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Mechanism of Ethyl Palmitate and Cobra Venom Factor Enhancement of Heterologous Erythrocyte Survival (38290)

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(Introduced by Patrick J. Mulrow)

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The intravascular survival of human erythrocytes (RBCs) transfused into rats is extended significantly when the animals are pretreated with ethyl palmitate (EP) and cobra venom factor (CVF) (1). Ethyl palmitate blocks the reticuloendothelial system (RES) (2, 3) and thus delays RES phagocytosis of the transfused human RBCs, whereas CVF, a potent complement inhibitor (4), enhances the RBC survival through the inhibition of intravascular hemolysis. This animal system allows investigation of the behavior of abnormal human erythrocytes *in vivo*, and it has been applied to the study of sickle hemoglobinopathies (5, 6) and hereditary spherocytosis (7).

Our objective in the present experiments was to examine more closely the mechanism of prolonged human RBC survival in this animal model. For this purpose, the ^{51}Cr organ uptake in rats transfused with ^{51}Cr-labeled human RBCs was measured and the changes in organ radioactivity induced by pretreatment of the animals with EP and/or CVF were determined.

Methods. A total of 43 male Sprague–Dawley rats weighing between 150 and 250 g was used. Of these, 7 rats were pretreated with EP alone, 10 with CVF alone, 18 rats were treated both with EP and CVF, and a group of 8 untreated rats served as controls. Pretreatment of the animals with EP and CVF and the heterologous transfusions were carried out with a modification of a previously described method (5, 8). Briefly, each animal was injected intravenously with 0.5 g/kg of EP (Eastman Kodak Co., Rochester, NY) and/or with 10 anticomplementary units of CVF (Cordis Laboratories, Miami, FL) 2 hr prior to the transfusion study. Four-milliliter blood samples from 5 normal human subjects were collected in tubes containing acid–citrate–dextrose solution and incubated with 200 μCi of ^{51}Cr (sodium chromate, New England Nuclear, Boston, MA). After washing twice with isotonic saline, the suspension of labeled RBCs was adjusted to a hematocrit of 33%. Three milliliters per kilogram of the human RBC suspension was then transfused into the anesthetized animals through the dorsal vein of the penis. An aliquot of the labeled RBCs was also radioassayed to determine the total radioactivity injected. At periodic intervals after transfusion, 10-μl blood samples were taken either from the cut tail surface or from blood obtained by cardiac puncture and their ^{51}Cr activity determined. Also at various times after transfusion, a minimum of 2 animals in each pretreatment group was killed with ether. Liver, spleen, and one kidney were removed from the carcasses, and excess blood was blotted. The organs were weighed and their total ^{51}Cr radioactivity determined. The radioactivity contributed to each organ by trapped blood was estimated as follows:

$$\text{cpm in organ blood} = \text{cpm in 1 ml blood} \times \text{organ wt (g)} \times N/100,$$

where N is the number of milliliters of blood contained in 100 g of tissues. For liver, spleen, and kidney, N was 4.7, 16.6, and 4.9, respectively, according to published values for blood volume in rat organs (9). All organ activity was corrected by subtracting the amount contributed by the blood at the time of death. This correction was always less than 10% of total organ activity. Organ specific activity was obtained by dividing the total number of counts by the weight of the organ.

Results. The specific ^{51}Cr activities of the tissues of rats transfused with ^{51}Cr-labeled human red cells are shown in Table I. In the untreated animals, liver and spleen specific activities were similar (spleen/liver ratio about 1/1) so that the total liver activity was more than 20 times greater than that of the spleen. In animals treated with EP only and sacrificed 1.5 hr after transfusion, liver specific activity was reduced to one-seventh and spleen specific activity to one-fifth that of the control value of the untreated rats. The animals treated with EP alone had the highest renal specific activity at all times following transfusion. Rats treated with CVF only showed little change in hepatic activity, but spleen specific activity rose to the highest value observed for any organ both at 24 and 48 hr following transfusion (spleen/liver ratios > 10/1). In these animals, renal ^{51}Cr activity was the lowest. The lowest liver and spleen specific activities immediately after transfusion were found in the animals treated with both EP and CVF.

Figure 1 shows the effect of treatment with EP alone, CVF alone, and EP plus CVF on the total liver and spleen uptake of ^{51}Cr. The posttransfusion RBC recovery and ^{51}Cr RBC survival is also shown. In control animals and in those treated with either EP or CVF alone, less than 50% of the injected RBCs remained in circulation 15 min after transfusion. The group of rats treated with EP alone showed at all times a profound inhibition of liver and spleen ^{51}Cr uptake. The animal group treated with only CVF showed the greatest values for total splenic ^{51}Cr uptake. Both at 24 and 48 hr, the amount of radioactivity present in the spleen was almost equal to that in the liver despite gross differences in the weights of these organs. Treatment with both EP and CVF resulted not only in profound initial depression of organ uptake but also in marked enhancement of ^{51}Cr survival of human RBCs in the animal's blood.

Discussion. The patterns of ^{51}Cr liver and spleen uptake in rats transfused with ^{51}Cr-labeled human RBCs are consistent with the mechanisms by which EP and CVF are thought to prolong human red cell survival. In the untreated animals, the fate of the majority (63–80%) of the transfused RBCs appears to be phagocytosis by the liver RES cells. The spleen and kidney activities are low in untreated animals, suggesting that splenic uptake and intravascular hemolysis are not as important as liver RES phagocytosis for the immediate clearance of human erythrocytes from the animals' blood. We have not excluded, however, the possibility that in these animals a small proportion of the liver ^{51}Cr activity might represent uptake of haptoglobin–hemoglobin complexes generated as a result of intravascular hemolysis.

Administration of EP alone resulted in two important changes: (a) total hepatic and splenic uptake of human RBCs was reduced to one-tenth that of the untreated control rats, a finding that is consistent with profound blockade of RES phagocytic function, and (b) renal specific activity was the highest observed in any of the groups of animals studied. Intravascular survival of human RBCs, however, was only slightly prolonged in the EP treated rats. This suggests that the high renal specific activity observed in these

TABLE I. Effect of Ethyl Palmitate (EP) and Cobra Venom Factor (CVF) on Organ ^{51}Cr Specific Activity [a] of Rats Transfused with ^{51}Cr-Labeled Human RBCs.

Organ	Hours after transfusion	Control	EP	CVF	EP+CVF
Liver	1.5	60.77	8.68	53.38	9.40
	24.0	24.35	19.62	35.57	42.54
	48.0	30.48	6.82	37.68	67.26
Spleen	1.5	59.10	10.85	68.28	6.55
	24.0	17.99	30.06	378.60	192.00
	48.0	24.63	6.45	423.09	302.22
Kidney	1.5	9.09	60.66	2.55	9.96
	24.0	23.62	87.45	2.55	38.46
	48.0	31.83	34.52	10.28	15.71

[a] Mean cpm/mg of tissue. 2–4 animals were used for each determination.

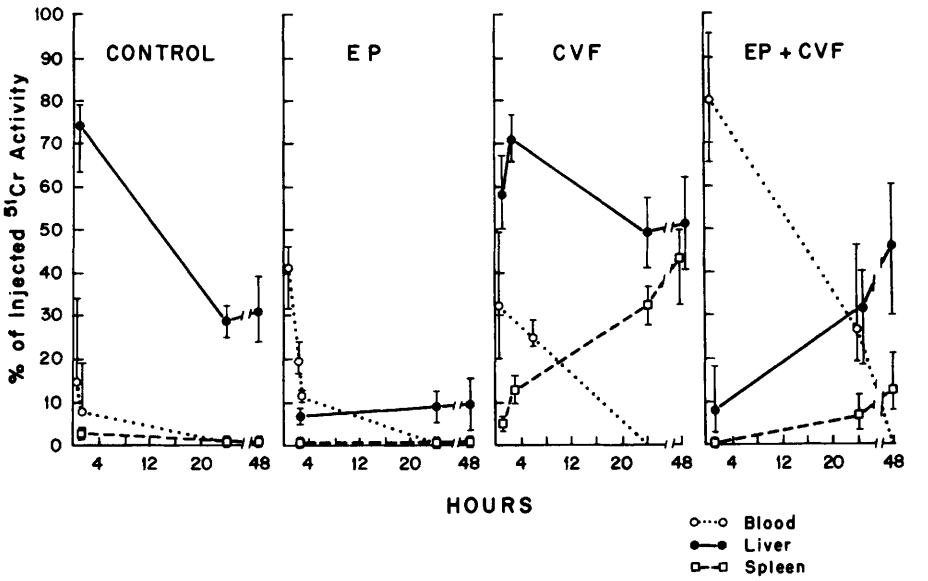

FIG. 1. This figure shows the liver and spleen ^{51}Cr localization and blood survival of ^{51}Cr human erythrocytes at various intervals for control rats as well as those pretreated with ethyl palmitate (EP) and cobra venom factor (CVF) or both. The ordinate measures percent of the injected ^{51}Cr activity found in the organs and the abcissa time after transfusion. Points represent mean values and the bars cover the ranges for total organ activity.

animals was due to increased excretion of labeled hemoglobin and/or of unbound ^{51}Cr, probably derived from nonphagocytized, intravascularly lysed RBCs. Intravascular hemolysis remained unchecked and actually appeared to play a greater role in the destruction of the RBCs in circulation.

In rats injected with CVF alone, renal specific activity was less than one-third that of the control rats at 1.5 hr and about one-tenth their level at 24 hr posttransfusion. The reduction in renal activity in these animals is consistent with decreased excretion of ^{51}Cr RBC breakdown products probably because of CVF inhibition of complement-dependent intravascular hemolysis. Liver ^{51}Cr uptake was essentially the same as that in control animals but splenic activity was increased to levels which were higher than that recorded for any organ in the entire study. The ^{51}Cr survival of transfused human RBCs in animals treated only with CVF was minimally prolonged over control values since overall RES activity remained intact and continued to rapidly remove circulating human RBCs. The liver pattern of high initial activity followed by a decline during the next 24 hr suggests high initial RES phagocytosis of the transfused human RBCs with rapid attainment of saturation of total organ phagocytic activity for these cells. In contrast, the spleen continued to gradually increase its radioactivity during the 24-hr period, suggesting sequestration as the major mechanism for organ accumulation of labeled RBCs with little metabolic turnover.

The most important change observed in the animals treated with the combination of EP and CVF was the significant enhancement of the intravascular survival of human RBCs. The combined effect appeared to be synergistic because the ^{51}Cr posttransfusion recovery and survival of the heterologous RBCs in EP plus CVF treated animals was far greater (more than 25% of the transfused RBCs circulated after 24 hr) than the sum of the values in animals treated with either CVF or EP alone (less than 50% of transfused RBCs were present in the blood 15 min after transfusion). This apparent synergism could be explained by our organ findings which suggest that reticuloendothelial phagocytosis and intravascular hemolysis are mechanisms competing for blood clearance of the transfused human RBCs. Suppression of only one of the removal mechanisms did not result in the expression of its full effect in the improvement of intravascular human RBC survival because it probably allowed enhancement of the unblocked

competing mechanism. The full effect of EP and CVF on the extension of the survival of heterologous RBCs became apparent only when both substances were administered in combination.

Experiments in this study were conducted with RBCs from human subjects without hematologic abnormalities. Therefore, the patterns of organ uptake described provide a baseline for future investigation of the mechanisms and sites of removal of abnormal human RBCs in this model.

Summary. In this study the blood survival and organ localization in rats of intravenously injected ^{51}Cr-labeled human erythrocytes was determined in controls and following pretreatment with ethyl palmitate, a cobra venom factor, or both. Organ distribution of labeled cells was consistent with a reticuloendothelial blockade effect for the ethyl palmitate and inhibition of hemolysis for the cobra venom factor. Erythrocyte survival was enhanced only moderately following the injection of either of these substances alone. The combined administration of ethyl palmitate and the cobra venom factor, however, resulted in marked prolongation of human erythrocyte survival consistent with a synergistic effect. This suggests that in the untreated animal, both complement-dependent hemolysis and reticuloendothelial phagocytosis compete for the removal of circulating heterologous erythrocytes. Both must be blocked simultaneously for maximum heterologous erythrocyte survival.

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