A CRITICAL REVIEW OF KALA AZAR AND TROPICAL SORE.¹

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(Continued from p. 20.)


Since the first observation of the presence of *Leishmania* parasites in a tropical sore by Wright, in 1903, a great number of accounts have appeared, confirming their presence in the tropical sores peculiar to particular countries or districts. Up to this time there was considerable doubt as to the identity of the various sores common in many parts of the Tropics, and this doubt is reflected in the innumerable synonyms by which the lesions are known. One after another these lesions were searched for the new parasites, and, practically without exception, they were readily detected. Without attempting to give a complete list of the various names, the following may be mentioned: Aleppo boil, Armenian boil, Persian boil, Delhi boil, Frontier sore, Bagdad sore, Biskra boil, Gafsa boil, Nile sore, and innumerable others. The more general terms of tropical sore, Oriental sore, and *bouton d’Orient* have also been freely employed by those who believed in their identity on clinical grounds. Of these general terms, the two latter are no longer appropriate, since the affection is now known to exist in the New World as well as in the Old, and even the term tropical sore, adopted in the present instance, is no longer strictly accurate,

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since their presence on the northern shores of the Mediterranean
has been recognized.

Besides confirming the identity of the sores, so long familiar
to tropical physicians in many parts of the world, the easy demon­
stration of the parasites has led to the detection of tropical sores
in countries hitherto unsuspected. Of these, the following may be
mentioned: Asia, Bettman and Wasielewski describe a case from
Central Asia, and Nicolas thinks it is common in New Caledonia;
Marzinowsky also reports more cases from Transcaucasia in
addition to those in which he first identified the parasites, inde­
dependently of Wright. In Europe chief interest attaches to the
discovery of the existence of this form of Leishmaniosis on the
Calabrian coast of Italy by Gabbi and Lacava, while Cardamitis
and Melissidis have also found it in Crete, and Reinhardt in
Constantinople. In Africa new foci have been demonstrated in
Algiers and other parts of the northern coast, and at Zinder in
Northern Nigeria, where Stevenel has shown it to be identical
with the disease known locally as "Cro-Cro." Benoit-Gonin
also speaks of it as common on the upper reaches of the Niger
itself. America, too, has been shown to be no longer exempt,
as has been believed for so long, and apparently cases are far
from uncommon in several parts of Brazil and on the Amazon,
as has been pointed out by Paranhos and Marques, Carini, Lindenberg
and others. In this country the local name of "Bauru ulcer"
appears to have been used for some time, and the majority of the
cases have been found in the province of Sao Paulo. Trinidad
and the Canal Zone of Panama have been found by Darling and
Connor to harbour the disease, and there can be little doubt that
further extensions will soon be made to the geographical limits
of the American form. It has been suggested that the disease
may have only been recently imported into Central and Southern
America, and Carini and Paranhos mention that their first case
was that of a Syrian who had recently come from Beirut, but
at the same time they add that a similar type of sore has been
known in Brazil at least since 1895.

Morphology of the Parasites.—If differences exist between
Leishmania tropica and the other species they must be very slight,
since the great majority of observers who have had the opportunity
of comparing them are agreed that they are morphologically in­
distinguishable. The description of one holds good for the others.
The few special points which may be noted are that Nattan-Larrier
and Bussière have sometimes seen a delicate filament connecting
the blepharoplast and the nucleus which they do not think corresponds to the rhizoplast described by Novy in *L. infantum*, and by Christophers in *L. Donovani*. Several observers have studied the fresh parasites, and all are agreed that they show no motility. In most instances the parasites are intracellular, and only rare free forms are to be seen, but Cardimitis, in his study of the Cretan cases, found the reverse, namely, that they were almost all free and isolated, and only a few intracellular; it may be noted that the sores he examined were not ulcerated. The size of the parasites in comparison with that of the other species has been the subject of some difference of opinion, some holding that they are larger on the average than those of the constitutional disease, and others that they are smaller. The point is not one of value in differential diagnosis, since it is abundantly clear that there are wide variations in this respect, even in single species, and that the size and shape may be influenced by the site, and probably also by the rapidity of growth and the stage of development. As far as the experience of the writer goes, he has found the parasites somewhat larger in tropical sore than in the other species, but he is not inclined to lay any stress on this.

Forms which apparently indicate a multiple division of the parasites have been described by Lindenberg and others, but Marzinowsky, from his observation of living specimens, does not agree with this, and considers they are due to compression within the cells, since he has noted such forms on escaping from the cell to resume their usual shape and appearance.

*Cultures and Cultural Forms.*—Artificial cultures of *L. tropica* are as readily secured as those of *L. infantum*, but, apparently, both of them are much more sure of success than in the case of *L. Donovani*. As to the medium employed, there does not appear to exist here the distinction between *L. Donovani* and *L. infantum*, since it has been found possible to secure good flagellate development of *L. tropica* in citrated human blood, as has been done both by Row and by Marzinowsky, and also in Novy’s medium, as recorded by Nicolle and his colleagues and also by Marzinowsky.

Considerable differences, however, are to be noticed in the temperatures which have been observed for cultivation. While Nicolle and others cultivate at 22° C., Row reports good development between 25° and 28° C., and Marzinowsky’s cultures were maintained at 37° C. This difference is certainly noteworthy, since little evidence of growth can be obtained in the case of the two kala azar parasites above 25° C. At the same time, it is
only at the lower temperature that successful sub-cultures can be secured and a strain kept going through many generations. Nicolle, for instance, mentions a culture, of human origin, of the thirty-third generation, while Marzinowsky, working at 37° C., was only once successful in obtaining a sub-culture.

In the cultural forms, as in the intracellular forms, no distinction can be observed between the three species. Nicolle in his earlier work was inclined to think that small differences, which he described in detail, did exist, but in a later communication he states that the cultural forms of *L. tropica* are absolutely identical with those of *L. infantum*. At the same time, Nicolle and Manceaux have noted that at the end of the optimum period of growth, eight to ten days, cultures of *L. tropica* are always more abundant than cultures of *L. infantum*.

Marzinowsky's studies of these cultural forms have, however, led him to conclusions widely differing from those of other observers. He believes that they afford evidence of a sexual process and he describes as male forms comparatively small parasites with a large nucleus and pale-staining protoplasm, and as females larger parasites with a small nucleus and a protoplasm which stains deep blue. As development advances he says these distinctions, with the exception of the difference in size, are lost. He also thinks that he has observed a process of conjugation between a male and a female parasite in which the two merged into one and their nuclei became fragmented and its particles distributed throughout the common mass of protoplasm. After this fusion he says that the flagellum disappears, the parasite lengthens out and becomes motionless, and the blepharoplast also vanishes, while a single large nucleus makes its appearance and is situated centrally. This he thinks is the end of the cycle, as observable in cultures, though it probably is continued in the body of the intermediate host, whatever that may prove to be. These views will, of course, need full confirmation and all that need be said at present is that the temperature at which these experiments took place was far in excess of that used by any other worker and may have had a bearing upon the appearances which he has described.

*Animal Experiments.*—The virus of tropical sore, like that of infantile kala azar, has been successfully inoculated into both monkeys and dogs with the production of local lesions, closely resembling those which are found in man, in which the parasites are found in the cells and from which they may be cultivated.
Nicolle and Manceaux have succeeded in infecting these animals not only with material derived directly from a human source but also by means of the inoculation of the flagellated cultural forms, the lesions produced by these two methods being identical. They found, however, that it was impossible to produce any pathogenic effect by the intravenous inoculation of cultures which were only infective when rubbed into the skin. In the case of the monkey the site they found best was either the eyebrow or the skin at the root of the nose, and in this animal the duration of the lesion was only twenty-one days. The incubation period in the dog was about a month, and they were able to prove that one attack of the sore in this animal as a rule gave complete immunity against a subsequent inoculation of the virus; on the other hand, the intraperitoneal inoculation of even 100 cultures of the parasite failed to produce any disease or to give any immunity to those animals.

Row, working in India with material from human sores in Cambay, was also successful in infecting Macacus sinensis by rubbing the human material directly into a scarified surface, but he was not successful with the inoculation of cultures. He finds some differences in his monkey results as compared with those of Nicolle and Manceaux, in such points as the incubation period, the number of parasites in the lesion, the characters of the lesion, and in the fact that he found the animals susceptible to another infection during the progress of the first lesion. None of these differences, however, appear of great importance, and it is probable that extended experience will attribute them to differences in the susceptibility of the experimental animals or other variants.

A few of the other facts determined by animal experiments with L. tropica will be referred to later in connexion with the question of immunity.

Pathological Anatomy.—The histological characters of tropical sores and the distribution of the parasites therein has been the subject of elaborate studies by a number of observers, but no analysis of this side of the affection could be undertaken without unduly prolonging this article, and it appears the less necessary since the work of Bettmann and von Wasielewski, of Nattan-Larrier and Bussière, and numerous others, is readily available to those who would wish to go further into the subject. No more will therefore be attempted than reference to a few of the points recently brought to light, especially to such as have any relation to the problem of etiology.

In almost all instances the parasites are found inside cells and
only a few of them free, but since the studies on which such observations are based were mostly sections of tissue, it must be very hard to be certain as to whether a particular parasite is really extracellular or has merely been pushed out of position by the action of the knife. The nature and origin of the cells which harbour the parasites occupies a large portion of the contributions dealing with the histology of tropical sore, and all are agreed that most are mononuclear cells, though wide differences of opinion are held as to their nature and origin. Giant cells have been noticed in many cases, and in some material sent from Guiana parasites have been found in such cells by Nattan-Larrier, Touin, and Heckenroth. No mention of the occurrence of parasites in plasma cells has been made. As to their presence in leucocytes, a point of obvious importance in connexion with etiology, they are commonly seen in what are called large mononuclear leucocytes, and have also been found by several observers in polynuclear leucocytes. The latter site at once suggests the possibility of the parasites being carried by such cells into the circulating blood, but, although very careful search has been made for them in the peripheral blood, there is at present only one observation of their being found, Neumann recording their presence in blood drawn from the finger in a patient who had a tropical sore on the forearm of the same side.

The distribution of the parasites in the lesion differs according to the stage of the sore, and the common experience is to find them in greatest number in the cells at the margin of the lesion and in its depths, when the boil is young and especially before it has ulcerated. In contrast to this, however, Marzinowsky states that he found them most numerous in older lesions, and especially in those which showed a tendency to cicatrization.

The gross characters of the lesions have been often described, but, now that the presence of the parasites gives certainty of diagnosis, the differences which are being recorded assume a greater importance, and there is an obvious tendency nowadays to believe that it will be necessary to subdivide these cutaneous forms of Leishmaniosis. The non-ulcerating form described by Thomson and Balfour in the Sudan may perhaps be taken as the type of one of these future subdivisions, and there is much in support of the views of those who think that the American cases will also prove to be due to a different species. At the present moment, however, there is no evidence strong enough to admit of any such subdivision of *L. tropica* into sub-species, or new species.
Another point of interest is that the sores may sometimes be found on mucous membranes as well as on the skin; this has been recorded by Cardamitis and Melissidis in a case which suffered from no less than thirty-five boils on the face and arm and in which the mucous membrane of the lip was also involved, while Carini, in connexion with the American form, says such cases are not uncommon and that ulcerations of the palate and the buccal and nasal mucous membranes, in which *Leishmania* can be demonstrated, are graver in their nature than similar lesions of the skin.

**Etiology.**—Recent knowledge, although full of facts which are suggestive, has afforded no definite explanation of the etiology of tropical sore. In this instance we know neither the alternative host, if one exists, nor the transmitting agent, which almost certainly must exist. The developments in connexion with infantile kala azar and its apparent mode of infection naturally direct attention to the dog and the dog-flea, especially since the two diseases are found side by side in many places, but, in spite of the fact that it has been found possible to transmit the disease to the dog by inoculation, there is no evidence of the existence of tropical sore as a spontaneous infection of these animals. The only exception is a very doubtful one. A large number of sores were found on an emaciated and moribund dog by Dschunkowsky and Luhs, and after death they found the organs heavily infected with *Leishmania*. Although some parasites were found in the sores it may only have been a case of canine kala azar.

The well-known fact that the sores are almost always on exposed parts of the body has influenced the many theories which have been advanced as to the transmitting agent, and there is hardly a biting parasite or insect which lacks an advocate.

The house-fly is thought by Row to be the carrier, chiefly on account of the coincidence of its seasonal prevalence with the most frequent period of infection at Cambay, and a similar view is expressed by Cardamitis and Melissidis, who support it by the results of some experiments in which they allowed *Musca domestica* to feed on a sore containing the parasites and believed that they got evidence of a certain degree of development of the parasites in the gut of such flies.

*Phlebotomus* has the support of Thomson and Balfour, and of Sergent (commenting on a paper by Cambillet), while Wenyon, who has made a very careful study of the question in Bagdad, also states that he is unable to exclude this insect.
Simulium has its advocate in Fink, and mosquitoes have frequently been suggested. In connexion with the latter, Wenyon quotes many facts observed by him at Bagdad pointing in the direction of Stegomyia as the possible carrier; among others, that he has by feeding experiments seen a certain degree of development of the parasite in the mid-gut.

Bugs cannot be said to have been altogether excluded, although the fact that they seldom bite exposed parts of the body is strongly against them. Thomson and Balfour suggest them as an alternative to Phlebotomus, as the agent in the causation of the non-ulcerating form they found in the Sudan, and there is an interesting observation of Billet’s of a man in Algiers who was bitten at night on the face by a bug, which he caught in the act, and who subsequently developed a sore at the exact spot.

It will be obvious from the above that we have still much to learn in connexion with the etiology of tropical sore and its possible relationship to kala azar. Nicolle and Manceaux sum up the situation well, speaking of the local reactions which sometimes result from inoculation of L. infantum, by saying, “Aucune conclusion définitive ne pourra être portée avant que nous ne connaissions par quel hôte intermédiaire s’opère le transport des deux Leishmania, et que nous puissions par la même juger expérimentalement des caractères de la lésion locale d’inoculation, déterminée par chacun de ces hôtes avec chacun des deux virus.”

Treatment.—In the past this has been notoriously unsatisfactory, there being no method which could be relied upon to produce rapid healing of the sores. It is fortunate that they have a natural though dilatory tendency towards spontaneous cure, but the average duration is about six months, and the scarring and pigmentation which they often leave behind them is very disfiguring. It is much to be hoped, therefore, that further trial may be given to some of the methods which have been recently advocated and for which better results are claimed.

The drastic procedure of excision of the sore has still its advocates, including Nicolas and Wenyon, but it is agreed that this should only be practised if the sore is single and that it should be made to include apparently healthy tissue round the sore. If this is not done the sore may reappear in the cicatrix in a few days, as happened to Marzinowsky. The method has also the disadvantage that, if practised too soon, the individual is left without protection and may be re-infected. Free curetting should be carried out on the same principles and combined with the application of powerful disinfectants.
A new line of treatment has been recommended by Billet. The sore should be dusted with potassium permanganate, with the object of killing pyogenic organisms and, a few days later, a 10 per cent solution of methylene blue should be applied, with a view to the destruction of the protozoa. This method has also been found good by Nicolas, who recommends in addition the use of picric acid as an antiseptic, cicatrisant, and keratoplastic agent.

Another method is that of Gueyat, who advises that the crusts should be softened and the exudates eliminated by hot starch poultices kept on for twelve to twenty-four hours; next day the wound should be carefully cleaned of all debris with swabs and forceps and touched with oxygenated water or a 1 per cent solution of permanganate of potash. This is repeated until the wound is perfectly clean, when the following ointment is applied, covered with dry gauze and protected:

Salicylate of methyl  ...  ...  ...  ...  ...  5 grm.
Salicylate of bismuth  ...  ...  ...  ...  ...  2''
Subnitrate of bismuth  ...  ...  ...  ...  ...  1''
Oxide of zinc  ...  ...  ...  ...  ...  20''
Glycerine  ...  ...  ...  ...  ...  10''

Healing is usually very rapid, and he has never seen a case resist longer than three weeks.

Finally, Nicolle and Manceaux speak highly of the use of arsenobenzol. They tried it in two cases; to the first they gave 30 cg. with a partial improvement, and to the second 60 cg. with very rapid improvement.

In concluding this review of kala azar and tropical sore it has been thought well to deal separately with two points whose interest is common to each form of infection. These are, the demonstration of the parasite, and immunity.

The Demonstration of the Parasite.—In tropical sore this is a matter of comparative ease; the lesion lies open to investigation and it is merely a question of a little experience and a satisfactory technique to stain and identify L. tropica. This is, however, far from being the case in Indian or infantile kala azar, and however convincing the clinical picture, there can be no certainty of diagnosis until the parasites have actually been seen.

The means at our disposal for such demonstration are as follows:
(1) Examination of the peripheral blood. (2) Vesication. (3) Spleen
puncture. (4) Hepatic puncture. (5) Marrow puncture. (6) Cultivation. Each of these will be briefly considered.

(1) Examination of the Peripheral Blood.—The explanation for the general failure of this method in kala azar is twofold: the parasites, if present at all, are very rare and, since they are encountered in cells and not free, the intense leucopenia which is usually found adds greatly to the difficulties of the search. In the Indian form very few positive results have been mentioned, with the striking exception of Donovan's success at Madras; as already mentioned, he has demonstrated their presence in the peripheral blood in 93.2 per cent of his recent Madras cases. On the other hand, Prashad records 213 examinations of finger blood with only one positive result, and the latter's experience is in accord with that of most observers. Donovan's technique, therefore, demands consideration and imitation. He compresses the pulp of the finger-tip for a minute before puncturing, in order to increase the number of the leucocytes, and then prepares several films in such a manner that the end of the film ends as a straight line and not in the customary "tails." The leucocytes will be found congregated in this zone, which facilitates their examination. Several slides should be searched exhaustively, and if negative the examination must be repeated at intervals of a day or two.

Nicolle and Comte advocated the collection of 1 c.c. to 2 c.c. of blood, which should be citrated and centrifuged, as in the usual opsonic technique, and the examination of the superficial layer of cells which contain an abundance of leucocytes. In a later communication, however, they record that this has yielded them no better results.

(2) Vesication.—The production of a blister by means of a vesicant applied to the skin was originally suggested by Cummins, and the writer is glad to take the opportunity of emphasizing this since it has erroneously been attributed to him by Nicolle and Comte. The object is to secure the examination of a larger number of leucocytes than can be done even by prolonged blood examination. It has not been very extensively tried, but several positive cases have been recorded in the infantile disease as well as in the experimental disease of the dog.

(3) Spleen Puncture.—This is certainly the method most commonly in use and the most reliable one, but it has the grave disadvantage that it is not altogether free from danger. A certain number of instances of fatal haemorrhage have followed its practice, and these have led some workers to abandon it altogether. At the
same time many who have extensive experience of it consider it free from danger if certain precautions are observed. Nicolle and his colleagues have not mentioned any accident; Jemma and di Cristina have punctured 200 spleens and Bousfield 120 without trouble, and many others report similar favourable results. The following precautions should be observed: the spleen should be fixed as far as possible during a deep inspiration, if dealing with an adult; a fine, and preferably new, steel needle should be employed; both syringe and needle should be perfectly dry (to obviate haemolysis), and no more than a drop or two of blood should be taken; even if no blood appears to enter the syringe there will almost always be sufficient in the needle of the syringe to make films for examination. The operation should be carried out rapidly and the syringe should not be held too tightly lest a sudden expiration should threaten laceration of the splenic tissue. Pressure may be kept up with the finger on the site of puncture for a few minutes and the patient made to lie down for some hours. Preliminary treatment with calcium chloride is advisable if there is any reason to think that the blood is defective in coagulability.

(4) Hepatic Puncture.—This method is recognized as being freer from risk than splenic puncture, but it has the disadvantage that it may yield negative results even when parasites are abundant in the liver. The syringe fills as a rule only too readily with blood, and it may safely be said in these operations the more blood the less chance of finding parasites. What is desired is a fragment of hepatic tissue, and it is not easy to obtain this by simple puncture. At the same time, it is sometimes advisable to carry out hepatic puncture, and there is no objection to this provided that too much reliance is not placed upon a negative result.

(5) Marrow Puncture.—This method, though largely employed in the case of experimental animals, has been little used in man. Donovan, however, advocates the perforation of the head of the tibia or of the rib with a small gimlet, which enables a sample of the marrow to be withdrawn for examination. An anaesthetic, however, is necessary, and the procedure does not appear free from danger.

(6) Cultivation.—This has been very little tried, but, in view of the ease with which cultures are obtainable from the spleen, at all events in the infantile disease, it might be more widely tried as a means of detecting parasites in the blood, when none can be found by direct examination. Novy, for instance, got a positive result in an infected dog when he failed to find parasites on microscopic examination.
Immunity.—In common with other protozoal diseases we possess no precise knowledge of the nature of the immunity in *Leishmania* infections. That such immunity does occur has long been known in connexion with tropical sore, in which recovery results in a very marked and durable protection against subsequent infection; but the avenues of research which are open to us in investigating bacterial immunity are here closed or non-existent. Whatever changes occur in the tissues or fluids of the body in an immunized animal or man, we are not yet able to demonstrate or measure them by any of the methods used in bacterial investigations. Agglutination experiments with cultures are negative in their results, on account of the tendency of the parasites to spontaneous clumping, and experiments to demonstrate the presence of antibodies by complement deviation, using extracts of organs rich in parasites as antigen, have in Cannata’s hands given negative results.

What information is available, then, has been derived from observation of the diseases in man and animals and from immunization experiments on animals. As regards natural immunity, acquired by a previous attack, this, as has been said, is a well-established fact in the case of tropical sore, and has also been borne out by experiments on dogs, where it has been proved by Nicolle and Manceaux that, after complete recovery from an experimental infection, the animal is no longer susceptible. At the same time, they have found that it was not possible to induce this immunity by the inoculation intraperitoneally of large doses of the cultural forms. Apparently, the monkey, when recovered from an experimental sore, has not attained a similar degree of immunity, since it has been found possible to re-infect it soon after the attack. For the establishment of this acquired immunity sufficient time must elapse, and attempted re-infections shortly after recovery, or before this was complete, have shown that there exists a degree of hypersensitivity to infection.

Recovery is unfortunately so rare in human kala azar that we have little knowledge of natural acquired immunity in this instance. Still, cases undoubtedly do recover spontaneously, which proves that there must have been sufficient immunity produced in such cases to destroy the parasites and bring the disease to an end. The only observation as to the duration of such immunity is that of Muir, whose successful treatment of Indian kala azar has already been mentioned; he states that recovered cases are liable to re-infection but have a milder attack. It is open to doubt whether such cases were true recoveries and whether it may not have been possible that the disease was only in abeyance.
The part played by the phagocytic cells of the host is obviously one of great importance, and it appears legitimate to attribute to phagocytosis an important rôle in both protection and recovery from infection. It is evident that all forms of the parasite, flagellated as well as non-flagellated, are readily taken up by all cells which possess this power, and it is equally evident that these cells are, in most cases, unable to destroy them. The essential factor, then, in successful immunity may be an improvement in the activities of these cells, which enables them not only to ingest the parasites but also to destroy them by intracellular digestion. The writer has mentioned the experiments in which he observed in vitro phagocytosis of the flagellate forms of Leishmania infantum and their complete disintegration within human leucocytes, and this is quite consonant with the general failure to cause experimental infection by means of this stage of the parasite; it would be interesting to try similar experiments with the flagellate forms of L. tropica, with which experimental infection is possible.

Very interesting results have been obtained by Nicolle and his colleagues in cross-immunization experiments with L. infantum and L. tropica. They find that recovery from an attack of kala azar protects an animal against infection with the virus of tropical sore, and have also found evidence of some degree of immunity against kala azar in a monkey which had recovered from an experimental tropical sore. They have also numerous observations on the effects of passage of each of these viruses through dogs and monkeys upon the virulence of the strain, but the varying degrees of susceptibility of these animals naturally makes them guarded in their conclusions.

The general trend of these experiments shows a close relationship existing between the different species, and it is possible that, with further experience, we may be able to take advantage of this, both for prophylactic and therapeutic purposes.

[For the references, 250 in number, the original paper should be consulted.—Ed.]