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NOTES EXPLANATION OF USE FOR P-32, CR-51, K-42 TO LABEL CELLS FOR BLOOD VOLUME MEASUREMENTS STUDIES ON NORMAL MALES & FEMALES pg 1/8
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Sent to Dr. Berlin

not - this paper is still work on problem of nature of anemia

Has we more data on short red cell life and a unusual case of ? Gaucher's + other showing short red cell life and spleen destruction

To be reviewed for JAMA

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RECENT ADVANCES IN METHODS FOR STUDY OF RED CELL MASS, RED CELL PRODUCTION AND DESTRUCTION

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With the development of the cyclotron and nuclear reactors, it ~~has~~ became possible to produce radioactive isotopes of elements associated with normal metabolic processes. ~~As a result it has become possible to follow the metabolism of a given element or substance by the introduction of a labeled isotope into the body.~~ Recent great improvements in the methods for measurement of radioactive isotopes, particularly the developments in scintillation counters, have made it possible to carry out tracer studies in laboratories or hospitals with relative ease. For most of these studies it is not necessary to have expensive or complex equipment.

In the evaluation of erythropoiesis, one must know the total volume of circulating red cells, the rate and site of production of red cells, and their life span. ~~life span of red cells.~~ Knowing these three values, one can then determine whether an individual is normal, polycythemic, or anemic, and also whether an increase or decrease in the total circulating red-cell volume is due to a change in the life span ~~of red cells~~ or in the rate of production of red cells, or to both.

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METHODS

Blood Volume and Red Cell Volume.

The availability of radioactive isotopes of iron, phosphorus, chromium, potassium, and thorium makes it possible to measure the total circulating red-cell volume with accuracy and ease. The isotopes phosphorus-32,[1] chromium-51,[2] potassium-42,[3] and thorium-C[4] may be incorporated into the red cell *in vitro*. This permits the labeling of an individual's own red cells. Iron, on the other hand, must first be administered to a donor, whose red cells are subsequently injected into the individual whose blood volume is to be determined. Although the radioisotope method would be the ideal method for determining blood volume, it has not found wide usefulness in clinical work because of the necessity for maintaining a pool

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of donors from various blood groups and the need for cross-matching, blood typing, and the various serological studies that are required before transfusion. Furthermore, the dose of radioiron to be administered to the donor is so large that the radiation to the bone marrow approaches tolerance values. In experimental animals, however—particularly in the rat, where inbreeding results in a uniform strain of animals in which cross-matching is no problem—the iron method has been of great value in the determination of blood volume.

For the P^{32}, Cr^{51}, and K^{42} methods in which red cells are labeled *in vitro*, it is necessary to remove only a small quantity of blood (10 to 15 cc) from the individual and to "incubate" these cells with the isotope at 37°C with constant mixing. In general, more than 50% of the isotope will have moved to ~~been incorporated into~~ the red cells at the end of one hour. The red cells are then washed to remove the excess, unincorporated isotope and are resuspended in either saline or plasma. The reconstituted whole blood containing the labeled red cells is then administered to the patient, and after adequate time for mixing a sample is withdrawn. An aliquot of the blood injected into the patient is kept and the total radioactivity administered is determined from it. Thus, knowing the total radioactivity administered and the radioactivity per milliliter of whole blood withdrawn from the patient, one may calculate the blood volume. The total red-cell volume is determined from the blood volume and the peripheral hematocrit corrected for trapped plasma. Actually, this method is precise. The plasma volume can be calculated from the total red-cell volume and the hematocrit. Although there has been some objection to this method of calculation of the plasma volume (because several studies indicate that the hematocrit of the large-vessel blood may not be the same as that in the smaller vessels), we do not consider this to be a significant factor and believe that the plasma volume can be calculated with a high degree of precision from the total red-cell volume and the venous hematocrit.[5]

~~Of P^{32}, Cr^{51}, and K^{42},~~ The isotope of choice for the determination of blood volume is Cr^{51}. It is easily measured in a well-type scintillation counter; the rate of elution of Cr^{51} from the red cell is relatively slow compared with that of P^{32}, and the physical half life is long compared with K^{42}. While many valuable studies have been made using P^{32}, most workers have now adopted the Cr^{51} method. The usefulness of K^{42} is limited by its short half life (12.4 hours), which means that the worker must have access to a facility for its production. The ThC method has been explored by Hevesy and Nylin, and should prove valuable. It has not been used extensively in other laboratories although it does offer some promise for greater ease of labeling red cells than K^{42}, Cr^{51}, and P^{32}. The radiation dose involved in the use of ThC remains to be established. The rapid physical decay of the other isotopes presents no radiation hazard to the individual receiving the isotope.

The results obtained with these methods show

for normal males (71 subjects):[6] ? reference

average total blood volume	69.0 cc/kg of body weight,
average plasma volume	38.7 cc/kg.
average total red-cell volume	29.9 cc/kg;

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for sixteen normal females studied in this laboratory: (6)

average total blood volume	64.4 cc/kg of body weight,
average plasma volume	37.0 cc/kg,
average total red-cell volume	27.0 cc/kg.

(This was the first study of the blood volume of the normal female by isotopic methods.)

Subsequently, Ashworth reported, (reference) (7) for a series of eight normal women,

average total blood volume	66.5 cc/kg,
average plasma volume	43.1 cc/kg (by the Evans-Blue method),
average total red-cell volume	23.4 cc/kg (by the P^{32} method).

The value for the total red-cell volume in the males, 30 cc/kg, appears to be well confirmed.

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Red-Cell Production

At ~~the~~ present time there is only one method for the direct and destruction determination of the rate of red-cell production after intravenous injection and simultaneous ~~and that~~ is the measurement of the rate of disappearance of radioactive iron from the plasma and subsequent uptake of radioiron in the red cells.(8) ~~An analysis of the rate of disappearance of radioiron from the plasma indicates that~~ Approximately 80% to 90% of the iron is removed by a first-order process. That is, for each given time period a constant fraction of the radioiron remaining in the plasma is cleared, largely by the bone marrow and to some extent by the liver, spleen, and other tissues. From the rate of disappearance of radioiron from the plasma, the turnover of iron in the plasma can be calculated, as follows:

$$\frac{(0.693) \times (\text{uc Fe/ml in plasma}) \quad (\text{plasma volume in ml}) \times (24 \text{ hours})}{\text{Plasma radioiron half time (in hours)}}$$ 9

The plasma iron concentration, is determined by the method of Kitzes et al.(10) The plasma volume is determined from the blood volume by use of labeled cells, as described above. After the determination of the rate of removal of radioiron from the plasma, a study is made of the uptake of iron in the red cells. At approximately 7 to 10 days, most of the iron that is going to appear in the red cells has been cleared from the plasma to the bone marrow and has been incorporated into hemoglobin, and the mature red cells containing the radioiron are in the peripheral blood. Thus, the percentage of iron that passes through the plasma, ultimately to reach the peripheral blood in red cells, may be determined at approximately 7 to 10 days following the original injection of the isotope.

This now completes the data required for determination of the rate of production of red cells. In general it is found that approximately 0.26 milligram of iron is utilized for red-cell production per kilogram per day in a normal male. Much higher values are found in polycythemia. In leukemia these values are usually normal or increased to two to three times normal, whereas in certain anemias the values may be normal, increased, or (in case of failure to produce red cells) as low as one-fifth to one-third normal.(8) 8

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Finally, providing a good volume of

The use of scintillation counters placed over the spleen, liver, and sacrum—the latter ~~representing~~ bone marrow—makes it possible to determine the time course of the distribution of radioiron in these tissues.~~11)~~ (Fig. 1). This shows that there is a rapid clearance of iron by the bone marrow and a later release of the iron from the marrow to the peripheral red cells. Some iron reaches the liver and the spleen and thereafter is slowly removed from these tissues. It is this slow release from the spleen and liver that accounts for the slight rise of radioiron in the peripheral blood after 7 to 10 days. In pathologic conditions - sites of red cell ~~production~~ & elsewhere may be focused - in the spleen or liver, using either radio iron or radio chromium, but iron is preferable since it measures production also.

Red-Cell Life Span ~~and Red Cell Destruction~~

Prior to the introduction of isotopes into the study of red-cell physiology, it was possible to measure the life span of the red cell by the Ashby differential agglutination technique.~~12)~~ To obtain valuable data by use of the Ashby technique, one must administer a large volume of blood (approximately 500 cc) from a suitable donor. This method has usually been applied by the transfusion of normal red cells to an individual with a disease state, and yields information regarding only the fate of normal cells in an abnormal environment. Some studies have been carried out by transfusing cells from a donor having sickle-cell anemia to a normal individual, and in this experiment the survival of sickle cells in a normal environment could be observed. The Ashby method, however, does not permit the determination of the life span of the red cells in ~~its~~ their own environment.

With the demonstration by Shemin and Rittenberg~~13)~~ 12 that the nitrogen atom of glycine is specifically used for the four nitrogen atoms of hemin and the preparation of N^{15}-labeled glycine, it became possible to study the life span of the red cell in its own environment. This study demonstrated that the normal life span of the red cell was approximately 120 days, and valuable information on the red-cell life in sickle-cell anemia and in pernicious anemia was also obtained. In our laboratory C^{14}-labeled glycine was introduced for such studies~~14)~~ 13 because it has been shown that the alpha-carbon of glycine atom is the source of 8 of the 34 carbon atoms of glycine~~15)~~ 14 and ~~N^{15} is much more difficult to measure.~~ C^{14} is easier to measure and the sensitivity of the method is greater than that of N^{15}.

Some indication of red-cell life span may also be obtained by following the activity of radioiron in the peripheral blood. This method is less satisfactory than the C^{14} method, however, since 70% to 90% of red-cell iron liberated at the end of the life span of a red cell is reutilized for the formation of new red cells. As the red cells reach the ends of their life spans, the radioiron is released and reutilized, and there is no sharp drop in the curve of Fe^{59} hemoglobin activity. This disadvantage can be overcome in experimental animals by loading the animal with non radioactive iron after the red cells are labeled.~~16)~~ 15 Thus, when the red-cell iron is released, it is released into a large pool of iron, and the specific activity becomes considerably lower, so that when it is reutilized ~~in~~ for red-cell formation it is of a much lower specific activity than that originally present in the first labeled cells. A sharp drop in the curve for the Fe^{59} hemoglobin specific activity is noted, and the red-cell life can be determined.

Recently, Cr^{51} has been introduced as the labeling agent for study of the life span of the red blood cells. With the demonstration that the rate of elution of Cr^{51} from the labeled red cells *in vivo* is very slow, it has

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now become possible to obtain ~~a measure~~ an estimate of the red-cell life span by following the in vivo rate of disappearance of Cr^{51} following the labeling of the cells; these studies do not give an absolute value for the red-cell life span, but they may be used to compare a red-cell life span in a given state with that of a normal.[18-19] It should be emphasized that this is a valuable method for clinical determination of alterations in the life span of the red blood cell, but that the values obtained—even with the present methods of analysis—are not comparable with the actual value of red-cell life span, and must be compared with the normal as determined with this method. Various methods of analysis have been proposed for interpretation of the curves of the graphs obtained. At the present time, however, we feel that these have not been completely substantiated.

In comparing the various methods for study of red-cell life, the Ashby method might be criticized because normal cells are placed in a pathologic environment or pathologic red cells in a normal environment, and red cells are never studied in their normal environment. In most normal individuals, the Ashby, C^{14}, and Fe^{59} methods give comparable results. We have seen one discrepancy, however, in which the values by the Ashby technique indicated a longer red-cell life than those techniques using the C^{14} and Fe^{59} methods. There was another case in which the Ashby technique showed a normal red-cell life, but the C^{14} and Fe^{59} results showed a shortened red-cell life. The conclusion might be drawn from these findings that the individual's own cells were qualitatively poor, while the destructive mechanisms were normal, and therefore the normal donor cells survived their normal life span.

DISCUSSION

With the development of methods for determining total red-cell volume and cite the rate of production of red cells, and the life span of the red cell, it has come possible to ~~assay the erythropoietic state~~ assess the state of erythropoiesis. Anemia or polycythemia can only arise from some change in the rate of production or in the life span of the red cell. This may even apply to bleeding states. Although the rate of production of red cells could conceivably be unaffected, the life span of the cells would be shortened in the bleeding state in the sense that cells did not survive their normal life span. The resulting anemia would then be due to a decrease in the red cell life span.

The methods described have been applied simultaneously in the study of various hematopoietic disorders, such as polycythemia, leukemia, aplastic anemia, hemolytic anemia, and hyperoplenism.

Polycythemia

~~With P32 labeled red cells it can be shown that~~ There is ~~an~~ a marked elevation of the blood volume in polycythemia (Fig. 1A).[19,27] This figure clearly indicates that the hematocrit cannot be used to predict the total red cell volume, although in general, as the hematocrit increases the total red cell volume increases. In 32 patients with a hematocrit above 55, the total red cell volume was elevated in all but two, ranging from 30.8 to 93.9 cc./kg. of body weight. The plasma volume was low in 22 patients, in the lower range of normal in 8, and high normal in 2. Thus, in polycythemia vera in relapse with an elevated hematocrit, there is usually an increased total

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