitters of the specific flagellate.

It is well-known that there come to Rio a number of patients of leishmaniosis, who usually remain in parts of the town and of this suburbs in which there is no lack of mosquitoes, simulidía and bugs, but in which phlebotomi, on the other hand are rather more scarce. Now these patients do not give rise to foci of the disease, and, on this account, there are about half a dozen cases, at most, of infection reported in Brazilian medical literature, whilst abroad one case has been published, that of WERNER, in the Archiv für Schiffs- und Tropenhgiene of 19910.

There comes however a patient to a part in which phlebotomi are very much more plentiful than in any other quarter of the town, and immediately there is formed a focus which in 3 or 4 months originates 4 times ten cases and which would certainly have progressed had it not been discovered in time and the necessary steps taken, with the treatment and isolation of the patients.

It is owed to Professor EDUARDO RABELLO, one of the most prominent

---

(1) Professor LAROUSSE se has found a *Phlebotomus migonei*, on material e that we had sent to him from this site.
men in this field, to have made a clinical diagnosis, in July of 1921, of the first case belonging to the focus of leishmaniosis in Rio de Janeiro. I had the satisfaction of confirming this diagnosis by microscopical examination and was thus placed on the track of other cases existing in the same quarter. It was possible to make sure the existence of a great number of cases spread over the same part of the town. They had arisen 2 or 3 months before, which added to the one to three months of incubation, which is allowed for the disease, leads one to suppose that they began in the first months of the year and became numerous in the rainy season up to June, exactly at a time when phlebotomi are most plentiful.

Anyway, our colleague Dr. ARTHUR NEIVA, was well aware of the abundance of phlebotomi on Santa Thereza Hill and on the slopes which lead up to it and he had already foreseen, a long time ago, that a focus of the disease might arise here as came to pass reality. For this it sufficed that there should arrive one patient, and it is not at all improbable that the origin of the cases of Aguas Ferreas is to be found in a small focus, which, since 1919, was maintaining a few cases in Santa Alexandrina. Santa Alexandrina is a quarter located in a valley also leading up to Santa Thereza Hill, but on the slope opposite to that of Larangeiras although quite near to the latter. In 1919 3 cases were observed in the Rua Santa Alexandrina, in persons of the same family, and, in 1921, yet another 2 cases were pointed out in persons of the same family, which shows that the focus remained in activity during this time, giving in all 5 known cases.

The existence of this focus came to be known through the appearance of one of the cases in Out-Patient Department for Skin Diseases of the Rio de Janeiro Policlinic, so excellently directed by Professor WERNECK MACHADO. The information we owe to Professor WERNECK MACHADO and to Professor PARREIRAS HORTA, whose courtesy in giving us a clue to the possible origins of the focus of Aguas Ferreas, we have great pleasure in acknowledging.

One cannot admit for the formation of the focus of Aguas Ferreas the hypothesis suggested by some that leishmanias are specific and normal parasites of certain blood-sucking insects, with the property of adaptation to the human body, when inoculated by the stinging of the insect. This hypothesis might be called in to explain the appearance of cases of tegumentary leishmaniosis among workmen employed in destroying forests and in more or less uninhabited zones, as happened near Bauru and other parts of the North-East of Sao Paulo.

But even so the appearance of the disease has nothing extraordinary if is be remembered that the incubation lasts from one to three months and that these workmen prior to arriving in the uninhabited places, where they were to work, went through places in which cases of leishmaniosis are plentiful and must certainly have got infected by the transmitting insects found in these places.

In the case of the focus of Aguas Ferreas it should be borne in mind that in this place there have always existed the same blood-sucking insects and especially phlebotomi and that there never occurred a case of leishmaniosis there before 1921. It was necessary that a carrier of disease should first arrive, before a focus was formed and the disease spread to about 50 cases in the space of 6 months. No sooner had the disease been discovered and the patients treated or removed, the disease disappeared within a short space of time, although the blood-sucking insects remained the same. If they had it in them to
become reservoirs of virus, or if normally they played this rôle, once infected they would have continued to transmit the disease.

What happened however was that the insects were only able to transmit the disease while there was human material capable infecting them.

The hypothesis advanced by SÉR- GENT, LEMANE and SENEVET in 1915 that certain lizards, Tarentola mauritanica, for instance, are often stung by phlebotomi, might be reservoirs of the virus is not to be upheld. Although these writers obtained the cultivation of a flagellate by inoculating appropriate culture-media with the blood of this lizard, this does not prove anything, since the flagellates did not display the characteristics of forms of evolution of Leish- mania tropica and were inoculated without success into men and laboratory animals.

WENYON, who found in Egypt flagellates of the Herpetomonas type parasitizing the intestinal cloaca of Camelco vulgaris, ascertained later that the same thing happens in parts in which oriental sore is unknown.

What makes it still more unlikely that lizards should act as reservoirs for leishmanias is the fact that leishmanias are very little adaptable to other homothermic vertebrates much lower in the zoologic scale.

Besides man, the only natural host of the virus known is the dog. BRUMPÈR believes that a sore seen in an age out of the region of Baurú (State of São Paulo) was of a similar nature, but there is no microscopic proof of the fact.

For lack of experimental evidence the hypothesis suggested by OLYMPIO DA FONSECA Fe and later by STRONG of a connection between flagellates of plants and the leishmanias of man cannot for the moment be accepted. The serum agglutinations made by NOGU- CHI have demonstrated a great difference between Leishmania and plant flagellate.

It is not possible to give an exact statement of the number of cases of the focus of Aguas Ferreas or of the smaller ones of Lagoinha and Santa Alexandrina.

Between the cases seen by us and those seen by the committee appointed by the Public Health and comprising Dr. AMARILIO DE VASCONCELLOS and Dr. ALEXANDRE CERQUEIRA, whose excellent report we may recommend to those interested in the subject, 41 cases were counted. We also had notice of a few more, not seen, and it is probable as happens in light cases, or by treatment that some of these either got well alone, as happens in light cases, or by treatment, or else left the focus before it became known.

Thus it would not seem exaggerated to estimate the number of cases of this focus of leishmaniosis in Rio de Janeiro at about 50 in the six or eight months it lasted.

As regards the clinical forms and the persons affected, the study of what happened here is of considerable interest. Once again a confirmation is obtained of the small proportion of cases affecting the mucous membranes, as compared to the total number of cases of the disease. Thus we only had the opportunity of seeing three cases of this from of the disease. Of these, one had a localisation on the mucous membrane of the nose, another had got sores on his arm and showed a typical leishmaniotic rhinophyma besides, with pronounced lesions of the mucous membrane, and finally the third case was one of special interest, about which a few words must be said.

This was the case of a Pressman who looked me up one day, saying that for some time he had been noticing a roughness on the roof of his mouth,
every time he touched it with his tongue, a lesion which he ascribed to syphilitic infection.

Examination of the patient showed that in reality he had in the middle of the palate a small zone of congestion, showing slight vascularisation but no ulceration. Some material was obtained by scraping the congested area with the point of a bistoury and some smear preparations were made with this material. These after fixation with alcohol showed numerous typical leishmanias, thus showing that this was a mucous lesion from the outset.

I do not believe however that it was a primary mucous form, seeing that the patient informed that a month before he had had, at the edge of the nasal orifice, what appeared to be a small boil and healed alone. This might have been the initial leishmaniotic lesion. The patient treated with tartar emetic according to the approved method had his lesion, as yet so undeveloped, rapidly cured.

Thus once more it was demonstrated that the number of cases of leishmaniosis involving the mucous membranes is always inconsiderable, confirming what Prof. BRUMPT and Prof. ALEXANDRINO PEDROSO had pointed out concerning this form of leishmaniosis in the North-West of the State of São Paulo. If the observation of patients found within the Hospitals gives an opposite impression, this is due to the fact that the patients, who ask for admission, are just the ones affected with the more severe lesions and are looking for treatment.

The great majority of the lesions shown by the patients of the focus of leishmaniosis was made up of the ordinary sores which are agreeing with the typical appearance of lesions produced by *Leishmania brasiliensis*.

These sores were single or multiple and were found on lower and upper limbs, on the neck, on the face and on the head, whilst lesions on the body were rare. Recent lesions were always very plentifully provided with parasitic forms, which could be shown up by examination of smears and of sections. It was also possible to obtain, in one instance abundant and typical cultures of the parasite.

Another interesting fact made evident in the focus of leishmaniosis of Aguas Ferreiras, was that here the disease eased to be what it is nearly everywhere in Brazil, a disease of rural labourers, of those working on the obtaining of rubber or on coffee plantations, in the clearing of forests, in hewing timber etc. In this instance something very different occurred, owing to the kind of people living in the infected zone. As was already pointed out, the place is inhabited by rich and poor, the former in good houses with extensive gardens, the latter in cabins and cottages in the woods going up the slopes to Sylvestre and Santa Thereza.

There are no rural labourers. The population is made up of doctors, lawyers, business men, workmen of the Corcovado Back Railway, tramway-conductors, servants and so forth, Brazilian and foreign.

As the dwellings of the rich and the poor stand by side the disease spread indiscriminately amongst the dwellers and left aside any predilection for rural labourers and attacked doctors, lawyers, business-men, servants, street-car conductors, children, adults and old men, anyone living in the zone. The seat of the lesions also varied, but in persons going about completely clothed and with shoes, they were more often found on the head and neck, which were less protected.

As the proportion of men to women in this place is about equivalent, the number of cases was approximately equal in both sexes. Thus 14 cases in men and 12 in women are mentioned in
the report of the Public Health Dept. In the regions of the North-West of São Paulo were the gangs of labormers are generally made up of men alone, the proportion is of 94% cases in males against 6% in females.

As may be seen, from what has been said, a change of location sufficed to give new aspects as yet unsuspected to leishmaniosis as it was known from the inferior.

As this focus of leishmaniosis was at my disposal under conditions which made research-work easy, I decided to attempt something in connection with the transmission of the disease, a subject of great interest from several points of view, practical and theoretic.

The presence of phlebotomi in great numbers in the locality, led me to some experiments purporting to demonstrate the part played by these insects in the transmission of the disease.

It was not a difficult task to persuade a few patients who showed a little more good will, to catch some phlebotomi at home. Of these insects, with which they proved to be acquainted, we asked them specially to catch those which stung the inflamed parts surrounding the ulcers; these, according to one of the patients, seemed to be preferred by the insects. With the help of the patients I was able to obtain 207 phlebotomi under favourable conditions. Some of them it was possible to maintain alive for 4 days in the laboratory. For the rest, it is easy to catch in this region large numbers of Phlebotomi, not only in the houses, but also in the poultry-houses, the places, where goats use to sleep, specially at night, but also in the day-time, though in smaller numbers. As a rule the insects refuse to sting after they have been caught, but sometimes it was possible to in duce them to sting rabbits.

Experiments were made with a suspension in water of a certain number of phlebotomi inoculated each time in the snout and ears of puppies. Only once in a suspension made with two phlebotomi, was I able to see a few flagellates with the appearance of the forms of evolution of Leishmania. The material was inoculated in a puppy, but the animal already emaciated at the time, died in 9 days without showing any lesion at the site of the injection.

Besides this puppy, another ten were inoculated, of which seven died at different times up to one month and a half after inoculation without any of them having contracted the disease.

Of the survivors, the one inoculated on the snout on Oct. 28, 1921, with a suspension of 8 phlebotomi, which three days before had stung a patient, showed nothing but a very slight reaction at the site of inoculation, up to the end of January 1922. From this date on, i. e. 3 months after inoculating a small nodule became noticeable at the site of injection, on the snout of the animal.

The nodule was incised and the surface of incision scraped so as to make some smears, but nothing was seen in this first examination. On Feb. 10, after the nodule had grown some more, a deeper incision was made and material was obtained for smears, which were stained with the Giemsa stain. In these preparations a few absolutely typi al Leishmanias were seen, and this also happened in later examinations.

The parasites were almost always isolated in the preparations.

On Feb. 16, the puppy showed a small depressed ulcer (Fig. 2) on the snout, as is seen on the photograph given. This was the only lesion shown. A small fragment was obtained for section by biopsy and the material imbedded for section. After this biopsy the lesion regressed, until towards the end of March it had practically disappeared.
Smears made at this time, after incision, showed no more parasites.

Such were results of experiments on the transmission of leishmaniosis by phlebotomii.

I believe they give a satisfactory proof that in Brazil, *Phlebotomus intermedius* LUTZ and NEIVA is capable of harbouring *Leishmania braziliensis* VIANNA, in conditions permitting the transmission of the disease.

It is altogether probable that the other species of phlebotomi found in Brazil should also be able to transmit cutaneous leishmaniosis.

At the time these experiments were being made here, the brothers SERGENT and their fellow-workers PARROT, DONATIEN and BREGUET (Comptes rendus de l'Académie des Sciences) showed how the first time in an undetectable way that the virus of leishmaniosis remains alive and infections in phlebotomi. These research-workers, brought a lot of phlebotomi from Biskra the well-known focus of leishmaniosis, to Algiers, where the disease does not exist. There they made suspensions of the insect, and by inoculating them in healthy soldiers, obtained in one of them, in less than three months, a typical lesion with plenty of parasites.

Quite recently, in a paper published this year, ADLER and THEODOR (Ann. of Trop. Med., V. 20, n. 2, 1926) report a series of experiments, of the most interesting nature and very conclusive, showing the part played by phlebotomi in the transmission of the well-known cutaneous leishmaniosis of the Old World.

They found that, in Jericho, *Phlebotomus papatasii* collected in foci of leishmaniosis was infected with flagellates of the type of the forms of development of Leishmanias (Herpetomonas). The insects were infected in 1% of cases. They further showed that these forms of development are found in the proboscidea, pharynx, oesophagus as well as in the other segments of the digestive tube of the insects. Already in 1921 WENNON had pointed out that phlebotomi collected in ALEPPO, another place where leishmaniosis prevails, harboured herpetomonas in the digestive tract.

ADLER and THEODOR made use of the material containing plenty of parasites obtained from phlebotomi in Jericho and inoculated it in scarifications made on the arms of three volunteers in Jerusalem, where the disease is not prevalent. In from one to three months all the cases broke out with lesions in which absolutely typical leishmanias were to be seen.

The experiments of ADLER and THEODOR bring a new argument in favour of the part played by phlebotomi in the transmission of cutaneous leishmaniosis and confirm the results obtained by SERGENT and his collaborators as well as our own.

The part played by phlebotomi does not limit itself to cutaneous leishmaniosis as Old and the New World.

Just as the different species of plasmodia of malaria are transmitted by Anophelines, everything leads one to believe that phlebotomi are responsible not only for the transmission of cutaneous (cutaneous) but also of the other leishmania-diseases involving the viscera. Evidence is now beginning to accumulate in favour of this interpretation.

Results obtained by the British Commission entrusted with the study of Kala-azar in India are of great interest.

This commission made up of CHRISTOPHERS, SHTOT, BARRAUD, KNOWLES, NAPIER and SMITH, made sure that phlebotomi of the species *Phlebotomus argentipes* ANNANDALE and BRUNETTI, stinging patients with Leishmanias in the circulation, get infected in a degree of 30%, so that flagellates (herpetomonas forms) become plentiful in their intestinal tract a few days after the infecting meal.
The commission, which is still at work, has not as yet made experiments on the transmission of kala-azar to Man, by means of phlebotomi harbouring herpetomonas, but everything would seem to indicate that if this were tried, positive results would be assured, in view of the case with which the development of the parasites is obtained in the digestive tube of the insects, in which they are found in pharynx, oesophagus, stomach and rectum.

Taking into consideration the results obtained up to now in the transmission of Leishmania-disease, not only in the tegumentary form, as in the visceral form of the type of Indian Kala-Azar, everything is conducive to the belief that infantile leishmaniosis, whose mode of transmission is not yet ascertained, should also have phlebotomi as transmitters. I feel sure that if research be directed towards this particular, it cannot fail to give most interesting results, and, needless to say, results of great practical value.

It is to be hoped that something more may yet be done on the part played by phlebotomi in the transmission of leishmaniosis and I would be fully repaid, if the present paper should encourage, in some way, further research on this important and momentous subject of tropical pathology.

**SUMMARY**

From all that has been ascertained up to now, it would seem that the transmission of tegumentary leishmaniosis is carried out by different species of Psychodids amongst them Phlebotomus papaiasi, Phlebotomus intermedius and Phlebotomus argentipes.

The epidemiology, the geographic superposition between the area in which these insects exist and the area in which the disease is found, together with the results of the attempt to transmit experimentally the disease by phlebotomi, all speak very much in favour of this.

Phlebotomi must not only transmit tegumentary leishmaniosis, but also Indian Kala-azar, as is demonstrated by experiment made up to date and very probably they are the transmitters, as yet unproven, of leishmaniosis infantum. It is always possible for a very active focus of tegumentary leishmaniosis to arise in a zone in which phlebotomi are plentiful, as happened in Aguas Ferreus, at the end of the valley of Laranjeiras and well within the town of Rio de Janeiro.

Facts observed in Aguas Ferreus entirely exclude the idea that leishmanias might be normally harboured by phlebotomi.

Other blood-sucking invertebrates (mosquitos, flies, ticks, bugs etc.) up to now accused of possibly transmitting tegumentary leishmaniosis do not appear to be able to do this.

Besides dogs, no other animal is proved to be able to act as a reservoir of the virus of tegumentary leishmaniosis and it is altogether unlike why that lizards should be able to act as such.
EXPLANATION OF PLATE 104.

Fig. 1.—Plant of the city of Rio de Janeiro on white the focus of leishmaniosis are signalated.

Fig. 2.—Dog presenting on the nose a leishmaniotic ulcer obtained by the inoculation of an emulsion of phlebotomi of the focus of leishmaniosis of Rio de Janeiro.