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## Diagnosis of Leishmaniasis by Slide Agglutination.\*

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Numerous serological studies on leishmania infections have provided information on the following reactions: Agglutination,<sup>1-4</sup> complement fixation,<sup>4,5</sup> reversed complement fixation,<sup>6</sup> and intradermal tests.<sup>4,7-11</sup> Moreover, complement fixation,<sup>12,13</sup> precipitin,<sup>14</sup> agglutination,<sup>15</sup> and lysis,<sup>16,17</sup> as well as slide agglutination,<sup>17</sup> have been obtained in *Trypanosoma*

*cruzi* infection. In the present study slide agglutination in leishmaniasis as well as the relationship of homologous and heterologous immune sera to this reaction have been tested.

Antigens for immunization were prepared according to the writer's method.<sup>17</sup> Two rabbits each were immunized with killed *Leishmania brasiliensis*, *L. donovani*, *L. tropica*, and *T. cruzi* by intravenous injection 3 times a week. The initial dose of 0.5 cc was increased by 0.1 cc to 1 cc, which amount was then maintained until a total of ten injections had been given. A week later each animal was bled from the marginal ear vein and the titer of agglutinins determined. Immunization with living cultures 3 times a week was then carried out. The initial dose of 1/4 culture slant 4 to 6 days old was increased to 1 1/2 slants, with a total inoculation of 5 1/2 slants. A week later, the animals were again bled and the agglutinin titers were determined.

Slide agglutination antigen and "H" antigen were prepared as previously described;<sup>17</sup> "O" antigen, by adding gradually with constant shaking 70% alcohol to a saline suspension of organisms until the final concentration of alcohol was 50%. The suspensions were left overnight at room temperature, washed 3 times in physiological saline solution and then standardized. Slide and tube agglutination tests were performed according to approved technics.<sup>17</sup>

**Results**—Table I shows the titers of slide agglutination, and of "H" and "O" agglutination of rabbits immunized with killed and living cultures of the organisms tested.

**Conclusions.** Rapid diagnosis of visceral, cutaneous, and mucocutaneous leishmaniasis may be made by slide agglutination. "H" and "O" agglutinogens are present in all species tested. The homologous organism is agglutinated to a high titer, but co-agglutinins

\* Aided by a research grant to the Department of Tropical Medicine of Tulane University by Eli Lilly and Co. Thanks are extended to Dr. E. C. Faust for his valuable comments in the preparation of this manuscript.

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TABLE I.  
Agglutination Test in Rabbits.

| Rabbits immunized<br>with | <i>L. brasiliensis</i> |                 |       | <i>L. donovani</i> |                 |        | <i>L. tropica</i> |                 |       | <i>T. cruzi</i> |                 |       |
|---------------------------|------------------------|-----------------|-------|--------------------|-----------------|--------|-------------------|-----------------|-------|-----------------|-----------------|-------|
|                           | Slide                  | Antigen<br>"H," | "O"   | Slide              | Antigen<br>"H," | "O"    | Slide             | Antigen<br>"H," | "O"   | Slide           | Antigen<br>"H," | "O"   |
| <i>L. brasiliensis</i>    |                        |                 |       |                    |                 |        |                   |                 |       |                 |                 |       |
| 1. Killed                 | 1:640                  |                 |       | 1:320              | 1:400           | 1:200  | 1:80              | 1:400           | 1:200 | 1:80            | 1:200           | 1:100 |
| Living                    | 1:640                  | 1:800           | 1:200 | 1:320              | 1:400           | 1:200  | 1:80              | 1:400           | 1:200 | 1:80            | 1:200           | 1:100 |
| 2. Killed                 | 1:320                  |                 |       | 1:80               |                 |        | 1:40              |                 |       |                 |                 |       |
| Living                    | 1:1280                 | 1:1600          | 1:400 | 1:320              | 1:400           | 1:200  | 1:80              | 1:400           | 1:200 | 1:20            | 1:200           | Neg.  |
| <i>L. donovani</i>        |                        |                 |       |                    |                 |        |                   |                 |       |                 |                 |       |
| 3. Killed                 | 1:20                   |                 |       | 1:640              | 1:12800†        | 1:1600 | 1:20              | 1:200           | 1:200 | 1:2             | 1:400           | 1:100 |
| Living                    | 1:40                   | 1:100           | 1:100 | 1:2560             |                 |        | 1:20              | 1:200           | 1:200 | 1:40            | 1:400           | 1:100 |
| 4. Killed                 |                        |                 |       |                    |                 |        |                   |                 |       |                 |                 |       |
| Living                    | 1:40                   | 1:100           | 1:200 | 1:320              | 1:6400          | 1:1600 | 1:20              | 1:100           | 1:200 | 1:20            | 1:200           | 1:100 |
| <i>L. tropica</i>         |                        |                 |       |                    |                 |        |                   |                 |       |                 |                 |       |
| 5. Killed                 | 1:160                  |                 |       | 1:2                |                 |        | 1:160             |                 |       | 1:40            |                 |       |
| Living                    | 1:160                  | 1:400           | 1:400 | 1:20               | 1:100           | 1:200  | 1:640             | 1:800           | 1:400 | 1:80            | 1:200           | Neg.  |
| 6. Killed                 | 1:640                  |                 |       | 1:2                |                 |        | 1:20              |                 |       | 1:20            |                 |       |
| Living                    | 1:640                  | 1:800           | 1:200 | 1:40               | 1:200           | 1:200  | 1:640             | 1:800           | 1:400 | 1:40            | 1:400           | 1:100 |
| <i>T. cruzi</i>           |                        |                 |       |                    |                 |        |                   |                 |       |                 |                 |       |
| 7. Killed                 | 1:40                   |                 |       | 1:2                |                 |        | 1:40              |                 |       | 1:160           |                 |       |
| Living                    | 1:40                   | 1:100           | 1:100 | 1:20               | 1:200           | 1:200  | 1:20              | 1:200           | 1:200 | 1:1280          | 1:3200          | 1:200 |
| 8. Killed                 | 1:40                   |                 |       | 1:160              |                 |        | 1:20              |                 |       | 1:320           |                 |       |
| Living                    | 1:40                   | 1:400           | 1:200 | 1:320              | 1:400           | 1:400  | 1:40              | 1:200           | 1:200 | 1:1280          | 1:3200          | 1:200 |

are present in low titer to the heterologous organisms. *L. donovani* and *T. cruzi*, responsible for systemic infections, produce higher

titers than *L. tropica* and *L. brasiliensis*, which have only cutaneous or mucocutaneous manifestations.

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### Effect of Adrenocorticotrophic Hormone on Leucocyte Picture of Normal Rats and Dogs.\*

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Experimental evidence is available which indicates that stimulation of the adrenal cortex by injection of adrenocorticotrophic hormone (ACTH) produces a marked decrease in the weight of the thymus and lymph nodes in the mouse<sup>1</sup> and in the rat.<sup>2</sup> Subsequent studies by Dougherty and White on the white blood cells in the mouse and rat<sup>3</sup> and dog<sup>4</sup> after such treatment have demonstrated a typical change in the leucocyte picture characterized by transient leukopenia with an absolute lymphopenia and an absolute polymorphonuclear leucocytosis. The reaction was thought to be specific in that the effects were not observed in the treated adrenalectomized animal, and were not duplicated by the injection of control substances. The same response, moreover, was produced by the injection of adrenal cortical extract into normal and adrenalectomized mice.

Certain of the above findings studied in this laboratory have been confirmed. Acute and chronic injections of purified ACTH in normal rats of the Long-Evans strain have resulted in the production of an absolute lymphopenia and

polymorphonucleosis. A typical experiment is presented. Purified ACTH<sup>5</sup> was injected into normal 26-day-old male rats in doses IP (intraperitoneal) of 1 mg every 4 hours. Four hours after the second injection, leucocyte counts and differential counts were made from tail blood samples. The findings are presented in Table I. It is apparent that ACTH treatment is followed by a mild leukopenia with a definite absolute change in the ratio of polymorphonuclear to mononuclear cells.

Injections of ACTH were carried out on fasted adult normal dogs to ascertain the type of reaction which might be obtained in this animal form. Purified sheep ACTH was injected IP in dosages of from 10 to 100 mg. Duplicate white cell counts and differentials were made at 2-3-hour intervals until counts and differential percentages returned to normal control levels. Specimens of blood were obtained from the femoral artery in unanesthetized animals. Control experiments were carried out using similar amounts of purified lactogenic hormone,<sup>6</sup> boiled (inactivated) interstitial cell stimulating hormone<sup>7</sup> (ICSH), and boiled inactive pituitary gland residue. The experiments are summarized below. Peak leucocytoses were noted in all animals within a period of 5-10 hours. The white cell counts returned to normal within

\* Aided by grants from the Research Board of the University of California and the Rockefeller Foundation, New York City.

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