circulation from auxiliary stores, during hibernal sleep might be considered. In contrast to the hibernating hamster, the rabbit displays a decreased concentration of serum *beta*-globulins and an elevation of *gamma*-globulin during simple cold exposure(6).

Summary. 1) Alterations in various fractions of serum proteins and hematocrits were studied in hibernating and non-hibernating hamsters. 2) During hibernation the hematocrit, serum albumin and *beta*-globulins increased to a significant degree. The increase in *beta*-globulins was too large to be accounted for on the basis of simple hemoconcentration. Since concentrations of neither $alpha_1$ nor $alpha_2$-globulin increased, the data were interpreted to mean that total circulating quantity of these globulins decreased. A marked decline in concentration of *gamma*-globulin occurred.

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Metabolism of Human Leukocytes *in vitro* V. Inhibition by Human Serum of Formate and Glycine Incorporation.*† (24218)

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Human leukocytes can be isolated, incubated *in vitro*, and observed to incorporate labeled precursor compounds into cellular protein, nucleic acid, and acid-soluble components at rates characteristic of cell type and maturity(4). Incorporation of formate(4) and glycine(1) has been previously shown to be significantly more rapid in Krebs-Ringer-Bicarbonate-Glucose media than in normal human serum, suggesting that serum may contain inhibitors or dilutors of metabolic processes in which these precursors are involved. These findings may suggest the presence of a substance or substances in normal circulation which regulate metabolic processes in leukocytes. The physiological and clinical implications of such regulators might prove to be of great interest. Further studies of the effect of serum on formate and glycine incorporation into normal and leukemic leukocytes *in vitro*, therefore, were undertaken.

Materials and methods. Separation, incubation, and fractionation of leukocytes. Leukocytes were collected in citrate-fibrinogen, washed in saline, suspended in 1 ml of Krebs-Ringer-Bicarbonate-Glucose (KRBG)§ me-

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§ The following abbreviations have been used in this paper: KRBG = Krebs-Ringer-Bicarbonate supplemented with 3 mg/ml glucose; KRB = Krebs-Ringer-Bicarbonate; GPF = gross protein fraction; ASF = acid soluble fraction; CLL = chronic lymphocytic leukemia; CGL = chronic granulocytic leukemia; and AL = acute leukemia.

TABLE I. Effect of Normal Human Serum and Serum Derivatives on Formate Incorporation into Intact Human Leukocytes.

Incubated cells (0.5 to 1.0×10^8 cells/beaker)	(Final vol = 2.2 ml/beaker)	Gross protein fraction, % inhibitions of formate uptake*
(A) Chronic lymphocytic leukemia	Krebs Ringer bicarbonate	0 †
	Fresh normal serum	37 §
	Serum ultrafiltrate‡	40 §
	Boiled serum ultrafiltrate¶	30 §
(B) Chronic granulocytic leukemia	Krebs Ringer bicarbonate	0 †
	Serum ultrafiltrate‡	64 §
	Ether extract of S.U.F.**	6 †
	Ether residue of S.U.F.**	78 §
(C) Acute granulocytic leukemia	Krebs Ringer bicarbonate	0 †
	Fresh normal serum	30 §
	Serum ultrafiltrate‡	30 §
	Dialysate††	17 ‡
	Residue††	0 †

* 100 (1 − incorporation in test media/incorporation in K.R.B.).

† $p < .01$ in comparison with fresh normal serum or serum ultrafiltrate.

‡ $p < .05$ in comparison with K.R.B. of same experiment using pooled replicate variance for experiment.

§ $p < .01$ for same.

‡ Fresh normal serum ultrafiltrated at 4°C through cellophane membrane under pressure.

¶ Serum ultrafiltrate kept at 100°C for 30 min. and reconstituted to the original volume.

** Serum ultrafiltrate extracted with equal volumes of diethyl ether, the ether and aqueous layers brought to dryness and each reconstituted to original vol.

†† Fresh normal serum dialyzed against an equal vol of Krebs Ringer bicarbonate medium and the dialysate tested. Residue was then dialyzed against a large vol of water and the residue tested.

dium(3) to which was added either 1 ml of serum or serum ultrafiltrates or Krebs-Ringer-Bicarbonate (KRB) media(3) and 0.2 ml of a saline solution of formate-C^{14} (.67 μM, 2.0 μC), or glycine-1-C^{14} (1.41 μM, 2.0 μC). All incubations were carried out in triplicate incubations, usually for 4 hours. Each beaker contained potassium penicillin G and streptomycin sulfate in concentrations of 0.15 mg/ml each. Separation of the gross protein fraction and calculation of specific activity and per cent inhibition were performed as previously outlined(4).

Results. Table I summarizes the results of 3 typical experiments showing that the presence of fresh normal human serum decreased formate incorporation into the gross protein fraction (GPF) of various types of leukemic leukocytes by 30 to 64%. This effect is due to low molecular weight components of the serum since the activity passes through a cellophane membrane during dialysis or ultra-filtration. It is ether-insoluble and heat-stable.

The time course of formate incorporation into leukocyte GPF in the presence and absence of serum dialysate was found to be linear with time in both instances, but to be 30% slower in the presence of the serum dialysate (Fig. 1a). Fig. 1b shows incorporation of formate in a 4-hour incubation period as a function of this amount of normal serum in the incubation medium.

Table II shows that normal serum ultrafiltrate decreases incorporation of glycine as well as formate into the gross protein fraction of chronic granulocytic leukemia leukocytes. Studies with other protein and nucleic acid precursors will be required to determine whether the effects of serum are general or limited to formate and glycine.

Table III summarizes the data on the effect of normal human serum on formate uptake by normal and leukemic leukocytes. It is apparent from these data that all cell types are decreased by normal serum to approximately the same degree.

It was of interest to determine whether there are differences between the effect of serum from various normal and pathological

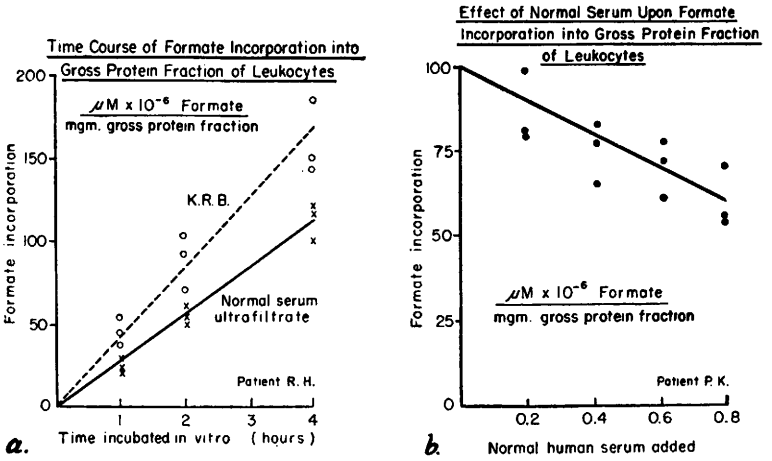

FIG. 1.

human sera on formate incorporation into different types of leukemic leukocytes. Preliminary data bearing on this question have been plotted in Fig. 2. These data include sera from normal subjects and from patients with various types of leukemia, lymphoma, lupus erythematosus, and other hematologic diseases. The categories tend to overlap considerably. However, 17 serial observations using one normal subject's sera on various cell samples representing the 3 major typs of leukemia have a mean inhibition of formate incorporation into leukocyte gross protein fraction (46.1%) which is significantly ($p < .05$) more pronounced than that of the 16 serum samples from patients with various types of leukemia (33.0%). Rather low levels of inhibition were obtained with serum from occasional cases of leukemia and lymphoma. Formate uptake appeared to be inhibited to a greater than normal extent in sera from 2 patients with lupus erythematosus. Such inhibition might, of course, be of a different type than the inhibitory activity noted in normal sera. Inhibition by sera from a number of other patients of various types was within normal limits.

Discussion. The present studies must be considered preliminary, but they strongly suggest the presence in normal serum of a heat stable, ether insoluble substance or substances

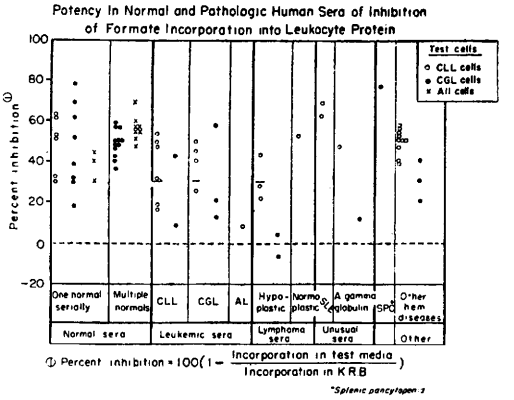

FIG. 2.

TABLE II. Effect of Normal Serum Ultrafiltrate on Precursor Incorporation into Gross Protein Fraction of Chronic Granulocytic Leukemia Leukocytes.

Precursor compounds (.1 mg/beaker)	$\mu M \times 10^{-6}$ incorporation precursor/mg G.P.F. in K.R.B.G.*		% inhibition in serum ultrafiltrate†	Significance of difference from K.R.B.‡
Formate-C^{14}	240	60	56	<.01
Glycine-1-C^{14}	4440	275	30	<.05

* Mean ± stand. dev. of 3 replicates. † % inhibition $= 100\left(1 - \frac{\text{Incorp. in test media}}{\text{Incorp. in K.R.B.}}\right)$.

‡ Calculated from pooled intraprecursor stand. deviations.

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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