Summary. When the isolated rat liver was perfused with a solution containing whole rat's blood diluted 1:3 with a solution of electrolytes, crystalline albumin, amino acids, and vitamins, bile was continuously formed over a period of 2 to 11 hours. The initial perfusate level of cholesterol averaged 13.8 mg %, and the initial perfusate level of cholate averaged 1.2 mg %. The final perfusate levels of cholesterol averaged 18.0 mg %, and of cholate 1.4 mg %. The differences between initial and final concentrations are not significant for these data. The bile contained an average of 6.3 mg % of cholesterol and 117 mg % of cholate. These data demonstrate that the isolated perfused rat liver maintains its ability to synthesize and degrade cholesterol.

The authors wish to express their thanks and appreciation to Ralph W. Brauer, for his assistance and advice.

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Received May 4, 1953. P.S.E.B.M., 1953, v83.

Mechanical Fragility of Erythrocytes from Leukemic Patients.* (20311)

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The theory that an excessive rate of hemolysis might be a causative factor in the anemia of leukemia is not new(1). Jaffe's(2) observation of hemosiderosis in the organs of leukemic patients who had not been transfused led him to emphasize the probability of an increased red cell destruction in this disease. In the past few years R. Berlin(3) and Aas(4) and Brown *et al.*(5) have reported that donor erythrocytes have a sub-normal life span in leukemic patients, even when the latter failed to exhibit the usual signs of hemolytic anemia. Ross and his colleagues(6) have demonstrated increased fecal urobilinogen and shortened red cell life in patients with leukemia. The red cell survival was estimated in the latter report by radioactive iron tagging of the patient's erythrocytes, while in the study by R. Berlin the survival of transfused cells was studied by the Ashby technic. N. I. Berlin *et al.*(7) have demonstrated a shortened red cell life in some leukemic patients by glycine C^{14} tagging of their red cells. Watson and Hagen(8) have reported some instances of frank hemolytic anemia in patients with leukemia.

The mechanical fragility (M.F.) of the erythrocytes is increased in several varieties of hemolytic anemia, *e.g.,* congenital spherocytosis(9), thermal burns(9), some cases of acquired hemolytic anemia(10), methemoglobinemia with hemolytic anemia(11), malaria, and the sickled cells of sickelemia(9). A similar increase in the M.F. of the erythrocytes from leukemic patients is reported in this paper.

Methods. One-half ml of oxalated(12) venous blood with the hematocrit adjusted to 35% by adding or removing plasma was rotated at 44 RPM at room temperature for one hour in a 50 ml Erlenmeyer flask containing 10 glass beads 4 mm in diameter. The radius of the circle through which the flasks turned was 8.7 cm. The apparatus and method are similar to that described by Shen, Fleming, and Castle(8). The hemoglobin appearing in

* Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

the plasma of the rotated sample was measured in an Evelyn colorimeter and expressed as a per cent of the total hemoglobin present in ½ ml of blood. Blood from 43 leukemic patients was studied. The "control" values were determined on 43 persons, either healthy people or hospitalized patients with illnesses considered unrelated to the present study. The controls were selected to match the patients in age. In the leukemic group there were 16 patients with chronic lymphocytic leukemia, 13 with chronic granulocytic and 14 with acute leukemia. Fecal urobilinogen values were determined by Watson's technic (13), utilizing a 4-day stool collection.

TABLE I. Mechanical Fragility of Erythrocytes in Leukemia, in 43 Cases.

Age	Sex	Diagnosis	WBC $\times 10^3$	HGB, g/100 ml	M.F. %
63	♂	AL(?)	5.0	7.3	2.2
63	♂	CGL	30.4	9.9	2.6
65	♂	"	30.7	10.0	2.7
2	♂	AL	96.2	6.8	3.0
85	♀	CLL	49.6	11.9	3.1
46	♀	"	9.4	11.5	3.4
37	♂	CGL	18.1	16.5	3.5
65	♂	CLL	23.6	14.0	3.7
67	♂	"	24.0	15.4	4.1
69	♂	"	70.5	14.0	4.1
68	♂	"	66.5	12.3	4.1
40	♀	AL	5.9	10.8	4.4
19	♂	"	14.3	9.5	4.7
64	♂	CGL	28.3	11.5	4.8
2	♂	AL	7.4	10.2	5.0
43	♀	CGL	809.1	10.0	5.2
18	♀	AL	7.0	12.0	5.7
22	♀	"	64.3	14.0	5.8
55	♀	CLL	20.5	11.3	5.9
53	♂	AL	220.1	7.5	5.9
57	♀	CLL	103.5	12.7	5.9
14	♂	"	113.0	6.8	5.9
50	♂	"	68.2	16.0	6.1
68	♀	CGL	119.5	11.1	6.2
62	♀	"	297.0	10.5	6.2
61	♂	CLL	282.1	7.1	7.0
59	♂	"	25.1	12.2	7.1
65	♀	CGL	257.0	11.8	7.7
45	♀	"	79.6	13.7	7.8
49	♂	CLL	221.5	4.8	7 9
75	♂	AL	84.0	10.6	7.9
59	♀	"	4.9	11.8	8.0
23	♀	CGL	78.4	8.8	8.3
62	♂	AL	1.1	6.4	8.9
50	♀	CGL	72.5	13.2	9.1
66	♂	"	141.8	6.7	9.1
64	♀	AL	5.9	9.1	9.5
3	♂	"	6.9	6.7	9.6
39	♀	"	33.5	7.6	9.8
59*	♂	CLL	55.9	4.8	10.0
66	♂	"	59.5	11.0	10.8
59*	♂	"	11.3	3.7	11.9
30	♂	CGL	184.5	12.5	13.0
		Avg		10.3	6.46

* Positive Coomb's test.

TABLE II. Summary of Statistical Analysis of Mechanical Fragility of Erythrocytes.*

	Avg M.F. %	Range	S.D.†
43 leukemic patients	6.46	2.2–13.0	± 6.91
(26 male)	6.4		
(17 female)	6.6		
43 controls	4.38	2.1– 6.4	± 1.26
(19 male)	4.6		
(24 female)	4.2		

$t = 4.698$—significant at the 1% level of significance.

* The University of Wisconsin Computing Service obligingly analyzed the data summarized in Table II.

† S.D. = Stand. deviation.

Results and discussion. Tables I and II show the pertinent data. The average M.F. for the control group was 4.4% and for the leukemic group it was 6.5%. The average for the 14 patients with acute leukemia was 6.5%, for chronic granulocytic 6.6%, and for chronic lymphocytic 6.1%.

The Coomb's test was positive in 2 of 10 bloods studied from patients with chronic lymphocytic leukemia. The M.F. of these 2 bloods was 10% and 11.9%, and the patients excreted 663 mg and 348 mg of fecal urobilinogen per day.

The average hemoglobin level for the 43 leukemic patients was 10.3 g/100 ml. The 12 having an M.F. of at least 8% had an average hemoglobin of 8.5 g/100 ml, while those with an M.F. of less than 8% had an average hemoglobin level of 11.1 g/100 ml. Interpretation of these data is difficult because transfusions had been given to some patients before the M.F. was determined, but the average M.F. of blood from 20 patients who had received no transfusions was 6.2%, not significantly lower than the average of 6.5% for the total group of 43.

Eighteen of the 43 leukemic bloods (42%) had an M.F. value which exceeded the highest value in the control group.

The M.F. of erythrocytes from one woman with chronic granulocytic leukemia was determined 5 times over an interval of 19 months

with the following results:

Date	M.F. %	Hematocrit
7/51	6.2	35
10/51	8.3	30
6/52	3.9	36
8/52	8.5	31
3/53	1.9	49

In March, 1953, at the time of the most recent M.F. determination the patient was enjoying a remission following therapy.

An increased M.F. is common to several varieties of hemolytic anemia, and the finding of an increased M.F. of the red cells from leukemic patients is consistent with previous studies of a different nature indicating an increased rate of red cell destruction as a contributory factor in the anemia of leukemic patients.

Summary and conclusions. The average mechanical fragility of the erythrocytes from 43 leukemic patients was 6.5%, while that of the control group was 4.4%. Statistical analysis of the reported data indicates that this difference is not due to chance.

It is a pleasure to acknowledge the help of Miss Margaret Thompson with some of these determinations.

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Received May 4, 1953. P.S.E.B.M., 1953, v83.

Evidence for Lipolytic Action by Human Plasma Obtained after Intravenous Administration of Heparin.* (20312)

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The action of heparin *in vivo* has become a focal point in the study of serum lipoproteins and their relationship to the development of atherosclerosis. It was observed by Hahn(1) that intravenous injection of heparin could effectively clear lipemic blood in humans. More recently, it has been shown by Graham *et al.*(2) in this laboratory, using ultracentrifugal technics, that drastic alterations in the serum lipoprotein distribution could also be produced by heparin *in vivo*. These effects have not been observed when heparin was allowed to react with serum *in vitro;* but plasma obtained from a human subject 15-30 minutes after the administration of heparin can evidently interact with certain classes of lipoproteins on incubation to cause changes in their ultracentrifugal pattern and other observable effects. For brevity, such plasma will be referred to as post-heparin plasma. Attempts to isolate or concentrate an active component by chemical fractionation of post-heparin plasma have been reported by other workers(3), and ultracentrifugal fractionation is being carried out in this laboratory toward a similar goal. The most pronounced transformations that occur as a result of reaction

* This work was supported in part by the U. S. Atomic Energy Commission and by the Lederle Laboratories Division, American Cyanamid Co.