Differences in Distribution of Erythropoietic and Reticuloendothelial Marrow in Hematologic Disease

By D. Van Dyke, C. Shkurkin, D. Price, Y. Yano and H. O. Anger

In normal animals and man, reticuloendothelial cells are a constant constituent of red bone marrow. The ratio of phagocytic cells to hematopoietic cells is relatively constant, so that the skeletal distributions of myeloid, erythroid, and reticuloendothelial elements in normal animals are the same. Since knowledge of the distribution of the active marrow may be needed in investigative work on the marrow and may be helpful in the care of patients whose treatment or primary disease involves the bone marrow, tracer methods for visualizing the distribution of active marrow have been developed. The reticuloendothelial elements can be labeled with radiocolloids, and the erythropoietic elements can be labeled with radioiron. Then their distributions can be determined with an isotope scanner or scintillation camera. There is at present no method to effectively label the granulocytic precursors in the bone marrow for clinical scanning.

In normal animals or human beings, similar marrow distribution patterns can be expected by either of the above tracer methods. However, since it is not in the normal subject that such information is most needed, and since the various components of the marrow may be quite differently affected by treatment or disease, the two methods of labeling (phagocytic and hemoglobin synthesis) may give quite different distributions, each providing useful but different information. Since few direct comparisons of the two methods have been made in abnormal subjects, the present study was undertaken. All patients on whom a marrow distribution study was indicated were investigated simultaneously with Tc99m-sulfur colloid and Fe52. The results of the comparison are presented here.

Materials and Methods

For labeling the erythropoietic portion of the marrow, the best results are obtained with the short-lived (T-1/2, 8 hours) positron-emitting isotope, Fe51. The distribution of activity is recorded with the positron scintillation camera or with the whole-body scanner, Mark II. Iron-59 may be used in place of iron-52, but scanners specially designed for high-energy gamma-ray emitters must be used, and the images obtained so far are not as satisfactory.

The reticuloendothelial portion of the marrow can be labeled with any colloid of the proper particle size. The most satisfactory at present—because of availability, cost, and low radiation dose to the patient—is the short-lived (T-1/2, 6 hours) technetium-99m-

From the Donner Laboratory of Medical Physics, Lawrence Radiation Laboratory, University of California, Berkeley, California.

This work was supported in part by the United States Atomic Energy Commission and in part by grant #5ROCA03370-02 from the National Cancer Institute of the National Institutes of Health.

First submitted Jan. 9, 1967; accepted for publication April 5, 1967.

ERYTHROPOIETIC AND RETICULOENDOTHELIAL MARROW

After labeling with this colloid, the marrow, liver, and spleen can be visualized with a conventional radioisotope scanner or with the gamma-ray mode of the scintillation camera. The scintillation camera, a nonscanning electronic instrument for making pictures of the distribution of gamma-ray and positron-emitting nuclides in vivo, has been described in detail in a previous publication. The Donner Laboratory whole-body scanner, Mark II, has 64 scintillation counters, each with a 1- by 1-inch sodium iodide crystal, in a massive lead shield. The length of the array of counters is 27 inches, wide enough to cover the width of the body. The patient lies on a table that moves at a constant rate over the counters. With a single sweep of the table, a 64-line scan of the subject results. Nine feet above the patient is a 500 millicurie source of Americium-241, which emits 60 KeV gamma-rays. By detecting these rays and displaying them, one gets a transmission picture of the body outline of the patient. The final readout is obtained with a Polaroid scope camera. The patient table and the film cassette are mechanically coupled together, so that the photographic film moves up or down in synchrony with the patient table. Flickering points on the cathode-ray tube screen become lines of varying density on the final image. The usual scanning time for either Fe or Fe is 5 to 15 minutes for head-to-toe pictures. For Tc the scanning time is 0.75 to 3 minutes.

In our laboratory the Mark II whole-body scanner and the positron scintillation camera are located in the same area so that a quick whole-body scan can be taken first to be used as a guide in taking the smaller but more detailed positron camera pictures.

The radiation doses to the bone marrow in a 70-Kg. man for the three isotopes used have been estimated to be as follows: 2.5 rads to bone marrow for a 100 mc. dose of Fe, 1.0 rad for a 20 mc. dose of Fe, and 0.09 rad for a 3 mc. dose of Tc-sulfur colloid.

The methods of preparation of Fe and Tc-sulfur colloid have been described in the literature.

All patients were evaluated by members of the Donner Laboratory medical staff, and in most cases therapy and long-term follow-up were managed through the Donner Laboratory outpatient clinic. Complete records were kept of all procedures and therapy performed throughout the entire course of the patient's disease. All patients with polycythemia rubra vera were in the early stages of the disease, classes I and II as defined by Pollycove et al.

Method of Marrow Labeling

When using radioiron, pictures of the bone marrow must be taken at the time of maximum marrow uptake, after the iron has cleared the plasma and before it has passed from the marrow to the peripheral blood. For most patients this means 10–24 hours after intravenous administration of Fe. The patient is injected in the afternoon with 100–150 mc. Fe as ferrous citrate, and pictures are taken the following morning. The specific activity of the Fe was 0.5 mc. per mcg. in the earlier studies, subsequently improved to 12 mc. per mcg.

When using a colloidal preparation of sulfur labeled with Tc, most of the colloid localizes in the liver, spleen, and hemopoietic marrow within 5 minutes. Picture-taking can be begun very soon after intravenous administration of the labeled colloid and the study completed during a single visit. In the present studies 2.5–3.0 mc. of Tc-sulfur colloid were given.

In each study, scintiphotos of Fe distribution are made first with the camera in the positron mode, taking 5–10 minutes per 9-inch diameter field. At the completion of the Fe picture, the technetium colloid is injected, and 10–15 minutes later the same areas are photographed with the scintillation camera in gamma mode, using a thin-septum multichannel collimator, an appropriate narrow energy window, and exposures of one minute or less.

RESULTS

Direct comparison of the distributions of erythropoietic and reticuloendothelial marrow in patients with diseased marrow demonstrates a range from...
identical to totally dissimilar. Table 1 summarizes the results and gives an indication of the degrees of correlation between the distributions of the two marrow functions in 25 patients studied to date. Arbitrary estimates of correlation ranging from "no correlation" to "identical" are given in the table to provide an indication of the frequency of marked or minor differences in results using the two techniques. Examples of the different categories are given in the figures.

Figure 1 ( #23 in the table) shows identical distribution of hemoglobin synthesis and phagocytic activity in the marrow of a patient with polycythemia associated with renal vascular anomalies and an increased erythropoietin production. Urinary excretion of erythropoietin was 10 times normal and easily measurable concentrations of erythropoietin were found in plasma. Except for slight extension down the humerus, the pattern is that found in normal adults.

Figure 2 shows a normal marrow distribution pattern as judged by Tc⁹⁹ᵐ-sulfur colloid in a 20-year-old patient ( #1 in the table) with congenital absence of erythropoietic marrow. Iron kinetics and Fe⁵² positron camera studies showed complete absence of erythropoiesis. A summary of this case has been published elsewhere.³

Figure 3B shows hypertrophied, peripherally extended marrow as seen with Tc⁹⁹ᵐ in a middle-aged man with severe idiopathic hypoplastic anemia ( patient #2 in the table ). Virtual absence of erythropoietic marrow is demonstrated in
Fig. 1.—Distribution of erythropoietic marrow (A) and phagocytic marrow (B) in a patient with increased red cell production due to increased erythropoietin production.

The radioiron scintiphotos (Fig. 3A), although a very faint outline of residual peripheral erythropoiesis is seen in the femur and humerus.

Figures 4 and 5 have been selected to illustrate various degrees of difference in functional distribution. Figure 4A shows the erythropoietic marrow (Fe₅₂) in a patient with long-standing Hodgkin’s disease treated with irradiation and chemotherapy (patient #3 in the table). Erythropoietic marrow was widely, but irregularly, distributed throughout the skeleton. The irregularities are attributed to the presence of tumor and previous irradiation therapy. Figure 4B shows the uptake of Te⁹⁹ë-sulfur colloid by the reticuloendothelial cells of the same patient on the same day. Splenic and hepatic uptake of most of the colloid is demonstrated with rather poor uptake by the marrow. Comparison of Figures 4A and 4B shows similarities in the distribution in the knees and dissimilarity in the shoulders and elbows. The patchiness apparent in the erythropoietic pat-
Fig. 2A

Fig. 2B

Fig. 2.—Distribution of erythropoietic marrow (A) and phagocytic marrow (B) in a patient with congenital absence of erythropoietic marrow. There was no medullary uptake of Fe²⁺; all the iron being deposited in liver and spleen. Uptake and distribution of colloid in marrow, liver, and spleen was normal (B).

tern in the pelvis and right arm and leg was not apparent in the phagocytic pattern.

Figure 5 compares the distributions of hemoglobin synthesis and phagocytic activity in the marrow of a second patient with long-standing Hodgkin’s disease treated with a combination of irradiation and chemotherapy (patient #4 in the table). In this case distribution of the two elements is essentially the same except in the feet and ankles, where phagocytic activity was unassociated with erythropoiesis. Free technetium excreted into the bladder can be seen. As can be seen in Figures 5C and 5D, the Mark II whole-body scanner can give an adequate assessment of isotope distribution. However, fine and often important detail may be missed.

DISCUSSION

In normal subjects the skeletal distribution of radioiron and radiocolloid is
Fig. 3.—Distribution of erythropoietic marrow (A) and phagocytic marrow (B) in a patient with idiopathic hypoplastic anemia. Hypertrophied and peripherally extended marrow is seen with the Tc\(^{99}\)m sulfur colloid, whereas the erythropoietic marrow is essentially absent except for slight residual activity in the femur and humerus.

identical and corresponds to the distribution of hematopoietic marrow as determined by autopsy examination.\(^{17}\) Erythropoietic and reticuloendothelial marrow are invariably found throughout the central portion of the skeleton (ribs, spine, pelvis, scapula, and clavicle), with the exception of the caudal half of the sacrum. In the extremities some marrow is always found in the head of the humerus and the area of the lesser trochanter of the femur. There is considerable individual variation in the amount of marrow in the skull, and in the extent to which it extends down the shafts of the humerus and femur. Marrow is usually confined to the proximal one-fourth of the humerus and femur in the adult, and extension beyond the proximal one-third is considered abnormal.

Marrow characteristically expands peripherally on increased demand for red cells,\(^{18}\) but there are great variations in detail.\(^{3}\) The bones of the legs may be
Fig. 4A

Fig. 4B

Fig. 4.—Distribution of erythropoietic marrow (A) and phagocytic marrow (B) in a patient with Hodgkin’s disease treated with irradiation and chemotherapy.

more involved than those of the arms, or vice versa. Heavy concentrations of marrow may extend to the distal ends of the femur without extension of marrow into the tibia. Conversely, the tibia may be densely filled, with little or no marrow in the distal femur. The marrow is not always distributed with complete bilateral symmetry, even in the absence of any known bone injury or disease. A given bone may be filled quite unevenly; that is, the distal end of the femur may contain large amounts of marrow with little in the shaft, or the shaft may be filled evenly down to the lower one-fourth, where it stops abruptly. Within an otherwise completely filled femur or humerus, there may be local areas devoid of marrow.  

Te^{99m}-sulfur colloid used to label the reticuloendothelial portion of the marrow has the advantage of being inexpensive and readily available. It delivers an extremely low dose of irradiation to the patient, and it has the added advantage
ERYTHROPOIETIC AND RETICULOENDOTHELIAL MARROW

Fig. 5A

Fig. 5B

Fig. 5.—Positron scintillation camera pictures of distribution of erythropoietic marrow (A) and phagocytic marrow (B) in a patient with Hodgkin's disease treated with irradiation and chemotherapy.

that rapid uptake by the reticuloendothelial system makes it possible to complete the study during a single visit.

Major disadvantages of the use of colloid to visualize the marrow are that one does not label the usually clinically important marrow functions by this method, and that 96 per cent of the dose goes to liver and spleen, making it impossible to visualize the large fraction of the marrow in the spine behind these organs. Loss of marrow from the spine, even in the presence of peripheral extension, may be an important finding of any individual study.

Since granulocytopoiesis and erythropoiesis are almost always accompanied by phagocytic cells, the colloid method is useful in demonstrating marrow extension within the skeleton. Phagocytic activity may persist in the presence of partial or complete failure of erythropoiesis, however. Therefore, the colloid method is not suitable for the study of patients with hypoplastic or aplastic anemia.
Fe\textsuperscript{52}, on the other hand, must be produced in a cyclotron and is not widely available. Because of this and its short half-life, it can be used in few locations for scanning of erythropoietic marrow. Invariable correlation of reticuloendothelial and erythropoietic marrow would make the technetium-99m-sulfur colloid the ideal agent for bone marrow scanning. Unfortunately, the present study demonstrated that these two marrow functions do not always coincide.

**SUMMARY AND CONCLUSIONS**

Although reticuloendothelial cells are an invariable component of normal marrow, the distribution of phagocytic activity may or may not correspond to the distribution of erythropoietic activity in the presence of disease affecting the marrow. A direct comparison of the distributions of reticuloendothelial marrow (using Tc\textsuperscript{99m}-sulfur colloid) and erythropoietic marrow (using Fe\textsuperscript{52}) in patients receiving treatment for various diseases affecting the bone marrow has been made. In the presence of hematologic disease, the correlation between the distributions of these two marrow functions has varied from identical to totally dissimilar.

Unless one is studying normal subjects, knows *a priori* that marrow composition is normal, or is specifically studying the reticuloendothelial component of the marrow, one must be cautious in equating marrow distributions obtained with colloidal material with erythropoietic marrow distribution.
SUMMARIO IN INTERLINGUA
Ben que cellulas reticuloendothelial es invariabilemente un coml)onente dcl normal medulla ossee, le distribution de activitate phagocytic pote sed non debe corresponder al distribution de activitate erythropoietic in le presentia de morbo afficiente le medulla. Esseva effectuate un comparation directe del distributiones de medulla reticuloendothelial (con le utilisation de colloide sulphuric a Te') e de medulla erythropoietic (con le utilisation de Fe') in patientes sub tractamento pro varie morbos afficiente le medulla ossee. In le presentia de morbo hematologic, le correlation inter le distributiones del mentionate functiones medullari ha variate inter le extremos de “identic” e “totalmente dissimile.”

Excepte quando on studia subjectos normal o quando on sape a priori que le composition del medulla es normal o studia specificamente le componente reticuloendothelial del medulla, on debe esser conscie del facto que distributiones medullari constatate con le uso de materiales colloide non 1)ote esser identificate Con distributiones medullari erythropoietic.

ACKNOWLEDGMENTS
The authors are indebted to the following for referral of patients: Dr. Fudenberg, University of California Medical School, San Francisco (Fig. 3), Dr. Cooney, Palo Alto, California (Fig. 4), and Dr. Barkin, Stanford Medical School (Fig. 5); and to Dr. Leonard Schaer, Donner Laboratory, for permission to use Figure 2.

REFERENCES
14. Pollycove, M., Winchell, H. S., and Lawrence, J. H.: Classification and


Differences in Distribution of Erythropoietic and Reticuloendothelial Marrow in Hematologic Disease

D. VAN DYKE, C. SHKURKIN, D. PRICE, Y. YANO and H. O. ANGER