Exchange of Injected Labeled Red Cells in Liver Tissue During Hemorrhagic Shock.* (32005)

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Reproducible blood volume estimations by indicator dilution methods are thought to approximate closely the true blood volume. However, plasma protein indicators, T-1824 or RHISA may leave the confines of the intravascular space along with plasma proteins and their volumes of distribution are not identical with the true plasma volume. Use of plasma indicators as measurements of plasma volume gives rise to a systematic error, which Reeve *et al*(1) have described as the "extra plasma" volume. Labeled red cells more accurately define the limits of the vascular space, but their application to blood volume measurements is not without problems. Direct observations of the microcirculation by Chambers *et al*(2), Knisely and associates(3) and others(4,5) have demonstrated rapidly moving cells which are distributed evenly in the flowing stream, as well as slowly moving cell clumps or aggregates, and cell aggregates which do not appear to move at all. The nonmoving cells are still within the vascular space(2). Further, studies from this laboratory with labeled red cells have demonstrated delayed red cell equilibration after hemorrhagic shock without concomitant delay in mixing of the plasma label (6). This was taken to mean that, in hemorrhagic shock, some red cells were not circulating sufficiently rapidly to be mixed with the injected labeled red cells within the appropriate time allowed for mixing(6,7). Furthermore, a noncirculating red cell component, which may be regarded as equivalent to a nonexchangeable fraction, was also observed in both experimental and clinical shock series(6,7).

In both normal circumstances and under conditions of shock from hemorrhage and trauma, it must be assumed that the indicator is distributed evenly within the circulating blood volume. A second assumption implicit in this type of estimation is that the measured volume is the functionally active blood volume; that is, the volume which is circulating effectively throughout the vascular system. Red cells which are occupying space but not actively circulating, or red cells which are not circulating at normal velocities obviously will affect the circulatory status of the patient differently than normally circulating cells.

Possible abnormal distribution of the blood volume within various anatomic areas of the body are also of import in the analysis of blood volume measurements. Sequestration of blood in the splanchnic area is said to occur in shock from hemorrhage. Further, the concept of the liver as a reservoir of blood was entertained by Krogh(8,9) as early as 1912; the liver was thought able to discharge significantly large volumes of blood into the general circulation in man on going from rest to exercise.

Measurements obtained with the usual intravenous sampling sites cannot possibly reveal changes in the blood volume within various organs and tissues. The present studies, therefore, were undertaken in an attempt to elucidate some of these problems by measurement of tissue red cell content before and during hemorrhagic shock in the dog. A colorimetric method for analysis of tissue hemoglobin was devised(10) to measure red cell content in tissue biopsies. This method was compared with an independent radioassay method. Serial determinations on biopsies were taken over a period of time after hemorrhage and reinfusion of shed blood. By combining both the colorimetric method and the radioassay method on the same biopsy specimens, information on the equili-

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bration of red cells in tissues with the circulating red cells was obtained.

Methods and materials. 1. Methods (a) Animal preparation. Twelve apparently healthy, splenectomized mongrel dogs were lightly anesthetized by intravenous administration of sodium thiopental. Polyethylene tubes (Clay-Adams PE 240) were inserted in the superficial femoral artery and the femoral vein using aseptic techniques.

(b) Red cell chromation. On the morning of the experiment, approximately 20 ml of blood was withdrawn from each animal for red cell chromation. The red cells were washed and labeled with Cr^{51} using the Read(11) modification of the method of Sterling and Grey(12).

2. Protocol. (a) Reinjection and equilibration of labeled red cells. An aliquot of the dog's own labeled cells was injected through the femoral venous catheter after which 4 ml blood samples were withdrawn from the femoral arterial catheter at 10 minute intervals. Equilibration of injected, labeled red cells in the circulating blood stream was demonstrated by constant radioactivity of packed red cells in 3 to 4 successive 10 minute samples.

(b) Tissue biopsy technique. The abdomen was opened through an upper abdominal midline incision, and a biopsy sample of the liver was obtained rapidly with a sharp knife without previously placing hemostatic sutures in the liver substance. Particular care was taken to avoid crushing or manipulating the liver tissue before and during the biopsy procedure. The biopsy tissue was immediately plunged into liquid nitrogen. Then hemostatic sutures were used to close the biopsy site.

(c) Method for production of hemorrhagic shock. Following control samples of peripheral blood and liver biopsy, hemorrhagic shock was produced by the standard Wiggers technique(13). Sufficient quantities of blood were removed to lower the mean arterial blood pressure to approximately 50 mm Hg. The pressure was maintained at this level for 2 or more hours by removal or replacement of additional blood.

(d) Subsequent sampling. Peripheral blood samples, hepatic and muscle biopsies were obtained during the hypovolemic stage. After return of the shed blood, blood samples and biopsies also were taken at intervals. In some cases additional blood transfusions from unlabeled crossmatched donor dogs were given to replace blood loss from biopsies and to sustain blood pressure, and after time was allowed for equilibration, repeated blood samples and hepatic biopsies were taken.

3. Analyses. (a) Radioassay of circulating red cells. The blood samples were centrifuged at 2000 *g* for 20 minutes. The plasma was removed; 0.5 ml aliquots of packed red cells were laked in 1.0 ml distilled water. The radioactivity was assayed in a NaI well scintillation counter. Radioactivity was expressed in CPM per ml red cells.

(b) Radioassay of liver biopsy for red cell content. Frozen biopsy samples were pulverized in the cold. Approximately 1 gram was carefully weighed and homogenated in 5 ml distilled water. Radioassay of aliquots of homogenated tissues was made under comparable counting conditions.

(c) Colorimetric analysis of red cell content in liver. Chemical assay of tissue hemoglobin was determined by a previously described method(10). Briefly, this method consists of extraction of a weighed sample of liver tissue (approximately 1 gram) in 1 ml 2N HCl and 8.0 ml water. The acid separates the protoporphyrin ring from the globin part of the hemoglobin molecule, the soluble porphyrin part is transformed to ferriprotoporphyrin which is distributed in the water phase while most of the tissue proteins are precipitated in the acid solution. Most of the proteins are separated by centrifugation at 2000 *g* for 30 minutes. However, after centrifugation the supernatant fluid frequently may remain cloudy. Seven ml of the supernatant fluid is removed and 2.0 ml of 1N NaOH are added to this solution. By adding an excess of base and shifting the solution to alkalinity much of the protein is dissolved leaving a slightly turbid fluid. A few crystals of NaCN are added to the alkaline solution; cyanferriprotoporphyrin is formed. The remaining turbidity is removed by treating with 1 ml of a 10% solution of sodium lauryl sulfate. After standing for 36

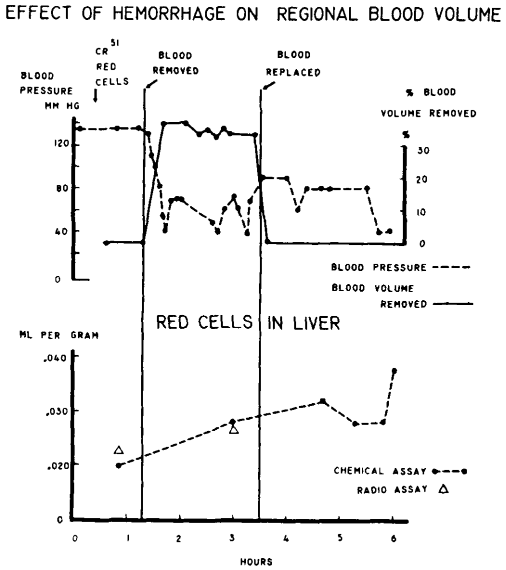

FIG. 1. Effect of hemorrhage on liver red cell content is illustrated by data of a representative experiment. Upper section shows blood pressure (dotted line) and volume of blood removed (solid line); lower section shows red cell content of liver biopsies as measured by chemical and radioassay methods throughout the experiment. Time of injection of labeled red cells is indicated by first arrow. After equilibration of labeled red cells in the peripheral blood, biopsy of liver showed .020 and .023 ml red cells/gram liver tissue respectively by the two different assay methods.

Following biopsy, 35% of the dog's blood volume was removed (represented by the second arrow and vertical line). This brought the blood pressure down to about 40 mm Hg.; as the pressure rose to 50 or 60 mm Hg additional blood was removed. A second liver biopsy was obtained after about 2 hr of shock. Then, the shed blood was returned to the animal at time indicated by third arrow and vertical line. This partially and temporarily restored blood pressure. Four liver biopsies at this time showed liver tissue red cell content consistently elevated over control levels.

or more hours at 4°C a voluminous white precipitate forms. The precipitate is removed by centrifugation and leaves a clear supernatant fluid; there is no loss of color at the desired wave length. The absorbency of the solution is read at 545 mμ in a Beckman spectrophotometer. This value is compared with known hemoglobin standards, treated in an identical manner. The content of red cells in the tissue is calculated from curves made from these standards.

The mean percentage of recovery from 12 biopsy samples with various amounts of red cells was 103 ± 2.2 (SE)% for the chemical assay and 102 ± 2.4 (SE)% for radioassay(10).

Results. 1. Concentration of blood in liver under control conditions. During the control period the mean concentration of red cells in the liver of this series of 9 dogs was 0.027 ± .002 (SE) ml/gram liver tissue. The data of a representative experiment is illustrated in Fig. 1.

2. Content of red cells in the liver after hemorrhage and retransfusion. After hemorrhage, the red cell content usually remained near control levels. With retransfusion of the shed blood, the liver red cell content increased markedly. After replacement of the shed blood, there was an average of 0.052 ± 0.006 ml/gram liver tissue. When the posttransfusion values were compared with their own control values using the *t* test for differences between correlated means, the difference was significant($P<0.01$).

In 2 additional experiments, the animals were not entirely normal prior to the initial biopsy; following anesthesia and laparotomy, there was a modest fall in arterial pressure as well as some swelling and congestion of the liver. In these 2 experiments the tissue red cell content averaged 0.114 ml/gram liver tissue before hemorrhage and 0.320 ml/gram after hemorrhage and replacement of the shed blood.

3. Changes of circulating and tissue red cell radioactivity after hemorrhage and transfusion. There was no significant change of red cell radioactivity in blood samples obtained from the femoral arterial catheter after blood removal and replacement of the shed blood. However, with transfusions of unlabeled, crossmatched donor dog blood, the radioactivity of these circulating red cells decreased abruptly as the dog's labeled cells were diluted progressively by the added unlabeled red cells in the transfusions (Fig 2).

The radioactivity of red cells in the liver tissue also was unaffected by blood removal and replacement of the shed blood. But, in contrast to the circulating red cells obtained from the arterial catheter, the subsequent addition of unlabeled, donor transfusions did not give rise to a marked lowering of the tissue red cell radioactivity (Fig. 2). The pro-

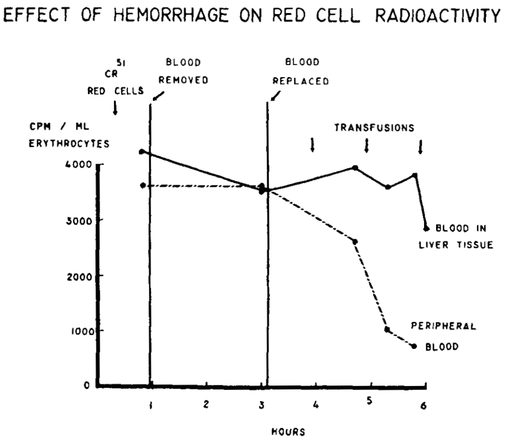

FIG. 2. Effect of hemorrhage on red cell radioactivity in liver biopsy is illustrated in a representative experiment. The dog's own cells labeled with Cr^{51} were injected prior to hemorrhage at time indicated by first arrow. Radioactivity of red cells in femoral arterial (peripheral) blood samples is indicated by dotted line and radioactivity of red cells in the liver biopsies indicated by solid line. Minor differences occurred before and immediately after blood removal, probably explicable in terms of methodological errors. After three 200 ml transfusions of unlabeled donor blood, indicated by arrows on the right, the radioactivity of the circulating red cells decreased markedly since they were diluted by the transfused cells. However, red cell radioactivity in the liver biopsies remained distinctly higher than the circulating red cells, indicating a lack of uniform mixing of red cells in liver tissue with transfused blood.

nounced difference between circulating and tissue red cell radioactivity after unlabeled donor transfusions indicated a failure of the transfused blood to equilibrate completely with the red cells of the liver tissues. This supports the view that sequestration or pooling of red cells in the liver observed during shock represents slowly circulating and noncirculating fractions of the red cell mass (6).

Discussion. The proposed method of tissue hemoglobin analysis was used to estimate red cell content of tissues, the obvious assumption being that the amount of red cells in tissues may be accurately reflected in hemoglobin measurements. It is conceivable that hemoglobin breakdown products from lysis of red cells in microthrombi may be carried to the liver and produce spuriously high values. However, analysis of plasma hemoglobin levels in these and other comparable experiments showed no appreciable increase. Further, there was agreement of repeated measurements over varying periods of time under control conditions. This suggests that although these factors may be a problem in experiment of longer duration, the likelihood of major errors from this source in short term experiments is remote. The assumptions also were tested by the independent assay of red cells by radioactive labeled techniques; good agreement of the two methods was found.

The results of experiments using two independent methods for measurement of red cell volume in tissues cast doubt upon the conventional interpretation of blood volume measurements based on a single early blood sample. In the present studies on hemorrhagic shock in dogs as well as previous experiments (6,7) there was uniform mixing of tagged red cells under normal conditions. In shocked animals, after transfusions with unlabeled red cells, there was decreased mixing between red cells sampled from the arterial tree and red cells of hepatic tissues. During shock, a slowly exchangeable or nonexchangeable fraction of erythrocytes which escaped detection by conventional dilution techniques was found in the liver. This failure of equilibration of the red cell label within the total red cell mass in shock suggests that the total red cell mass may not be measured correctly when samples are taken at 10 or 20 minutes after injection of the red cell indicator. When blood volumes are calculated from radioactivity values of circulating red cells at a given time after the injection of labeled red cells, the derived calculation will reflect the volume in which the red cell label is distributed at the instant of the sampling time but not necessarily the volume of the total red cell mass.

The present experiments also have demonstrated an increased content of red cells in the canine liver in shock as compared with normal conditions. Such internal redistribution of the red cell mass also will not be disclosed by conventional blood volume measurements. Acute congestion and engorgement of hepatic sinusoids, which was manifest in these experiments as an increase in tissue red cell content, were observed in human autopsy material(14). Moreover slowly circulating and noncirculating red cell volumes were documented in shock patients who had sustained acute blood loss and trauma(7).

The vascular arrangement in the hepatic sinusoidal system, which has been studied by direct observations in several species, is particularly vulnerable to cellular aggregation(4, 5,14). Moreover, aggregation of red cells which have been observed in vascular beds other than the liver(2,3) may also increase the error in blood volume measurements by conventional indicator dilution methods.

Methods using a plasma label, if properly performed, will give a satisfactory measurement of the plasma volume, even in shocked animals(16). However, measurement of total blood volume based on plasma volume and arterial hematocrit determinations may not be precise, especially when aggregation and pooling of red cells produce differences between hematocrit of blood in tissues and large vessels. Krogh(17) demonstrated variations in the hematocrit in small vessels under normal conditions. Moreover, Gibson *et al* (18) showed abnormal distribution of blood between large and minute vessels which were not reflected in the arterial hematocrit. The latter believed that in shock, widespread trapping of red cells in small vessels reduced capillary blood flow through all organs(18).

Summary. Red cell content in liver was measured in normal and in shocked dogs using two independent methods of measurement. The mean concentration of red cells in the liver of a series of dogs under control conditions was 0.027 ± 0.002 (SE) ml/g liver tissue. After hypovolemic shock and retransfusion of the shed blood, the red cell content in liver tissue increased to an average of 0.052 ml/g. Under control conditions, the red cell label of peripheral blood was mixed adequately with the red cells in liver tissues. After shock, differentially labeled red cells mixed more slowly with the red cells in the liver and in some instances did not become equilibrated during the course of the experiments. This suggests a pool of noncirculating or slowly circulating red cells in the liver after shock. These changes may affect adversely the precision and interpretation of blood volume measurements made by conventional techniques and sampling schedules.

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1. Reeve, E. B., Gregersen, M. I., Allen, T. H., Sear, H., Wolcott, W. W., Am. J. Physiol., 1953, v175, 211.

2. Chambers, R., Zweifach, B. W., Lowenstein, B. E., ibid., 1943, v139, 123.

3. Knisely, M. H., Eliot, T. S., Block, E. H., Arch. Surg., 1945, v51, 220.

4. Gelin, L-E., Shoemaker, W. C., Surgery, 1961, v49, 713.

5. Shoemaker, W. C., Brunius, U. Gelin, L-E., ibid., 1965, v58, 518.

6. Shoemaker, W. C., Am. J. Physiol., 1962, v202, 1179.

7. Suzuki, F., Baker, R. J., Shoemaker, W. C., Ann. Surg., 1964, v160, 263.

8. Krogh, A., Skand. Arch. Physiol., 1912, v27, 126.

9. ———, ibid., 1912, v27, 227.

10. Andersen, D., Shoemaker, W. C., Clin. Chem., 1965, v11, 422.

11. Read, R. C., New Eng. J. Med., 1954, v250, 1021.

12. Sterling, K., Grey, S. J., J. Clin. Invest., 1950, v29, 1614.

13. Wiggers, C. J., Physiology of Shock, Commonwealth Fund. New York, 1950.

14. Shoemaker, W. C., Szanto, P. B., Fitch, L. B., Brill, N. R., Surg. Gyn. Obst., 1964, v118, 828.

15. Prentice, T. C., Ollney, J. M., Jr., Artz, C. P., Howard, J. M., ibid., 1954, v99, 542.

16. Noble, R. P., Gregersen, M. I., J. Clin. Invest., 1946, v25, 58.

17. Krogh, A., The Anatomy and Physiology of Capillaries, Yale Univ. Press, New Haven, 1929.

18. Gibson, J. G., Seligman, A. M., Peacock, W. C., Fine, J., Aub, J. C., Evans, R. D., J. Clin. Invest., 1947, v26, 126.

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