14.5 mg and the thymus 101 mg. Twelve milligrams of pure compound E lost 6.5 mg weight. The adrenal glands weighed 19 mg and the thymus 188 mg. Pellets containing 20% cortin (containing all of the compound E and some of compounds A and B which were present in the original total extract) and 80% cholesterol were prepared as in Experiment 2. These pellets were implanted into 4 rats. One pellet weighing 54 mg lost 6.5 mg during one week. The adrenals weighed 30 mg and the thymus 433 mg. Two pellets having a total weight of 95.5 mg lost 26.5 mg in one week. The adrenals weighed 27 mg and the thymus 425 mg. Four pellets weighing 220 mg lost 37 mg in one week. The adrenals weighed 17 mg and the thymus 68 mg. Six pellets having a total weight of 302 mg lost 52 mg in one week. The adrenals weighed 15 mg and the thymus 63 mg.

Our data suggest that cortin compounds may be more efficiently utilized when administered to the rat in this manner than when given by the other usual methods. However, there may be appreciable errors in our evaluations of the extent of loss of cortin material by weighing. There may have been a differential absorption of the cortin compounds and of the cholesterol from the pellets and there may have been a cellular infiltration of the pellet which masked the true weight loss to some degree.

**10128**

## Phagocytosis of Leishman-Donovan Bodies by Leukemic Blood Cells.

CHIA-TUNG TENG AND HUEI-LAN CHUNG. (Introduced by F. R. Dieuaide.)

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Since kala-azar produces characteristically a marked leukopenia in patients, it seemed interesting to know what effect this infection might produce on the blood picture of patients with chronic myelogenous leukemia. As we had no opportunity to observe this in patients, we thought it worthwhile to study the reaction of leukemic blood cells *in vitro* on Leishman-Donovan bodies, *i. e.*, their phagocytic activity. Incidentally it is also of interest to find out what the relative phagocytic activity of various types of leukemic blood cells is, especially in view of the fact that leukemic patients in general do not

resist infections well in spite of the increased number of circulating leukocytes.

The spleen, aseptically removed from a Chinese hamster *(Cricetulus grieseus)* heavily infected with *Leishmania donovani,* was emulsified in 5 cc sterile physiologic saline. The emulsion was centrifuged for a few minutes at a low speed (500 rpm), and the sediment discarded. The supernatant suspension of Leishman-Donovan (L.D.) bodies was then ready for use. Twenty cc of leukemic blood were obtained from a cubital vein of a patient* with chronic myelogenous leukemia, 1 cc of 3.8% sodium citrate being used to prevent coagulation. The blood was allowed to sediment spontaneously at a temperature of 36°C in an incubator. The plasma was removed and discarded, the middle "buffy" layer was separated from the red cells and used for the experiment. A small drop of this leukocytic cream was mixed with an equal drop of the L.D. suspension on each of 12 pieces of mica cover-slips, mounted separately on 12 hollow-ground glass slides as hanging drops, sealed with sterile vaseline and incubated in an incubator at 36°C. The hanging drops were examined after various periods of incubation, and ordinary smears were made and stained with Wright stain. A differential count was then made on these smears. A summarized result is as shown in Table I.

From the table it is clear that neutrophils from the leukemic patient have very strong phagocytic activity on L.D. bodies, metamyelocytes have less and myelocytes still less activity. On the other hand none of the lymphocytes, basophils, basophilic and eosinophilic myelocytes, and myeloblasts showed such an activity, while no monocytes were seen. Eosinophils only occasionally phagocytosed the parasites. It must, however, be pointed out that the number of such cells observed is too few and the presence of basophilic and to some extent eosinophilic granules made the identification of L.D. bodies, if any, in these particular cells rather difficult.

Besides the stained smears, supravital preparations were also made from the mixtures of the L.D. suspension and the leukocytic cream, and examined in a warm chamber. Active phagocytosis was found as early as within 5 to 10 minutes. The relative activity of the various types of cells was same as observed in the Wright-stained smears. We also found that the cells which phagocytosed L.D. bodies tended to degenerate and disintegrate more rapidly than those which did not.

* The patient was a Chinese male of 34, having symptoms of the disease of 7 months' duration. The total white blood cell count at the time of the experiment was 164,000 per $mm^3$. He was later treated with a course of deep X-ray irradiation over the spleen with prompt remission.

TABLE I.
Phagocytosis of Leishman-Donovan Bodies by Various Types of Leukemic Bloo Cells.

| No. L.D. in each cell | Phagocytosis | | | | | | | | | | | | No phago-cytosis | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 4+ | | Total | | | | | |
| Cells | No. Cells | % | No. Cells | % | No. Cells | % | No. Cells | % | No. Cells | % | No. Cells | % | No. Cells | % | No. Cells | % |
| Lymphocyte | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 100 | 8 | 0.8 |
| Monocyte | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Neutrophil | 57 | 27.2 | 47 | 22.5 | 33 | 15.8 | 18 | 8.6 | 15 | 7.1 | 170 | 81.3 | 39 | 18.7 | 209 | 20.9 |
| Basophil | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27 | 100 | 27 | 2.7 |
| Eosinophil* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 100 | 5 | 0.5 |
| N. Meta-myelocyte | 20 | 22.7 | 7 | 8.0 | 3 | 3.4 | 3 | 3.4 | 1 | 1.1 | 34 | 38.6 | 54 | 61.4 | 88 | 8.8 |
| N. Myelocyte | 18 | 2.9 | 12 | 1.9 | 5 | 0.8 | 2 | 0.3 | 3 | 0.5 | 40 | 6.5 | 578 | 93.5 | 618 | 61.8 |
| B. Myelocyte | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 100 | 4 | 0.4 |
| E. Myelocyte | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 100 | 30 | 3.0 |
| Myeloblast | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 100 | 11 | 1.1 |
| | | | | | | | | | | | | | | Total | 1,000 | 100 |

*After examining more cells, we found that eosinophils also occasionally phagocytose L.D. bodies.

The same experiments were repeated, using the blood of another patient† with chronic myelogenous leukemia and also that of a normal individual, and very similar results were obtained. (Lymphocytes and monocytes in normal blood were never seen to show any phagocytic activity, but the number of the latter cells encountered was too small to permit a conclusion).

We also tested the phagocytic activity of various types of leukemic blood cells on *pneumococcus type I* and *streptococcus hemolyticus* and our findings were in agreement with those reported by Strumia and Boerner.[1]

*Conclusion.* Neutrophilic leukocytes beyond the myeloblastic stage, and occasionally the eosinophils, from the blood of patients with chronic myelogenous leukemia phagocytize L.D. bodies *in vitro*.

## 10129

## Intracutaneous Immunization of Rabbits with Photodynamically Inactivated Type I Pneumococcus.

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Julianelle,[1] employing the endermal route of immunization of rabbits with heat-killed type I pneumococcus, could not demonstrate any significant agglutinative or precipitative response to the homologous organism and specific soluble substance respectively. He ascribed this failure to the fact that the pneumococcus undergoes disintegration in the skin of rabbits and this results in the destruction of the type-specific antigen. The rapid extracellular digestion by tissue-ferments of heat-killed pneumococci when injected into the skin, as demonstrated by Dubos and Macleod,[2] lends some support to this idea. In view of the fact that the pneumococcus inactivated by the photodynamic action of methylene blue is much more resistant to

† The second patient was a Chinese male of 28, who had been suffering from the disease for one year and 8 months. The total leukocyte count at the time of the experiment was 179,000 per mm³. He was also subsequently treated with deep X-ray irradiation which brought about a satisfactory remission.

1 Strumia, M. M., and Boerner, F., *Am. J. Path.*, 1937, **13**, 335.

1 Julianelle, L. S., *J. Exp. Med.*, 1930, **51**, 441.

2 Dubos, R. J., and Macleod, C. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 696.