This discrepancy is probably associated with the greater incubation time used in the recent experiments. Thus, a substantially larger amount of hydrolysis of the proteins incubated in sodium hydroxide may have taken place with prolonged standing.

Summary. 1. Titration and swelling curves of the human aortic albuminoid were determined and analyzed with respect to possible effects of increasing age and atherosclerosis. Except for previously determined differences in acid-binding, no marked changes were observed. 2. Titration curves were characterized by extension of the isoelectric point into an isoelectric range between approximately pH 5 and 10. Maximal swelling was observed at approximately pH 12.

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Cholesterol and Cholate Concentration of Perfusate and Bile of Isolated Liver Preparation.* (20310)

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A perfusion apparatus has been described by Brauer, Pessatti, and Pizzolato(1) in which the isolated rat liver can be maintained in a viable state for many hours, and its functional capacity demonstrated by the continuous formation of bile. The present report describes the cholesterol and cholate content of the perfusate, and of the bile, in 12 perfusion experiments on the isolated rat liver.

Methods. Young, male, non-fasted, Long-Evans rats in a weight range of 220-350 g were utilized. Ether inhalation anesthesia was employed in all instances (in some cases, intraperitoneal nembutal (12 mg) was given initially). After opening the abdomen, 1.0 mg of heparin was injected into the inferior vena cava. The bile duct, portal vein, and thoracic vena cava were cannulated in turn with polyethylene catheters. The liver was excised and placed in the perfusion apparatus, with perfusate entering the portal vein cather under a constant, non-pulsatile pressure of 21.5 cm. Clean, but not sterile, surgical technic was employed. The operative time during which circulation through the liver was interrupted, *i.e.*, from ligation of the portal vein until placement of the excised liver in the perfusion apparatus, was under 10 minutes in all cases, and averaged 5 minutes. The perfusate consisted of one part of heparinized whole rat blood diluted with 3 parts of salt solution, prepared according to Brauer(1). Dextrose sufficient to achieve a concentration of 80 mg % was added to the complete perfusate. In all but the first 4 experiments, an additional 4.0 cc of 10 "essential" amino acids was added, as prepared by White(2). In the last 4 experiments 4.0 cc of 9 of the B vitamin group, and 4.0 cc containing carotene, Vit. A, ascorbic acid, glutathione, and cysteine were added, also as prepared by White(2). An infusion containing the same salt and albumin† concentrations as the perfusate with the addi-

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TABLE I. Cholesterol and Cholate Concentration of Perfusate and Bile of Isolated Liver Preparation.

	Duration of perfusion Hr	Min.	Perfusate: Flow* (cc/min.)	Perfusate: Cholesterol Initial (mg %)	Perfusate: Cholesterol Final (mg %)	Perfusate: Cholate Initial (mg %)	Perfusate: Cholate Final (mg %)	Bile: Flow (cc/hr)	Bile: Cholesterol (mg %)	Bile: Cholate (mg %)
Avg:	7	25	49	13.8	18.0	1.2	1.4	.24	6.3	117
Range:	3 to 11	40 to 10	7–95	8.3–17	8.3–30	.0–2.6	.0–3.0	.10–.61	3.5–11.6	59–240
S.E. mean†:			4.6	1.6	2.0	.42	.32	.04	.86	17
No. of observations:	12		12	5	11	5	11	12	8	10

* Avg flow values calculated as mean of values observed at beginning, middle, and end of perfusion period.

† Stand. error of mean calculated by formula $\text{S.E.} = \sqrt{\frac{\Sigma x^2}{N^2}}$.

tion of .5% of dextrose, was injected at a rate of .29 ml/hour. To control bacterial growth, terramycin was added, first to the infusion, later directly to the perfusate in a concentration of 0.01 mg/ml. Samples of perfusate and bile were removed for analysis of cholesterol and cholate according to technics previously described from this laboratory(3,4). The average initial volume of the perfusate was 95 ml. The perfusate gave an hematocrit reading of about 10%; the pH varied from 7.1 to 7.3; the proteins were 2.5 g %. Oxygen saturation was maintained at 92%, temperature at 37°C.

Results. The following data were compiled from 12 perfusion experiments.

I. *Perfusate.* The average flow maintained through the hepatic circulation during the period of perfusion was 49 cc per minute. The rate of flow through the liver generally increased, during the first half-hour, from a minimum to the average value and continued unabated at this rate until the experiment was terminated at the cessation of bile flow. The cholesterol content of the perfusate at the start of the perfusion averaged 13.8 mg % (range: 8.3-17 mg %). At the end of the perfusion, the cholesterol content averaged 18.0 mg % (range: 8.3-30 mg %). The plasma cholesterol of intact rats averages 48 mg %) (range: 32-76 mg %(7)). The difference between initial and final perfusate cholesterol levels is within three standard errors of the mean and is not considered significant. The initial level of perfusate cholate averaged 1.2 mg % (range: 0.0-2.6 mg %). The final concentration averaged 1.4 mg % (range: 0.0-3.0 mg %). The average cholate concentration of intact rats recently obtained in this laboratory is 3.5 mg % (range: 0.5-4.5 mg %(7)).

II. *Bile.* The average bile flow was 0.24 cc per hour. This is 41% of the average rate of 0.59 ml/hour secreted by the intact animal (5). The total amount of bile collected from each preparation averaged 2.0 cc (range: 0.68-4.9 cc). The average cholesterol content of the bile was 6.3 mg % (range: 3.5 mg % to 11.6 mg %). The cholesterol concentration of bile obtained from intact rats averages 55 mg % (range: 42-69 mg %). The amount of cholesterol secreted per hour was 0.0183 mg. This is 22.5% of the 0.075 mg per hour secreted by the intact rat(7). The average cholate level in the bile was 117 mg % (range: 59-240 mg %). In the intact rat biliary cholate concentration averages 184 mg % (range: 98-290 mg %(7)).

Discussion. The above studies demonstrate that the isolated perfused rat liver not only can be maintained in a viable state over a period of many hours as demonstrated by the continuous formation of bile, but will continue to secrete cholesterol and cholate into the bile. Since biliary cholesterol has been shown to be an index of hepatic synthesis of cholesterol(5) and biliary cholate to be the major excretion product of the hepatic destruction of cholesterol(6), these data demonstrate the integrity of these vital processes of cholesterol synthesis and degradation in the isolated perfused rat liver.

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.

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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

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