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Distribution of the Erythron and the RES in the Bone Marrow Organ[1]

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INTRODUCTION

The recent availability of ^{99m}Tc-labeled colloids (1) has made radiocolloid photoscanning of the bone marrow organ a relatively simple and safe procedure. However, several fundamental questions must be carefully examined in order to utilize and interpret the information derived from a radiocolloid bone marrow scan. What functional or physiologic significance can one attach to the pattern of distribution of radiocolloid in the RE cell of the marrow organ? To what extent is the spatial distribution of colloid quantitatively similar to the distribution of the active erythropoietic or white cell elements in the marrow? How is this relationship altered by disease affecting the bone marrow organ, such as polycythemia, aplastic anemia or leukemia? The evidence accumulated thus far supports the concept that in normal and many disease states the pattern of RE cell distribution portrayed by radiocolloid photoscanning may be closely associated with the functional ditsribution of the hematopoietic cells of the marrow (2-6).

The purpose of this report is to present a critical examination of one of these fundamental questions—the relationship of the distribution of the RE cells to the erythropoietic cells, the erythron. Using the rabbit as an experimental model, the quantitative distribution of the RE cell and the erythron was studied throughout the marrow organ. There was a remarkably similar distribution of these two cell systems in the normal situation and under limited conditions of stimulated and suppressed erythropoiesis.

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MATERIALS AND METHODS

Two kg white New Zealand rabbits were used in these studies. To label the functioning erythropoietic cells, rabbits were first injected intravenously with 10 to 20 μC of ^{59}ferrous citrate[1] containing 40 μC of ^{59}Fe/μg Fe. After 5½ hours, when a majority of the ^{59}Fe has entered the marrow (7), the rabbit was given a second intravenous injection containing 1 to 2 mg per kg body weight (average particle size of 450 mμ) of sulfur colloid labeled with 1 to 2 mc of technetium-99m. The Tc colloid was prepared according to the methods previously reported in this journal (1). Thirty minutes later, when the colloid was essentially completely removed by the RE cells, the animal was anesthetized with pentobarbital (30 mg/kg body weight). The inferior vena cava was cannulated and the animal was exchange transfused with 150 to 200 ml of isotonic saline. Using this procedure, any circulating ^{59}Fe or ^{99m}Tc was reduced to negligible amounts. Prior to death, hematocrits were routinely reduced to from 5 to 10%. After exchange transfusion, ^{59}Fe activity in the total vascular compartment was calculated to be from 4 to 15% of the injected dose and ^{99m}Tc activity was less than 1%. Following exchange transfusion, animals were sacrificed and autoclaved at 115°C for 60 minutes. After autoclaving, the total skeleton was removed from the carcass.

Each individual bone was placed in a 30 ml glass counting vial and dis-

[1]Prepared by adding .05 ml of 4% sodium citrate to 4 ml of ^{59}ferric chloride in 0.9% saline to give a final concentration of 10 to 20 μC of ^{59}Fe per ml.

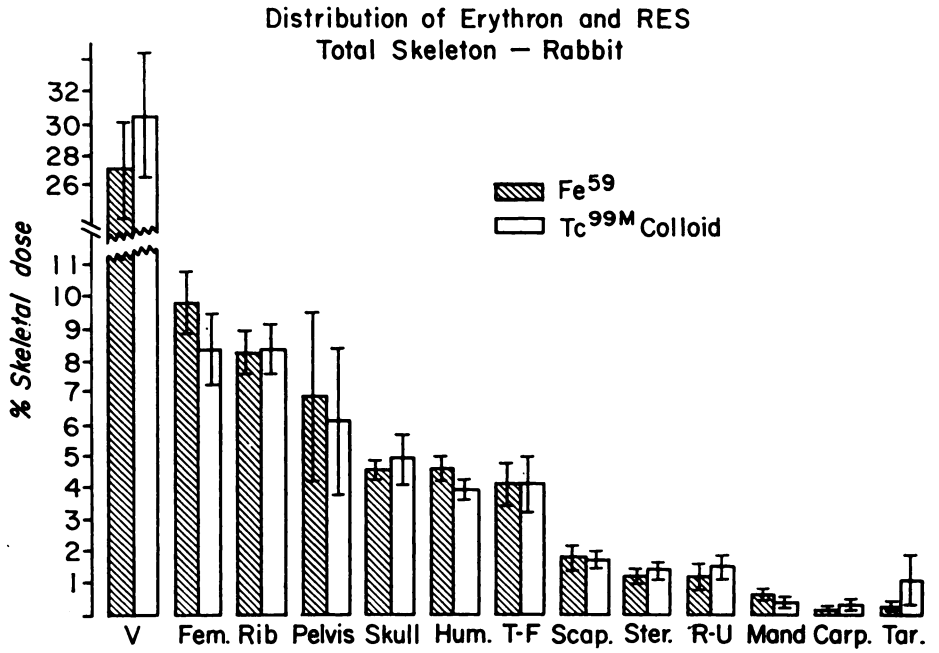

Fig. 1. The RES and Erythron distribution in the skeleton of the rabbit. Mean of six animals. See text for details.

solved in 20 ml of concentrated nitric acid. The ^{59}Fe and ^{99m}Tc radioactivity in each bone was counted, using a sodium iodide scintillation detector and dual channel gamma ray spectrometry. Each sample was counted to less than a three per cent relative error according to the criteria of Loevinger and Berman (8). By comparison with an aliquot of the injected dose of ^{59}Fe and ^{99m}Tc, the fraction of the dose of each isotope in each bone and in the total skeleton was calculated.

After the similarity of the distribution of ^{59}Fe and ^{99m}Tc-colloid was apparent in the control group, additional distribution studies were performed following acute stimulation and suppression of hematopoiesis. Hematopoiesis was enhanced in three rabbits by producing acute hemolytic anemia. One hundred to 250 mg of phenylhydrazine (9) were injected intraperitoneally three to four days prior to a ^{59}Fe, ^{99m}Tc-colloid distribution study. This treatment provoked a 30 to 50% reduction in hematocrit and produced an average reticulocyte elevation to 25%. Erythropoiesis was inhibited in two additional rabbits by hypertransfusion for 15 and 27 days with 140 and 200 ml of blood. Hematocrits were chronically elevated to between 50 and 60% and plasma iron disappearance times were greatly prolonged.

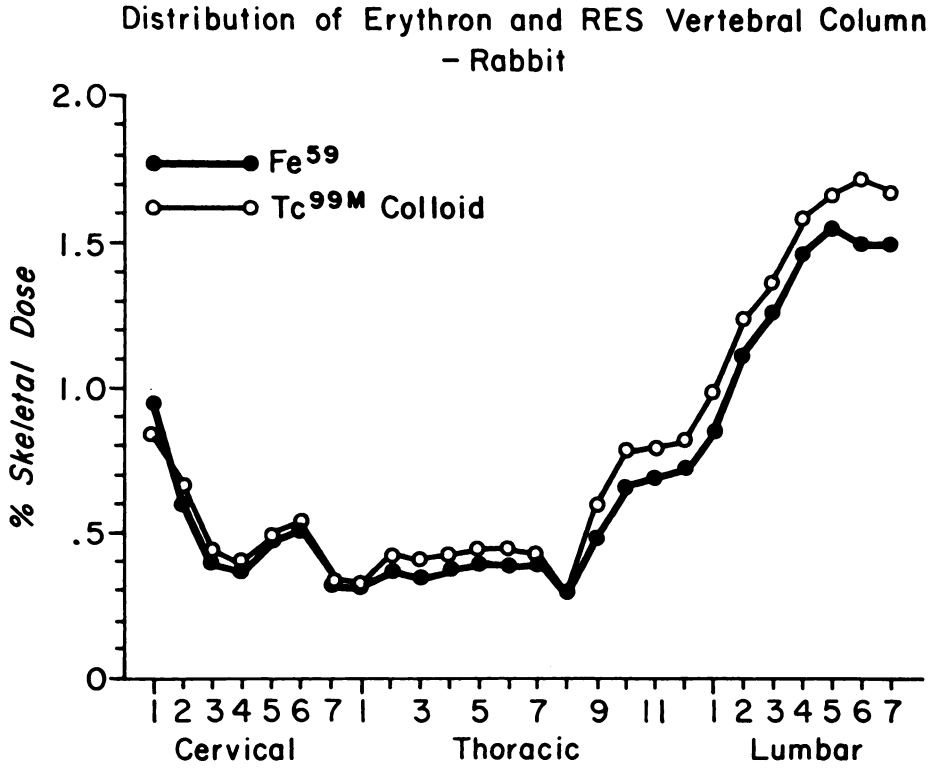

Fig. 2. Striking similarity of the quantitative distribution of the RES and Erythron in the individual vertebrae. See text for details.

RESULTS

RE Cell and Erythron Distribution in the Normal Rabbit

The skeletal distribution of ^{59}Fe and ^{99m}Tc was studied in six normal animals (Fig. 1). An average of 59% of the injected dose of ^{59}Fe and 11.5% of ^{99m}Tc-colloid was recovered in the total skeleton. The amount of radioactivity in each bone unit was expressed as a per cent of the amount of ^{59}Fe or ^{99m}Tc in the total skeleton. For example, of the ^{59}Fe that localized in the skeleton, an average of 27% was in the vertebral column. Of the colloid which went to the bone marrow, an average of 30% was localized in the vertebrae (Fig. 1). One can note the striking similarity of the relative distribution of ^{59}Fe and ^{99m}Tc-colloid throughout the skeleton. The vertical bars in Figure one represent mean values and the extended lines indicate one standard deviation. Statistical analysis of the data indicated that although of small magnitude, the difference in mean values were significant ($p<.05$) for the femurs, humeri, mandibles, carpals and tarsals.

The similarity of the distribution, of the erythron and the RE cell is well illustrated by Figure two. The per cent of the skeletal dose of ^{59}Fe and ^{99m}Tc-colloid in each of the 27 vertebrae from one experiment is plotted. This same relationship was noted in all six rabbit vertebral columns.

Effect of Acute Hemolytic Anemia

Total skeletal distribution studies were repeated in three additional rabbits after erythropoiesis was stimulated by inducing a severe hemolytic anemia with phenylhydrazine. Despite the marked increase in the rate of erythropoiesis (see methods), there was no alteration in the pattern of distribution of ^{59}Fe or ^{99m}Tc-colloid throughout the skeleton.

Effect of Hemotopoietic Stimulation

In two rabbits, erythropoiesis was suppressed by hypertransfusion for 16 to 28 days. Despite the reduced utilization of iron (see methods), the relative distribution of ^{59}Fe and ^{99m}Tc-colloid was again unchanged from the normal pattern.

COMMENTS

It is apparent from this animal model that the relative distribution of ^{59}Fe in the erythron and Tc-colloid in the RE cell is strikingly similar throughout the functioning marrow. Thus photoscanning of radiocolloids in the bone marrow organ in the absence of disease will depict the distribution of the functioning erythropoietic marrow. A recent study in dogs by Greenberg, Atkins and Schiffer (5), in which the total skeleton was analyzed after ^{59}Fe and ^{99m}Tc-colloid injection, also showed the same relative distribution of the two isotopes.

In the rabbit there was no alteration in the distribution of erythron or RE cell activity following limited erythropoietic stimulation or inhibition. In these studies, plasma iron turnover was increased two to fourfold after the acute

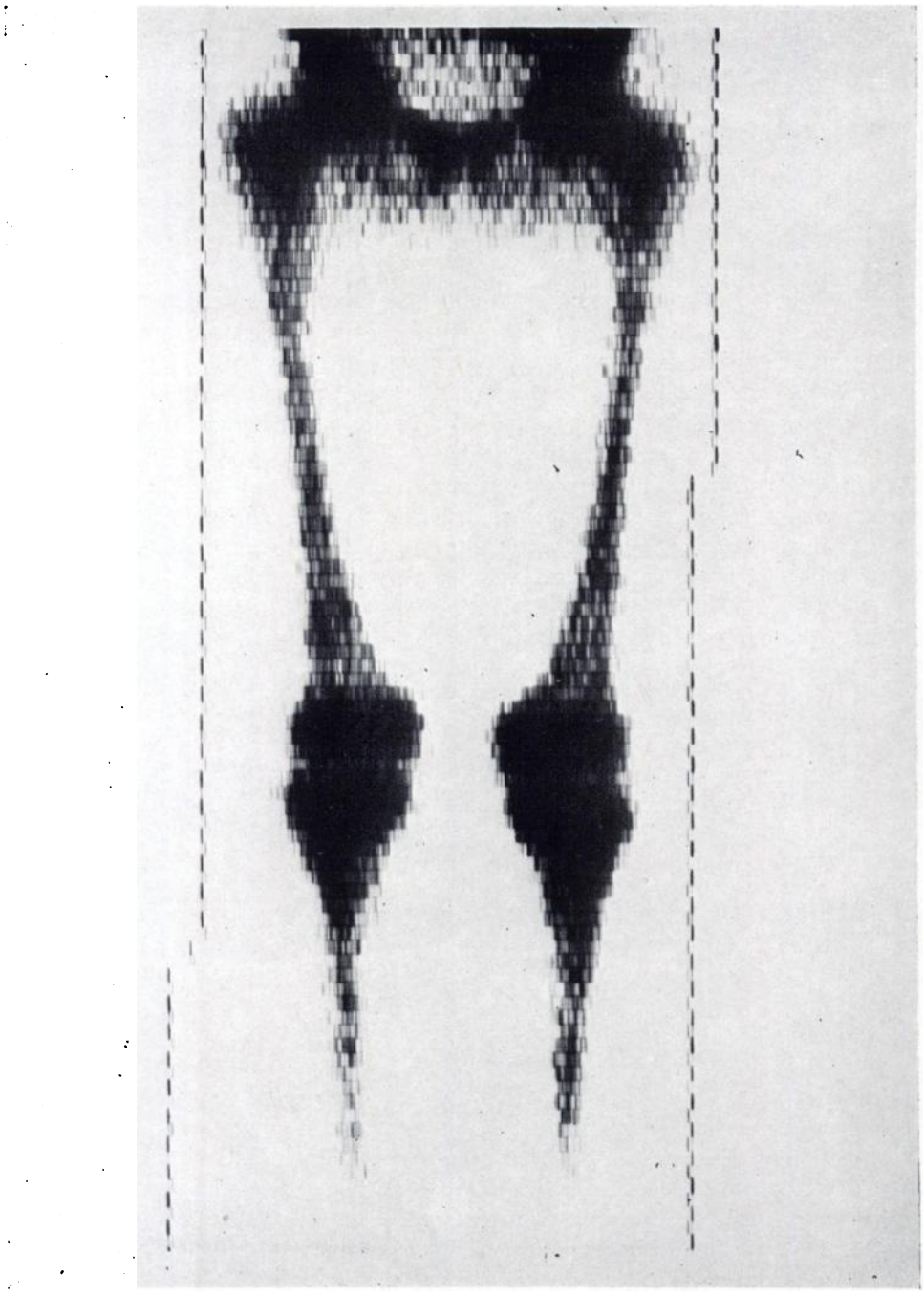

Fig. 3. Photoscan of lower pelvis and legs in a patient with polycythemia vera showing dramatic expansion of functioning marrow organ. Normally RE cell activity does not extend beyond the upper one third of the femur. Patient received 10 mC of ^{99m}Tc-colloid.

hemolytic anemia and was reduced approximately 75% following hypertransfusion. On the other hand, Atkins and Associates demonstrated that chronic bleeding in adult dogs produced an extension of the functioning erythropoietic marrow space into the distal portions of the femurs. This phenomenon was accompanied by concomitant extension of RE cells into the same areas (6). In our experience, as well as that of others (3,6), radiocolloid photoscanning in patients with increased erythropoietic activity due to polycythemia or chronic hemolytic anemia often reveals dramatic extension of RE cell activity into the distal portions of the long bones of the extremities where normally little or no marrow activity is noted (Fig. 3).

Considerable evidence suggests that trace amounts of colloid are cleared from the vascular space by the RES in relation to the relative blood flow through the various portions of the RES (10,11). Thus, in the bone marrow organ, the remarkable similarity of the relative distribution of the RE cell and the erythron suggests that the functioning erythropoietic mass is also distributed in close relationship to the relative blood flow throughout the marrow.

Additional experience and experimentation is needed to determine to what extent changes in the distribution of red cell elements in the marrow in disease will be associated with similar changes in the distribution of radiocolloid as depicted by photoscanning. When might dissociation of the distribution of RE cells and the erythron occur? For example, after exposing one hind limb of the rabbit to 250 to 5000 rads of x-radiation, there is a period of two to seven days when RE cell distribution in the marrow of the limb is near normal yet the localization of ^{59}Fe in the erythron is reduced to 20% or less of the control limb. Currently studies are underway in our laboratory to inspect the extent and duration of this dissociation, as well as the long-term effect of x-radiation on both cellular systems.

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