hormones from the myocardium due to degeneration of adrenergic nerves. A working hypothesis is that the activity of lipoprotein lipase is stimulated by free (or "released") norepinephrine and/or epinephrine in heart tissue. In this connection, it was found (studies in progress) that pretreatment of rats with monoamine oxidase inhibitors (Marplan and Iproniazid) increased lipoprotein lipase activity released from heart tissue and the effect of heparin on enzyme activity was increased 2-fold.

Summary. The lipoprotein lipase activity released from heart slices of normal *ad lib.* fed and 20-hour fasted rats was the same. Heparin elevated equally the activity of tissues from both types of rats. Pretreatment with reserpine reduced the amount of activity of lipoprotein lipase released by incubated heart slices. The effect of heparin was negligible when heart tissues were used from rats treated with reserpine once daily for 4 days. The possibility of a relationship between reserpine-induced decrease in activity of lipoprotein lipase and depletion of catecholamines from heart tissue was discussed.

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Nucleic Acid Synthesis by Leukocytes in Presence of Anti-Leukocyte Factors.* (26609)

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A variety of evidence(1) indicates reactions between antinuclear substances in sera from patients with systemic lupus erythematosus and cell nuclei, nucleoprotein, and DNA.† There appear to be at least 2 factors: one which reacts with cell nuclei and is responsible for the "L.E. phenomenon," and another, present only in certain sera, which reacts with purified DNA from different sources and species. Both factors migrate with the gamma globulins on zone electrophoresis, and it has been hypothesized that the described interactions are antigen-antibody reactions.

Calabresi, Edwards, and Schilling(2), using a fluorescent antibody technic, have detected antinuclear globulins in the sera of all patients with SLE or Felty's syndrome of rheumatoid arthritis which they studied. They also obtained evidence, by plasma transfusions, that a leukopenic factor was present in the plasma from 2 patients with

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† The following abbreviations are used: DNA, deoxyribonucleic acid; SLE, systemic lupus erythematosus; RNA, ribonucleic acid; CGL, chronic granulocytic leukemia.

Felty's syndrome. In conjunction with their work, we have tested some SLE and Felty sera for possible effects, as compared to normal sera, on DNA, RNA, and protein synthesis by human leukocytes. To evaluate the results, we also compared normal rabbit serum and rabbit serum containing agglutinins to human leukocytes for effects on synthesis by human leukocytes.

Materials and methods. Sera: To minimize any effects of different salt concentrations, sera to be used in incubations with leukocytes were dialyzed for two 2-hour periods against 100× volume portions of the glucose-salts incubation mixture.

Incubations with human leukocytes. Leukocytes (number indicated in tables) obtained from patients with chronic granulocytic leukemia were incubated for 1 hour under 95% air + 5% CO_2 in Robinson's salts mixture(3) supplemented with 100 mg% glucose and 209 mg% sodium bicarbonate. Experiments with 25% serum were carried out in 60-ml Warburg vessels containing 1.0 μM radioactive precursor in 12.0 ml medium or in 9.0 ml medium + 3.0 ml experimental serum. Experiments with 100% serum were carried out in 25-ml Warburg vessels containing 0.5 μM radioactive precursor in 3.0 ml medium or serum. Methodology for preparation and incubation of leukocyte suspensions and separation of nucleic acid fractions has been described(4). Differences in the present procedure are as follows: Instead of perchloric acid addition immediately after incubation, contents of the chilled flasks were centrifuged in the cold and the sera were poured off. In measuring incorporations of glycine-2-C^{14} into cell protein, the pellets were washed once with a 10× volume of cold nonradioactive medium and recentrifuged before addition of perchloric acid. Specific activity of all radioactive compounds was 2×10^6 cpm/μM.

Preparation of rabbit antileukocyte serum. Three rabbits were immunized for another study by injection of human leukocytes prepared from (1) chronic myelogenous leukemic, (2) chronic lymphocytic leukemic, and (3) normal blood. Each rabbit was given an intravenous injection of 1.5×10^6 leukocytes and a subcutaneous injection of 3.5×10^7 leukocytes one month later. Ten ml immune serum from each rabbit were harvested 2 months after initial injection and pooled for the present study. After inactivation at 56°C for 30 minutes, the serum was incubated with an equal volume of washed human red cells in a 37°C water bath with gentle agitation for 2 hours, and then at 4°C for 8 hours, after which serum and red cells were separated by centrifugation. Normal rabbit serum was treated by the same procedure. The sera were then dialyzed against the glucose-salts incubation mixture as described above.

Results and discussion. The effects of 25% normal serum are shown in Table I, Exp. 1 and 2. Compared to the control without serum, incorporation of adenine-8-C^{14} into RNA adenine was higher, into RNA guanine lower or unchanged, into DNA adenine lower, and incorporation of thymidine into DNA was lower. Serum from a patient with chronic granulocytic leukemia, one with SLE, and one of 2 patients with rheumatoid arthritis showing Felty's syndrome had the same effects as normal sera. Another serum from a patient with Felty's syndrome gave an inhibition instead of a stimulation of adenine incorporation into RNA adenine (but no marked effect as compared to the control), and a greater inhibition of thymidine incorporation into DNA than did the normal serum tested in the same experiment (although not greater than the inhibition by the normal serum of Exp. 3). This sample was also run undialyzed as a check on possible loss of activity upon dialysis, but values did not differ greatly from those with the dialyzed serum.

In Exp. 3, Table I, a smaller number of cells was suspended in 3.0 ml undiluted serum, thus increasing the ratio of inhibitory or stimulatory factors to cells. Serum (or glucose-salts control) was preincubated with cells for 30 minutes at 3°C before addition of radioactive precursor. As with 25% serum, incorporation into RNA was higher and into DNA lower than the control, and results

TABLE I. Effect of Human Sera on Incorporation by Human Leukocytes from CGL.

	Serum	Specific activity with serum * / Specific activity without serum			
		Adenine-8-C^{14}			Thymidine-H^3
		RNA adenine	DNA adenine	RNA guanine	DNA
Exp. 1: 25% serum in 12.0 ml incubation medium; 5×10^6 cells C.L.	F.S. Normal	1.31	.87	.44	—
	A.W. "	—	—	—	.73
	D.C. CGL	1.16	.71	1.00	—
	R.S. Felty	.82	.91	.46	.37
	" " undialyzed	.86	.98	.57	.48
Exp. 2: 25% serum in 12.0 ml incubation medium; 5×10^6 cells A.M.	F.S. Normal	1.17	.64	.76	—
	S.C. "	1.32	.51	.99	.72
	I.B. Felty†	1.13	.48	1.01	.77
	A.T. SLE	—	—	—	.88
Exp. 3: 100% serum in 3.0 ml incubation medium; 2×10^6 cells S.C.; cells & serum pre-incubated 30 min. at 3°C before addition of radioactive precursor	R.W. Normal	1.30	.52	—	.39
	I.B. Felty†	1.35	.37	—	.50

* Specific activity values were averages of duplicate flasks which varied by less than 10% from the mean.

† Also positive LE preparation.

with a SLE serum were similar to those with normal serum.

The experiments shown in Table I tested a few sera with each of 3 suspensions of leukocytes from 3 different patients. To determine the variation in effect of a larger number of sera on one leukocyte population, the experiment shown in Table II was performed.

TABLE II. Effect of Human Sera on Incorporation of Adenine-8-C^{14} by Human Leukocytes from CGL.

Serum	Sp. act. with serum * / Sp. act. without serum	
Incubation 1†		
J.S. Normal	1.10	1.04
J.V. "	.98	1.03
P.P. SLE (4.1)‡	1.03	1.18
R.S. Felty (1.7)	1.04	1.09
Incubation 2§		
L.S. Normal	1.13	1.27
M.E. "	.96	1.30
E.B. Felty (1.6)	1.10	1.17
D.W. Felty (1.6)	1.13	1.31

* Specific activity values were avg of duplicate flasks which varied by less than 10% from the mean.

† Flasks without serum: RNA adenine = 26,430 ± 210 cpm/μM; DNA adenine = 480 ± 22 cpm/μM.

‡ Wbc ($\times 10^3$/mm^3) when serum was withdrawn.

§ RNA adenine = 28,740 ± 1360; DNA adenine = 563 ± 6.

Sera from 4 normal subjects, 3 leukopenic patients with Felty's syndrome, and 1 patient with SLE were tested with leukocytes from G.Z. under the conditions described for Exp. 3, Table I. Blood was withdrawn from G.Z. twice on the same day for the 2 incubations, each of which included 2 normal and 2 pathological sera. As in the previous studies, pathological sera had no marked effects over those from normal sera. In this experiment, incorporation into both RNA and DNA adenine with serum was either somewhat higher than the control or was not significantly different.

Frenster *et al.*(5) have reported a decrease in rate of incorporation of formate and glycine into the protein plus nucleic acid fraction of human leukemic leukocytes when normal or pathological human serum was added to the incubation mixture. In 3 experiments of the present study, there was an inhibition or no marked change of incorporation at 60 minutes into RNA guanine and into DNA, but a stimulation of that into RNA adenine. In one experiment, incorporation into both RNA and DNA adenine either was not significantly affected or was stimulated by serum. Thus, results with both normal and pathological sera were variable. It was

TABLE III. Effect of Rabbit Serum Containing Agglutinins to Human Leukocytes on Incorporation by Human Leukocytes from CGL.

Cells B. G. 3.0 ml medium	Glycine-2-C^{14}, 2×10^7 cells, cpm/mg protein	Adenine-8-C^{14}, 4×10^8 cells cpm/μM RNA adenine	cpm/μM DNA adenine	Thymidine-H^3, 10^8 cells, cpm/mg DNA
Glucose-salts	650 ± 42*	19,260 ± 650	459 ± 18	980 ± 60
Normal serum 1:2	562 ± 24	18,260 ± 860	511 ± 15	876 ± 60
Immune " "	546 ± 36	20,960 ± 470	514 ± 14	1164 ± 10
Normal serum 1:50	958 ± 62	18,230 ± 750	706 ± 59	988 ± 96
Immune " "	1150 ± 32	16,760 ± 250	438 ± 8	1052 ± 40

* Variation from mean of duplicate flasks.

somewhat surprising to us that the pathological sera tested did not exert additional effects on *in vitro* incorporation into leukocytes. Plasmas from R.S. and I.B. caused definite, transient depressions of the leukocyte count when transfused into other human recipients (2). Antinuclear globulins were detected, by means of the fluorescent antibody test described in (2), in the sera of all patients with SLE or Felty's syndrome used in the incorporation studies. These antinuclear globulins combined with nuclei of both granulocytes and lymphocytes. Sera were withdrawn for incorporation tests while the patients were in an active state of their disease.

To determine what effect on incorporation sera known to contain antibodies would have, serum was harvested from rabbits immunized against human leukocytes. Normal rabbit serum prepared as described under *Methods* caused no macroscopic or microscopic agglutination when the relative proportions of white cells and serum used in incubations were mixed together. Immune rabbit serum, in dilutions up to 1:10, caused macroscopic agglutination within 15 minutes or less, at 3°C or room temperature. In the incubation experiments, cells and serum (or glucose-salts mixture) were preincubated 30 minutes at 3°C before addition of radioactive precursor. The sera were used at 1:2 and 1:50 dilutions; at the latter dilution, agglutination did not occur with the immune serum. When the vessels containing immune serum in the 1:2 dilution were removed from the shaker, the cells were observed to be agglutinated.

However, results for normal and immune sera differed markedly only for incorporation of adenine-8-C^{14} into DNA with the serum diluted 1:50, where normal serum was stimulatory and immune serum caused no significant change (Table III). At this dilution, there was no significant difference between normal and immune sera for incorporation of thymidine-H^3 into DNA, and at the 1:2 dilution, incorporation of the latter precursor was lower than the control in presence of normal serum and higher in presence of immune serum. There was thus no consistent difference in effect of immune serum compared to normal serum.

Holman *et al.*(1) have reported that the antinuclear factors present in SLE sera did not interfere with growth of normal or tumor cells in tissue culture. From these and other data they concluded that the factors could not gain access to the nucleus of a viable cell. This possible nonpenetration to the nucleus may at least partly explain the failure in the present study to demonstrate an effect of anti-leukocyte factors on incorporation *in vitro*. In addition, since rabbit sera with antibody concentrations which caused macroscopic agglutination did not cause marked differences in incorporation, it perhaps should not be too surprising that no pronounced effects were obtained with the human pathological sera, in which postulated antibodies would be present in lower concentrations.

Summary. Normal and pathological human sera were tested for effects on incorporation of nucleic acid precursors into RNA and DNA during 60-minute incubations. Sera from systemic lupus erythematosus or Felty's syndrome of rheumatoid arthritis did not differ markedly from normal sera. With serum (compared to glucose-salts control),

incorporation of adenine-8-C^{14} into RNA adenine was higher or unchanged, into RNA guanine lower or unchanged, and into DNA adenine variable; incorporation of thymidine-H^3 into DNA was lower. Normal rabbit serum and immune rabbit serum containing agglutinins to human leukocytes were tested for effects on incorporation of precursors into nucleic acids and protein. Immune serum at a concentration causing macroscopic agglutination of the cells gave results similar to those with normal (non-agglutinating) serum.

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Studies on Bile Acids in Rat Systemic Blood. Bile Acids and Steroids 107.* (26610)

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For several decades many attempts have been made to estimate blood bile acid levels (Sobotka(1)). The results of these studies have varied greatly and even during the past 10 years values ranging from 0 to 40 mg per 100 ml plasma have been reported(2). Most values have been obtained using direct colorimetric methods of doubtful specificity. During the last few years chromatographic methods have been applied prior to spectrophotometric or fluorometric measurements(3,4). With these methods no bile acids could be detected in normal human serums. Although bile acids have never been isolated from blood, Carey(5) has observed spots corresponding to bile acids on paper chromatograms of purified human serum extracts. He also found through spectrophotometric methods trihydroxy- and dihydroxycholanic acid levels of 0.14 and 0.08 mg/100 ml respectively(6).

Byers and Friedman have reported the cholate level in rat serum to be 2.1 mg/100 ml(7). Since bile acid metabolism has been studied extensively in the rat we have further investigated the bile acids in the systemic blood of this animal. This work is a continuation of a previous study of the portal bile acids(8).

Experimental. Cholic acid was labelled by exposure to one curie of tritium gas(9) for 14 days in the apparatus described by Bergström and Lindstedt(10). To remove labile tritium the cholic acid was repeatedly evaporated with ethanol-water, then heated for 16 hours at 120°C in 2 N NaOH. After ether extraction of the acidified solution the acid was chromatographed twice with phase system C described below. The cholic acid peak was diluted with twice the weight of inactive cholic acid and recrystallized twice giving a specific activity of about 6.9×10^8 cpm per mg when counted in an infinitely thin layer in a methane gas-flow counter (Frieseke Hoepfner FH 51).

Sprague-Dawley rats weighing 200-300 g were used. 1-2 mg of labelled sodium cholate was administered either orally or intraperitoneally in one or 3 ml of saline respectively. The rats were fed regular stock diet and water *ad lib.* Twenty-four or 48 hours after

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