Erythrocyte Survival After Experimental Portacaval Shunt.* (30417)

EMANUEL RUBIN, NORMAN R. GEVIRTZ, PAUL RICHTER AND JULIUS H. JACOBSON II
(Introduced by Louis R. Wasserman)
Departments of Pathology, Hematology and Surgery, Mount Sinai Hospital, New York City

Among the complications attributed to the construction of portacaval shunts for portal hypertension is hemolytic anemia, as evidenced by increased circulating indirect reacting bilirubin and a shortened erythrocyte survival following the operation(1). The observation of hepatic siderosis and hemochromatosis following portacaval shunts in patients with cirrhosis(2), and the demonstration that experimental diversion of the portal flow from the liver can result in hepatic siderosis in the absence of cirrhosis(3,4) suggested that diversion of the portal blood flow *per se* might lead to shortened erythrocyte survival and a consequent accelerated turnover of hemoglobin iron. To investigate this possibility the survival of *in vivo* labeled erythrocytes and labeled homologous erythrocytes in rats subjected to portacaval shunts was studied.

Materials and methods. The experimental group consisted of 10 Fisher CDF rats obtained from the Charles River Laboratories with an initial weight of 180-200 g. These highly inbred animals were used because of their genetic homozygosity. End-to-side portacaval shunts were constructed by microsurgical techniques in 7 animals and sham operations were performed in 3 controls. The rats were housed in individual cages and pair fed during the experiment.

Cr 51 labeling: Six weeks following surgery, one shunted and one control animal were sacrificed by exsanguination from the aorta. The blood in ACD solution was incubated for one hour with Cr^{51} at 37°C. At that time ascorbic acid was added to reduce the chromate(5). The supernatant was removed and 0.5 ml packed erythrocytes was injected into the tail veins of recipient animals according to the following scheme: Group 1) One rat. Control blood into control recipient. Group 2) Three rats. Control blood into shunted recipients. Group 3) One rat. Blood from shunted rat into control rat. Group 4) Three rats. Blood from shunted rat into shunted rats. At various intervals, 0.02 ml blood was withdrawn from the tail vein and diluted in 1 ml distilled water. This was counted in a well-type scintillation counter (3″ sodium iodide crystal) preset to 20,000 counts.

Di-isopropyl-fluorophosphate-P^{32} (DFP^{32}) labeling. A dose of 8 microcuries DFP^{32} was injected intramuscularly into all animals, with the exception of those sacrificed to obtain Cr^{51} labeled erythrocytes. At suitable intervals progressive amounts of blood, from 0.02 ml to 0.2 ml, were obtained from the tail vein and evenly distributed on lens filter paper. For the final count, 1 ml of blood was obtained on day 49, at time of sacrifice. The filter paper was wrapped around a Victoreen 1B85 Geiger tube and counted with the scaler preset to 3200 to 6400 counts.

Results. The body weights of the rats subjected to portacaval shunts and their pair fed controls had increased about 10% by the end of the experiment. The initial hematocrits and measured red cell masses are given in Table I. The half-life of Cr^{51} labeled erythrocytes in all groups was about the same, with a range of 12 to 14 days (Table I). The life span of erythrocytes labeled *in vivo* with DFP^{32} was substantially the same in all animals, with a range of 55 to 66 days.

Discussion. Studies of erythrocyte survival utilizing *in vivo* labeling with DFP^{32} in rats show no evidence of decreased erythrocyte life span after portacaval shunts. Cr^{51} labeled erythrocytes from shunted rats injected into controls and into other shunted rats have a normal apparent half-life, while erythrocytes from control rats injected into shunted rats have the same normal survival. The eryth-

* This investigation was supported in part by Research Grants AM-03846 and AM-01063 from Nat. Inst. of Arthritis & Metab. Dis., HE-07707 U.S.P.H.S., and Albert A. List, Frederick Machlin and Anna Ruth Lowenberg Funds.

TABLE I. Survival of Cr^{51} Labeled Homologous Erythrocytes and Erythrocytes Labeled *in vivo* with DFP^{32} in Rats Subjected to End-to-Side Portacaval Shunts and in Normal Rats.

Group	Recipient	Donor	RBC volume Cr^{51} (ml)	Initial hematocrit (%)	Cr^{51} survival (T-½) (days)	DFP^{32} survival (days)
1	Control	Control	4.0	47	14	63
2	Shunt	"	4.4	49	14	58
2	"	"	6.6	54	14	55
2	"	"	7.3	58	14	55
3	Control	Shunt	6.6	55	13	62
4	Shunt	"	6.2	58	12	Died at 9 days
4	"	"	6.6	54	14	62
4	"	"	Not done	59	—	66

rocyte life span obtained with DFP^{32} is similar to that found by other investigators (6), while the half-life of Cr^{51} labeled erythrocytes is slightly longer. Thus, it is unlikely that the clinically observed decrease in erythrocyte survival following portacaval shunts is directly caused by diverting the portal flow into the systemic circulation. Many types of liver disease, including portal and postnecrotic cirrhosis(7-9), biliary cirrhosis and hepatitis(10) are accompanied by increased hemolysis. This is ascribed usually to hypersplenism, but suggestive evidence for an alteration in the shape of erythrocytes, possibly resulting from liver damage, has been presented(8). The degree of hemolysis has also been correlated with the severity of liver cell damage in one series(11). In view of the demonstration of hepatic cell damage induced by diversion of the portal flow(12), increased hemolysis following clinical portacaval shunt may result from a relatively mild insult to the integrity of the hepatic cells produced by portal blood deprivation superimposed on the pre-existing intrinsic liver damage. The hepatic siderosis following portacaval shunt also cannot be attributed to hemolysis but is rather a reflection of the diffuse hepatocellular damage produced by lack of portal blood, probably with increased absorption of dietary iron(13).

Summary. Studies of erythrocyte survival using Cr^{51} and DFP^{32} labeled red cells in rats following end-to-side portacaval shunts disclosed no change in erythrocyte longevity. It is suggested that the clinically observed decreased erythrocyte survival and hepatic siderosis following portacaval shunts may result from hepatocellular damage produced by the diversion of the portal flow, which is superimposed on pre-existing liver damage.

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Received May 17, 1965. P.S.E.B.M., 1965, v119.