

validity of this ratio as the governing factor of osmotic activity.

On the basis of the assumption that the above laws are valid for blood, mathematical expressions have been derived which indicate the distribution of electrolytes and water between cells and serum, and the manner in which the distribution is affected by changes in P_H (CO_2 tension) and oxygen content. The effect of varying CO_2 tensions has been investigated, and the results have been found to accord with those calculated on the basis of the above assumption.

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Leishmania donovani in the peripheral blood.

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Donovan¹ who found *Leishmania donovani* first in the living patient also discovered the parasite in smears from the peripheral blood. Many have advocated the examination stained blood smears for diagnosis of the disease (Patton,² Marshall,³ Cannata,⁴ Knowles⁵) but all agree that the organism is present in small numbers and that many slides must be carefully studied. Spleen smears obtained by aspiration has been the usual method for diagnosis. As the bleeding time is prolonged in all advanced cases this procedure is not devoid of danger (Donovan,¹ Wylie,⁶ Knowles,⁷ Rogers,⁸ Bramachari⁹). Blood culture has been suggested as a substitute but results have been inconstant (Mayer and Werner,¹⁰ Wenyon,¹¹ Row,¹² Korke,¹³ Knowles⁵). The last named author made 128 cultures from 34 patients with two posi-

¹ Donovan, *Lancet*, 1904, ii, 744.

² Patton, W. S., *Indian Jour. Med. Res.*, 1914, ii, 492.

³ Marshall, W. E., Fourth Report, Wellcome Trop. Res. Labs., Khartoum, 1911, 157.

⁴ Cannata, S., quoted by Wenyon, *Trop. Dis. Bull.*, 1922, xix, 1.

⁵ Knowles, R., *Indian Jour. Med. Res.*, 1920, viii, 162.

tive cultures from one patient. At first Rogers merely citrated the patient's blood but Nicolle was much more successful with his "N.N.N." medium, a rabbit blood agar. The experiments of Rogers¹⁵ and of Cornwall and LaFrenais¹⁴ showed that culture media made with human blood were not favorable to the growth of *Leishmania donovani*.

Two series of experiments were made using salt agar as in N.N.N. medium but with washed red cells and serum, both unheated and heated at 56 C° for one-half hour, from man, horse, sheep and rabbit. Human red cells were somewhat inhibitory; human serum much more so. Rabbit cells and serum were less unfavorable to growth but were inferior to whole defibrinated rabbit blood. In action the cells and serum of the horse and sheep lay between those of man and the rabbit. As a result of these experiments, a technic for blood culture was devised as follows: 10 c.c. of blood was drawn from a vein into 2 c.c. of 1 per cent. citrated Locke's solution in a syringe and immediately expelled into 50-70 c.c. of the same fluid in a flask. On return to the laboratory, the diluted blood was divided between two 50 c.c. centrifuge tubes and spun slowly (about 750 r.p.m.) for five minutes. The blood was thus divided into a supernatant cloudy fluid and a sediment of red cells. The cloudiness of the first is caused by blood platelets and some red cells. Extracellular Leishman-Donovan bodies were occasionally found in this fraction. After rapid centrifugation (about 1400 r.p.m. for five minutes) the sediment from this upper portion was planted into N.N.N. medium of about P_H 7.6. Using this technic, 115 culture tubes were made from 34 blood specimens from untreated cases of kala-

⁶ Wylie, J. H., *China Med. Jour.*, 1920, xxxiv, 593.

⁷ Knowles, R., *Indian Jour. Med. Res.*, 1920, viii, 177.

⁸ Rogers, Sir Leonard, Fevers in the Tropics, Third Ed., p. 48.

⁹ Bramachari, A. N., *Kala-azar and Its Treatment*, Butterworth, 1920, p. 99.

¹⁰ Mayer, M., and Werner, H., *Deutsch. Med. Wchnschr.*, 1914, xl, 67.

¹¹ Wenyon, C. M., *Jour. Trop. Med. and Hyg.*, 1914, xvii, 49.

¹² Row, R., *Indian Jour. Med. Res.*, 1914, ii, July editorial.

¹³ Korke, V., noted in Cornwall and LaFrenais, *Indian Jour. Med. Res.*, 1915-16, iii, 398.

¹⁴ Cornwall, S., and LaFrenais, H. M., *Indian Jour. Med. Res.*, 1915-16, iii, 398.

¹⁵ Rogers, Sir Leonard, *Lancet*, 1919, i, 505.

azar. Thirty-one of the specimens showed flagellates (91 per cent.), 82/115 culture tubes being positive. 134 cultures were also made from forty specimens of blood from kala-azar patients treated with several forms of antimony. When these were arranged in ascending order of doses of milligrams of antimony per kilogram weight of patient it was found that up to a total dosage of 12 mg. (0.162 to 11.96 mg.) positive cultures were obtained in 60 per cent. of the fifteen cases and in 22/59 of the culture tubes. Beyond that point, *i. e.*, doses of 20.66 to 77.64 mg., seventy-five cultures from twenty-five specimens were uniformly negative. Three similar series were made from spleen juice. The first showed twenty out of twenty-two (91 per cent.) specimens positive (68/108 tubes) in untreated cases. The nine specimens from patients treated up to 12 mg. (0.05 to 10.2 mg.) per kilogram of their weights had seven positive (77 per cent.) with 30/56 of the tubes showing growth. Only three spleen cultures were made from patients who had received more than 12 mg. (12.9, 30.7 and 95.87 mg.) All were negative. Comparison was made between the results obtained by the author's technique (Series A) and that of Row¹⁶ (Series B). A third series (Series C) was made from the cloudy fluid above Row's sediment after centrifugation. Series A showed 30/69 (43.5 per cent.) tubes positive; Series B, 16/46 (34.8 per cent.) and Series C 10/43 (23.3 per cent.) The only obvious difference between Series A and C was that in the latter, the material had stood in contact with the diluted human serum for from 15 to 20 hours. The greater number of positive cultures in Series B than in Series C suggested a search of the layers of the red cell sediment obtained after slow centrifuging of the diluted blood specimen.

In the bottommost part of this sediment both mononuclear and polymorphonuclear leucocytes were found and in a few of them, Leishman-Donovan bodies. Of twenty-seven smears made from nine blood specimens from five untreated kala-azar patients twenty-one showed intracellular parasites. Of twenty-six slides from seven specimens from four cases treated with 0.49 to 5.13 mg. of antimony per kilogram of patient's weight fifteen gave

¹⁶ Row diluted $\frac{1}{2}$ -2 c.c. of blood in 20 c.c. of citrated saline and after sedimentation over night, planted the sediment in N.N.N. tubes.

positive results (*i. e.*, 5/7 specimens positive). None of the seven blood specimens from six who had received 28.68 to 77.64 mg. of antimony per kilogram of their weights showed any Leishman-Donovan bodies. The method followed was to withdraw a column of blood 1 to 2 cm. high in a fine capillary pipette from the bottom of the conical tube in which the diluted blood specimen was centrifuged. The smears were made on slides in the usual manner. Wright's or Leishman's stain was used.

1. The effect of human red cells and serum on the growth of *Leishmania donovani* has been studied and a blood culture method devised, based on these findings.

2. The distribution of blood platelets and of the different varieties of leucocytes in the strata of centrifuged diluted blood has been investigated and also the distribution of Leishman-Donovan bodies, extracellular and intracellular in these strata.

3. The effect of treatment with antimony on the cultivability of *Leishmania donovani* has been studied.

Conclusions

1. Human red cells in a culture medium permit only a feeble growth of *Leishmania donovani*.

2. Human serum, whether inactivated or not is distinctly inhibitory to the growth of this parasite.

3. If an adequate amount of blood is taken (10 c.c.) and most of the serum and red cells removed by centrifugation, positive cultures may be obtained in about 90 per cent. of untreated cases of kala-azar.

4. By this technic positive cultures may be obtained as frequently from peripheral blood as from spleen punctures.

5. Extracellular Leishman-Donovan bodies are approximately of the size and specific gravity of platelets and are found in the "platelet fraction" of centrifuged diluted blood.

6. Mononuclear and polymorphonuclear leucocytes with the contained intracellular forms are usually heavier than the other blood cells and go to the bottom of the tube on centrifugation or sedimentation.

7. In the series of cultures made, the blood of patients treated intravenously with antimony salts became free from cultivable *Leishmania donovani* after they had received about twelve milligrams of antimony per kilogram of their body weights.

8. Cultures from spleen punctures show similar results.