expect that the smaller particles of such a dissociated toxin might dialyze easier than the coarser aggregates of the crude toxin. The experimental evidence indicates the reverse to be the fact. While the crude toxin dialyzes with a comparative ease, the acidified toxin remains quantitatively inside the parchment thimble.

May not this phenomenon be explained on the basis of the theories offered by some physical chemists[3] namely, that while the coarser aggregates of protein carry no charge, the increase in the dispersion resulting upon dilution and especially upon acidification confers the electrical charge on such dissociated particles. In virtue of this charge these smaller particles are adsorbed to the membrane whereas the coarser particles of undissociated substance were able to go through the pores of the membrane.

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The diagnosis of kala-azar by blood culture.

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The usual method for diagnosis of kala-azar by means of cultures has been from spleen juice obtained by puncture and aspiration with a syringe and needle. Prolonged bleeding-time is fairly constant in advanced kala-azar. The risk of bleeding from the needle wound in the spleen together with the possibility of tearing that organ by a sudden movement of the patient during puncture, makes a safer method for cultural diagnosis desirable.

Meyer and Werner[1] reported in 1914, five successful cultures from a single specimen of blood. Row[2] and Korke[3] each report one. Cornwall and LaFrenais[4] succeeded in seven cases; however,

[3] Robertson, T., Brailsford. The Physiological Chemistry of the Proteins, 1918, p. 153.

[1] Meyer, M., and Werner, *Deutsch. Med. Wchnschr.*, 1914, xl, 67.

[2] Row, R., *Indian Jour. Med. Res.*, 1914, July (quoted in (4)).

[3] Korke, V., idem.

[4] Cornwall, J. W., and LaFrenais, H. M., *Indian Jour. Med. Res.*, 1915–16, iii, 698.

Knowles[1] in attempting to repeat the work using the methods of Row and of Cornwall and LaFrenais only obtained two positive cultures out of 129 tubes from thirty-four cases. Meyer and Werner, Cornwall and LaFrenais, and Knowles (54 cultures from 12 cases), simply added small amounts of blood to tubes of Nicolle-Novy-MacNeal medium ("N.N.N."). Row and Knowles (34 cultures from one case) added $\frac{1}{4}$ to 2 c.c. of blood to 20 c.c. of "citrated saline" and then distributed the sediment into N.N.N. medium after incubating the tubes for 24 hours at 22° C.

The low percentage of successful culture by Knowles shows that neither method can be relied upon to give constant results. We have confirmed the findings of Cornwall and LaFrenais[2] that the blood of man is unfavorable to the growth of *Leishmania* and have found that this is true not only for whole blood but for washed red cells and for serum whether fresh or heated for $\frac{1}{2}$ hour at 56° C. There was only an occasional feeble growth with blood and red cells, none with serum alone.

In order to free the blood from red cells and serum the following method was used: 10 c.c. of blood was drawn from a vein at the elbow into one or two cubic centimeters of citrated Locke's solution and immediately expelled into a flask containing 50 c.c. of the same fluid. This diluted blood was centrifuged at a low speed to throw down the red cells only. The supernatant fluid was transferred to another sterile 50 c.c. tube and centrifuged at a high speed. The sediment from this was distributed into tubes of buffered N.N.N. medium adjusted to varying hydrogen-ion concentrations and incubated at 22° C.

Culture Medium.—This was the Nicolle-Novy-MacNeal medium[3, 4] with the addition of 0.2 per cent. dipotassium phosphate (K_2HPO_4) as a buffer. The salt-phosphate agar was adjusted to hydrogen-ion concentrations varying from P_H 6.8 to P_H 8.2. Defibrinated rabbit blood was added in the proportion of one part of blood to three of agar. Leishman-Donovan bodies in spleen pulp and peripheral blood develop into flagellates throughout the range tested with little detectable difference. Cultures from

[1] Knowles, R., *Indian Jour. Med. Res.*, 1920, viii, 140.

[2] Cornwall and LaFrenais, *loc. cit.*, p. 299.

[3] Novy and MacNeal, *Jour. Inf. Diseases*, 1904, i, 1.

[4] Nicolle and Comte, *Bull. de la Société de Path. Exotique*, 1908, i, 299.

spleen and blood have not yet been attempted at higher and lower concentrations than those indicated. Flagellates grow on medium at least as alkaline as P_H 9.0. In all of these cultures post-flagellate forms may be found including definite Leishman-Donovan bodies. Other forms suggesting Cornwall's second type of "thick tails" [1] are occasionally met with. Cultures on buffered medium remain viable for a considerable time. A successful subculture has been made after 56 days although no flagellates were found in a drop of the fluid used for inoculation. Growth took place on the surface of the medium above the water of condensation, to a height of four centimeters in one instance. The surface of the medium was slightly dulled. Such surface growth seemed especially rich in rosettes and active flagellates.

The results from peripheral blood thus far cultured are as follows: Ten samples of blood have been taken from five different patients and distributed into forty tubes of buffered N.N.N. medium of various hydrogen-ion concentrations and incubated at 22° C. Twenty-nine of these cultures were positive (72.5 per cent.). One or more tubes from nine of the ten samples showed flagellates (90 per cent.). At least one culture was positive from each patient. One case gave positive results after having received 20 c.c. of 0.2 per cent. colloidal antimony sulphide eight hours before the blood was taken. Before the culture she had received in all 121 c.c. of the suspension, equivalent to 0.173 gram of metallic antimony injected intravenously over a period of fourteen days. Another patient had received intravenously 377 c.c. of a similar suspension, equivalent to 0.539 gram of metallic antimony, over a period of thirty-three days. The last dose of 30 c.c. was given three days before the culture was taken.

SUMMARY.

1. Human red cells and serum are unfavorable to the growth of *Leishmania donovani*.

2. A method is given for removing the red cells and serum from blood before planting.

3. By this method blood cultures have been obtained from nine out of ten samples of blood from five patients, some of them after considerable antimony treatment.

[1] Cornwall and LaFrenais, *loc. cit.*, p. 299.

4. A modified "N.N.N." medium is suggested.

5. On this medium the Leishman-Donovan bodies from spleen punctures or peripheral blood develop into flagellates at all hydrogen-ion concentrations tested, *i.e.*, between P_H 6.8 and P_H 8.2 and the flagellates grow at least to P_H 9.0.

6. Cultures on this modified medium show post-flagellate forms and perhaps Cornwall's "thick tails."

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Further observations on anaphylactoid phenomena from different agents, including histamin.[1]

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Of the twenty-five different agents which were injected intravenously into guinea pigs, the following caused anaphylactoid symptoms: Colloidal arsenic, kaolin, blood charcoal, colloidal iron, ten per cent. sodium chloride (?), tragacanth, toxified agar, lung extract, glacial acetic acid, copper sulphate, fuller's earth, sodium oxalate, sodium citrate, tannin, tartar emetic and histamin. Especially noteworthy were the results after injection of histamin, which produced symptoms with the very small dosage of 0.00011 mgm. per gram of animal. All of these agents, except the chloride and citrate, produced thrombi in the pulmonary blood vessels. The appearance of pulmonary thrombi (platelet) after the injection of histamin agrees with the observation of Dale and Laidlaw, who detected the presence of platelet thrombi in the blood of

[1] This investigation is supported by a grant from the Therapeutic Research Committee of the Council of Pharmacy and Chemistry of the American Medical Association.