Method for Tissue Hemoglobin Analysis

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A colorimetric method for analysis of the red cell content of tissues is described, and compared with an independent radioassay method. The introduced method is based upon acid extraction of tissue, treatment with base, and adsorption of the remaining turbidity with lauryl sulfate. Preliminary data are presented on the reproducibility of the colorimetric method on multiple biopsies of the canine liver. Red cell content of biopsies obtained from a series of normal dogs was 0.031 ml./gm. of liver tissue with a standard deviation of ± 0.011. The red cell content of the liver of dogs in various physiologic conditions ranged up to 0.096 ml./gm. of liver tissue.

In recent years there has been increased awareness of the importance of changes in total blood volume as well as in blood distribution or red cell volume during circulatory and other disorders. Methods for extraction and quantitative colorimetric measurement of red cells in tissue have been reported by Lowry and Hastings (1) and by Cohn (2). These methods involved spectrophotometric assays on turbid solutions with attempts to correct for the error resulting from turbidity. Goodman et al. (3) have used a method for simultaneous measurement of plasma volume in tissue by the dye T-1824, and of red cell volume by hemoglobin assay. They treated a tissue homogenate with phosphotungstic acid and ether. T-1824 was measured in the water phase and hematin in the ether phase. They reported good recovery of added T-1824 but gave no recovery results for the hemoglobin assay. Klein (4) has reported a tissue hemoglobin method in which hemoglobin is extracted from tissue with water and the turbidity removed by the addition of an ammonium sulfate-phosphate solution. After the solution clears, the hemoglobin is transformed to cyanmethemoglobin and measured colorimetrically.

We have been unable to obtain complete extraction of hemoglobin from liver tissue with distilled water as the extraction medium, and have there-

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fore proposed a method based upon acid extraction, treatment with base, and adsorption of the remaining turbidity with lauryl sulfate. The method has been compared with an independent radioassay method.

Methods

Collection of Liver Samples

Dogs were anesthetized by rapid intravenous injection of thiopental. Immediately after a suitable level of surgical anesthesia was reached, the abdomen was opened through an upper abdominal midline incision as quickly as possible. An entire hepatic lobe was clamped, severed, removed in toto, and immersed in liquid nitrogen. Every effort was made to obtain tissue samples in as near a normal state as possible and to prevent escape of blood during the sampling process. It was found that time delays, manipulation of the tissue during the biopsy procedure, and struggling of the animal during induction of the anesthesia produced congestion of the liver. The time from anesthesia to freezing of the hepatic lobe was often less than 2 min.

Colorimetric Assay of Tissue Hemoglobin

A 1-gm. portion of liver tissue was homogenized in 1 ml. of 2N HCl, and water was added in sufficient quantity to give a total volume of 10.2 ml. Since liver tissue contained approximately 20–25% solids, the total water phase in the homogenate was 10 ml. The homogenate was centrifuged at 2000 g for 45 min. After centrifugation, the supernatant fluid was usually still very cloudy; 2 ml. of 1N NaOH were added to 7.0 ml. of the supernatant fluid, and by shifting the solution to alkalinity much of the protein was dissolved, leaving a slightly turbid fluid. A few crystals of NaCN were added to the alkaline ferriprotoporphyrin solution to produce cyanferriprotoporphyrin. The turbidity remaining was treated by adding 1 ml. of 10% (w/v) sodium lauryl sulfate and allowing the solution to stand 12–36 hr. at 4°C. Under these conditions, a voluminous white precipitate formed. This precipitate was removed by centrifugation of 2000 g for 30 min. in a refrigerated centrifuge. The supernatant fluid was removed before the solutions warmed, as the precipitate would have dissolved slowly and again formed a turbid solution on standing at room temperature. The supernatant is almost always clear; however, a weak opalescence frequently will disappear after a short time at room temperature. The absorbance of the solution was measured at 545 mµ in a Beckman DU spectrophotometer.

A set of standard solutions, prepared from known hemoglobin solutions or from aliquots of packed red cells, was analyzed as above. The
tissue hemoglobin or the tissue red cell content was calculated from the values obtained with these standard solutions.

Radioassay of Cr\(^{51}\)-labeled Red Cells in Tissue

Results of the colorimetric analysis of tissue hemoglobin were compared with those of an independent method using labeled erythrocytes in dogs. Canine red blood cells were incubated with 50–100 \(\mu\)c of radiochromate, after Read’s (5) modification of the method of Sterling and Grey (6). After reinjection of an aliquot of the labeled cells, 30–60 min. were allowed for equilibration. Then liver biopsies were obtained and aliquots of the liver tissue homogenized. The radioactivity of the homogenate aliquots was assayed in a NaI well-type scintillation counter. Blood samples obtained from the femoral vein at the time of hepatic biopsy were centrifuged and known volumes of packed red cells were assayed for radioactivity. Tissue red cell content was calculated by dividing the radioactivity per gram of liver tissue by the radioactivity per milliliter of red cells, and expressed as ml. red cells/gm. tissue.

Results

Recovery Experiments

Dog and rat livers were removed from anesthetized animals and the liver perfused exhaustively with saline until the hepatic efflux was clear. Analysis of tissue hemoglobin from sections of the saline-perfused liver revealed negligible quantities of blood. Recovery experiments were performed by adding known amounts of Cr\(^{51}\)-labeled red cells to the tissue homogenate. Aliquots were analyzed by both chemical and radioassay methods. The mean percentage of recovery from 12 samples with various amounts of red cells was 103\% \(\pm\) 2.2 (S.E.) for the chemical assay and 102\% \(\pm\) 2.4 (S.E.) for radioassay. The data are illustrated in Fig. 1.

Reproducibility in Multiple Biopsies

The variations in red cell content of different parts of the liver were studied in 17 biopsy sections from all parts of the liver of a dog. These were taken through a standard, upper abdominal, midline incision immediately after induction of anesthesia. The mean red cell content of the biopsy sections by chemical assay was \(0.019 \pm 0.001\) (S.E.) ml./gm. of tissue.

Red Cell Content of Liver Tissue from Normal Dogs

As soon as possible after induction of anesthesia, 5–7 replicate analyses were made on biopsy sections from each of 9 dogs. The average red cell content was 0.031 ml./gm. of liver tissue; the standard deviation of
the series, calculated from mean values of each of the 9, was 0.011 ml./gm. of liver.

Comparison of Colorimetric and Radioassay Methods

Liver biopsies were performed after laparotomy in dogs given an injection of labeled red cells 30-60 min. previously. The tissue was analyzed for red cell content by both methods. Analysis was made of liver tissues from dogs considered normal and from a few animals with undue stress incident to anesthesia, or inadvertent manipulation of the tissues. The comparative results are given in Table 1.

Discussion

A major problem in measuring the volume of red cells in tissues lies in obtaining clear hemoglobin solutions from the tissue extract. When the latter are obtained, standard methods of chemical analysis are available. The present communication describes a method for obtaining clear solutions from a tissue extract with satisfactory recovery of added hemoglobin, and good agreement with an independent radioassay method.

Addition of acid to tissue homogenates separates the protoporphyrin ring from the globin part of the hemoglobin molecule. The soluble, porphyrin part is transformed to ferriprotoporphyrin (7) which is dis-
tributed in the water phase while most of the tissue proteins are precipitated in the acid solution. The turbidity remaining is cleared by treatment with base and adsorption with lauryl sulfate.

Cyanferriprotoporphyrin has absorbance maxima at 415 and 545 m$\mu$. We have chosen to measure the absorbance at 545 m$\mu$ because preliminary investigation suggested that a certain number of interfering substances were to be found at 415 m$\mu$. The absorbance of the solutions is independent of pH when pH is higher than 8. The color is stable over several days at 4°C or at room temperature.

We have used this colorimetric assay in conjunction with the radioassay method to measure the rate of exchange of the blood in various tissues after the injection of labeled red cells. In this way, the rapidity of tissue red cell movements in normal and disturbed circulatory states may be investigated with an approach analogous to the measurement of exchange rates with specific activity-time curves.

References