indicated a breakdown of the coagulation mechanism.

Postmortem examination of the animals killed by asphyxia revealed firm clots in the heart and great vessels. The blood of the animal killed by electrocution was also coagulated.

Conclusion. It could not be demonstrated that severe asphyxia has any effect on the clotting properties of the blood of the dog.

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A Concomitant Change in Mitochondria and Virulence of a Transplanted Lymphoid Leukemia.

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Potter and Ward[1] published the numbers of mitochondria in lymphocytes from the blood of C58 mice inoculated with several of the transplantable leukemias which had originated from different spontaneous cases in this laboratory. Two highly virulent leukemias, Lines I and MLiv which had then been transferred 800 or more times, killed the mice 4 days after inoculation with a standard number of cells. Two relatively less virulent leukemias, Line S transferred about 90 times and Line U transferred 2-9 times, killed the mice 7 and 15 days after inoculation.

Since that study was made Line I and Line MLiv have passed through about 500 additional transfers and the interval before death is still about 4 days.[2] In Line U and Line S, however, during about 350 additional transfers, the interval between inoculation and death has decreased to about 4 days. The present study was undertaken to determine what changes if any had occurred in the mitochondria of these leukemias. Lines U and I were selected as representative of leukemia which in one instance had greatly increased in virulence, and in the other had shown no change in the interval between inoculation (with a standard number of cells) and death, since the previous study.

Materials and methods. Virtually the same procedures were used as in the earlier study.[1] Blood from the tails of C58 mice (4 to 6 weeks old) inoculated with either Line I or Line U was studied during the terminal stages of the disease when the number of leucocytes in the peripheral blood was great. Control data were provided by counts of mitochondria in animals just before inoculation with leukemia as well as in their untreated littermates. Mitochondria stained with Janus green were counted in 500 to 1000 lymphocytes (50 or 100 cells selected at random from each mouse) from each of the leukemic groups and from control animals. Slides were prepared by flooding them with a solution of Janus green and neutral red in absolute alcohol, after which they were drained and allowed to dry dust free. Each 10 cc of this solution contained 14 drops of 1% neutral red in absolute alcohol and 27 drops of 1% Janus green in absolute alcohol. A drop of blood was placed upon such a slide, covered with a glass slip and sealed about the edges with vaseline.

The diameter of lymphocytes was measured with a calibrated ocular micrometer in blood smears stained with Wright's solution. If the lymphocytes were oval rather than round the diameter was assumed to be the average of the length and breadth.

Observations. Table I presents the mean

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1 Potter, J. S., and Ward, E. N., *Cancer Research*, 1942, **2**, 655.

2 MacDowell, E. C., *Cold Spring Harbor Symp. Quant. Biol.*, 1946, **11**, 156.

TABLE I.
Average Number of Mitochondria in Lymphocytes of Normal C58 Mice and Mice Inoculated with Line I and Line U Leukemia.

	No. of transfer	Leucocytes per cu mm	No. of lymphocytes counted	Avg No. of mitochondria	Standard deviation	Range
Normal		17,309	550	32.55 ± .55	12.85 ± .39	8- 89
Line I	1510-1513	147,476	500	43.35 ± .62	13.95 ± .44	8-115
" U	357-370	101,180	1,000	73.16 ± .70	22.00 ± .49	6-143
Normal*			1,000	18.77 ± .20	6.40 ± .14	5- 42
Line I*	1017-1046		1,000	32.07 ± .27	8.55 ± .19	12- 60
" U*	1-9		1,000	34.42 ± .31	10.04 ± .22	6- 67

* Data of Potter and Ward, '42.

number of mitochondria for the three groups of C58 mice. Normal mice have the fewest mitochondria; in mice inoculated with Line I the number is increased by approximately a third; in mice inoculated with Line U the number is more than doubled. The modes and upper limits of the 3 frequency distributions (Fig. 1) differ in the order of the means, but the lower limits coincide.

Data from the earlier study of Potter and Ward[1] are presented for comparison (Fig. 1, Table I). Although the relative position of normal, Line I, and Line U with respect to the number of mitochondria in lymphocytes is the same, the present counts are consistently greater than the previous counts. This appears in the means, modes and upper limits of the frequency distributions. The total range for each of the two leukemic groups is now more than double the previous ranges but the lower limits are still nearly the same as before.

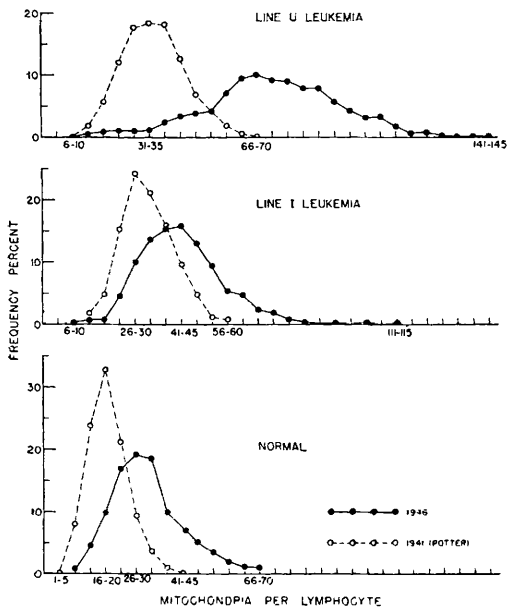

FIG. 1.

An explanation for the discrepancy in the number of mitochondria in normal lymphocytes could not be established. Theoretically there are two possibilities. Either there has been an actual change in the number of mitochondria or an apparent one resulting from differences in technic. The fact that C58 has long been a highly inbred strain argues against the first possibility. Although the procedure of Potter and Ward was followed as closely as possible, with one known exception, unrecognized differences in technic may have affected the results. The present counts were made on slides coated with a stronger solution of dyes since, in a few preliminary counts on slides prepared according to their method, the mitochondria faded so rapidly that only a few cells could be studied. Although these preliminary counts average slightly lower than later ones, they were higher than the 1941 counts, and it is felt that the difference in the preparation of the slides cannot account for the discrepancy.

Whatever may be the explanation of the present greater number of mitochondria in normal lymphocytes, the difference in the number of mitochondria between the 2 counts of Line U is greater than the difference between the 2 normal counts. The average number of mitochondria in normal lymphocytes is now 73% greater than previously, whereas the mean of Line U is now 113%

TABLE II.
Average Diameter of Lymphocytes of Normal C58 and Mice Inoculated with Line I and Line U Leukemia.

	No. measured	Avg diameter, microns	Standard deviation	Range, microns
Normal	550	9.87 ± .06	1.49 ± .04	6-15
Line I	500	12.85 ± .09	1.96 ± .06	7-18
" U	1,000	12.17 ± .07	2.24 ± .05	7-18

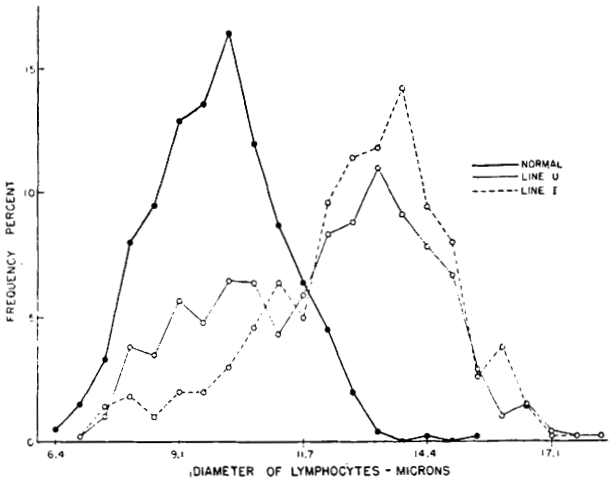

FIG. 2.

larger than the previous count. This notable increase in the number of mitochondria of Line U is most readily appreciated by comparing the 2 frequency distribution curves of the 2 normal counts with the 2 counts of Line U (Fig. 1). While the 2 normal curves overlap in large measure, the area of overlapping of the 2 Line U curves is small. In contrast, comparison of the Line I curves with the normal curves does not reveal an increase in the number of mitochondria in Line I which exceeds the increase between the 2 counts in control mice.

No change in the morphology of the mitochondria could be detected in a comparison with the descriptions and photographs of the earlier study. Now, as in the past, most of the mitochondria of normal lymphocytes are small distinct granules but there are some rods, filaments and indistinct granules. In Line U granules also predominate but have a greater range of size than those of normal lymphocytes. Rods, filaments and indistinct small granules are also seen. As in the previous study, the proportions of the several shapes and sizes varied from animal to animal inoculated with Line U. Mitochondria in lymphocytes of Line I have maintained the characteristic morphology previously described. The large granules of swollen appearance and the large and small discrete granules are so distinctive that they easily identify lymphocytes of this line.

Lymphocytes of the 3 groups also differ from each other in mean cell diameter (Table II). The range of cell size in the leukemic groups is greater than in the control group but the lower limits are almost the same. In range, the 2 leukemic groups are exactly the same but Line U has a greater proportion of the smaller lymphocytes (Fig. 2). Similarly, in the previous study the average diameter of lymphocytes of Line U was smaller than that of Line I.

Summary and conclusions. New data presented in conjunction with data published by Potter and Ward indicate that a marked increase in the number of mitochondria in lym-

phocytes of C58 mice inoculated with Line U leukemia has occurred in the course of 350 transfers. This increase in the number of mitochondria has occurred concomitantly with a decrease in the killing time, following a standard number of cells, from about 15 days to 4 days. The number of mitochondria in lymphocytes of Line I has not shown a similar increase in the course of 500 transfers made during this same period of time, and the interval between inoculation and death has remained unchanged at approximately 4 days. In making these new counts it was not possible to follow in every respect the technic for staining of mitochondria used by Potter and Ward.

Certain other differences between Line U and Line I have not changed since the time of the previous study of these leukemias. In spite of the increase in mitochondria, the lymphocytes of Line U remain smaller than those of Line I. In morphology, the mitochondria have retained the characteristics which distinguished them previously. Since the range in size of lymphocytes is the same in the two leukemias, although the means are different, if size is inversely correlated with the age of the lymphocyte as has been suggested,[3,4] then the 2 groups of lymphocytes share the same ages and stages of differentiation. The persisting differences between Line U and Line I, therefore, may be characteristics of the lines of leukemia and not differences due to the age and developmental stage of the lymphocytes.

[3] Wiseman, B. K., *J. Exp. Med.*, 1931, **54**, 271.

[4] Potter, J. S., and Richter, M. N., *Arch. Path.*, 1933, **15**, 198.

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Distribution and Rate of Disposition of Myanesin in Dogs.

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One of the most interesting characteristics of the action of myanesin* (3-(orthotoloxyl)-1,2-propanediol) is its shortness of action.[1-3] By the use of 3 different analytical methods the duration of pharmacological effect has been correlated with concentration of free myanesin in plasma.[3,4] It has also been demonstrated that less than 1% of myanesin administered intravenously can be recovered in urine as free myanesin. After hydrolysis with acid, material has been obtained from the urine which may be nitrated or coupled with diazotized dinitro-aniline to an extent which accounts for at least 30 to 40% of the total myanesin administered. It would appear, therefore, that myanesin is rapidly degraded in the body and that its short action is due almost entirely to rapid metabolic change. If myanesin is to be used intelligently, more information is needed as to the character of its absorption, excretion, distribution, and disposition. This report summarizes certain aspects of this problem.

Methods. Of the 3 methods proposed for the determination of myanesin in body fluids and tissues,[3,4] the coupling reaction with diazotized dinitro-aniline described by Titus, Ulick and Richardson is most suitable since

* We wish to thank Squibb Institute for Medical Research for the sample of myanesin used in these studies.

[1] Berger, F. M., and Bradley, W., *Brit. J. Pharmacol.*, 1946, **1**, 265.

[2] Burke, J. C., and Linegar, C., to be published.

[3] Titus, E. O., Ulick, S., and Richardson, A. P., *J. Pharm. and Exp. Ther.*, to be published.

[4] Wyngaarden, J. B., Woods, L. A., and Seevers, M. H., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 256.