whereas cephalin phosphorus from the same livers averaged 363-735.[5]

The changes in the % P32 dose per mg of liver lecithin and cephalin paralleled each other. The values increased during the first 2 days of fasting, fell on the third day, and increased again markedly on the fifth day. With the exception of the value obtained on the third day of fasting, the % P32 dose per mg total liver phospholipid represented approximately the average of the values for the % P32 dose per mg lecithin and cephalin.

During the first 2 days of fasting when depot fat was being rapidly mobilized, the increased phospholipid metabolism in the liver was evidenced by an increase in the % P32 dose per mg liver phospholipid. As previously observed[6] the liver total lipid reached a maximum on the second day of fasting. In some respects a similar condition obtains on a low choline diet, *viz.*, the liver neutral fat is increased and the lecithin level falls below normal; the administration of choline reverses this relationship.[7]

The accumulation of fat on the second day of fasting might account for the decrease in the % P32 dose in the liver lecithin and cephalin on the third day. Later, as the fat moved out of the liver (third to fifth day of fasting) the phospholipid metabolism presumably increased and, by the fifth day, the value for the % P32 dose in the liver phospholipids attained very high values (4 times normal). In the light of recent transmethylation studies it seems reasonable to suppose that more of the choline precursors were made available when protein metabolism was accelerated during the last days of fasting.

Summary. Young, adult, male albino mice lost almost half the liver total phospholipid during a 5-day fast. The liver weight also decreased in such a fashion that the phospholipid concentration remained approximately constant. The amounts of lecithin and cephalin decreased in proportion. The % P32 dose per mg of phospholipid increased markedly during fasting and, although the % P32 dose per mg lecithin is normally higher than that of cephalin, during fasting the values increased markedly for both fractions.

5 Riley, R. F., *J. Biol. Chem.*, 1944, **153**, 535.

6 Hodge, H. C., MacLachlan, P. L., Bloor, W. R., Welch, E., Kornberg, S. L., and Falkenheim, M., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 137.

7 Fishman, W. H., and Artom, C., *Federation Proc.*, 1945, **4**, 90.

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Effect of Asphyxia on Blood Coagulation in Dogs.

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Several manuals on postmortem examinations contain statements to the effect that a fluid state of the blood in a body is suggestive of oxygen deficiency at the time of death. For example, in describing the conditions found after death from asphyxia, Ross[1] stated "The blood is fluid and very dark in color owing to its being chemically almost completely reduced." Under the caption "Drowning," the same author said "The blood on the right side is dark in color and fluid (asphyxia)." In the section on asphyxia, Warthin's "Practical Pathology"[2] contains the following: "The lips, skin of the face and neck, and the finger nails may be deeply cyanotic; the blood is dark and fluid."

This experimental study was undertaken to determine the effect of severe asphyxia on the clotting mechanism as judged by *in vitro* tests on blood withdrawn from living animals

1 Ross, J. M., *Post-mortem Appearances*, New York and London, Oxford Univ. Press, 1939.

2 Warthin, A. S., *Practical Pathology*, Ann Arbor, George Wehr, 1928.

TABLE I.
Results of Coagulation Time Determinations in First Series.

Exp. No.	Blood coagulation time (min.)			Period of asphyxia (min.)
	Normal	During asphyxia	Recovery	
1	8	6	7	5
2	7	7	7	7
3	7	9	7	5
4	6	5	5	5
5	7	6	7	6
6	6	5	5	6
7	9	9	8	5
8	7	6	5	7
9	6	6	*	5
10	7	7	10	6
11	10	9	*	5

* Expired.

and by observations of the postmortem state of the blood in animals killed by asphyxiation.

Method. A common method for determining the coagulation time of the blood is that of Lee and White.[3] A sample of venous blood is drawn and placed in 3 test tubes. One of these is inverted at 30-second intervals until a clot has formed which retains its position in the bottom of the tube. The second and then the third tubes are treated in a similar manner. The time from the drawing of the blood until the clot forms in the third tube is taken as the coagulation time. In this investigation, it was necessary to prevent contact between the blood sample and the oxygen of the air. We tried putting the blood under a layer of mineral oil in the test tube, but the greasy inner wall of the tube and the slight emulsification caused by the repeated inversions of the tube made it difficult to judge the end-point. A more satisfactory method involved the use of rubber capped tubes filled with the inert gas helium. The blood sample was placed in the tube by piercing the diaphragm of the cap with the needle on the syringe.

The first series of experiments was carried out on 11 healthy dogs. The animals were rendered unconscious with ether, after which a catheter with a distensible balloon was inserted into the trachea. Ether-oxygen anesthesia was continued through this closed system. Both femoral veins were exposed through surgical incisions to permit quick and accurate vein punctures. A sample of blood was removed from the right femoral vein and divided into 3 helium tubes. The clotting time of this sample was noted. The endotracheal catheter was then clamped for about 5 minutes or until the animal was almost dead of asphyxia. At this point, the tongue would be purplish black in color, the heart action feeble and the respiratory movements spasmodic or absent. A second sample of blood was removed from the left femoral vein and its clotting time determined. With 2 exceptions, the prompt administration of oxygen by artificial respiration with the anesthesia apparatus permitted the animals to recover, after which a sample of the re-oxygenated blood was obtained.

In the second series of experiments, 3 anesthetized dogs were killed by asphyxiation, the endotracheal tube being clamped until there was no sign of life. Autopsy was performed after 2 hours and the condition of the blood in the heart and great vessels was noted. A fourth animal was killed instantly by electrocution, one electrode being placed over the heart (non-asphyxial death).

Results. The results of the 11 experiments in the first series are shown in Table I. It will be noted that there were no significant differences in the clotting times of the specimens removed before, during and after the periods of severe asphyxia. There was certainly no tendency for the second specimen, obtained during asphyxia, to show a prolongation of clotting time which would have

[3] Lee, R. I., and White, P. D., *Am. J. Med. Sc.*, 1913, **45**, 495.

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.