different from the others. This difference was noted not only in the analysis of sera from patients who were presumably infected with a type other than *L. sejroe* but also in the serum of the rabbit immunized with this type.

Certain strains of *L. sejroe* are known to be strongly cross-reactive when tested against sera from patients(12). Since *L. sejroe* reacted with all the patients' sera tested, one might have expected a reaction with the rabbit anti-*L. pomona* serum. The relative specificity of the rabbit anti-*L. sejroe* serum is also remarkable considering that the same strain was used in the agglutination tests and for immunizing the rabbit. No explanation for these seemingly paradoxical findings is apparent.

Summary. Chromatography on DEAE cellulose and susceptibility to inactivation by 2-mercaptoethanol indicate that the major portion of the agglutinins in patients' sera for 7 types of leptospirae are 19S macroglobulins even at 4 to 8 weeks following the onset of symptoms. A distinctly greater proportion of the agglutinins for *L. sejroe* was found in the 7S fraction of patients' sera. The agglutinins produced in rabbits following the injection of *L. pomona* and *L. sejroe* were initially almost entirely in the 19S fraction of serum but appeared also in the 7S fraction as time progressed. *L. sejroe* showed a greater tendency to elicit the production of 7S agglutinins in the rabbit than did *L. pomona.*

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Differences in Sensitivity of Human Blood Cells to Fresh Rabbit Serum.* (30655)

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Previous work(1) has shown that 50% fresh rabbit serum had little or no cytocidal effect on the survival of normal human lymphocytes. In this study, the cytocidal effects of fresh rabbit serum were determined on normal granulocytes, monocytes and erythrocytes and on the blood cells from patients with chronic and acute granulocytic leukemia.

Methods. Heparinized blood from normal individuals or leukemic patients was allowed to sediment for 30 or more minutes at 37°C. The plasma and leukocytes were removed and

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TABLE I. Cytocidal Effects of 50% Fresh Rabbit Serum on Human Blood Cells from 7 Normal Individuals and 9 Patients with Acute or Chronic Granulocytic Leukemia.

	Viable cells/mm³						
	In 50% human serum			In 50% fresh rabbit serum			
	Avg "H"			Avg "R"			% of avg "R" to avg "H"
	No.	%	Range	No.	%	Range	
Normal							
Lymphocytes	615	45.5	307- 805	514	86.7	304-691	83.6
Neutrophils	602	44.6	341-1005	1	.2	0- 2	.2
Eosinophils	38	2.8	8- 104	25	4.2	5- 88	64.4
Monocytes	96	2.1	37- 226	53	8.9	8-120	54.8
Total	1351	100.0		593	100.0		
Erythrocytes	1604		722-4220	13		0- 51	.8
Granulocytic leukemia							
Lymphocytes	122	14.4	13- 251	102	27.7	12-185	83.3
Mature and immature cells with neutrophilic granules	424	50.1	180- 734	27	7.3	0- 77	6.2
Mature and immature cells with eosinophilic granules	23	2.7	0- 125	20	5.4	0-131	84.8
Monocytes	19	2.2	4- 51	11	3.0	0- 18	56.1
Myeloblasts	259	30.6	11- 678	208	56.5	14-800	80.4
Total	847	100.0		368	99.9		

the cells washed and resuspended in equal parts of Fischer's medium No. 147G (Grand Island Biological Co., Grand Island, N. Y.) and fresh normal human serum. Preliminary counts were made and the cells diluted to give approximately 1,000 leukocytes per cubic millimeter. Aliquots of the suspension were then centrifuged and the fluid replaced with 50% fresh rabbit serum in Fischer's medium and, as a control, with 50% fresh human serum. The fresh rabbit serum was usually used within 24 hours and the human serum, within 4 days after preparation. The age of the human sera was not an important factor in these experiments. After incubation for one hour, 0.2 ml of the mixtures was transferred to a special slide chamber(2) which consisted of 2 large cover slips separated by a metal plate, 1.0 mm in thickness. The cells deposited in a single layer on the lower cover slip and were examined with an inverted phase contrast microscope. The various types of viable cells were counted in a measured area (10 × 0.04 mm) of the chamber and the number of cells per cubic millimeter was calculated. The cytologic characteristics of the various types of leukocytes as seen with phase contrast microscopy are described and illustrated in Rind's Atlas(3).

Results. In 7 experiments with normal human blood cells, the fresh rabbit serum had little or no cytocidal effects on lymphocytes, eosinophils and monocytes (Table I). In contrast, the neutrophils were rapidly killed by the fresh rabbit serum and the erythrocytes were lysed. As seen in phase contrast microscopy, the dead neutrophils were perfectly round and had a thin cytoplasmic wall, a peripheral zone of somewhat coarse granules, and an irregular shaped pyknotic or lysed nucleus.

With heat inactivated (56°C for 30 minutes) sera of rabbits, the human red blood cells and neutrophils formed small and large clumps which usually consisted of one type of cell. The erythrocytes did not undergo lysis even after incubation for several days. The neutrophils in the periphery of the clumps remained viable but the cells in the center could not be visualized sufficiently to determine viability. Addition of fresh human or guinea pig sera failed to restore the cytotoxicity to the heat inactivated rabbit sera.

A similar study was made on the effect of

TABLE II. Concentration of Myeloblasts from Blood of 2 Patients with Granulocytic Leukemia by Incubation of Blood Cell Suspensions in 50% Fresh Human Serum in T-flasks and Then Incubation of Cells of the Supernatant in 50% Fresh Rabbit Serum.

	Patient L				Patient K			
	Before purification		After purification		Before purification		After purification	
	Cells/mm³	%	Cells/mm³	%	Cells/mm³	%	Cells/mm³	%
Lymphocytes	165	19.3	185	36.9	104	10.2	108	16.4
Mature and immature cells with neutrophilic granules	452	52.8	51	10.2	180	17.7	6	.9
Mature and immature cells with eosinophilic granules	6	.7	0	0	3	.3	0	0
Monocytes	0	0	0	0	51	5.0	15	2.3
Myeloblasts	233	27.2	265	52.9	678	66.7	530	80.4
Total	856	100.0	501	100.0	1016	99.9	659	100.0

50% fresh rabbit serum on the blood cells of 9 patients with chronic or acute granulocytic leukemia. The fresh rabbit serum killed nearly all the mature and immature cells with neutrophilic granules, including promyelocytes, myelocytes and neutrophils (Table I). In contrast, the myeloblasts, eosinophils and eosinophilic myelocytes were resistant to the cytocidal effects of fresh rabbit serum.

A comparison of the average percentages of cells from granulocytic leukemic patients shows (Table II) that the fresh rabbit serum concentrated both the lymphocytes and myeloblasts. In 2 patients, L and K, with granulocytic leukemia, the routine differential count showed a high percentage of myeloblasts, 17 and 58, and a low percentage of lymphocytes, 4 and 8, respectively. The blood cells of these patients were used to determine whether it would be possible to obtain a concentrated suspension of myeloblasts. The method consisted, first, in preparation of a leukocyte suspension in 50% fresh human serum. The suspension was incubated in a T-flask for 30 minutes at 37°C. Previous work(4) had shown that under these conditions the monocytes and the mature neutrophils and eosinophils adhered to the glass, but not the myelocytes, blasts, and lymphocytes. The supernatant from the T-flask was then removed, centrifuged and the cells were incubated for 30 to 60 minutes in 50% fresh rabbit serum in order to kill the myelocytes and promyelocytes. Differential counts were made of the viable cells in the 50% rabbit serum. It is seen that the purification process concentrated the number of myeloblasts by eliminating, to a large extent, the neutrophilic granulocytes and myelocytes.

Discussion. Fresh rabbit sera were found to kill rapidly human neutrophilic granulocytes, myelocytes and promyelocytes and to lyse red blood cells. The sera had little or no toxicity to lymphocytes, monocytes, eosinophils or myeloblasts. The sensitivity of neutrophilic cells as contrasted to the resistance of other leukocytes to rabbit sera suggests that the neutrophilic granules may be the site of action of rabbit sera.

The difference in susceptibility of human leukocytes to rabbit serum and the difference in the capacity of leukocytes in fresh human serum to adhere to glass provided a means to obtain concentrated suspensions of myeloblasts.

Summary. Fresh sera of rabbits caused lysis of human erythrocytes and killed rapidly human neutrophilic granulocytes, myelocytes, and promyelocytes. The sera had, however, little or no effect on lymphocytes, monocytes, myeloblasts and eosinophilic granulocytes and myelocytes. The cytocidal action of fresh rabbit sera to cells with neutrophilic granules was used to prepare concentrated suspensions of myeloblasts from the blood of two patients with granulocytic leukemia.

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Propensity for Lunar Periodicity in Hamsters and Its Significance For Biological Clock Theories.* (30656)

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Two alternative hypotheses exist for the timing system of the rhythmic daily activity patterns (circadian rhythms) persisting in animals shielded from all ordinary environmental variations. The endogenous hypothesis is that the basic timer is a very stable autonomous oscillation which in constant illumination and temperature runs freely revealing its individual inherited period. This postulated inherent rhythm is assumed to depend upon the daily light and temperature variations (Zeitgeber) for its synchronization to the day. The exogenous hypothesis is that organismic responsiveness to pervasive rhythmic geophysical variations constitutes the basic timer for the persisting circadian rhythms, and that the diverse frequencies observed even among individuals of the same species under the same conditions of unvarying light and temperature are derived through intraorganismic variable frequency transformation. Both rational biological explanations (1,2) and theoretical models(3) for such frequency transformation have been suggested. Thus, either timer hypothesis is now tenable, with personal preference and plausibility determining the choice between them for interpreting any given observed circadian characteristics.

The following two reports of observations of mice with accurate mean 24-hour periodicities illustrate two very different possible interpretations.

It has been noted(4) that in blinded mice held under controlled environmental temperature (24 ± 0.5°C) and on a 24-hour cyclic lighting regimen—12 hours of light alternating with 12 hours of darkness—several circadian rhythms that were desynchronized from the lighting schedule during the first few months after the operation returned to an average 24-hour period about 5 months after blinding. The interpretation favored was that the mice had become responsive to 24-hour variation in such subtle potentially effective factors as unidentified laboratory noises and perhaps odors which, for the same mice, were initially ineffective as synchronizers of postulated inherent rhythms.

In another study of mice(5) under conditions of constant illumination and temperature, and within the same enclosure, in which some of the mice were capable of displaying each its individual odd circadian period there was a tendency for the other mice to maintain an apparent precise mean 24-hour period, sometimes continuing over several months. This tendency was much greater than would have been expected from a random distribution of circadian periods. In mice displaying a circadian period different from 24 hours, there was demonstrated to be present simultaneously a characteristic underlying variation in activity related to hour-angle of the sun. This became evident when one examined units of data just long enough to randomize the circadian component. It was postulated that those mice displaying a 24-hour cycle were locking their circadian activity pattern to the 24-hour, presumably geophysically dependent component.

The two foregoing proposed explanations

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