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RADIOISOTOPES IN HEMATOLOGY

Rollin K. McCombs, and John H. Lawrence

March 29, 1955

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ABSTRACT

This paper is a review of radioisotopes in hematology, with particular reference to the literature since 1950.

Red-cell life-span studies may be performed by labeling the cells with $Na_2Cr^{51}O_4$ or administering N^{15}- or C^{14}-labeled glycine. Patients with polycythemia vera produce two red-cell populations---one with a life span of only a few days and one with a normal life span. In chronic lymphatic leukemia the life span is sometimes considerably shortened and has a pattern of random destruction. In chronic myelogenous leukemia the red cells have a shortened by finite life span, suggesting an intrinsic defect in the cell. In congenital hemolytic anemia, thalassemia minor, myeloid metaplasia, acquired hemolytic anemia, hypoplastic anemia, and untreated pernicious anemia the red-cell survival time is shortened. The anemia of cancer may be attributable in part to a shortened red-cell survival time.

Plasma volume may be measured with radioiodinated human serum albumin; and total red-cell volume may be measured with red cells labeled with P^{32}, K^{42}, Cr^{51}, or radiothorium. Total blood volume may be calculated if either the plasma volume or total red-cell volume is known. In polycythemia vera and in secondary polycythemia the total red-cell volume is elevated, and the plasma volume is diminished. In relative polycythemia the total red-cell volume is near normal; but the plasma volume is diminished, giving a falsely high hematocrit. A relatively large number of cancer patients with anemia have normal total red-cell volumes with expanded plasma volumes, accounting for the clinical impression that anemia is widespread in cancer and showing the unreliability of the red-cell count and hematocrit in estimating total red-cell volume.

Plasma radioiron turnover in polycythemia vera is about five times normal; treatment with radioisotopes with satisfactory remission restores the plasma iron turnover values to normal. In secondary polycythemia the plasma iron turnover is elevated to a lesser extent than in polycythemia vera and is regulated to a rate compatible with the red-cell mass. In the anemia of myelogenous leukemia the plasma iron turnover is not diminished; in pernicious anemia it is elevated but tends to return to normal upon treatment. Iron turnover studies in cancer patients show that blood production proceeds at an actually increased rate though at lower levels of total circulating hemoglobin.

In vivo radioiron turnover studies employing scintillation counters placed over the liver, spleen, and sacrum (bone marrow) in normal individuals and in patients with various hematopoietic disorders have led to the establishment of four patterns, which are described.

Radioiron studies show that reticulocytes, but not mature erythrocytes, will assimilate iron and synthesize heme in vitro; uptake by marrow normoblasts is even greater. Patients with fever, untreated pernicious anemia, and refractory anemia absorb from the gut more iron than they use for hemoglobin production. Animal studies show that the hemoglobin iron of new red cells is derived from the iron of broken-down old cells rather than from reserve stores.

Isotopic studies show that the nitrogen atom of glycine provides all four nitrogen atoms and the methylene carbon of glycine provides eight of the thirty-four carbon atoms of protoporphyrin.

The incorporation of P^{32} into the desoxyribonucleic acid of leukocytes and the separation of granulocytes from lymphocytes by differential sedimentation establish for the granulocytes a mean age of 9 days. Two groups of lymphocytes --- one with a mean age of 3 to 4 days, comprising 15 percent of the lymphocyte population, and the other with a mean age of 100 to 200 days, comprising 85 percent of the lymphocytes in the blood --- are demonstrated by this method. P^{32} from labeled leukocytes or platelets disappears more rapidly from the blood than does inorganic phosphate solution. Parallel studies in animals show that transfused leukocytes are arrested selectively by the lungs, whereas platelets are captured selectively by the spleen.

P^{32}-labeled red cells have been used to show the dilution of bone-marrow aspirate by peripheral blood, indicating that sternal puncture data are not quantitative.

P^{32} and As^{76} in the treatment of Hodgkin's disease and mycosis fungoides give results no better than those obtained by types of therapy previously employed. The results of treatment of multiple myeloma with P^{32}, Sr^{89}, and Y^{90}, singly or in combination, give results as good as after x-ray, stilbamidine or urethane, P^{32} is the agent of choice in the treatment of polycythemia vera, leading to a nearly normal life expectancy; and the incidence of leukemia among patients treated with P^{32} is no greater than among patients treated by other means. P^{32} occupies a prominent and well-deserved place in the management of chronic leukemias. The dosages and the criteria used by various writers to assess the success of treatment of both polycythemia vera and chronic leukemia are discussed.

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INTRODUCTION

The development of the cyclotron and the discovery of artificial radioactivity heralded the approach of a new era in physics and medicine, one which was to see the advent of the synchrotron, the synchrocyclotron, betatron, linear accelerator and, finally, the nuclear reactor. From the cyclotron and the nuclear reactor has come a wide assortment of isotopes, among which C^{14}, N^{15}, Na^{24}, P^{32}, S^{35}, K^{42}, Cr^{51}, Fe^{55}, As^{76}, Fe^{59}, Sr^{89}, Y^{90}, and I^{131} are most commonly used today. These isotopes have been used in a vast number of biochemical, physiological, and medical investigations and in the treatment of several diseases. In the space allotted to this review only the general aspects can be considered.

INVESTIGATIVE APPLICATIONS OF ISOTOPES

Red-Cell Life

N^{15} is a stable isotope of nitrogen whose presence can be detected by means of the mass spectrograph. Administration of N^{15}-labeled glycine results in the production of red cells containing labeled heme. Plotting the atom percent N^{15} excess in the hemoglobin as a function of the time after feeding the labeled glycine yields a curve which attains a maximum about the 30th day, remains constant until about the 60th day, then decreases. This decrease is not exponential, as it would be if the destruction of the labeled erythrocytes were a random phenomenon. Rather, mathematical analysis of the curve shows that the erythrocytes are destroyed as a function of their age.[1] Allowance must be made for the fact that the cells are not labeled all at once but, instead, over a period of a few days; this results in "tailing" at the decreasing end of the curve. The use of probability theory results in a value for the life span of the erythrocyte, in a normal subject, of around 127 days.

In patients with polycythemia vera the N^{15} curves have been found by London and his colleagues to be nearly identical with normal curves. The average life span of the erythrocyte is around 131 days, probably within normal limits. There is, therefore, an abnormally high rate of hematopoiesis with normal erythrocyte life span. Berlin and his colleagues studied patients with polycythemia vera following administration of C^{14}-labeled glycine, and found that there was a rapid rise in the specific activity of the hemoglobin followed by a fall and secondary rise.[2] The initial rise and fall may be attributed to the delivery into the peripheral circulation of a class of red cells with a life span of only a few days. These short-lived cells are present in addition to a cell population with normal life span.

Berlin and his colleagues have studied patients with chronic leukemia after administration of C^{14}-labeled glycine.[3] In two patients with chronic

lymphatic leukemia who were not anemic the red-cell life span was normal, while in a third patient with chronic lymphatic leukemia, who was in the anemic terminal phase, the red-cell life span was considerably shortened and had a pattern of random destruction. In this case there was classical evidence of a hemolytic process as demonstrated by marked reticulocytosis and increased fecal urobilinogen excretion. In five patients with chronic myelogenous leukemia the red cells had definite life spans ranging from 70 to 100 days, suggesting an intrinsic defect in the red cell in chronic myelogenous leukemia.

Berlin and his colleagues feel that the anemia of leukemia, when not due to hemorrhage, is the result of shortening of the red-cell life span and not the result of crowding out of red-cell precursors from the bone marrow, for radioiron studies in these and other patients have demonstrated that the bone marrow is producing a normal or greater than normal number of red blood cells.

It has recently become possible to obtain a measure of red-cell life by labeling a subject's red cells with radioactive sodium chromate, $Na_2Cr^{51}O_4$.[4,5,6,7,8] This method affords ease of labeling and of measuring radioactivity, and avoids the problem of compatibility of donor and recipient bloods and of differential agglutination techniques encountered in the Ashby method. There is no recycling of the radiochromium, as there is when radioiron Fe^{59} is used to label the red cells. Use of the mass spectrometer, required if N^{15} is employed to label the cells, is avoided. However, the Cr^{51} elutes from the red cells at a constant rate which approximates 1 percent per day. If correction is made for this, the apparent survival curves obtained with this method can be adjusted to agree with those obtained by the Ashby method or by the isotopic methods using N^{15}, C^{14}, or Fe^{59}.[7]

Studies by the Cr^{51} method of patients with chronic lymphatic leukemia have shown shortened red-cell survival times in patients who have other evidences of increased red-cell destruction.[8]

Four patients with congenital hemolytic anemia were studied by Read and his colleagues by the Cr^{51} method before and after splenectomy.[6] All patients showed shortened Cr^{51} survival before splenectomy and normal Cr^{51} survival after splenectomy.

Two patients with myeloid metaplasia studied by Read and his colleagues showed shortened Cr^{51} survival times; after splenectomy the Cr^{51} survival times increased, one to normal and the other to slightly less than normal.[6]

Two patients with acquired hemolytic anemia studied by Weinstein and LeRoy showed shortened Cr^{51} survival times; on cortisone therapy one patient's hemogram became normal, and his Cr^{51} survival time likewise became normal.[8] The other patient experienced some improvement of her anemia, but more particulars are not given.

One patient with hypoplastic anemia studied by Weinstein and LeRoy had shortened Cr^{51} survival time.[8] Three patients studied by Read and colleagues showed some shortening of Cr^{51} survival time; but the interpretation is obscured by the fact that two of the patients had previously been given transfusions, while the third developed lymphatic leukemia six months after study.[6]

One patient with thalassemia minor studied by Read and colleagues showed shortened Cr^{51} survival time; after splenectomy the survival time was increased.[6]

Heme is synthesized *in vitro* in erythrocytes of patients with sickle-cell anemia, as shown by incorporation of N^{15} into hemin by incubation with N^{15} glycine. This occurs at the rate of 0.1 to 0.2 percent of red-cell heme per day and is not observed in normal or sickle-trait erythrocytes. Studies by London and his colleagues of a patient given N^{15}-labeled glycine have shown that the erythrocytes are destroyed indiscriminately rather than as a function of age. The mean survival time is about 42 days. The rates of hemoglobin and red-cell production are about 2.5 times normal. The bone marrow is markedly hyperplastic, with reticulocytosis of the peripheral blood. The defect in the erythrocytes appears to reside in the red-cell membrane.

In pernicious anemia the diminished production of red cells capable of reaching the peripheral blood, and the diminished mean survival time--about 82 days--are consistent with the view that the red cell of untreated pernicious anemia is intrinsically defective. The amounts of bile pigment produced are very large compared with the rate of hemoglobin production and degradation. It has been suggested by London and his co-workers that this excess bile pigment is derived from (a) hemoglobin of red cells destroyed shortly after reaching the peripheral blood or which never reach it and are utilized in the marrow, (b) porphyrins not utilized for hemoglobin production, or (c) direct synthesis of bile pigments via a pathway which does not involve degradation of a porphyrin ring. After treatment with liver extract the destruction of red cells is a function of age only, and the mean life span is normal.

Administration of N^{15}-labeled hematin to a normal dog has led to highly labeled stercobilin in the dog's feces, thus demonstrating that hematin can be converted into bile pigment in the mammal.[9] In normal man breakdown of hemoglobin appears to occur principally in the reticuloendothelial system with the formation of bilirubin, which is excreted into the bile by the liver. Finding, in the first week of administration to man of N^{15}-labeled glycine, a high concentration of N^{15} in stercobilin indicates that a portion of the bile pigment is derived from sources in addition to mature circulating erythrocytes.[10] In disease states such as pernicious anemia and congenital porphyria the proportion derived from these alternative sources may be increased.

Blood Volume

A variety of methods for determining blood volume, using several different radioisotopes, has been described; and the literature abounds with papers describing, comparing, extolling, and criticizing the individual methods.

Requirements that must be fulfilled by the test substance are (a) that it remain in the vascular system for a relatively long time, (b) that it mix readily with normal blood constituents, (c) that it be easily identified and measured, and (d) that it be nontoxic.

In 1940 Hevesy described a method of measuring blood volume by introducing into the blood stream a known amount of P^{32}-labeled red cells and calculating the red-cell mass from the dilution of the labeled red cells. The labeled red cells were at first obtained by administering P^{32} to a donor subject and allowing time for incorporation into the donor's red cells. Disadvan-

tages of this method were that the preparation of the donor was long, that it was necessary to administer heavy doses of P^{32}, and that account had to be taken of blood types and incompatibilities. In 1942 Hevesy and Zerahn showed that it was possible to label red cells *in vitro*, thus simplifying the procedure. Incubation at 37°C of whole blood with P^{32} leads to uptake by the red cells of about 30% of the radioactivity in one hour and of about 50% in two hours. At 4°C there is no appreciable uptake of P^{32}, indicating the metabolic nature of the incorporation of P^{32} by the red cells.[11] The half time of disappearance has been found to be between 12 and 18 hours. The loss of P^{32} in 1 hour amounts to not more than 4 percent, and error from this cause can be reduced to 1 to 2 percent by drawing the postinjection blood sample at, say, 15 minutes.[12] This method of determining total circulating red-cell mass has been refined and applied to a variety of clinical conditions.[13, 14, 15, 16, 17, 18, 19, 20]

It has been found possible to tag human albumin with radioiodine I^{131}, which is bound to the albumin molecule through the tryosine groups of the albumin. In 1950 Storaasli and his co-workers described the use of radioactive iodinated albumin in the determination of plasma volume.[21] The method consists in injecting a known amount of radioiodinated albumin into the blood stream and calculating the plasma volume from the dilution. Some writers block I^{131} uptake by the thyroid by administration of Lugol's solution for a few days prior to the test.[22, 23] Apart from uptake by the thyroid the disappearance of I^{131} from the vascular system occurs by urinary excretion and by diffusion of the iodinated protein into the extravascular spaces. At 1 hour 90 percent of the injected protein is still in the blood stream. In the absence of thyroid binding, I^{131} as iodide ion or as diiodotyrosine is almost quantitatively recovered in the urine within 48 to 72 hours. The mechanism of splitting of the I^{131} albumin linkage in the body probably occurs by splitting off by peptide-bond hydrolysis of the iodotyrosine fragments, although the possibility that simple deiodination occurs *in vivo* in this abnormal protein cannot be dismissed. Appearance of measurable amounts of radioactivity in bile soon after administration suggests that much of the breakdown of albumin occurs in areas where exchange is rapid, as in the liver.

Sterling found in six normal subjects that in the disappearance of iodinated albumin from the blood stream there was a rapid-turnover component with a half time of 0.5 day, attributed to distribution in the body's exchangeable albumin pool (including extravascular sites), and a slow-turnover component with a half time of 10.5 ± 1.5 days.[22] The mean albumin turnover rate for 21 normal subjects was 6.7 ± 0.93 percent per day. The exchangeable albumin pool, obtained by isotope dilution, using the zero-time extrapolation of the slow component, was 259 ± 40 grams or 232 ± 34 grams/1.73 m^2/day. The mean turnover amounted to 17.2 ± 2.7 g/day or 15.4 ± 2.0 g/1.73 m^2/day. Approximation of the circulating plasma, estimated by extrapolating the rapid component and using the isotope-dilution principle, gave a value of 0.45 for the ratio of circulating albumin pool to exchangeable albumin pool.

Studies of the thoracic duct lymph in dogs given intravenous radioiodinated albumin have shown a rapid rise in lymph radioactivity during the first hour, followed by a more gradual rise.[24] From these studies it was concluded that total lymph volume is not measurable and that a negligible amount of the injected tagged protein is present in the lymph during the first 10 minutes.

Radioactive potassium, K^{42}, has been used to label red cells.[25, 26, 27, 28] The mean loss of K^{42} to the plasma by tagged red cells amounts to about 2 per-

cent per hour. Experiments in which red cells were tagged simultaneously with P^{32} and K^{42} have given essentially identical values for total red-cell volume. The short half life of K^{42} poses some problems in procurement and utilization.

Radiochromium, Cr^{51} (with a half life of 26.5 days, disintegrating by K-capture and emission of 4.29-kev x-rays plus a few percent of 0.237-Mev gamma rays), in the anionic hexavalent form ($Na_2Cr^{51}O_4$) labels red cells and in the cationic trivalent form ($Cr^{51}Cl_3$) is firmly bound by plasma proteins. Chromic chloride, in contrast to sodium chromate, is not taken up by the red cell, presumably because of the relative impermeability of the red-cell membrane to the cation.[29,30]

Hemoglobin has demonstrated a significantly greater binding capacity for chromic chloride than for sodium chromate. It has been suggested that anionic hexavalent chromium diffuses through the red-cell membrane and is bound by the hemoglobin within the cell, probably after reduction to the cationic trivalent state, resulting in the firm tagging of the erythrocyte. Once it is bound, there is no exchange with chromate outside the cell. Stromafree hemoglobin from cells previously tagged with sodium chromate retained some 68 percent of its activity after 3 days' dialysis against saline and retained its activity after 24 hours' mixing with a combination of anionic and cationic exchange resins capable of removing all the chromium present.

Albumin tagged with chromic chloride has shown little loss of radiochromium by prolonged dialysis. The binding may involve initially a polar attraction by carboxyl groups. The firm attachment may be explained by coordinate covalent bonds of the type $Cr^{51}(NH_3)_6^{+++}$ between Cr^{51} and terminal amino groups of lysine or other basic groups.[31]

Radiochromium is thus rather well suited to the measurement of either circulating red-cell mass or of plasma volume.[5,29,30,32] The advantage of Cr^{51} over P^{32} in measuring red-cell mass lies in the fact that whereas the latter emits only beta rays and is thereby cumbersome to measure, the former emits gamma rays, so that it is possible to measure very easily blood specimens in small vials in a well-type scintillation counter.

Thorium B fulfills all the conditions of a label for red cells.[28] Thoron in oxygen is bubbled for a few minutes through a blood sample. Thoron is converted with a half life of 55 seconds into Th B, whose half life is 10.6 hours. Th B is taken up by the red cells. Plasma Th B rapidly disappears, so that the activity is due almost exclusively to the red cells. Loss of Th B is almost negligible during the first two hours. Th B $\xrightarrow[\text{Beta}]{\text{Soft}}$ Th C $\longrightarrow$ Hard Beta Ray, measurements being mainly by the hard beta ray of Th C.

Thus far little mention has been made of the sources of error in measurements of blood volume. Sufficient time must be allowed for mixing after introduction of the labeled material, yet this time should be kept as short as practicable to minimize error because of loss of the labeling substance from the vascular system, as by diffusion of P^{32} or K^{42} from the red cell or by passage of labeled albumin through the capillary wall. In patients in congestive heart failure, where the circulation is slowed, 30 minutes or longer may be required for mixing to be completed.[33] Taking the postinjection sample at this time will result in an error no greater than 1 to 2 percent by loss of radioactive material from the vascular system.

A second error, whose magnitude can be held to 2 to 3 percent by centrifugation for a sufficiently long time at speeds which provide adequate centrifugal acceleration, results from trapping of plasma within the red-cell and white-cell mass when the cells are separated from the plasma.[26,34]

The most serious and most discussed errors arise whenever the venous hematocrit enters into calculations. It is generally agreed that the total red-cell volume as measured by dilution of tagged red cells and the plasma volume as measured by dilution of tagged albumin are accurate. However, calculation of red-cell volume or total blood volume from plasma volume and venous hematocrit or, conversely, calculation of plasma volume or total blood volume from red-cell volume and venous hematocrit may entail an error of as much as 5 percent. In 1922 Krogh noted that in minute blood vessels there is a marginal layer of plasma through which an axial blood stream flows. Fåhraeus observed a similar phenomenon in capillary tubes. More recently Gibson and his co-workers have utilized radioactive iron to show that the average, or body, hematocrit of dogs is significantly lower than the hematocrit as determined from blood taken from peripheral vessels. Studies in human beings have shown that the body hematocrit, as measured by the ratio,

Red Cell Volume/(Red Cell Volume + Plasma Volume),

is likewise slightly less than the hematocrit as determined from peripheral blood, averaging about 90 percent of the latter.[17,18,19,20,26] If one takes a mean value of 0.925 for the ratio of body hematocrit to peripheral-vessel hematocrit, calculation of total blood volume from albumin or erythrocyte dilution alone is not likely to be in error by more than about 7 percent.[18] However, the differences are so small that various isotope methods of labeling the red cell or plasma are all satisfactory, and certainly they are superior to previously used methods such as the dye T-1824. The most accurate method of determining total blood volume consists in the separate isotope measurement of total red-cell and plasma volumes and the addition of the two.

Blood-volume studies for 71 male subjects and 16 female subjects give the following data:[35]

	Blood Volume cc/kg	Total Red Cell Volume cc/kg	Plasma Volume cc/kg	Hematocrit %
Male	69.0	29.9	38.7	43.0
Female	64.4	27.0	37.0	42.0

In a study of 66 cancer patients by Berlin and co-workers 32 percent were anemic by blood-volume determinations.[36] Of the anemic group 76 percent had metastases, while of the nonanemic group 80 percent had metastases. Thus in both the anemic and nonanemic groups the incidence of metastases was about the same. Of six patients who had bone metastases, however, five were anemic, suggesting that bone metastases are usually associated with anemia. A relatively large number of patients had normal total red-cell volumes with expanded plasma volumes, accounting for the clinical impression that anemia is widespread in cancer and showing the unreliability of the red-cell count and hematocrit in estimating total red-cell volume. Chodos and Moses, in the study of blood volume and of the utilization of iron in red-cell formation, conclude that in cancer patients blood production is not depressed but proceeds actually at an increased rate though at lower levels of total circulating hemoglobin.[37] The cause for the anemia occurring

in malignancy in the absence of hemorrhage, infection, or cachexia is not clear; but one possible explanation is an abnormally short red-cell life span, similar to that seen in leukemia.

Hematopoiesis

The unique rôle played by iron in hematopoiesis and hemoglobin metabolism has made it possible to use radioiron, Fe^{55} or Fe^{59}, in the study of these processes.

In vitro studies have demonstrated that mature erythrocytes do not take up iron from the surrounding plasma. Reticulocytes, however, assimilate iron and synthesize heme in vitro. Ferrous iron is taken up twice as fast as ferric iron. Iron in the iron-binding protein of serum is assimilated, but less rapidly than inorganic ferrous or ferric iron. Iron uptake is impaired by low temperatures and by lack of glucose.

Red cells have been fractionated by albumin flotation after incubation in vitro with radioiron; the largest amount of activity in the reticulocyte fraction is found in the stroma of hemolyzed cells; significant amounts of radioiron have also been demonstrated, however, in heme recrystalized from these cells. These observations indicate that assimilation of iron by developing cells is, first, attachment of iron to acceptors in the red-cell stroma capable of removing iron from the serum and, secondly, synthesis of heme. Reticulocytes are still capable of completing the process of hemoglobinization in the peripheral blood, in keeping with observations that tagged reticulocytes lead a normal life span. Radioactive heme has been demonstrable after incubation of radioiron with normoblasts of marrow; and, indeed, uptake by these cells is greater than that by reticulocytes.

During the past several years isotopic techniques have played a vital role in the study of the mechanism of the synthesis of heme.[38] It was first shown that acetate and glycine are utilized in the biologic formation of protoporpyhrin. The nitrogen atom of glycine provides all four nitrogen atoms, and the methylene carbon of glycine provides 8 of the 34 carbon atoms of protoporphyrin.[39] Acetate is utilized via a succinyl intermediate, δ-amino-levulinic acid, which is formed from the condensation of succinate and glycine.[40] Two molecules of δ-amino-levulinic acid combine to form a pyrrole whose structure is like that of porphobilinogen, which can spontaneously in vitro be converted to uroporphyrin III.[41] Evidence has been advanced for the conversion of uroporphyrin III to protoporphyrin.[42]

Radioiron has made possible studies of iron absorption and transportation. Iron has been found to be absorbed in the ferrous form.[43] Feeding a dose of iron results, several hours later, in a mucosal block which prevents further iron absorption for some 12 to 24 hours. The presence of iron in the mucosal cells stimulates their production of apoferritin, with which the iron combines to form ferritin.[43] The time sequence of increase and decrease of ferritin appears, in the guinea pig at least, to parallel the time sequence of the appearance and disappearance of the mucosal block. The stomach and duodenum seem to be most active in iron absorption, the colon absorbing very little. There is, thus, the suggestion of a gradient in the capacity of the gastrointestinal tract to absorb iron.[44]

Dubach and colleagues suggest that normal subjects given a 1 mg/kg dose may sometimes absorb more iron than is converted within a two-week

period into hemoglobin. They have shown that patients with fever, untreated pernicious anemia, and refractory anemia, absorb more iron than they use for hemoglobin. Their work indicates that patients with hemolytic anemia may absorb more iron than can be recovered at any one time in the peripheral blood. Thus, except in afebrile patients with hypochromic anemia, acceptance of the fraction of a given oral dose of radioiron which appears in the circulating hemoglobin as a measure of intestinal iron absorption must be made with caution.

In the anemia of iron deficiency two to ten times as much iron may be absorbed as in the normal, according to Hahn. In a number of the anemias, including pernicious anemia and refractory hypoplastic anemia, the limited data, largely obtained by Dubach and colleagues, indicate that the amount of iron absorbed is greater than normal. However, there is no evidence for the utilization of the iron in the correction of these anemias except in the case of the anemia of iron deficiency, where there may also be increased demands for replenishment of iron stores and tissue iron (enzymes and myoglobin).

Radioiron studies have shown that in dogs with tagged iron stores very little storage iron was used in the formation of new red cells following excessive red-cell destruction with phenylhydrazine.[45] In the normal animal only about 10 percent of iron used in the formation of red cells came from storage iron.

Not until the work of Huff et al., made fifteen years after the first use of radioiron in animal and human studies, did real insight into many phases of iron and red-cell metabolism begin. The original work was unique in that it was based on an understanding of how an isotope should be used.

This was of steady-state plasma and red-cell iron turnover in normal subjects and in patients with various hematopoietic disorders.[46] For this purpose the subject's plasma has been incubated at room temperature with radioiron and then reinjected into the subject. Samples of blood were taken frequently during the first hours after reinjection, then at least at daily intervals for 10 to 14 days. Plasma and red cells were analyzed separately for activity. Total plasma iron was measured by the method of Kitzes and colleagues, usually only once, since plasma iron was found to remain sensibly constant during the course of the studies. Red-cell mass was measured with P^{32}-tagged red cells. Plasma volume was obtained either by calculation from the red-cell mass or by calculation from the dilution of the Fe^{59}-tagged plasma; if the two values did not agree, the former was used in turnover computations.

The plasma radioiron concentration plotted on semilogarithmic paper as a function of time gives in general a straight line. This may be represented mathematically as

$$a_t = a_o e^{-Kt},$$

where

a_t = plasma concentration of tracer at any time t;

a_o = plasma concentration of tracer at time $t = 0$;

K = turnover constant.

Differentiating both sides of the equation gives

$$\frac{da_t}{dt} = -Ka_o e^{-Kt} = -Ka_t \quad \text{or} \quad K = -\frac{da_t}{dt} / a_t .$$

K is the negative of the slope of the above-mentioned plasma Fe^{59} concentration curve.

Now, since the Fe^{59} mingles indiscriminately with the ordinary species of iron, the rate of change of plasma iron with respect to time, $\frac{dFe_p}{dt}$, will be proportional to the rate of change of plasma radioiron with respect to time, $\frac{da_t}{dt}$; and Fe_p will be proportional to a_t, the constant of proportionality being the same in both instances. Hence, we may replace $\left(\frac{da_t}{dt}\right) / a_t$ by $\left(\frac{dFe_p}{dt}\right) / Fe_p$. Thus K, the turnover constant, is given by $K = -\left(\frac{dFe_p}{dt}\right) / Fe_p$; i.e., it is the fractional or relative time rate of change of plasma iron. If we define a new quantity $r = \frac{dFe_p}{dt}$, then $K = -r/Fe_p$.

Now, if A_o is the total amount of radioactivity injected and A_t is the amount of radioactivity in the circulating red-cell mass when equilibrium in the red cells has been attained, then $r\frac{A_t}{A_o}$ gives a measure of the fraction of the plasma iron turnover concerned with the red cells, i.e., the red-cell iron turnover. This equilibrium appears in many cases but not all; in the latter instances the highest value of A_t attained has been used. A_t is the product of the circulating red-cell mass and the radioactivity per unit mass of red cells.

For normal male subjects, Huff and colleagues have obtained the following values:

Average plasma Fe turnover = 0.35 mg/kg/da (0.31 - 0.39)

Average red cell Fe turnover = 0.26 mg/kg/da (0.22 - 0.28)

Average value of K = 0.41/hr (0.37 - 0.48)

If 120 days is taken as the average life span of the red cell, the fraction of red cells renewed per day is 1/120 or 0.0085. Isotope-dilution techniques give for normal human males a red-cell volume of around 30 ml of packed red cells per kilogram of body mass. Moreover, one milliliter of packed red cells contains one milligram of iron. Hence, the daily red-cell iron turnover is 0.0085/da x 30 ml/kg x 1 mg Fe/ml = 0.26 mg Fe/kg/da, which agrees well with the experimental value given above.

The bone marrow must remove most of the iron from the plasma, since the curve of concentration of radioiron in the marrow, obtained by in vivo counting plotted against time, is practically inversely identical to the curve of plasma radioiron concentration plotted against time. The marrow uptake of radioiron is a simple exponential growth curve. It is, of course, true that the pathway of iron from plasma to marrow to red cells is not the only pathway; so the method of measurement of iron metabolism and red-cell production is not quantitative but nevertheless is very useful in the study,

diagnosis, and therapy of various hematological disorders.

In polycythemia vera, nontreated or treated with unsatisfactory remission, Huff and his co-workers found a plasma iron turnover of about 1.8 mg/kg/da, about five times normal. Nearly all the high values for plasma iron turnover were associated with elevated values for red-cell iron turnover, to be expected since red-cell masses were elevated. However, red-cell iron turnover values were often inordinately high, being in some instances 10 times normal whereas the red-cell mass was 1.5 to 3 times normal. The previously described short-lived red cells present as a component of the total red-cell population of patients with polycythemia vera may explain these high red-cell iron turnover values.

In polycythemia vera treated with radioisotopes with satisfactory remission no high turnover values were noted in plasma or red cells. Fe^{59} studies can thus give indication whether the red-cell mass may be expected to decrease, thereby guiding therapy. Isotope therapy should be directed at excessive rate of production of erythrocytes, not at an excessive red-cell mass. Indeed, studies of suspected polycythemia vera patients have shown elevated red-cell masses with normal plasma and red-cell iron turnovers; and it appears that transient rises in red-cell mass can occur "normally."

In secondary polycythemia the plasma iron turnover is elevated, though less so than in polycythemia vera. The mean value for plasma iron turnover accordingly was 0.68 mg/kg/da, with less variation in individual cases. It appears that here the iron turnover is regulated so that it quite closely approximates a turnover rate nearly normal for the red-cell mass. The mean value of the fraction of red-cell iron renewed per day was 0.011 (5 studies). The apparently complete lack of regulation of iron turnover and possible lack of control of red-cell production in polycythemia vera, contrasted with secondary polycythemia, suggest the similarity of polycythemia vera to neoplasms.

In myelogenous leukemia, chronic except for one case, the average plasma-iron turnover was 0.84 mg/kg/da. These surprising data showing red-cell production to be normal suggested that the anemia of leukemia might be due to considerable shortening of the life span of the red cell; and this was shown to be true by Berlin, Lawrence, et al. and by other workers.[2,3,8] These findings are also true in chronic lymphatic leukemia.

In pernicious anemia two untreated patients showed plasma iron turnovers of 1.89 mg/kg/da and 2.70 mg/kg/da, values as great as those of some of the most severe cases of polycythemia vera. The red-cell uptake was low, but red-cell iron turnover was excessively high in relation to cirulating red-cell mass, the fraction of red-cell iron renewed per day being 0.340 and 0.650. In pernicious anemia there is good corroborative evidence of erythropoietic hyperplasia, formation of abnormal erythrocytes, and lack of staying power of red cells. The treated pernicious anemia patient showed a definite tendency to return to normal, although the predisposition toward accelerated iron turnover persisted. These findings are in harmony with the fact that the red cells in pernicious anemia have a short life duration. The low rise of blood iron level in untreated pernicious anemia compared with hemolytic anemia has been taken as presumptive evidence that hemolysis is not a prominent factor in the production of the anemia of pernicious anemia.

Patients with untreated pernicious anemia may absorb considerable amounts of radioiron administered orally, but the isotope does not appear in the peripheral blood in large amounts until after erythrocyte maturation arrest has been relieved by liver extract.

In some cases of refractory anemia where extremely rapid destruction of marrow cellular output occurs, the previously described method of computation of red-cell iron turnover breaks down, for the loss of Fe^{59} from the plasma is not a simple exponential process, but is complicated by rapid early loss with K = 4/hr and slow loss with K = 0.1 to 0.2/hr.

In hemochromatosis, a metabolic disease especially of the male, with hereditary tendencies, Fe^{59} utilization is profoundly depressed, although red-cell production proceeds normally.[47] Absorption of iron from the gut has been measured by the use of Fe^{59} given orally and has been found to be increased.[48]

In hemolytic anemias rise in radioactivity of blood during the first few days was rapid, usually stopping in a short time so that curves formed plateaux.

It is postulated that iron recently made available for storage from absorption, parenteral administration, or from hemoglobin liberated from destroyed red cells is retained in a form which can be more readily mobilized for use than can iron stored for longer periods of time. Recently stored iron seems to be used selectively for current metabolic needs.

Four patients with Addison's disease with mild normochromic anemia studied by Finch et al. have shown plasma radioiron disappearance curves in the normal range. One patient with postoperative myxedema approximated normal radioiron utilization. One patient with anterior pituitary hypofunction showed a definite decrease in radioiron utilization. In uremia the impairment of plasma radioiron disappearance roughly parallels the degree of azotemia. When the nitrogen retention is alleviated, utilization of radioiron improves, suggesting that some factor associated with retention of metabolic products interferes with blood production as measured by this method. It appears not to be an attendant disorder in iron metabolism, for the serum iron usually is within normal limits, according to Finch.

Similarly, Finch and his colleagues have found that two patients with subacute bacterial endocarditis showed rapid initial rise and early plateau like those in hemolytic anemias. In hemolytic anemia there is the suggestion either that the serum iron-binding protein is almost completely saturated with iron from destroyed red cells--with the result that injected radioiron is at once depositied in the inactive tissue stores--or that hemoglobin iron is used in preference to injected iron.

Patients with polycythemia vera and with secondary polycythemia have been studied before and after oxygen administration by the iron turnover technique.[49] Patients with secondary polycythemia demonstrated 28 to 35 percent diminution in plasma-iron turnover during and after oxygen administration. Such inhibition was not observed in the iron turnover of patients with polycythemia vera. Such studies show control of secondary polycythemia to be a normal physiological mechanism and polycythemia vera to be under no such control.

In vivo radioiron uptake studies employing scintillation counters placed over the liver, spleen, and sacrum (bone marrow) after intravenous administration of 5 to 30 microcuries of Fe^{59} have been carried out in various hematopoietic disorders.[50] In general four patterns have been observed:

1. Normal Pattern. Here the maximum of the marrow curve is considerably greater than that of either the liver or spleen, even though the counted volume is a far smaller fraction of the total marrow volume than the other counted volumes are of their respective tissues. The rise in marrow-site counting rate mirrors the decline in the plasma. The spleen-site curve rises to a low maximum and falls. The maximum fraction of the injected tracer present in red cells within 15 days varies between 75 percent and 90 percent.

2. Pattern M (Marrow Erythroid Hyperplasia). Here there is excessive plasma-iron turnover, and the estimated red-cell turnover is usually greater than normal. Such a pattern is observed (a) after hemorrhage, (b) in hemolytic anemias, (c) in chronic lymphatic leukemia, and (d) in pernicious and related anemias. The marrow radioiron-uptake curve rises to a subnormal maximum (dispersed erythroid tissue) with incomplete decline (recycling through abnormal iron compounds or short-lived cells). The liver radioiron-uptake curve at the time of marrow maximum is subnormal in amplitude (extraordinarily rapid depletion of plasma tracer by marrow), and the decline is less complete. The maximum fraction of the injected tracer appearing in the red cells during the first 15 days is characteristically less than normal. The "secondary" or "erythroclastic" spleen radioiron-uptake curve rises after the decline in marrow-site counting rate and is simultaneous with the appearance of labeled red cells in the circulation; the shape suggests random destruction of cells.

3. Pattern EM (Extramedullary Erythropoiesis). Here almost invariably there is greater than normal plasma iron turnover and usually greater than normal estimated red-cell iron turnover. This pattern is observed in chronic myelocytic leukemia and in myelofibrotic anemia with extramedullary cell production. The "primary" or "erythrogenic" spleen curve rises to an abnormally high maximum; the ascending segment is reciprocal to the decline in plasma Fe^{59} concentration and the descending segment is reciprocal to increasing red-cell radioactivity, --similar to the marrow curve in normal subjects. Here the marrow counting rate is always less than the spleen or liver counting rate. Occasionally the presence of an erythrogenic spleen curve with an overlapping erythroclastic curve suggests occurence of both production and destruction of red cells within the spleen. Usually the maximum fraction of injected tracer appearing in the red cells exceeds 50 percent.

4. Pattern 0 (Erythroid Hypoplasia). Here plasma iron turnover is normal or less; and the estimated red-cell turnover is less than that required to maintain normal red-cell mass and total circulating hemoglobin, as in erythroid hypoplasia and, possibly, acute stem-cell leukemias. The marrow curve may rise to a broadened, lower than normal maximum. Impairment of the plasma-to-marrow pathway is evidenced by a prolonged plasma-depletion half time and by failure of the marrow-uptake curve to rise normally. Despite the long plasma-depletion half life the high total circulating plasma iron may result in a plasma iron turnover that is only moderately reduced. The liver curve rises slowly as the plasma tracer declines, probably the result of nonerythrogenic turnover through the liver

pool. The maximum fraction of the injected radioiron appearing in the red cells during the first 15 days amounts to some 5 to 20 percent.

Treatment of pernicious anemia results in decrease of the disparity between iron turnover in the plasma and iron turnover in the cells, i.e., a reduction in recycling through short-turnover-time compartments, associated with normal maturation of erythroblasts.

In thalassemia minor the red-cell life is of the order of 85 days.[51] An erythroclastic spleen curve in the absence of signs of excessive destruction of hemoglobinated cells appears to be associated with splenic removal of defective iron-containing cells or cell fragments produced in addition to a longer-lived population. Turnover via imperfect, short-lived cells may account for some of the excess plasma turnover not attributable to maintenance of red-cell volume.

Recently Contopoulos et al. have utilized Fe^{59} in the study of the anemia which develops in rats after hypophysectomy.[52] They have separated from glacial acetic extracts of anterior pituitaries preparations which, given to hypophysectomized rats two months postoperatively for 14 days in 100-microgram daily doses, increased the hematocrit and total red-cell volume 110 percent above the values in the hypophysectomized controls and 45 percent above normal values.

Leukocytes and Thrombocytes

A method has been developed for estimation of the life span of leukocytes by measurement of the incorporation of P^{32} into the desoxyribonucleic acids (DNA) of the leukocytes.[53] The inertness of P^{32} in the DNA molecule after formation of the leukocytes has been found to be similar to the stability of N^{15} in hemin of the red blood cells.

Subjects were given orally 2.5 millicuries of P^{32} as inorganic phosphate. Every two days thereafter a sample of blood was drawn and the leukocytes separated by centrifugation. The cells obtained were weighed and the DNA fraction was isolated. The specific activity of the phosphorus in the DNA fraction was determined. Mathematical analysis of the curve of specific activity plotted against time in days yields an average life span of 12.8 days from the time of administration of P^{32} and a value of 8.8 days for the time the labeled leukocytes appear in the circulation in large numbers. The difference between these values may be interpreted as the time the bulk of the cells containing labeled DNA remain in the bone marrow. These values represent the average for all white blood cell types.

Recently, Ottesen has described a method for separating granulocytes and lymphocytes from human blood based upon differences in sedimentation rates and specific gravities.[54] Analysis of the curves of specific activity of the DNA fraction of the granulocytes or lymphocytes plotted against time after administration of P^{32} to the subject gives a mean age of about 9 days for the granulocytes. The main part of the granulocytes enters the blood stream at an age of about 6 days. Less than 5 percent of the granulocytes in the blood stream is younger than 5 days, and a negligible percentage is older than 3 weeks.

The lymphocytes form two groups, one younger than 10 days and with a

mean age of 3 to 4 days, and the other having a mean age of about 100 to 200 days. The short-lived fraction comprises around 15 percent and the long-lived fraction around 85 percent of the lymphocytes in the blood.

The transfusion of P^{32}-labeled leukocytes has been realized in man by a technique employing flotation separation and incubation in silicone-coated apparatus.[55] These experiments have demonstrated the rapid removal of the transfused leukocytes from the blood, the P^{32} disappearing more swiftly in the first few hours when bound to leukocytes than when injected as inorganic phosphate solution. The same technique has been utilized to label platelets, and disappearance of these also seems to occur in the first few hours after transfusion. No notable differences in urinary P^{32} excretion were noted between subjects receiving labeled leukocytes or platelets and control subjects receiving intravenous inorganic P^{32}.

Parallel studies involving transfusion into rabbits of P^{32}-labeled leukocytes from the exudate of other rabbits receiving intravenous P^{32} were performed. Transfused animals sacrificed at 30 minutes showed localization in the lung of an important fraction of the P^{32}. Rabbits sacrificed at 4 to 6 hours, however, showed marked activity in the spleen as well as in the lung; the liver, which weighs about 5 times as much as the lung, also showed considerable activity. The leukocytes appear to be arrested in the early minutes predominantly by the lungs, by a process of selective--not reticuloendothelial--filtration; for results are the same whether the injection is via the venous or arterial system.[56] The shift of radioactivity away from the lungs to the liver in 4 to 6 hours probably indicates destruction of the leukocytes, but may represent migration of intact leukocytes. It is unlikely, however, that transfused cells subsequently reappear in the circulation, since no change in radioactivity of the peripheral blood is noted. In the case of platelets the selective capture appears to occur in the spleen. P^{32} radioactivity of the kidneys is small and constant whether by inorganic P^{32} or by P^{32} tied to leukocytes or platelets.

The leukocytes here retained their normal staining, motility, and phagocytic activity to an extent comparable with leukocytes in stored transfusion blood. It is entirely possible that the mechanism here of removal of leukocytes accounts for the failure to elevate the leukocyte count of patients by blood transfusions. The accumulation of transfused leukocytes in the lungs has also been confirmed histologically.

Bone Marrow

Radiophosphorus has been used in the study of the extent of dilution of bone marrow aspirate by peripheral blood.[57, 58] Smears made from particles of marrow aspirate have failed to disclose radioactivity from peripheral P^{32}-tagged blood, thus demonstrating the freedom from the distorting influence of peripheral blood cells if only aspirate particles are used for making the smears. Aspirate fluid, on the other hand, is composed of a variable and unpredictable amount of peripheral blood in which are suspended bone marrow particles. Particle smear differentials never fail to reveal cellular elements, of diagnostic significance, which have been revealed in random aspirate fluid sample differentials. However, the reverse may be true, important cellular elements being found in particle differentials but not in aspirate fluid differentials. Neither gross appearance nor total nucleated count of aspirated fluid is a reliable estimate of bone marrow cellularity.

THERAPEUTIC APPLICATIONS OF RADIOISOTOPES

So far as therapy of hematopoietic disorders with radioisotopes is concerned, the only agent that has enjoyed any lasting success to date is P^{32}, which after studies in normal and leukemic animals, was first employed in 1936 by Lawrence and associates in the treatment of leukemia and polycythemia vera. Other isotopes will be discussed, however, in connection with the diseases in which they have been employed.

Dosimetry for Phosphorus-32

Low-Beer and his co-workers have formulated a method for estimating the dosage obtained from an intravenous addministration of P^{32}.[59,60] On the basis of experimental studies of relative phosphorus uptake and excretion in soft tissue and bone an initially uniform distribution of P^{32} in the body is assumed. After 3 days equilibrium is established between one compartment comprising the bone marrow, liver, and spleen and a second compartment comprising the remaining soft tissues, with the ratio of P^{32} concentration in the former to that in the latter being 10:1. From the third day on, bone and soft tissue are losing P^{32} through decay and excretion at the rate of 6.1 percent per day, corresponding to an effective half life of 11 days. The 12 percent of the administered dose that decays in the body during the first 3 days, added to the 58 percent of the adminstered dose that decays in the body from the third day on, gives a total of 70 percent decaying in the body. By calculation 34 percent of the administered dose decays in bone and 36 percent in soft tissue. The total intergral doses are 306 g r/μc for bone and 320 g r/μc for soft tissue, giving a total body integral dose of 626 g r/μc.

Lymphogranulomatoses

Hodgkin's disease and mycosis fungoides have been treated with P^{32} and As^{76}. There has been some preferential fixation of P^{32} in the skin lesions, the extent of fixation seemingly paralleling the intensity of the lesion.[61,62] A temporary amelioration has been followed by the rapid reappearance of symptoms more attenuated and more localized. The tumor elements were not influenced in dimensions or consistency; indeed, new lesions have appeared on previously uninvolved surfaces. Results with P^{32} often have not equalled those obtained with 300 to 500 r of nonpenetrating x-ray, which is generally sufficient to cause temporary regression of lesions in the regions irradiated.

As^{76}, a beta- and gamma-ray emitter with a half life of 26.8 hours, has been tried in the treatment of predominantly cutaneous generalized Hodgkin's disease and mycosis fungoides.[63] It is most effective against skin lesions and not to be counted upon for regression of adenopathies, splenomegaly, or pulmonary or osseous lesions. Fever has been reduced only to the extent to which it depends upon skin lesions. Hematopoietic alterations concern mainly the erythropoietic series; in two of four cases reported anemia, readily corrected with transfusions, occurred. Emphasis has been placed upon the absence of neutropenia and thrombopenia. It has been estimated that As^{76} predisposes less to blood alterations than radiotherapy, P^{32}, or nitrogen mustard.

The short half life as As^{76} is a disadvantage, posing problems of immediate administration. The gamma radiation creates hazard to the personnel. Finally, digestive troubles are very frequent, ascribable

both to irradiation and to the arsenic itself. Arsenical erythroderma can develop in very sensitive integuments.

It is necessary to grope in each case, using as small doses as possible in aiming at effacement of cutaneous lesions and pruritis. Adenopathies and polyvisceral lesions must be handled afterwards by other methods. In advanced mycosis fungoides As^{76} has temporarily blanched lesions and afforded unhoped-for easing in an implacable progression. In a study of As^{76}, given intravenously, in lymphogranulomatoses Block, Jacobson, and Neal have found no advantages over the types of therapy already in use.

Multiple Myeloma

In a series of 24 cases reported by Lawrence and Wasserman the age of onset ranged from 29 to 66 years, with a mean of 51.9 years.[64] Fifteen of the 24 patients were over 50 years of age at the onset of the disease. Nine patients received combined P^{32} and Sr^{89}; 11, P^{32} only; and 1, colloidal Y^{90} only. Seven were not definitely benefited by P^{32} or Sr^{89} or both, although five of these patients were in a very advanced stage of the disease when first seen. No benefit was noted in the one patient treated with Y^{90}. In 8 other cases the benefits were questionable. The length of life after onset of the disease ranged from 6 months to 9 years, with an average around 3 years.

There is no recommendation for a precise line of therapy. There is no evidence that Sr^{90} plus P^{32} is better than P^{32} alone. One millicurie of P^{32} intravenously, once or twice weekly for 4 to 6 weeks, has been employed, or about 5 to 10 mc per course, repeated in 3 to 4 months if necessary, provided the blood picture permits.

The results of treatment are not markedly better than with x-ray or with stilbamidine. Radiosensitive cases are influenced equally well by x-ray or radioisotope, although irradiation probably has little influence upon the course of the disease. Symptomatic improvement in some cases has been striking, and at times a combination of P^{32} and x-ray has been more effective than either one alone.[65] Because of its long half life (55 days) Sr^{90} may be unsatisfactory.

Polycythemia Vera

Polycythemia vera is a chronic, progressive disease of insidious onset characterized by an elevated red-cell count and usually accompanied by leukocytosis, thrombocytosis, splenomegaly, and rubrocyanosis. It is primarily a disease of middle age, with an average age of diagnosis around 52 years. Males are more commonly affected than females, the ratio being about 1.8 to 1.[66]

The etiology of the disease has not been established, although such mechanisms as hypoxemia, humoral agents, and hormonal agents have been suggested. Perhaps the best interpretation at present is that which regards the disease as neoplastic in nature.

Diagnosis can usually be made on the basis of the above-mentioned findings. However, total red-cell volume, measured with P^{32} or Cr^{51}, is of value, being elevated above the normal. Plasma volume may be normal

or reduced below normal. Blood oxygen saturation is normal, and plasma radioiron clearance is accelerated. The bone marrow may show hyperplasia of all marrow elements, and the percentage of nucleated red cells may be moderately elevated. These cells may be either ortho-chromatic normoblasts or of less mature type, but megaloblasts are not found. There is usually no extramedullary hematopoiesis. Peripheral blood shows at times some myelocytes but no erythroblasts or myeloblasts. There is rapid but defective clot retraction associated with the high hematocrit, elevated platelet count, and increased blood viscosity.[67]

Stroebel and his colleagues reported a series of 199 cases in 31 percent of which there was a history of some kind of vascular accident.[68] Peptic ulcer, renal calculi, and gout are commoner in polycythemia vera.[67,69] The increased incidence of peptic ulcer is attributed to thrombosis of vessels of the mucosa, since the ulcers disappear when remission of the polycythemia is achieved.[70]

Various modes of treatment have been employed in the past. Perhaps the oldest is bleeding; this is, even now, of value in urgent cases where the red-cell volume must be reduced rapidly. Bleeding alone, however, does not produce remission, because it stimulates hematopoiesis. Phenylhydrazine has been used not uncommonly, but it is toxic and destroys only adult red cells without inhibiting their production. Recently, triethylene melamine has been used, but without much success.[66] Fowler's solution has been used, but it is toxic and of doubtful efficacy. X-ray has been used in the past, but selective irradiation of the bone marrow alone is impossible. Marchal believes that teleroentgentherapy and P^{32} are comparable and interchangeable.[71]

Most workers in the field now agree that P^{32} is the treatment of choice in polycythemia vera. To be sure, the effect of the mode of operation of P^{32} is by 0.695-Mev beta irradiation and hence, basically, like x-ray, is radiotherapy. However, P^{32} is taken up preferentially by bone and cells with high phosphorus content and by cells that are undergoing rapid metabolism or mitosis. Thus, P^{32} is taken up selectively by the hematopoietic tissue, giving much more localized therapy than can be achieved by roentgen irradiation. Radiation sickness is extremely uncommon in P^{32} therapy.

Some writers prefer the oral route of administration; others, the intravenous route. The oral route has the advantage of convenience of administration and freedom from difficulties with pyrogens and bacteria. It is generally agreed, however, that P^{32} uptake from the gastrointestinal tract amounts to only around 80 percent of the administered dose, so that due allowance must be made for this. When given orally, the P^{32} is usually administered to the patient in the fasting condition, and nothing is taken by mouth for several hours after the dose. Intravenous administration circumvents the vagaries of intestinal absorption nicely. However, there is reason to suspect that some of the phosphorus becomes adsorbed onto particulate matter almost invariably present in solutions and that this colloidal material is taken up by the reticuloendothelial system rather than by the bone marrow for which it is intended.[72,73] Despite this possible objection, intravenous administration is the more commonly used route.

There is no universally accepted schedule for dosage. Most writers give an initial intravenous dose of 3 to 7 millicuries.[68,72,74,75,76,77]

Since the effect of P^{32} is not to destroy red cells but to decrease their rate of production, the rate of diminution of red-cell volume cannot possibly exceed that of natural red-cell destruction, 0.85 percent per day. Thus, the hematocrit does not fall until the fourth to sixth week or even later, and it is difficult to assess the results of treatment before at least two, and possibly as long as four, months after treatment. In urgent cases venesection can be employed during the latent period. This should, perhaps, be done in the first days after P^{32} administration, however, to minimize the stimulatory regenerative effects; for, in rats it has been shown that the reactive reticulocytosis after bleeding becomes greater as the interval after P^{32} administration becomes greater.[72]

According to Abbatt the platelets show the first depression, reaching a minimum at about four weeks and rising rapidly thereafter.[74] This platelet depression may be of remarkably short duration and may be missed unless frequent--at least weekly--blood counts are performed. The total white-cell count falls at a variable time, usually shortly after the platelet count falls, and recovers more slowly, but generally is much more indefinite in its behavior than the platelet count.

During the period of falling hematocrit, symptomatic improvement--which frequently begins before any objective evidence of response is observed--continues. The spleen, if enlarged, regresses; some patients at this time note left hypochondriac pain, provoked no doubt by retraction of a more or less fixed spleen.[76] The liver may decrease in size.[67] Hemorrhagic and thrombotic tendencies disappear and remission of variable duration ensues. Half of 73 patients hypertensive at initial examination showed significant drop in blood pressure after treatment.[67]

Abbatt has used the platelet count as a criterion of effective therapy, noting full remission in all cases in which the platelet count fell to 50,000 or less.[74] If this criterion be correct, then if no adequate platelet response has been achieved by the fourth to sixth week after treatment further P^{32} will be required for full remission.

Tubiana and Schapira regard the elevation of serum iron as an index of successful P^{32} treatment.[78] Serum iron is normal or low in untreated polycythemia vera. In cases treated without success the serum iron is also low or normal in some instances and elevated in others. In P^{32} treatment a rise in serum iron often precedes the drop in hematocrit. Conversely, sometimes there is a drop in serum iron when the hematocrit rises. The return of iron turnover to normal has already been discussed.

Bone marrow punctures are of little value in management except in cases of leukemic transformation of marrow. No significant changes have been noted in the bone marrow at varying times after the completion of therapy. However, when particles of bone marrow are studied where the architecture can be seen, the untreated cases present a characteristic picture, which after therapy, returns to normal.[67, 79]

Stroebel et al. have suggested in a study of some 170 patients that the basic criteria of (a) sustained red-cell count greater than 6 million, (b) sustained packed-cell volume greater than 55 percent in the presence of normal or increased total blood volume are diagnostic.[68] Leukocytosis, i.e., a white-cell count greater than 15,000, was considered to represent

a leukemoid reaction. Among cases of longer duration there was a higher incidence of leukemoid reaction, perhaps due to leukemoid changes in non-leukemoid types or to reversion to normal of nonleukemoid types. In patients with only the basic criteria treatment was successful in 90 percent of cases, being unaffected by such features as splenomegaly, hypervolemia, vascular complications, or history of previous treatment. If a leukemoid reaction was present, treatment was successful in 66 percent of cases. If there was a marked leukemoid reaction, treatment was successful in only 29 percent of cases. In 32 patients treated by means other than P^{32}, 56.3 percent died from vascular accidents and 12.5 percent from leukemia, with possible masking of the latter due to premature deaths from vascular accidents. In the group treated with P^{32} death from vascular accidents was less than 1/10 as great as in those not treated with P^{32}. Two females treated successfully with P^{32} subsequently bore apparently normal infants. Four patterns of successful remissions were observed: (a) ready response to small doses, (b) initial remission only after multiple injections but successive remissions with relatively small doses, (c) moderate doses (7 to 15 mc) for remission, and (d) large doses (greater than 15 mc) for remission. The duration of successful remission lasted 1 to 2 years in 50 percent, 2 to 4 years in 32 percent, and over 4 years in 4.5 percent. In otherwise successful cases remissions (13.5 percent) of less than 1 year were occasionally obtained. In patients treated with P^{32} leukemia has occurred in around 3 percent of the group, and other hematologic complications such as aplastic anemia and anemia with leukemoid reactions, bring the total incidence to 9 percent. The risk of leukemia is small compared to that of a vascular accident.

Studies on 263 patients by Lawrence et al. demonstrated that the life expectancy of their patients is only a few years under the normal for the age group in question, and is equal to or better than that reported for diabetics treated with insulin and of patients with pernicious anemia treated with liver extract.[67]

Aside from the possibility of inducing leukemia by using P^{32} which is neither well-founded nor of sufficient gravity to preclude treatment with this agent, the only danger is production of an aplastic bone marrow. No instance of a pancytopenia or fatality from bone marrow depression has been observed in any of the 263 patients studied by Lawrence et al.

A condition that must be differentiated from polycythemia vera is secondary polycythemia. These patients as a rule show elevated red-cell counts, normal white counts, elevated total red-cell volumes, decreased plasma volumes, oxygen saturations below normal, absence of palpable spleens, and red-cell iron-turnover rates several times normal but compatible with the total red-cell volume.[67] Attempts at reducing the red-cell volume usually would effect a disservice to the patient; for the red-cell volume has increased in a compensatory effort to provide greater oxygen-carrying capacity.

Residents at high altitudes show increased red-cell volumes (proportional to the decrease in oxygen tension at the altitude of residence) increased chest measurements in all directions, enhanced pulmonary second sounds, right heart enlargement, decreased arterial oxygen saturation, enhanced red-cell iron turnover, and absence of palpable spleen.[67]

Occasionally one encounters a patient who presents elevated red-cell

count, hemoglobin, and hematocrit in whom, however, the total red-cell volume is normal and the plasma volume is reduced.[67] Such patients are predominantly male, 50 percent being overweight and 50 percent being hypertensive. Red-cell iron-turnover rates are compatible with normal red-cell production rates. Blood oxygen saturation is normal. The picture resembles that observed in the hypoxic stress of high altitudes; indeed, a significant number of the patients manifest indications of nervous stress---psychoneurosis or mild anxiety state.

Leukemia

Irradiation for the generalized acute leukemic picture is usually of little value, but the use of x-ray therapy on localized lesions such as leukemic masses pressing on the central nervous system or enlarged kidneys is occasionally of value.[80]

Radiophosphorus, while only palliative and not curative, has in recent years come to occupy a prominent and deserved position in the management of chronic leukemias. The effects of P^{32} seem to be somewhat better than those obtained with classical transcutaneous roentgen therapy.[81,82] Success of therapy depends upon greater sensitivity of leukemic cells to irradiation, but often this is slight. Of 129 patients with chronic lymphatic leukemia seen at the University of California, the first series of patients treated there and followed for the longest time, 68 percent were males and 32 percent were females.[82] Age of onset ranged from 20 to 70 years, with an average of 55 years. No selection was made of patients. Nearly half the patients had had roentgen therapy prior to P^{32} or were given it afterward, as for local irradiation of enlarged lymph nodes or spleen.

In general enough P^{32} is given these patients to produce symptomatic relief. This, together with improved red-cell count and reduction in the size of the lymph nodes and spleen, was considered good response. The average patient received 1 to 2 millicuries of P^{32} per week for 4 to 8 weeks, this dosage being repeated when necessary. These small doses have sufficed to treat most patients for a longer or shorter time. One does not like to give more than 6 to 8 millicuries per course or more than 15 to 20 millicuries per year.

During the past 5 years it has been the practice to give these patients with chronic lymphatic leukemia 12 to 18 milligrams of testosterone per day orally during a good portion of each year. Some benefit in the form of a sense of well-being and an improvement in the total red-cell volume occurs. Many patients have been carried for months on testosterone with little progression of the disease and without recourse to other forms of therapy, such as irradiation.

There has been a suggestion of a favorable relationship between high original hemoglobin and duration of life. There has been no correlation between initial white-cell elevation and survival time. The average survival time has been in excess of five years.

Post-mortem examination of 21 of these patients dying of lymphatic leukemia showed generalized infiltration of the lymph nodes, spleen, liver, and marrow. In no case was there a report of aplastic marrow, although several of these patients had shown in the peripheral blood evidence of severe marrow depression. In no case has there been evidence at necropsy of an

induced neoplasm.

In some cases there appeared to be a gradual decrease in the radiosensitivity difference between normal and leukemic cells with lengthening period of treatment. Often it has been necessary to treat a patient, despite falling red-cell and thrombocyte counts, in hope that the disease can be corrected or alleviated by inhibiting leukemic marrow infiltration.

Sternal marrow studies may be of assistance in directing therapy, but leukopenia or thrombopenia due to irradiation may coexist with a hyperplastic marrow.

Coliez et al. note that all patients who have become refractory to roentgen irradiation, whatever the gravity of their hematological and clinical states at the time of treatment, have reacted favorably to P^{32}.[81] They predicate only one contraindication to undertaking P^{32} therapy: the rapid increase in the number of leukoblasts in the blood, representing the appearance of an acute leukosis against which P^{32} is ineffective. A chronic leukemia, roentgen- and chemoresistant, in a state of evolutive exacerbation but without sign of leukoblastosis may be ameliorated, at least temporarily, by P^{32}. These writers argue for using x-ray as long as possible, reserving P^{32} for the time when roentgen resistance develops.

Diamond et al. find that the presence of adenopathy and hepatosplenomegaly at the onset of the disease makes for poor prognosis.[83] Osgood and Seaman suggest that continued, titrated, regularly spaced doses of P^{32} should be used, and their results compare favorably with those obtained by the methods of Lawrence et al.[82,84]

Of 152 cases of chronic myelogenous leukemia studied by Lawrence and his co-workers, 56 percent were males and 44 percent were females.[82] Age of onset ranged from 10 to 71 years, with an average of 41 years.

On the average these patients received 1 to 2 mc of P^{32} weekly for 4 to 8 weeks. No remarkable differences in duration with age of onset were noted, but there was an indication that patients showing symptoms in the third decade tend to live longer than those in other ages. The average survival time has been about 3.2 years.

In chronic myeloid leukemia many patients enter the terminal phase with a picture of subacute or acute myeloid leukemia, i.e., with more than 1 to 2 percent of blast forms in the blood smear, fever, and usually an enlarged and rubbery spleen.[82] This terminal acute picture is rare in chronic lymphatic leukemia, where instead the picture is one of gradually increasing anemia, associated with more and more marrow infiltration by leukemic cells. Such anemia is usually associated with shortened red-cell life; and there is sometimes evidence of hypersplenism, in which case the anemia may be partially or completely relived by splenectomy.[85] Nearly all patients with chronic leukemia who are anemic have a greater-than-normal rate of red-cell production instead of the myelophthisic type of anemia classically referred to. The plasma volume is not uncommonly elevated (with normal total red-cell volume) in patients with chronic leukemia who have splenomegaly.

In only 1 of 36 necropsies of patients in the group of 152 cases of chronic myelogenous leukemia did the pathologist consider the marrow to be

depressed to the point of aplastic anemia. Radiation can affect cellular function, however, without producing morphological changes. There has been no evidence in this study of the emergence of bone tumors or other neoplasms with integrated total doses of P^{32} as high as 75 mc in 5 years.

In a study of 59 cases of chronic myeloid leukemia, Diamond and Craver observed that in 95 percent of their patients P^{32} produced at least a transitory fall in total peripheral white-cell counts.[86] In approximately 50 percent of this group the hemoglobin and red-cell count either remained insignificantly altered or showed rises in their levels. Hepatosplenomegaly was altered inconstantly by P^{32} alone, and local roentgen irradiation was employed. Gross myeloidization of lymph nodes as manifested by firm or bulky adenopathy gave an ominous prognosis; and rapid progression, regardless of the type of treatment, might be expected.

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