Studies on mitochondria in cases of chronic and acute lymphatic leukemia in man are not entirely in accord. In general, however, the reports indicate that the cells involved contain an increased number of very fine mitochondria in contrast to the moderately coarse structures found in normal lymphocytes (6). Lawrence and Todd (3) described 3 cases of lymphatic leukemia which were exceptions, in that 10 to 25 moderately coarse mitochondria were found in the mononuclear cells. These observations on number have been based for the most part on rough estimations, not actual counts. Wiseman (9), in attempting to correlate the number of mitochondria with the age of the lymphocyte, stated that “it is impossible to obtain accurate estimations of mitochondrial content, partly because of the physical difficulties involved in counting these bodies and partly because of the fact that some of the mitochondria occasionally fail to take the dye, while some fading of the stain occurs.” Although Wiseman’s paper is the only one dealing with the lymphocyte in particular, many others have shown that large numbers of mitochondria are present in young blood cells (2, 5).

As part of a comparative cytological study, this paper will present observations on the number, size, shape, and distribution of mitochondria in different populations of leukemic and normal lymphocytes in strain C58 mice.

Normal lymphocytes are defined as those found in the peripheral blood of mice showing no pathological condition. The data on normal cells were obtained from both young (average age 41 days) and older (average age 266 days) animals. The invaded peripheral blood was studied in cases of spontaneous leukemia (average age 479 days), and in 4 different lines of transplantable leukemias at the time of maximum invasion (hosts 4 to 6 weeks old). In the latter group, lines I and MLiv were both highly virulent with a survival time of 4 days, while lines S and U were less virulent, with a survival time of 7 to 15 days during the period of study.

Although fixed and stained material was also examined, the cells were studied for the most part in supravital preparations containing Janus green B and neutral red. Slides were prepared according to the following method after attempts had been made to find the most satisfactory combination for mouse blood: 9 drops of 1 per cent neutral red in absolute alcohol and 18 drops of 1 per cent Janus green (National Aniline, certified) in absolute alcohol were added to 10 cc. of absolute alcohol. Each slide was flooded with this mixture, drained, and allowed to dry dust-free. The blood films were made by touching a cover slip to a drop of blood and placing it on a prepared slide. Observations were begun immediately and counts made as soon as the dye had penetrated the cells. When a slide showed signs of cell death it was discarded and a fresh one prepared. For certain purposes dark field illumination was used, employing a Zeiss cardioi condenser and a 60 X, 1.05 mm. iris oil immersion objective.

The mitochondria were counted in a random sample of 100 cells from each of 10 animals in the various classifications, giving a minimum of 1,000 cells examined for each type of blood. In most cases the authors made independent observations on the same supravital preparation.

Observations

Number of Mitochondria

By counting the mitochondria in random samples of populations of lymphocytes it becomes possible to present numerical values for the 7 classifications chosen. Table I gives a summary of ranges, means, and standard deviations of the counts made and comparisons among the 7 groups. It is shown that the differences are statistically significant, except between the 2 highly virulent leukemia lines MLiv and I, and between the less virulent lines S and U. The differences among the transplantable leukemias are otherwise statistically significant. There was some individual variation which may have been due to the method of random sampling. However, the number of mitochondria for 100 cells when grouped in classes of 5 gave good distribution curves. For each indi-
individual classification, the mean of each sample of 100 usually occurred in the same class. The distribution curves for the number of mitochondria in 1,000 cells from 4 classifications are presented in Fig. 1.

There is evidence that the number of mitochondria is correlated with the average cell size of a given population, the normal population having the smallest average size, spontaneous leukemia next largest, and transmissible leukemias the largest. Individual observations within each sample indicated, however, that large cells sometimes had small numbers of mitochondria and small cells at times contained large numbers. Direct evidence that the largest cells did not always have the most mitochondria is found in comparing the 2 transmissible leukemias, lines U and I. The mean number of mitochondria for line U was 34.42 ± 0.31, and that of line I, 32.07 ± 0.27, yet the mean diameter of cells in the line I population was 11.44 microns (volume 1563.5 cu. microns), while the mean diameter for line U was 12.8 microns (volume 1929.9 cu. microns).

Perhaps the most significant correlation is between the age of the lymphocytes and the amount of mitochondrial material, a relationship already pointed out (9) for rabbit lymphocytes. In the present study and in earlier investigations (4) there seems little doubt that the larger cells in active lymphopoietic tissues of the mouse are the younger cells, judged by their ability to divide and the degree of basophilia. The proportion of immature cells and the rate of metabolism (7, 8) follows the same sequence in the 3 major classifications as did size; that is, normals lowest, spontaneous leukemias next, and transplatable leukemias highest. Within a virulent line of transplatable leukemia, samples taken daily during the progressive invasion of the blood by large, immature, and virulent cells showed an increase in the mean number of mitochondria (Fig. 2). A similar increase may be found in individual spontaneous cases with approaching death; in 3 cases where 2 counts were made on the same animal on different days, the mean of the 2nd count (3 to 9 days after the first) was the higher.

A sample of lymphocytes from a preparation of a normal lymph node teased in a drop of 0.9 per cent saline gave mitochondrial counts with a mean of 19.97 ± 0.53 and a standard deviation of 5.34 ± 0.37. This is not significantly different from the counts obtained on normal cells in peripheral blood. The mitochondrial content of a population of mononuclear cells from an active germinal center should be more nearly like that of a leukemic population, but sampling of that sort is at present technically impossible. Indirect evidence for this is the observation that immature normal cells contain a large number of mitochondria.

**TABLE I: MEAN NUMBER OF MITOCHONDRIA IN POPULATIONS OF LYMPHOCYTES, AND COMPARISONS AMONG THE DIFFERENT GROUPS STUDIED**

<table>
<thead>
<tr>
<th>Number</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (Y)</td>
<td>1,000</td>
<td>18.77 ± 0.20</td>
</tr>
<tr>
<td>Normal (O)</td>
<td>1,000</td>
<td>21.57 ± 0.21</td>
</tr>
<tr>
<td>Spont</td>
<td>1,000</td>
<td>27.93 ± 0.26</td>
</tr>
<tr>
<td>Line I</td>
<td>1,000</td>
<td>32.07 ± 0.27</td>
</tr>
<tr>
<td>Line MLiv</td>
<td>1,000</td>
<td>34.42 ± 0.31</td>
</tr>
<tr>
<td>S-Normal (O)</td>
<td>1,400</td>
<td>34.59 ± 0.29</td>
</tr>
<tr>
<td>Line U</td>
<td>1,000</td>
<td>34.42 ± 0.31</td>
</tr>
</tbody>
</table>

**SIZE AND SHAPE OF MITOCHONDRIA**

Certain variations in size and shape of mitochondria were seen within individual cells and among cells of a different population. A summary of the observations for each classification follows:

**Normal**—Small discrete granules for the most part, although some cells contained rather indistinct granules; scattered rods and filaments.

**Spont**—Both small and moderately large granules, discrete for the most part, but indistinct fuzzy granules were observed; scattered rods and filaments; a few ring-shaped forms.

**Line I**—Large granules of swollen appearance also large and small discrete granules; scattered rods and filaments; rings rarely seen.

**Spont-Normal (O)**

- Mean: 34.59 ± 0.29
- Standard deviation: 11.00 ± 0.20

**Normal (Y)-Normal (O)**

- Mean: 28.6 ± 0.20
- Standard deviation: 9.6

**Normal (Y)**

- Mean: 21.57 ± 0.21
- Standard deviation: 6.95 ± 0.15

**Spont**

- Mean: 27.93 ± 0.26
- Standard deviation: 8.40 ± 0.18
Line MLiv.—Large discrete granules for the most part; some small discrete granules; scattered rods and filaments; rings observed in 3 cases.

Line S.—A change occurred in the mitochondria of this line during the period of study. In transfers 85 to 90 the mitochondria were very small indistinct granules for the most part, although in some cases both moderately large and small granules and scattered rods and filaments appeared. In transfers 97 and 98 moderately large and small discrete granules and many long and short rods were seen.

Line U.—Great variability was observed in this line. In transfers 1 and 2 the mitochondria were very small indistinct granules. In transfer 3 large discrete granules, scattered rods, filaments, and rings appeared. In transfers 4 and 5 there were mostly large and small discrete granules and very few filaments or rods. In transfers 8 and 9 the mitochondria were small granules (not as discrete as in 4 and 5); a few rods were also seen.

Although in general the mitochondria in the lymphocytes of the mouse are small discrete granules, some rods and filaments were observed in each sample of the various populations. In all but lines MLiv and I a part of the mitochondria in each population was described as small and fuzzy or indistinct in outline. In the 2 highly virulent leukemias the mitochondria were consistently large sharply stained bodies which could be readily counted and photographed. This larger size seems to correct for the slightly higher mean number of the other 2 transplantable leukemias, and seems to permit a statement that, if not the actual number of granules, the total amount of mitochondrial material is correlated with size and physiologic age of the cell population. Fig. 3 reproduces photographs of typical normal cells, cells of spontaneous leukemia, and of lines I, MLiv, S, and U. The larger size of mitochondria in lines MLiv and I is apparent.

Individual cells with large mitochondria were seen in the leukemic lines and in normal populations, but...
large "swollen" mitochondria were noted particularly in line I transplantable leukemia. Since it has been reported that the enlargement of mitochondria sometimes accompanies cell injury (1), observations were made in both the supravital and dark field preparations to see whether any enlargement took place during the period of examination. Unless there was a change in the few seconds between removal from the mouse and the beginning of observation, it seems certain that no changes occurred. Neither did we find that there was any fading of Janus green during the time allotted for the examination of a supravital preparation. In unstained blood examined in a dark field, the larger size of the mitochondria in cells of transplantable leukemia MLiv as compared with line S was confirmed. It was therefore concluded that the consistently large mitochondria in the virulent leukemias were characteristic of the predominating immature cell type and not artifacts.

### DISTRIBUTION OF MITOCHONDRIA

The mitochondria in normal and leukemic cells apparently were not arranged in the cytoplasm or about the nucleus in a definite pattern. In focusing at different levels of a cell, mitochondria were usually found in each optical plane. Clumping of the mitochondria sometimes occurred but was not a usual pattern, nor was there any constant relation to nuclear indentation or to the nuclear membrane. Except during ameboid motion of a cell no movement of mitochondria in the cytoplasm occurred at an observable rate.

### SUMMARY

Differences have been demonstrated in the average number of mitochondria among populations of lymphocytes from the peripheral blood of normal mice and mice with spontaneous or transplantable leukemia. Table I and Figs. 1 and 2 are presented to show differences in number, while differences in size are shown photographically in Fig. 3.

It is concluded that these population differences indicate different proportions of cell types, and that particular stages of differentiation of the normal and leukemic lymphocyte have the same mitochondrial characteristics. Therefore the pathologic cell is not characterized by aberrant mitochondrial content.

The data presented here agree with Wiseman's statement concerning rabbit lymphocytes (9), that the amount of mitochondrial material is indicative of the age of the cell, large basophilic cells having the greatest amount.

### REFERENCES

Fig. 3.—Photomicrograph of supravital preparation (Janus green B and neutral red) showing the mitochondria in lymphocytes of normal and leukemic mouse blood. Note larger size of granules in cells of lines MLiv and 1. Mag. X 1000.
Mitochondria in Lymphocytes of Normal and Leukemic Mice

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