Tissue Red Cell and Plasma Distribution Changes Due to Irreversible Hemorrhagic Shock in Dogs (36890)

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Changes in blood volume indicator concentrations consequent to hemorrhage and shock observed in this laboratory (1) and in others (2, 3, 5) have been explained only incompletely by (a) capillary fluid exchange, or (b) redistribution of red cells and plasma. Also, studies on regional blood volumes have led to conflicting ideas concerning the presence or absence of blood pooling in various tissues and on the significance of such pooling, if it does indeed occur (6). Our experiments were designed to further define the relative changes in red cell and plasma distribution in skin and skeletal muscle tissues during irreversible hemorrhagic shock.

The red cells and albumin were labeled with isotopic tracers and radiation detectors were positioned over the tissues to monitor changes in radioactivity. These changes were then interpreted in terms of changes in red cell-^{51}Cr and albumin-^{131}I distribution.

Methods. Sixteen adult mongrel dogs averaging 13.6 kg were sedated with 10 mg/kg morphine sulfate (s.c.) and anesthetised 30 min later by intravenous administration of 15–20 mg/kg of sodium pentobarbital. Additional doses of sodium pentobarbital were given as needed to maintain anesthesia. After loosely tying the animal on its back, the spleen was removed through a mid-ventral abdominal incision. The trachea was cannulated, and the left carotid artery, left external jugular vein, left femoral artery and right cephalic vein were exposed. A period of one hour passed between the major surgical procedures and the beginning of the experiment in order to allow stabilization of the animal. Prior to cannulation of the vessels, heparin sodium was administered (10 mg/kg) to prevent clotting. This dose was supplemented with 2 mg/kg doses each hour.

Autologous red cells were labeled with ^{51}Cr (1) and plasma was labeled with albumin-^{131}I. Eight animals were injected with red cells-^{51}Cr and eight with albumin-^{131}I.

Radioactivity within selected tissues was monitored with a collimated probe having a one inch NaI scintillation crystal connected to a ratemeter (a 5 or 15 sec time constant was used). The probe was positioned directly upon a portion of the gracilis muscle from which the overlying skin had been removed (8 experiments) or over the hind paw (8 experiments). Adjacent tissues were covered with lead sheets to shield the probe from extraneous radiation. Each group of eight consisted of four animals that received red cells-^{51}Cr and four that were administered albumin-^{131}I.

All parameters were recorded on an Electronics for Medicine DR-8 recorder. A femoral artery was cannulated and connected to a reservoir bottle. The cannula was fitted with a sidearm to which was connected a Statham pressure transducer. Blood flow in the other femoral artery was continuously monitored with a Statham K-2000 electromagnetic blood-flow meter.

Control values were obtained for 60 min during which time stabilization of each parameter occurred. The animal was then hemorrhaged into the reservoir until the mean arterial pressure reached 30 mm Hg. This

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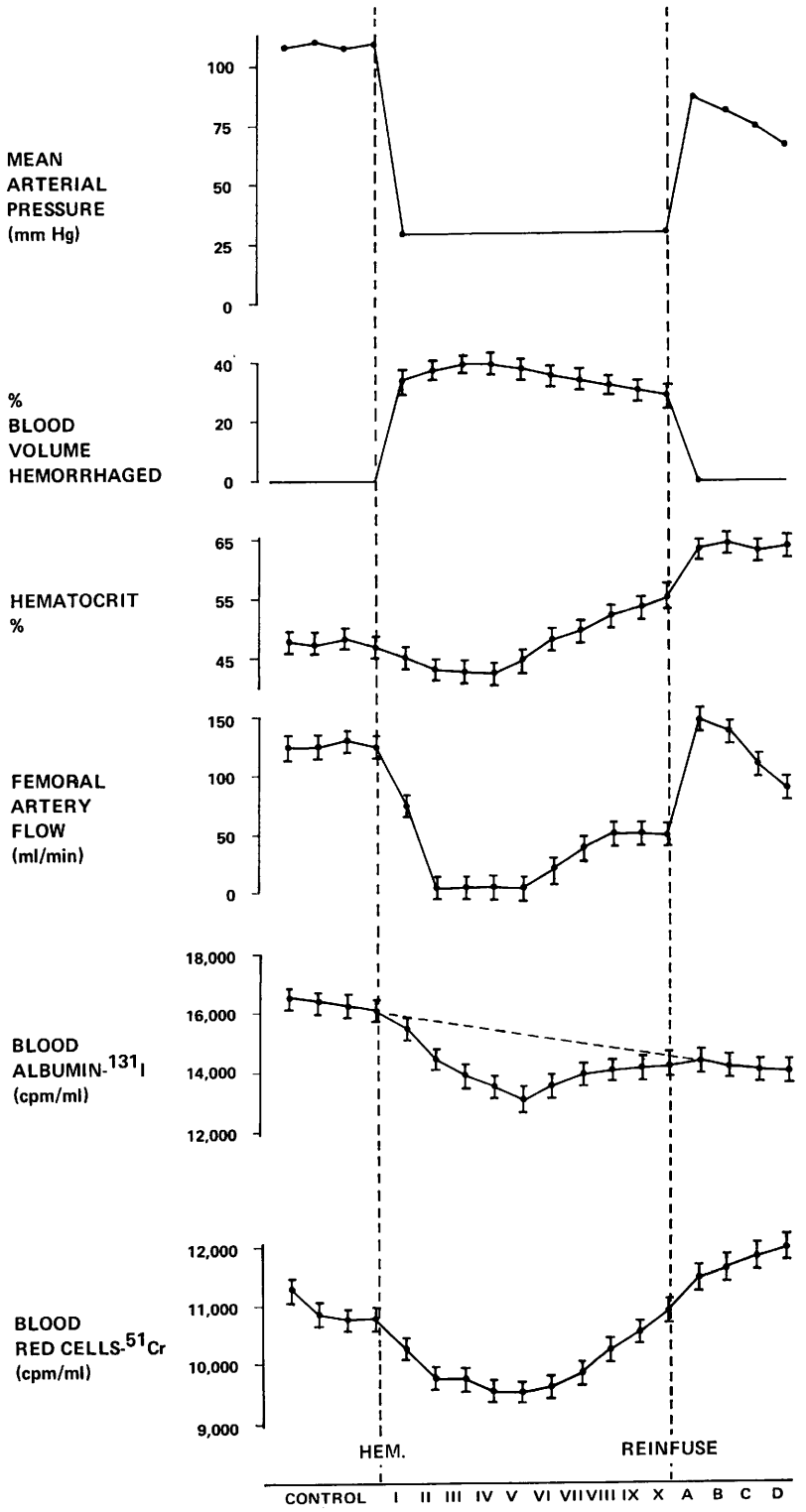
MEAN ARTERIAL PRESSURE (mm Hg)
100
75
50
25
0
% BLOOD VOLUME HEMORRHAGED
40
20
0
HEMATOCRIT %
65
55
45
FEMORAL ARTERY FLOW (ml/min)
150
100
50
0
BLOOD ALBUMIN-131I (cpm/ml)
18,000
16,000
14,000
12,000
BLOOD RED CELLS-51Cr (cpm/ml)
12,000
11,000
10,000
9,000
HEM.
REINFUSE
CONTROL I II III IV V VI VII VIII IX X A B C D

pressure was then maintained by adjusting the reservoir height as needed throughout the period of hypotension. The reservoir was open to the circulation so that as the signs of circulatory failure developed the animal could spontaneously take up the hemorrhaged blood as needed. When 25% of the bled volume had been taken up by the animal, the remainder was rapidly reinfused (5–10 min).

Arterial blood samples (3 ml) were drawn in syringes coated with heparin at 20 min intervals. Triplicate determinations of hematocrit by the microhematocrit method were made, and blood levels of radioactivity were measured using 1 ml samples in a well scintillation detector.

Results. Hemodynamics. Hemodynamic data from the 16 dogs subjected to hemorrhage are presented in Fig. 1. Six of these animals died from circulatory failure while the recordings were in progress and the remaining ten were sacrificed when the recordings indicating arterial pressure had fallen to 70% of the immediate post-infusion level. They were in the terminal phase of irreversible shock.

The individual experiments have been standardized by dividing the time from hemorrhage to reinfusion into ten equal time intervals. The time from reinfusion to the time when arterial pressure decreased to 80% of its maximum post-infusion level has been divided into four equal time intervals. This was necessary since the duration of the experiments was quite variable [hypotension: 3.4 $\pm$ 1.4 hr (SD), post infusion: 1.8 $\pm$ 0.6 hr]. It was, nevertheless, noted that the changes in the various parameters measured followed a pattern that was proportionally constant from animal to animal.

It should be noted in Fig. 1 that immediately following the hemorrhage there was a marked reduction in femoral artery flow that was maintained until period VI when it began to increase reaching a significantly higher plateau at interval VII. The hematocrit was also significantly reduced following the hemorrhage but by interval V of the oligemic period began to increase so that by the end of the hypotensive period it surpassed the control level.

Following reinfusion, the arterial pressure and femoral-artery flow increased to near control levels. However, by interval D these values had again decreased to levels approaching those during the oligemic period. The hematocrit increased further post-infusion and then plateaued.

Blood radioactivity changes. In the eight animals that received albumin-^{131}I (Fig. 1), there was a small disappearance slope as was to be expected during the control period. Following hemorrhage there was a highly significant ($p < 0.01$) decrease in activity until interval V. The values then gradually increased to the time of reinfusion ($p < 0.05$). This increase corresponded closely with the increase in hematocrit and femoral artery flow. Following reinfusion the level of activity was unchanged and then established a disappearance slope nearly approximating the one during the control period.

The eight animals that received the red cells-^{51}Cr (Fig. 1) reached the expected plateau concentration during the control period. Consequent to the hemorrhage there was a highly significant ($p < 0.01$) decrease in radioactivity until interval V. The radioactivity then gradually increased reaching the control level by the time of reinfusion of the shed blood. Following reinfusion the blood radioactivity continued to increase above the control level. This is markedly different from the albumin-^{131}I response as the red cells continued to concentrate (radioactivity increased) while the labeled protein did not.

The blood radioactivity values for albumin-^{131}I in Fig. 1 were expressed as cpm/ml plasma by dividing the blood radioactivity at each sampling interval by the comparable 1-hematocrit value. The blood red cell-^{51}Cr

FIG. 1. Relationships between the various time periods (see text for description) and the hemodynamic parameters and hematocrit of the experiments. Control period was one hour, period of hypotension averaged 3.4 $\pm$ 1.4 hr and the post-infusion period averaged 1.8 $\pm$ 0.6 hr. (Brackets indicate $\pm$ SEM).

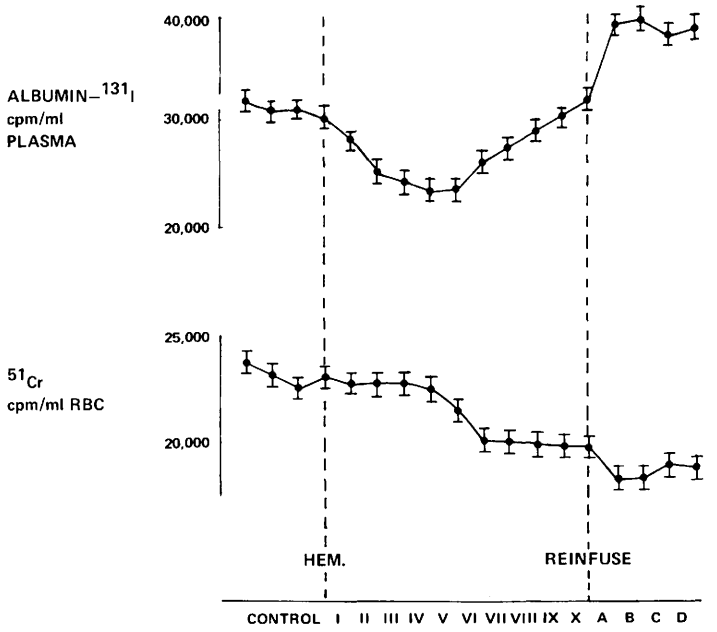

FIG. 2. Average albumin-^{131}I counts/(minute) ml of plasma and average ^{51}Cr counts/minute/ml of red blood cells. (Brackets indicate ± SEM).

values were expressed as ^{51}Cr cpm/ml RBC by dividing the blood values by the comparable hematocrit. These data are presented in Fig. 2.

The albumin-^{131}I cpm/ml plasma following hemorrhage decreased rapidly through period II and then gradually decreased to a minimal level by period V ($p < 0.01$). During periods VI to X, the amount of radioactivity increased to the control level. Following reinfusion the cpm of albumin-^{131}I per ml of plasma sharply increased to a level significantly above the control ($p < 0.05$).

The ^{51}Cr cpm/ml RBC following hemorrhage remained unchanged until period IV followed by a decrease through period VI and then plateaued through period X. Following reinfusion there was a small but insignificant further decrease.

Red cell and plasma distribution changes. Figure 3 is a composite graph of the averaged changes in radioactivity of the two tissues studied with each indicator.

Paw: The red cell-^{51}Cr and albumin-^{131}I radioactivity in the paw exhibited a marked decline post-hemorrhage ($p < 0.01$) and remained low during the oligemic period. Following reinfusion the albumin-^{131}I level increased to approximately one-third of the difference between the control and preinfusion level. The red cell-^{51}Cr level increased to about midway between the control and preinfusion level. Levels of significance were calculated by combining the two groups of four animals since the responses in each group were similar.

Skeletal muscle: There was a significant decline in radioactivity of both red cells-^{51}Cr and albumin-^{131}I after hemorrhage ($p < 0.01$) lasting until about interval IV or V. This was followed by a progressive increase in radioactivity in both the albumin-^{131}I and red cell-^{51}Cr administered animals lasting until the time of reinfusion of the shed blood ($p < 0.05$). This latter effect is distinctly different from that in the paw. Following reinfusion of the shed blood the albumin-^{131}I level increased abruptly and then plateaued near the control level while red-cells distribution increased more gradually to the control level and above. Levels of significance were calculated by combining the two groups of four animals since the responses in each group were similar. That is, the results appeared to

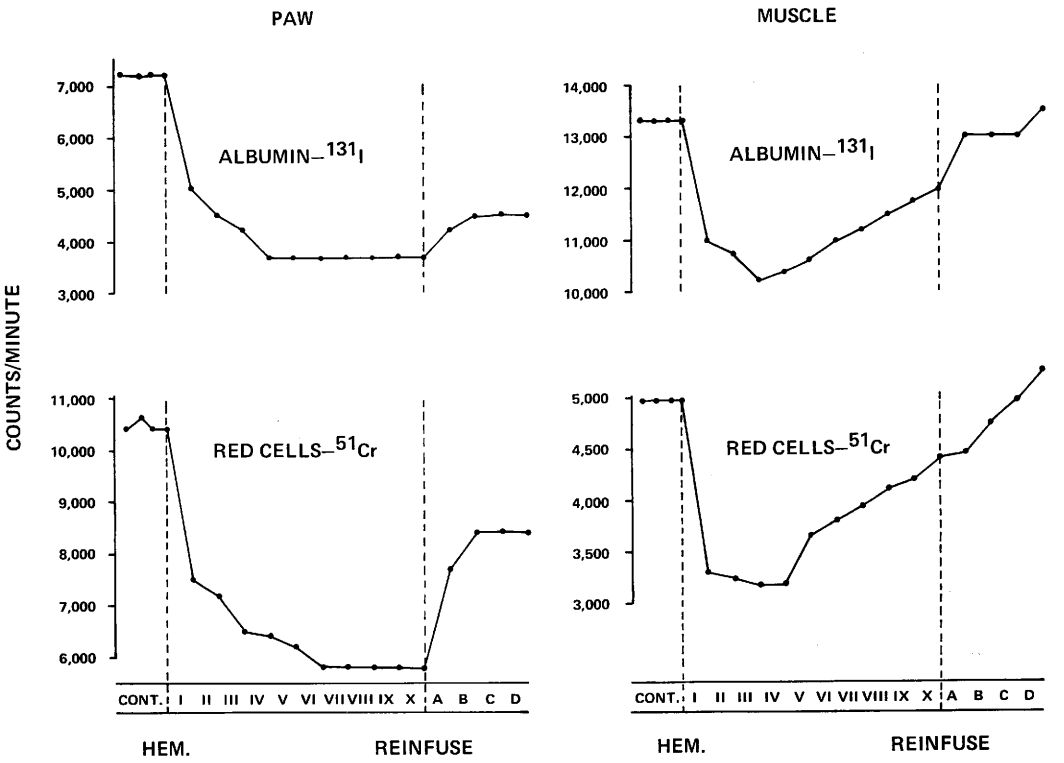

FIG. 3. Average red cell-^{51}Cr and albumin-^{131}I (plasma) distributions to (A) paw and (B) gracilis muscle. Each curve represents the mean of four experiments.

be independent of the indicator used.

Discussion. External monitoring of changes in red blood cell or plasma radioactivity of a tissue would seem to reflect changes in tissue distribution of each component. Factors such as ultrafiltrate shift (1), label loss from the circulation (4), mobilization and trapping of red cells (1, 6), changes in the size of the individual red cells and of density and geometry of the tissues would contribute to this distribution of red cells and plasma. We have tried to account for as many of these factors as possible. The splenic red-cell reservoir has been removed in these experiments, but other reservoirs are not so easily controlled. The other parameters listed have been measured for their effects.

An explanation for the rationale of this method of determining changes in blood distribution is in order. Gamma energies of either of two isotopes (^{51}Cr or ^{131}I) used as blood labels are monitored from a relatively small tissue area. This area is then taken as representing that region of the peripheral circulation, *e.g.*, skin, muscle, etc. Of course extraneous radiation from surrounding tissues must be minimized and this is the purpose of collimation. As the amount of labeled material in the vascular bed under the probe varies, there could be predicted that essentially proportionate change in the recorded activity from under the probe. Since we are not dealing with a point source of radiation, steric factors become a problem. The magnitude of this problem may not be great when the specimen is as small as a dog paw or an isolated strip of skeletal muscle which is relatively thin. However, if we were considering the steric problems of the abdomen for instance, where there is relatively large thickness, the difficulties could be major. This latter was pointed out in a paper by Hirschfield and Fell (7) using a tissue-monitoring technique where they studied thoracic and abdominal changes.

In a previous publication (8) it has been

shown that changes in tissue radioactivity (red cells-^{51}Cr) of the tongue and digital pad closely followed changes in tissue opacity in response to various hemodynamic alterations. This further supports the premise that changes in tissue radioactivity represented changes in tissue red-cell distribution. We have also observed that changes in red cells-^{51}Cr following injections of vasoactive compounds including epinephrine, norepinephrine and acetylcholine are quantitatively comparable to changes in tissue blood volume measured by plethysmography (Baker, C. H., unpublished observations).

The concentration of red cells in the central circulation is not equal to that of the peripheral circulation (1, 4). The assumption could be made that the relationship between central and peripheral hematocrit will remain relatively constant even during hemodilution or concentration. However, it has been reported that severe hemorrhage causes trapping of red cells (1, 9) and that the total body hematocrit to venous hematocrit ratio increases significantly (1). It has also been reported that this ratio does not change (4). In any event, these variations are small and may not drastically affect the trend of the results. The data in the report due to the necessity of grouping the dogs according to the isotope injected do not indicate if indeed there was any change in the ratio in these tissues.

It is not surprising that our findings indicate that skeletal muscle is a site of intravascular pooling of red cells and plasma in the oligemic phase of irreversible shock. This was predicted by Remington *et al.* (10) some years ago. It has also been indicated that changes in the skeletal muscle conductance or resistance are present in irreversible shock (11–13). They describe a progressive increase in blood flow conductance or decrease in resistance which could contribute to vascular pooling. Our data also indicate a significant increase in femoral-artery blood flow as irreversibility progressed. The report by Hollenberg and Nickerson (14) that small-vein wedge pressure increased during the development of irreversible shock would indicate that increased venous resistance could contribute to the pooling. These author's findings in conjunction with these in this report indicate a gradual failure of the primary skeletal-muscle resistance vessels allowing pooling in the capillaries and veins. Following reinfusion of the shed blood the distributions of cells and plasma in muscle returned to the control level.

In contrast the cutaneous area studied (hind paw) did not respond in a similar manner to the skeletal muscle area. The paw red cells and plasma decreased by nearly 50% soon after the hemorrhage and remained at this level to the time of reinfusion and then only partially returned toward control by the termination of the experiment. This would tend to indicate a marked reduction in vascular capacity that would seem to be mediated by the sympathetic nerves as well as catecholamines and was at least partially maintained even following reinfusion after 25% automatic takeup of the blood.

It should be noted that the changes in tissue red cells and plasma indicator distribution are due to factors other than transcapillary fluid shifts. This is evident when one compares the maximum decreases in blood radioactivity with the maximum decrease in tissue radioactivity. Tissue radioactivity changed approximately 50% in the paw for each isotope and 30–36% for muscle. However, the maximum decrease in blood radioactivity averaged about 11% for the red cells-^{51}Cr and 14% for albumin-^{131}I. The difference between the blood change and the tissue change should be indicative of changes in the amount of blood removed from the tissue, that is a decrease in vascular volume. It, therefore, can be concluded that the amount of indicator in the tissue was reduced by both dilution due to ultrafiltrate absorption and reduction in vascular volume.

Following the hemorrhage there was a reduction in the amount of albumin-^{131}I per ml of plasma indicating a movement of ultrafiltrate into the circulation. This trend was reversed at period VI and by the time of reinfusion an equal amount of ultrafiltrate had left the circulation as had previously entered. This would indicate an increased capillary pressure as has been reported by other au-

thors (14). The rate of loss of ultrafiltrate increased immediately post-reinfusion and then the concentration of albumin-^{131}I remained constant. It would appear that there was a new equilibrium reached where the colloid osmotic pressure had reached a level comparable to the elevated capillary pressure.

At the time of "reversal" there was also evidence of red-cell swelling as evidenced by the decrease in ^{51}Cr cpm per ml of RBC. This swelling persisted following reinfusion. The decline in ^{51}Cr cpm per ml of RBC could also be due to some red cells unlabeled with ^{51}Cr and previously sequestered entering the circulation after period IV. The data in this report cannot resolve this difficulty. However, we have found using another technique that small amounts of unlabeled cells do indeed enter the circulation following small and large hemorrhages (1). However, this was found to invariably occur within ten minutes. Period IV in this preparation was at least 30 min post-hemorrhage.

Summary. Experiments were designed to define the relative changes in red-cell and plasma distribution in skin and muscle during irreversible hemorrhagic shock. The blood of anesthetized, splenectomized dogs was labeled with red cells-^{51}Cr or albumin-^{131}I. Radioactivity in muscle and paw, was monitored by a collimated scintillation probe. Following a one hour control period dogs were hemorrhaged to and maintained at 30 mm Hg mean pressure until 25% of the shed blood was taken up. The remainder was then reinfused. The peripheral beds studied showed marked decreases in red cell and plasma following hemorrhage. Muscle red cells and plasma rose to levels about midway to the control level during the late hypotensive period and then increased to or above the control level after reinfusion. Cutaneous red cells and plasma remained at the low level to the end of the hypotensive period and then increased somewhat following reinfusion. Accumulation of red cells and plasma occurs in muscle and since it is the largest body-tissue mass, it could pool large volumes of red cells and plasma during the irreversible stage of shock. Evidence is also presented indicating plasma protein concentration increases and red blood-cell swelling during the last periods of hypotension and after reinfusion of the hemorrhaged blood.

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