LEISHMANIA DONOVANI AND LEISHMANIA TROPICA.*

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In 1904, a year after the discovery of the Leishman-Donovan body by Leishman¹ and Donovan,² Rogers³ grew this parasite for the first time in culture, and demonstrated its various developmental forms up to its flagellate stage. The results obtained by Rogers in Calcutta were confirmed subsequently by Chatterjee,⁴ Leishman and Statham,⁵ Christophers,⁶ and others. All these cultures were made in citrated blood with a variety of additions and modifications, but were not apparently available for successive cultures, the culture dying in the course of a week or two, although in one instance Leishman and Statham⁷ have recorded a successful subculture.

To Ch. Nicolle⁸ of Tunis belongs the credit of perfecting a technique by means of which it is possible to keep up indefinitely a series of successive cultures in a medium which is practically uniform in composition and available in any quantity. This medium has replaced in *Leishmania* culture work the original Novy-McNeal medium, in which Nicolle himself grew his first cultures of the *Leishmania* of infantile kala-azar of Tunis. It is not necessary to describe the details of preparing this well-known medium beyond stating that it is a simple agar without peptone or meat extract containing 25 per cent. rabbit's blood, and when prepared according to the formula⁹ given by Nicolle,⁹ has a fair amount of condensation fluid in which these parasites grow when planted. It is in the same medium that Nicolle cultured *Leishmania tropica* for the first time from material derived from Oriental sores in Tunis in 1908. Later, Row¹⁰ cultivated independently the parasite of Oriental sore of Cambay (India) in dilute human blood, and described the various developmental stages of the parasite up to the fully formed herpetomonas-like flagellate. Although this medium answers well for developing the various stages of this parasite as well as that of kala-azar (India), as will be seen presently, it is found difficult, from a practical point of view, to keep a regular supply of it in a constant, fresh, and sterile condition, such as would be available for successive cultures. It is on this account, I believe, that, as I stated in my last memoir¹¹ on this subject, I had failed to keep up a serial culture of the parasite. However, since reading this paper before you, I have again persevered with this attempt, but for success I have had to make use of the Nicolle-Novy-McNeal medium for continuation of successive cultures. Of late years the study of *Leishmania* cultures has been so repeatedly undertaken, and by so many workers all over the world, as you will see by a reference to the *Kala-Azar Bulletin* before you, that I felt a certain amount of hesitation to come before you with the same subject again, but the following one or two considerations may justify my doing so.

In the first place the parasite of Indian kala-azar has been pretty frequently cultured in the Novy-McNeal medium, and also in Nicolle-Novy-McNeal medium, both in India and in England, but always unsuccessfully (except in the one case of Leishman and Statham above referred to), and this constant failure to culture by workers in India on the Indian kala-azar in the Novy-McNeal-Nicolle medium, and also the like failure of Nicolle in culturing the Tunisian parasite of the infantile kala-azar in the medium recommended by Rogers, as also the failure to infect animals with the *Leishmania donovani* of Indian kala-azar, had led to the suggestion that there may be, after all, two species of this kala-azar parasite—one the Indian and the other that of infantile kala-azar. In the face of this suggestion it may be of interest to note that from the material of a splenic puncture in a case of kala-azar at present in the Jamsetjee Jeejeebhoy Hospital in Bombay, I have succeeded in growing in successive culture (four generations up till now) in the Novy-McNeal-Nicolle medium in the same way as I have done with the parasite of the Oriental sore of Cambay, thanks to Nicolle's technique. Not only is it, then, possible

to obtain the cultures of the Indian *Leishmania donovani* just as those obtained by Nicolle with the *Leishmania infantum*, in the Novy-McNeal-Nicolle medium, but also, contrary to Nicolle's observation, I find no difficulty in obtaining good developmental forms and flagellates in the citrated blood (2 per cent. sodium citrate in 0.8 per cent. NaCl). The result of the experimental infection in the monkey is being watched with interest, as a positive result would once for all remove one of the strong grounds for the supposition of the two parasites of kala-azar being distinct, and this result will be communicated at the first opportunity.

Further, it may be noted that these cultures, grown in identical conditions from the two different parasites, may help us to decide the identity or otherwise of our Indian parasite from those of North African and Mediterranean variety, and even to bring out further differences, if any, of the two kinds of *Leishmania* producing such two clinically different types of disease in India.

Secondly, it had been pointed out by myself¹⁰ and other workers that one of the points of difference between the *Leishmania donovani* and *Leishmania tropica* is the temperature conditions, the former thriving best at a lower temperature than the latter. In the light of the present observations, however, I find there is no need to lower the laboratory temperature to 22° C., as recommended by Rogers for his acidified citrated blood medium, nor for the range found best by Nicolle⁸ in Tunis, namely, 18° to 22° C. I find that the laboratory temperature in Bombay at all times of the year—including the hottest day of October or May, 28° to 32° C.—is quite good for *Leishmania tropica*, and that these as well as *Leishmania donovani* also do well during December and January temperatures, namely, 26° to 28° or 30° C. On the other hand, a lowered temperature, 18° to 20° C., in the ice box decidedly retarded the development of my culture of *Leishmania donovani*, so that what took place in four or five days took quite eight to ten days in the ice box at 18° to 20° C.

Thirdly, I find that my observations harmonize with those of Nicolle, inasmuch as that *Leishmania donovani* and *Leishmania tropica*, when planted in Novy-McNeal-Nicolle medium, develop practically in the same manner. I find no necessity of citrate nor of acidification¹² for my cultures, as recommended by Rogers. Acidification in a few tubes of my experiment has had a decidedly harmful effect on a vigorous growth; at any rate, it has been so with the few observations I have made on this point, and I have no intention just at present to test the utility or otherwise of the acid, as the cultures made in the plain Novy-McNeal-Nicolle medium are abundant and vigorous, as you will see from the living specimens under the microscope.

One other point of some difference, seen in the light of my present observation, to those already recorded by Nicolle and other observers, is in reference to the time limit. I find that for *Leishmania tropica* of the Cambay sore, from the fifth to the eighth day is the time when the parasites are at their best. They show decided signs of degeneration about the fifteenth day, and by the twenty-fifth day they are so far gone that I should not recommend this period for a reculture. The rosettes make their appearance soon after the flagellates are fully formed, and are fairly tired of dividing and subdividing, and I have seen them as early as the third day in subculture, but they are best seen, as stated above, between the sixth and eighth days. (Nicolle's cultures commence to grow about the seventh day, are abundant on the fifteenth, and live for six weeks.⁸) I believe the same rule applies to *Leishmania donovani*, but I am not able to give definite figures on this point, as I am engaged now with only young cultures, of which I have now reached the fourth generation, and my observations on this point are at present incomplete.

Comparison of Cultures of Leishmania Donovanii (India) and Leishmania Tropica Grown in Dilute Human Blood up to Flagellation.

Leishmania donovani grown in (non-acidified) diluted human blood (four times citrated saline) shows nothing different from what has been observed previously by other workers on this subject, and if I may compare these changes with those taking place in the same medium for *Leishmania tropica* in the early stages of development I

* Read at a meeting of the Bombay Branch of the British Medical Association on January 4th, 1912.

find nothing beyond a slower growth and a growth more or less in all dimensions than in any one particular direction. Thus in a twenty-four hours' culture of *Leishmania donovani* the best attempt at elongation results in the production of an ovoid body. One other feature noticeable, even at this early period, is the presence of a large number of fine vacuoles in the body protoplasm of the parasite, while, as I pointed out elsewhere, the parasite *Leishmania tropica* becomes distinctly oval or oat-shaped, pointed at one extremity and larger, and even in twenty-four hours shows distinct signs of division; it also looks less spongy. After this stage, from the third till the fourth day or longer the preflagellates of both the parasites grow more in the longitudinal direction and at last go into flagellates; the only difference noted, even here, is the slow nature of growth of *Leishmania donovani* and the stunted appearance of the flagellates in comparison with those of *Leishmania tropica*. All these cultures were grown at the laboratory temperature, 26° to 30° C. These slight differences, as I pointed out, may be only apparent and not real, and may be attributable to the slight differences of the culture medium, and this is why it is important to study the cultures of the same age grown in the same medium—for example, the Novy-McNeal-Nicolle medium.

Growth of the Parasite in Novy-McNeal-Nicolle Medium.

Here are the specimens of the growth of the two parasites in a five days' culture at the same temperature of the laboratory, and I think it best to compare the figures of the parasites drawn with the same magnification (Zeiss $\frac{1}{2}$ apochromat and No. VI eye-piece) with the aid of camera lucida. It think this is far more instructive than any amount of description. I may, however, summarize the differences as follows:

1. Abundance of the growth of *Leishmania tropica* during the same period compared with that of *Leishmania donovani*. It is clear that the latter is more delicate and offers greater resistance in artificial cultures than the former.

2. The size of *Leishmania donovani* is distinctly smaller than that of the other of the same age.

3. The posterior extremity of *Leishmania donovani* is distinctly more pointed than that of any other.

4. The abundance of fine vacuoles in the fully formed flagellates of *Leishmania donovani* in comparison with those of *Leishmania tropica*.

5. At certain stages of development *Leishmania tropica* is characterized by the appearance of fine chromatin particles distributed in the body of the parasites, just as *Leishmania donovani* is hollowed out by the presence of fine vacuoles.

These last two, however, may be purely accidental.

Post-flagellate Stage of *Leishmania tropica*.

In a former communication¹¹ I had pointed out that I found it impossible to obtain serial culture of these parasites, and nothing definitely could be made out after the parasite had once gone into the flagellate stages. Since reading this paper, however, I have succeeded, as stated above, in obtaining successive culture of this body in Novy-McNeal-Nicolle medium, and have now reached the twenty-fifth culture. No better conditions seem to be required than this for studying the post-flagellate stages. The culture is rich in flagellates about the fourth day; and even by this day small rosettes seem to come into view. It appears that after the flagellates have divided and subdivided a time is reached when the parasites find it impossible to continue this mode of multiplication, and the first thing observed is a tendency for the flagellates to agglomerate together and form smaller or larger rosettes. These are formed by the flagellates grouping themselves round a centre, a centre of attraction, with their flagella or their anterior extremities directed towards this centre, so that a colony of these agglomerated flagellates is so aptly described as a rosette by Nicolle. It is possible to see a small rosette consisting of six to eight flagellates, but the rule is to find rosettes of enormous size—about the sixth day of culture—made up of countless numbers of flagellates radiating from the centre. Once the rosette is formed, by some subtle influence the individual flagellates begin to fatten, so that each individual appears like a still amoeboid mass, the nucleus, the micronucleus, and

the vestige of the flagellum being still quite clearly made out. This may come about even as early as the fifth day, and then the parasite shows a fair number of fine chromatin particles. For further changes, however, older cultures, such as those of fifteen to twenty days, are required. In these it is not difficult to demonstrate a differentiation of the protoplasm of the amoeboid body into definite lumps of more deeply-staining material embedded in a lighter matrix, and from these seems to be derived a mass of what ultimately turn out to be the $\odot$ bodies of the parasite. Thus it would appear that even in cultures the original body stage is brought about. In some specimens, however, the division of flagellates into flagellates seems to be persevered in with such a pertinacity that, even when the flagellates fail to divide to reproduce their own former selves, flagellates of a stunted nature are produced, and even these stunted progeny of the original flagellates, though not much larger in size than that of the body described above, still continue to retain the vestige of the flagellum, as seen in the figures shown. How far these last contribute to form the $\odot$ bodies by dropping off the vestige of the flagellum, and to what extent the others described above contribute to the same end, is more than I can say at present.

The post-flagellate stages of *Leishmania donovani* are now being studied, but up to now my cultures have not grown strong enough to stand the test of being watched beyond ten days. The development up to ten days is shown to you both in the plates and in the specimens.

Should the post-flagellate development of the *Leishmania donovani* correspond with that of *Leishmania tropica* described above, it is needless to say that the two parasites run very close to each other in morphology and physiology outside the human body. It is remarkable that bodies so closely allied should be productive of such colossal clinical differences when they enter and get a foothold into the human system.

Before concluding I desire to express my deep sense of gratitude to Colonel C. H. L. Meyer, I.M.S., for his kindness in letting me have the material for work, and also for allowing me free access to the case of kala-azar under his care. But for this kindness this work could not have been undertaken. I also take this opportunity to thank Dr. Velankar, Dr. Date, and Dr. Soparkar for working with me at considerable self-sacrifice and personal inconvenience during the Christmas holidays, when this work had to be pushed, on account of the unique opportunity for work offered by this kala-azar case under the care of Colonel C. H. L. Meyer at the Jamsetjee Jeejeebhoy Hospital in Bombay.

Many microscopical slides and plates were shown, but owing to want of space they could not be embodied in this place.

REFERENCES.

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- ⁷ Leishman, Quarterly Journal of Medicine, September, 1911.
- ⁸ Ch. Nicolle, Annales de l'Institut Pasteur, May, 1909.
- ⁹ Ch. Nicolle, Archives de l'Institut Pasteur de Tunis, 1909, and Comptes Rendus de l'Académie des Sciences, 1908.
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At a special meeting of the West London Medico-Chirurgical Society on Friday, April 12th, at the West London Hospital, there will be demonstrations and a discussion on rheumatoid arthritis. The demonstrations will commence at 8 p.m., and will include the exhibition of cases illustrating: (1) Causation, (2) joint lesions, (3) trophic lesions, (4) skin affections, (5) eye affections; (6) diagnosis from (a) tuberculous joints, (b) neuropathic joints, (c) osteoarthritic conditions, (d) gonococcal arthritis, (e) syphilitic arthritis. Skiagrams illustrating: (1) Changes in cartilage, (2) changes in bone, (3) deformity. Museum specimens, illustrating: (1) Lesions of cartilage, (2) lesions of bone, (3) lesions of soft tissue. Bacteria illustrating: (1) Oral sepsis, (2) alimentary infection, (3) genital infection. In the discussion, which will commence at 9 p.m., the following will take part: The President (Mr. Wm. McAdam Eccles), Dr. F. J. Poynton, Dr. T. J. Horder, Dr. Reginald Morton, Mr. Lloyd Williams, Mr. W. J. Midelton, Dr. Bernstein, Dr. F. S. Palmer.