TABLE III. Inhibition of Formate Incorporation into Leukemic Leukocytes by Normal Human Serum.

	Cell type		
	CLL	CGL	AL
No. of cell donors	5	4	4
μM formate incorporated/mg protein*	266 11	295 105	709 152
% inhibition by normal serum	43 24	38 26	48 18

* Mean ± stand. dev.

of relatively low molecular weight, which inhibit incorporation of radioactive formate and glycine into the gross protein fraction of all types of leukemic leukocytes to an appreciable and approximately equal extent. It appears that sera from some cases of leukemia demonstrate a decrease in this inhibitory effect. Whether such a decrease is secondary to the disease process or plays some part in pathogenesis is unknown.

A possible explanation for the inhibitory effect is the presence in serum of formate, glycine, or other nucleic acid precursors which effectively dilute out the added radioactive precursors. On the other hand, it is also possible that a metabolic inhibitor or regulator may be present. If the observed inhibitor activity were ultimately proven to represent a substance active in the normal regulation of leukocyte production or activity, one could speculate further regarding a possible role in pathogenesis of leukemia, as has been suggested by Osgood(2)

Further studies are being made.

Summary. The presence of one or more chemical substances in normal and pathological human sera capable of decreasing the rate of incorporation of formate and glycine into human leukemic leukocytes *in vitro* has been shown. Human leukemia sera as a group demonstrated a slightly subnormal inhibitor activity.

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Preparation of a Functioning Frog's Heart Devoid of Ventricular Glycogen. (24219)

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In preparation for a study of factors relating to synthesis of glycogen by the ventricle of perfused frog's hearts, it was desirable to produce a functioning heart depleted of ventricular glycogen. The purpose of the present report is to describe the method used to produce such a heart and to call attention to a phenomenon which served to indicate when the ventricle was free of glycogen.

Methods. North American bullfrogs weighing 250-450 g were anesthetized by intraperitoneal injection of 20 mg sodium pentobarbital/100 g frog weight. The heart was exposed through a ventral midline incision, the circulation being left intact. After removal of the pericardium, the frog was placed in an airtight glass chamber where physiological salt solution(1) to which epinephrine was added in concentration of 1/500,000 dripped on the heart at a rate of 12-15 drops/min. The air in the chamber was then displaced by 100% N_2 and the chamber sealed tight. Though respirations ceased the heart continued to pump blood. After the desired time interval ventricular glycogen was determined by the method of Good, Kramer and Somogyi (2).

Results. After 0.5-4 hours in the chamber,

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TABLE I. Effect of Hypoxia on Cardiac Glycogen.

Experiment (all frogs fasted 48-72 hr)	No. animals	Ventricular glycogen* mg %
1. Controls	6	1213 ± 104†
2. After development of 3 × 3 mm white area and cannulation	7	0 ± 0
3. Perfused with glucose, 100 mg % for 6 hr after depletion of glycogen	6	588 ± 81

* All glycogen determinations were performed on ventricular tissue not involved by the white area.

† Stand. error calculated from the formula S.E. $= \sqrt{\frac{\Sigma x^2}{N(N-1)}}$.

a white, opaque, non-pulsatile area which grossly resembled scar tissue developed at base of ventricle. If air was then admitted to the chamber the white area disappeared, only to reappear again when oxygen was displaced, after which it continued to enlarge until it involved the entire ventricle. At this point the heart ceased contracting and could not be revived by admitting oxygen. However, when the white area was still small, about 3 x 3 mm, the frog could be removed from the chamber, the beating heart cannulated in preparation for perfusion, and the ventricle demonstrated to be devoid of glycogen (Table I).

The ventricles contained approximately 1200 mg % glycogen prior to placement in the relatively anaerobic chamber. Upon development of the 3 x 3 mm white area and cannulation of the heart, all ventricles analyzed were depleted of glycogen (line 2). Line 3 indicates that hearts treated as in line 2 but then perfused aerobically for 6 hours with physiological solution containing 100 mg % glucose synthesized about 600 mg % of ventricular glycogen.

Discussion. It is well established that hypoxia decreases glycogen content of cardiac muscle. That epinephrine has a similar action was demonstrated by Bogue, Evans and Gregory in the dog heart-lung preparation (3) and by Chang in the rat(4). A combination of hypoxia and epinephrine was utilized in this study to produce the glycogen-free frog's ventricle. The epinephrine dripped onto the external surface of the heart and appeared to strengthen the contractions. In all probability the hypoxia played the greater role in depleting the ventricle of its glycogen.

Grossly the white area appeared to consist of contracted non-functioning muscle which did not transmit the color of the blood in the ventricle as did the remaining myocardium. The stronger and more rapid the contractions the sooner the white area developed. Occasionally a heart would beat many hours in the presence of lowered oxygen tension without developing this sign; yet analysis showed the ventricle to be free of glycogen. Thus the appearance of the white area indicated that the ventricles were devoid of glycogen, but its appearance did not necessarily coincide with the exact time of glycogen depletion. In general the hearts functioned well in the chamber except in a few instances where hemorrhage occurred during preparation of the specimen and significantly decreased the amount of blood flowing through the heart.

To be certain no gycogen was synthesized from possible precursors within the myocardium between the time the frog was removed from the chamber and the completion of cannulation the glycogen determinations were carried out after the latter procedure rather than before. The ventricles therefore contained no glycogen at the time perfusion with glycogenic substances was begun. Of interest is the fact that hearts treated as described not only beat but also were able to resynthesize glycogen from glucose.

Summary. A method utilizing epinephrine and hypoxia to produce a viable frog's heart devoid of ventricular glycogen in preparation for studies on glycogen synthesis is described. The development of a 3 x 3 mm white area at the base of the ventricle served to indicate when the myocardium was so depleted. Hearts treated by this method were able to resynthesize glycogen from glucose.

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