It was also found that originally potent toxins became non-skin-necrotizing when injected endermally in undiluted form and non-lethal to rabbits when given intravenously in doses of 3 cc. per kilogram body weight.

Table II shows a good antigenic response in rabbits following intravenous or subcutaneous injections of staphylococcus toxin detoxified by the photodynamic action of methylene blue.

8741 C

Negative Effect on Blood of Normal Rabbits of Inoculation of Killed *Leishmania donovani.*

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Leucopenia is one of the outstanding features in kala-azar. It develops early in the course of the disease and is frequently out of proportion to the anemia. This fact has been well recognized, and its diagnostic value repeatedly emphasized. The mechanism of the development of this phenomenon is, however, by no means understood. By incubating leucocytes with blood serum, Maggiore and Sindoni[1] claimed to have demonstrated the presence in the blood serum of patients with visceral leishmaniasis, of a substance which is leucocytolytic. Arena[2] confirmed this finding. Their results were not at all conclusive, and there has been no further confirmation. Some workers[3, 4] believed that leucopenia as well as anemia in this disease is myelophthisic, the overgrowth of the macrophages ("clasmatocyte tissue") being comparable to the metastatic growths of carcinoma in blood-forming organs. This does not explain satisfactorily the early appearance of leucopenia when the infection is only beginning and the "crowding out" of the myeloid tissue hardly can have taken place.

It has been suggested,[5, 6, 7] in discussing the problem of leucopenia

1 Maggiore, S., and Sindoni, M., *Pediatrica,* 1917, **25**, 81.

2 Arena, G., *Pediatrica,* 1927, **35**, 465.

3 Hu, C. H., and Cash, J. R., *Trans. Far East. Assn. Trop. Med., 7th Congress, British India,* 1927, **3**, 62.

4 Keefer, C. S., Khaw, O. K., and Yang, C. S., *Nat. Med. J. China,* 1929, **15**, 731.

5 Doan, C. A., *J. A. M. A.,* 1932, **99**, 194.

6 Beck, R. C., *Arch. Int. Med.,* 1933, **52**, 239.

7 Beck, R. C., *Am. J. Path.,* 1934, **4**, 189.

in general, that there are possibly 2 factors concerned in the maintenance of a normal level of leucocytes in the peripheral blood, *viz.*, a maturation factor and a chemotatic factor. Whether or not such factors also operate in the production of leucopenia in kala-azar, one can only conjecture. Forkner and Zia[8] have shown that pentose-nucleotide, which was considered by Doan[5] as an example of a chemotatic substance for leucocytes, did not produce any significant change in the number of leucocytes when administered intramuscularly to patients with kala-azar. One may further postulate that there may be present in this disease an unknown factor which has a depressive effect on the myeloid tissue. The fact that the leucopenia and anemia begin to improve quite steadily soon after the institution of specific treatment may suggest the actual presence of some such factor which can be removed or counteracted by controlling the infection. It seems, therefore, worthwhile to investigate whether such a factor is present in the body of *Leishmania donovani* or its metabolites. It occurred to us that, by inoculation into animals of the killed organisms or their metabolites, it might be possible to bring about in the blood a picture comparable to that produced by actual infection.

Fresh cultures of *Leishmania donovani* were obtained from a puncture of the spleen of a patient with kala-azar. They were subcultured in a number of tubes containing Novy-MacNeal-Nicolle (N.N.N.) medium at a temperature of from 22 to 23°C. for from 2 to 4 weeks, at the end of which all the tubes invariably contained rich growths of the flagellates. The content of each tube was examined for the presence of bacteria and was discarded if contamination was present. The fluid portion of these tubes containing numerous flagellates in pure culture was then pooled. Tricresol was added in an amount equivalent to 0.5%, which caused the fluid to change its color from dark red to brown and the formation of brownish sediments. All the flagellates were killed by this treatment, as was demonstrated by the fact that they became unrecognizable under the microscope and could no longer be cultured in N.N.N. medium. The amount of the sediment in this suspension apparently depended on the richness of the culture, and it varied from 7 to 10% of the total volume as determined by centrifuging in a hematocrit tube. The suspension was kept in rubber-stoppered bottles in an ice chest. Immediately before use, it was thoroughly mixed.

Six normal rabbits, each weighing from 1,500 to 2,000 gm.,

[8] Forkner, C. E., and Zia, L. S., unpublished data.

were taken from stock without selection. They were kept in separate cages. Two of these animals (R.19 and R.20) were kept as controls, to which no suspension of *Leishmania donovani* was given. Two other animals (R.21 and R.22) received the suspension intraperitoneally in a short course of intensive treatment. The remaining 2 animals (R.23 and R.24) were given daily subcutaneous injections of the suspension for 48 days and, in addition, a short course of intensive treatment with intraperitoneal injections as for R.21 and R.22.

All animals were kept under the same environmental conditions, fed regularly at the same time each day and the studies were conducted at comparable periods throughout the experiment. All animals were allowed a period of control observations for 4½ months, during which time hemoglobin determinations, red blood corpuscle counts, white blood cell counts, and differential counts were made weekly from blood obtained by puncture of the ear vein. The body weight and temperature were recorded at the same time. During the period of continuous daily injections of the suspension in the case of R.23 and R.24, total white blood cell counts and differential counts were done daily, while the hemoglobin determinations and red blood corpuscle counts were made weekly. For the control animals, these observations were made once a week.

At the end of the experiment, all animals were killed by intravenous injections of air. The liver, spleen, lymph-nodes, lungs, kidneys, and the bone-marrow of the femur and the tibia were removed and fixed immediately in Zenker-formol solution (10% formalin). Sections were made and stained with hematoxylin and eosin. Comparative studies of the histologic pictures were made.

Results. 1. A slight decrease in the total leucocyte count and a relative decrease in the percentage of neutrophils occurred within 2 hours after the subcutaneous injection of the suspension of *Leishmania donovani.* Normal counts were restored about 3 hours after the injection. 2. There was no constant or significant change in the leucocyte and neutrophil counts in rabbits receiving either daily subcutaneous injections of relatively small amounts of the suspension or an intensive treatment with intraperitoneal injections of large amounts of it. (Chart 1. The findings in the case of rabbits Nos. 21, 22, and 23 were similar to those in rabbit No. 24.) 3. There was no decrease in the value of hemoglobin or in the number of red blood corpuscles following the injections of the suspension. The platelets remained normal in number. 4. The body weight and the temperature of the animals were not affected by the treatment. 5.

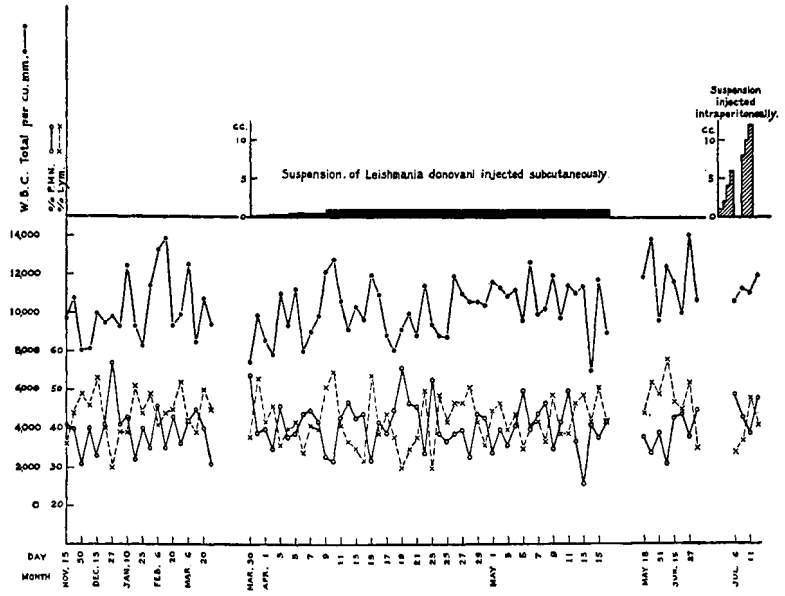

CHART 1.

Showing negative effect of parenteral inoculation of the suspension on the white blood cell counts of Rabbit No. 24 (R.24).

Studies of the histologic picture of the liver, spleen, lymph-nodes, lungs, kidneys, and the bone-marrow of the femur and the tibia did not reveal any striking difference in the animals treated as compared with those of the controls.

Rabbits were used in this experiment primarily because of the fact that they were more convenient for the study of changes in the blood. It is generally known that rabbits are relatively refractory to infection with *Leishmania donovani* and thus they may not be entirely suitable animals for this experiment. On the other hand, animals highly susceptible to the disease, such as Chinese hamsters *(Cricetulus griseus)*, are rather too small for repeated daily studies of the blood. It should be emphasized that what is true in rabbits might not be applicable to susceptible animals or human beings.

The suspension used for the injections was composed of killed flagellates of *Leishmania donovani* (not Leishman-Donovan bodies) in the fluid medium (N.N.N.) of the culture. It contained, therefore, both the substance of the organisms, the metabolites of them, and the medium. The physical nature of this suspension made it unsuitable for intravenous administration.

The decrease of leucocytes shortly following the injection of the

suspension was only slight and transient. The same phenomenon has been observed by others following inoculations of bacterial vaccines.[9] It is probably of no great significance.

The mechanism of leucopenia in kala-azar remains an unsolved problem.

8742 P

Liberation of Acetylcholine from the Perfused Human Placenta.

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A fresh human placenta was washed by an intra-arterial saline infusion until the fluid coming from the placenta became colorless. The wet weight having been taken, a piece weighing about 30 gm. was removed and extracted for the basal acetylcholine (hereinafter designated as a.c.) content. The placenta was supported on wire gauze fixed in the middle part of a large funnel, and perfused with a known volume (445-510 cc.) of oxygenated saline by means of a Dale-Schuster pump at a pressure of 90-100 mm. Hg. The whole outfit was kept in a small room which could be readily maintained at body temperature. At hourly intervals, the placenta was detached, weighed and a small piece removed for extraction. The perfusion-fluid was measured and 2 cc. were taken out each time for titrating its a.c.-activity. All the samples of tissue were extracted for 2 hours with 2 volumes of 96% alcohol. The different extracts and the samples of the perfusion-fluid were assayed on the same *rectus*-preparation.[1] The influence of body-temperature, potassium, and eserine has been studied in 5 experiments with uniform results; potassium was suggested for investigation by Feldberg.[2] Fig. 1 illustrates the essential points obtained.

It is evident that the potassium increases a.c. in the perfusion-fluid at the expense of the tissue-a.c., while eserine and an increase of temperature to 36°C. increase a.c. in the tissue as well as in the fluid.

Potassium may be considered to cause a transfer of a.c. from

[9] Wells, C. W., *J. Inf. Dis.*, 1917, **20**, 219.

[1] Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

[2] Personal communications.