MARKEDLY INCREASED BONE BLOOD FLOW IN MYELOFIBROSIS

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That the skeleton is markedly abnormal in advanced cases of myelofibrosis has been known since Vaughan and Harrison's well-documented study (*1*), and this is reemphasized each time the clinician observes the sometimes strikingly abnormal bones in roentgenograms. That the skeletal pathology is associated with an increase in bone blood perfusion such as that seen in Paget's disease of bone has not previously been suspected.

The rate of appearance of ^{18}F in the skeleton is principally determined by skeletal blood perfusion, making it possible to investigate the bone blood flow associated with diseases involving bone and bone marrow. The evidence and assumptions involved in interpreting skeletal uptake of ^{18}F ion as a function of bone blood perfusion rate have been presented previously (*2*). The distribution of positron-emitting isotopes such as ^{18}F in the human body can be accurately visualized using the positron scintillation camera (*3*) and a specially designed whole-body scanner (*4*). In this study scintigraphic and fluorokinetic data have been obtained showing a marked increase in skeletal blood flow associated with myelofibrosis, both idiopathic and secondary to polycythemia vera.

MATERIALS AND METHODS

Well-documented cases of myelofibrosis were chosen for complete fluorokinetic studies, but the duration, severity, and extent of extramedullary hematopoiesis varied from recently developed cases to others of long duration, completely compensated or requiring transfusions for maintenance of red cell count. Diagnosis was established by bone and marrow biopsy, by ^{52}Fe whole-body marrow distribution studies (*5*), and by ferrokinetic studies in some cases. Controls were volunteers, patients with blood conditions other than myelofibrosis, or hematopoietically normal subjects with various bone diseases.

Scintigraphy. Scintigraphy using the positron scintillation camera, the whole-body scanner, Mark II, and the method of preparation of ^{18}F are presented elsewhere (*3,4,6*).

The subjects were placed on the bed of the whole-body scanner, a plastic needle was inserted into the vein of one arm for blood sampling, 600 μCi of ^{18}F were given intravenously in the opposite arm, and scans were made at frequent intervals thereafter. At the end of the 3-hr period urine was collected to determine renal excretion of ^{18}F. Completeness of voiding was determined by taking a scan immediately after the urine collection. In some patients (see below) blood samples were taken frequently during the scanning procedure for 3 hr after ^{18}F injection. A graph of the activity profile was produced simultaneously with the whole-body scan.

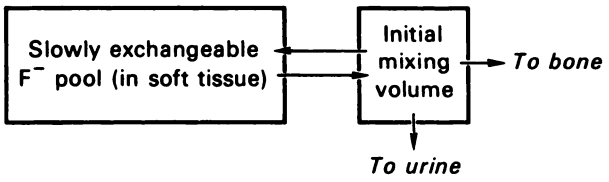

FIG. 1. Schematic representation of mathematical model of fluoride kinetics in humans.

Fluorokinetic analysis. Following a single injection of ^{18}F, blood samples were taken frequently, and urine was collected at 3 hr to determine renal excretion of the isotope. A two-component curve was fitted to the blood data, and blood and urine data were analyzed according to the two-compartment model of Fig. 1. The background for the choice of this model is given in the Discussion. Conceptually, minimum bone blood flow can be equated with the extrarenal fluoride clearance, and this to the rate of transfer from the initial mixing volume to bone in the model (Fig. 1). For purposes of report-

Received Oct. 27, 1970; revision accepted Feb. 3, 1971.
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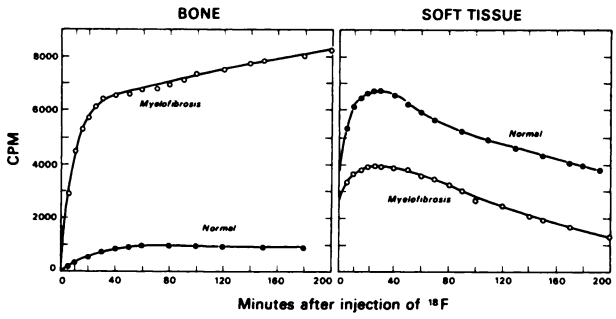

FIG. 2. Corrected bone and soft tissue pattern for typical normal subject and patient with myelofibrosis.

ing the results, we have simply labeled it "minimum bone blood flow," and clearly the flow could not be less than this figure. Total ^{18}F clearance was obtained as the reciprocal of the area under the blood curve normalized to fraction of injected dose per liter, the renal clearance from the relationship of the 3-hr renal excretion to the corresponding mean blood level given by the model, and the extrarenal clearance by subtraction of renal clearance from the total.

Results for "minimum bone blood flow" are given in terms of percent of cardiac output, using a standard figure of 72.5 ml/kg/min for cardiac output in man.

In addition to the above, detailed consideration was given to two other two-compartment models using a PDP-8 computer for calculation of rate constants and volumes of distribution. None of these exploratory considerations or the various values for volumes of distribution of the radioisotope is now felt to have much relevance to the present determination of extrarenal fluoride clearance, except to confirm us in the choice of the particular model of Fig. 1.

To obtain direct information as to the kinetic behavior of the bone and soft tissue compartments, area-of-interest cutouts were made in ½-in. lead disks positioned directly under the face of the image detector by being mounted in the "lazy Susan" collimator holder with which this specially built model of the positron scintillation camera is equipped. The focal detector head can be readily rotated out of position for ease of changing area-of-interest shielding. One area corresponding to the distal femur and one corresponding to the mass of muscle medial to the femur were cut out and alternately replaced during the 3-hr period of the study. The counting rate was recorded alternately for the two areas. With such a system the counts from muscle over and underlying the bone, as well as cross-talk between the adjacent areas, must be accurately subtracted to derive the true bone and muscle curves. These corrections were made by determining the contribution from soft tissue seen through the bone window in normal subjects in the first few minutes of the study before significant bone uptake had occurred. The correction for contribution from bone through the soft tissue window was determined at the end of the study of patients with myelofibrosis when bone was maximum and soft tissue was nearly cleared of activity. The corrected bone and soft tissure pattern for a typical normal subject and a patient with myelofibrosis is shown in Fig. 2.

Metabolic studies. To evaluate calcium and bone metabolism in patients with myelofibrosis, five were placed on a low phosphate (340-mg) diet for 3 days during which time daily 24-hr urine collections and appropriate blood samples were obtained to measure blood and urine calcium, serum and urine phosphorous, serum creatinine, creatinine clearance, phosphate clearance, and tubular reabsorption of phosphate (TRP). In addition, 4-hr calcium infusions were performed according to the method of Bhandarkar and Nordin (7). Endocrine studies included PBI and T_3 resin uptake, plasma and urinary 11-oxycorticosteroid, and growth hormone measurements following arginine and insulin stimulation.

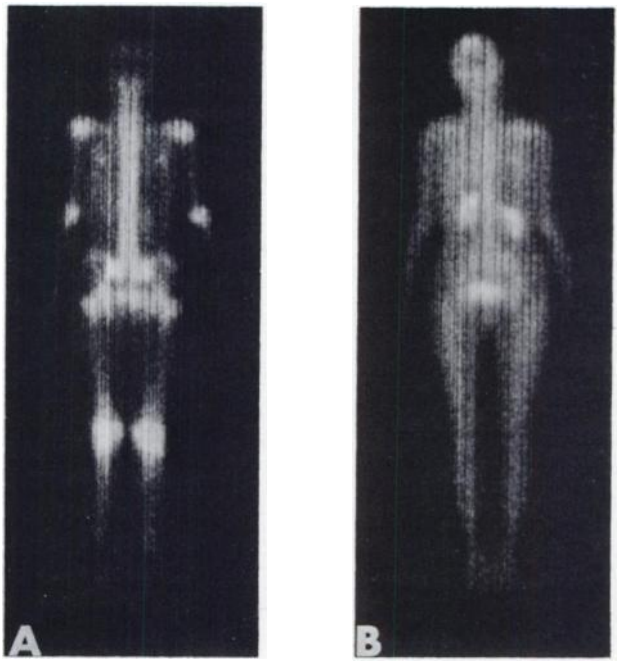

FIG. 3. Markedly increased bone blood flow in patient (MM) with myelofibrosis (A) as demonstrated with ^{18}F and the Donner Laboratory Mark II whole-body scanner. Subjects were each given 600 μCi of ^{18}F, and scan was started 15 min later (scan duration: 22 min). In the normal subject (B) kidneys, bladder, and general body outline are visible but skeleton has not yet accumulated sufficient isotope to be clearly evident. In patients with myelofibrosis skeleton is clearly evident within a few minutes after injection. Skeletal clearance of isotope is so rapid that renal clearance is relatively small.

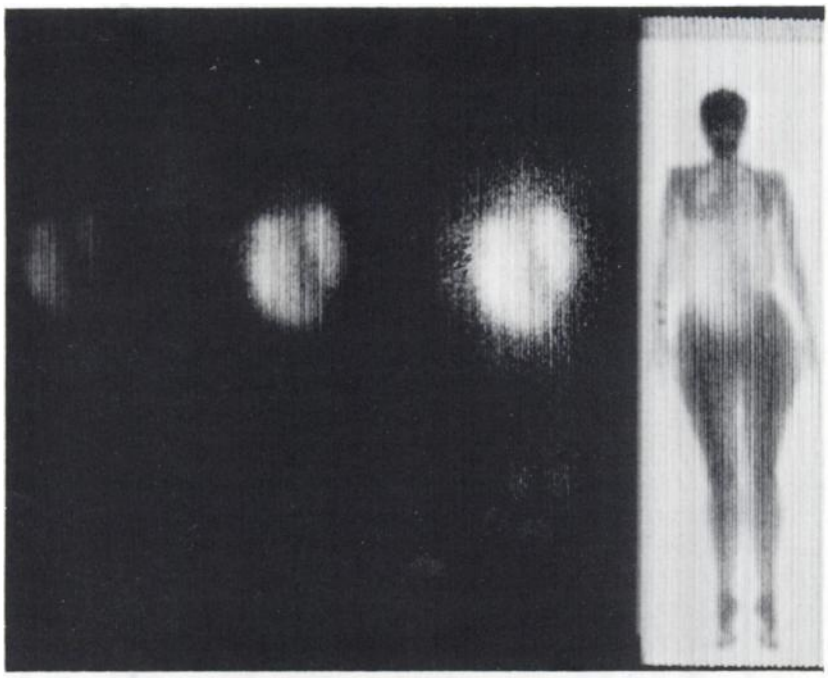

FIG. 4. Whole-body scan of ^{52}Fe distribution in patient in Fig. 3, showing complete loss of medullary erythropoiesis, which was well compensated by extramedullary erythropoiesis in spleen.

RESULTS

Scintigraphy. In the course of these studies of patients with myelofibrosis and of hematopoietically normal subjects, it became apparent that the most striking difference in appearance of the scans occurred 10–40 min after injection of the isotope. This follows from the fact that immediately after injection the distribution is essentially uniform in all subjects and at long times after injection the only appreciable ^{18}F concentration remaining is in the bone in all subjects. The only difference, then, is one of absolute intensity, which is difficult to judge visually in a scan. In the midtime period, however, a comparison between bone and soft-tissue concentration can readily be made by visual observation. The illustrations in Fig. 3 were made by taking scans starting 15 min after ^{18}F injection. Each scan required 22 min. Shorter scans were taken at intervals during a 3-hr period. In some cases, high-resolution positron scintillation camera pictures of ^{18}F distribution were taken as well. The Donner whole-body scanner takes four images simultaneously, only one of which is shown in Fig. 3 for the sake of simplicity. In normal subjects at this time (15–37 min), the skeleton has not yet accumulated enough isotope to be clearly visible, the distribution of isotope in soft tissues outlines the entire body, and the kidneys and bladder have relatively high uptake. In patients with myelofibrosis, the skeleton has accumulated most of the isotope at this time, and there is little in extracellular fluid, kidneys, and bladder.

The 54-year-old female patient (MM) shown in Fig. 3A had complete loss of medullary erythropoiesis as demonstrated by ^{52}Fe scanning (Fig. 4) but was well compensated by extramedullary erythropoiesis in a very large spleen. She did not require transfusions and had no evidence of renal disease. The diagnosis was agnogenic myelofibrosis with myeloid metaplasia.

To determine whether a generalized increase in bone blood flow occurred only in myelofibrosis, patients with other blood diseases as well as patients with bone disease but no evidence of disease involving the marrow were studied (Table 1). The table includes the 12 patients on whom complete fluorokinetic analysis was made as well as 30 classified by simple visual comparison of scans (22-min scan started 15 min after injection of ^{18}F). Since some degree of myelofibrosis is found in many patients with polycythemia vera (*8*), the distinction made in the table was based on whether the patient currently required phlebotomy or myelosuppressive therapy (polycythemia vera) or was in the "spent" phase requiring no therapy or transfusion (myelofibrosis secondary to polycythemia vera).

Roentgenograms of the femurs of several of the patients in this study have shown variable patterns ranging from no apparent abnormality (MM, Fig. 3A) to the markedly abnormal picture with areas of radiolucency and periosteal new bone formation found in a third to half such cases (*9*).

Fluorokinetic analysis. To rule out the possibility that differences in binding of ^{18}F in the blood (*10*)

were responsible for the differences in rate of uptake by bone or renal excretion, distribution of the isotope in red cells, plasma, and trichloroacetic acid-precipitable plasma proteins was investigated. Within the 3-hr period of study 30% of the isotope was consistently found in the red-cell fraction in equilibrium with the plasma. No significant amount was found bound to either red cells or TCA-precipitable plasma proteins.

TABLE 1. GROSS ^{18}F EVALUATION OF SKELETAL BLOOD FLOW IN VARIOUS CONDITIONS

Diagnosis	Total skeletal blood flow* Normal	Increased
Myelofibrosis, idiopathic		+
Myelofibrosis, idiopathic		+
Myelofibrosis, idiopathic		+
Myelofibrosis, idiopathic		+
Myelofibrosis, secondary to polycythemia vera		+
Myelofibrosis, secondary to polycythemia vera		+
Myelofibrosis, secondary to polycythemia vera		+
Myelofibrosis, secondary to polycythemia vera		+
Myelofibrosis, secondary to polycythemia vera		+
Myelofibrosis, secondary to polycythemia vera		+¶
Myelofibrosis, secondary to polycythemia vera		+¶
Polycythemia vera		+
Polycythemia vera	+	
Polycythemia vera	+	
Polycythemia vera	+	
Autoimmune hemolytic anemia		+
Congenital spherocytosis		+
Congenital red cell aplasia	+	
Pancytopenia	+	
Pancytopenia	+	
Chronic myelogenous leukemia	+	
Chronic myelogenous leukemia	+	
Lymphoma	+	
Hodgkin's disease	+	
Arterio-venous fistula	+	
Broken hip	+	
Hip pain	+	
Hip pain	+	
Hip pain	+	
Osteoporosis	+	
Hyperparathyroidism	+	
Acromegaly	+	
Acromegaly	+	
Acromegaly	+	
Normal volunteer	+	
Metastatic carcinoma	+	
Pneumatosis intestinalis cystoides		+
Generalized Paget's disease of bone		+
Generalized Paget's disease of bone		+
Generalized Paget's disease of bone		+
Generalized Paget's disease of bone		+
Voorhee's disease		+

* Not including limited areas of increase due to local disease.

¶ Intermediate increases.

Figure 5 shows results of fluorokinetic analysis from the subjects on whom 3-hr blood and urine collections were obtained. (Their general features are confirmed by a number of less complete sample collections obtained from other subjects and analyzed in less elaborate fashion in the preliminary stages of the study.) There was a clear-cut elevation in the "minimum bone blood flow" from a mean value of 3.3% of cardiac output in the control group to 7.8% in the patients with myelofibrosis. The mean value for renal ^{18}F clearance was not significantly different from the controls. One patient with myelofibrosis, the lowest on the graph (Fig. 6) was uremic. Despite a renal clearance comparable with the controls, the percent of the injected ^{18}F in the urine at 3 hr is low in the myelofibrotic patients (mean 9.4%, compared with control 22.5%), reflecting the increased competition of the bone for the circulating fluoride. Blood ^{18}F curves showed a consistent small difference between the two groups (Fig. 6); when extrarenal fluoride clearance was calcu-

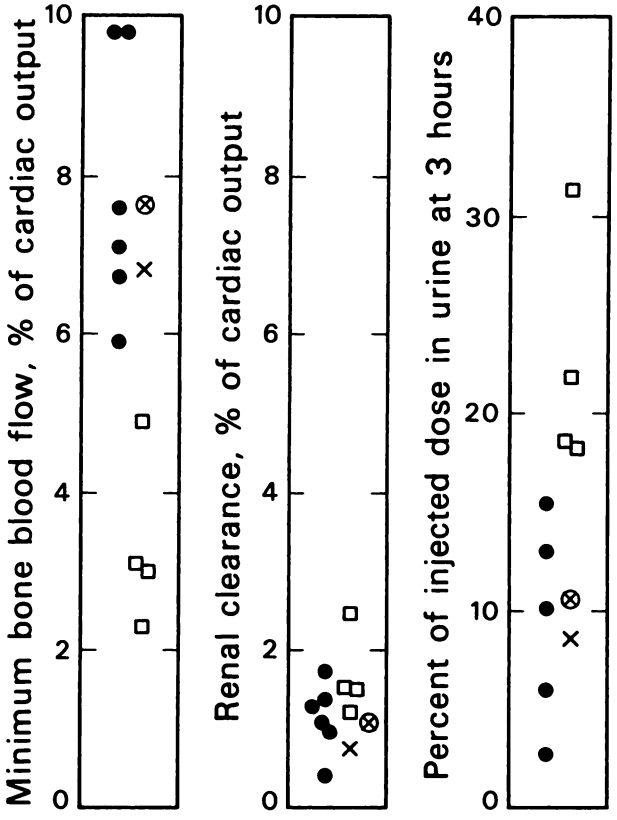

FIG. 5. Some selected parameters of interest from fluorokinetic analysis of blood and urine data. Patients with myelofibrosis are represented by solid circles and control group by open squares. Extrarenal clearance or "minimum bone blood flow" was clearly elevated in myelofibrotic patients. Urinary excretion of ^{18}F was reduced not because of reduced renal fluoride clearance, but from competition with bone. Patients represented by crosses are cases of far-advanced generalized Paget's disease of bone (Table 1).

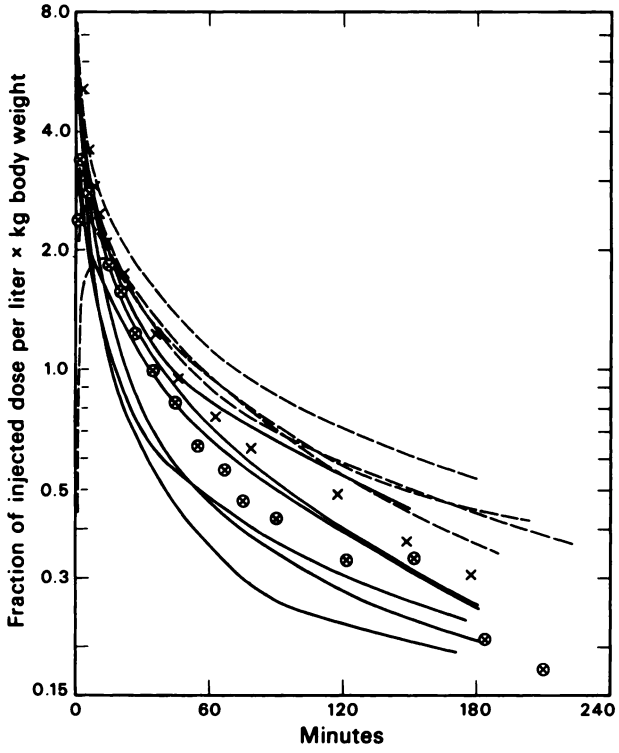

FIG. 6. Blood disappearance curves of ^{18}F. Patients with myelofibrosis are represented by solid curves, and those in control group by dashed curves. Patients represented by crosses are cases of Paget's disease and are not included in average values from fluorokinetic analysis given in text.

lated, this difference combined with the difference in urinary excretion to reveal a rather marked difference in bone blood flow, more nearly in keeping with the dramatic result seen on the scans and in the counting rate for bone and soft-tissue compartments (Fig. 2).

From the quantitative point of view, analysis of blood and urine data according to the methods outlined above has had to proceed largely independently of analysis of the leg counting data since a comprehensive model that can encompass both aspects of the problem has not been worked out.

While there is general confirmation from our quantitative studies, simple visual comparison of scintiphotos remains at present the most impressive and perhaps the most useful demonstration of the skeletal lesion.

Metabolic studies. The short-term balance studies were performed while most of the patients were on androgen therapy for myelofibrosis. The thyroid function was considered normal in all of these patients when changes in binding proteins produced by gonadal steroids were considered. Plasma and urinary adrenal steroids, and growth hormone responses to arginine or insulin were also normal. Three of the five patients showed mild degrees of hypocalcemia with serum calcium values ranging from 8.2 to 8.8 mg/100 ml. Hypocalciuria was present in all patients. One of the patients had mild hypoalbuminemia. Serum albumin was normal in the other patients but two of the four had hyperglobulinemia. Serum phosphorus measurements were slightly low or in the low normal range in all subjects but one (MM). Tubular reabsorption of phosphorus was normal in all patients and increased during phosphate restriction, reflecting a normal parathyroid response in these patients. The 4-hr calcium retention tests were abnormal in all patients, indicating a greater avidity for calcium than normal, which parallels the increased fluoride uptake in these patients.

DISCUSSION

The dominant microscopic features of the bone changes in far-advanced myelofibrosis are resorption of bone, altering the compacta into spongy bone and formation of numerous small trabeculae in the spongiosa on the inner surface of the cortex and throughout the medullary cavity. These alterations combine to increase the bone surface many times. One might suppose that the greatly increased surface could account for the rapid accumulation of ^{18}F. However, the extraction efficiency of normal bone is virtually 100%, and no additional accumulation of ^{18}F can occur unless there is greater blood flow past the vastly increased surface. Thus the greatly increased surface and capillary network must be accompanied by an increase in flow.

The fact that three of the patients in this study who showed markedly increased bone blood flow were not anemic (two with compensatory myeloid metaplasia and one in the active stage of polycythemia vera) rules out the possibility that the abnormality is secondary to anemia per se. The hypervascularity is then either primary or secondary to the poorly understood myeloproliferative process. Prolonged severe anemia from causes other than myelofibrosis is frequently associated with redistribution of bone blood flow, which accompanies redistribution of marrow (*11*). We have observed a generalized increase in total skeleton flow in one case of autoimmune hemolytic anemia and one case of congenital spherocytosis, but not in all patients with severe anemia. Presumably those blood diseases which are characteristically associated with roentgenographic changes in bone (*9*) can be expected to show fluorokinetic changes.

Because of the generalized involvement of the skeleton in myelofibrosis, an abnormality of the endocrine system influencing bone metabolism was considered. Growth hormone concentration in serum of seven patients with severe myelofibrosis was within

normal limits, and growth hormone response to insulin-induced hypoglycemia was not impaired. Adrenal and thyroid function was also normal. Increased calcium retention in all these patients resembled that described for osteomalacia and was quite distinct from the pattern usually seen in hyperparathyroidism, osteoporosis, Paget's disease, and other primary metabolic bone diseases. Parathyroid function was apparently normal, but some degree of secondary hyperparathyroidism could not be excluded. There is considerable recent interest in the role played by bone in marrow function (*12–15*). It is possible that the bone lesion is primary and that the grossly abnormal bone loses its ability to support hematopoietic marrow. If such is the case, attempts to determine the etiology and direct therapeutic management toward reversal of the bone lesion may lead to better results than restricting attention to the medullary aspects of the lesion. In a recent study of the response of one of the subjects to endosteal curettage (*13*), failure of curettage to induce an increase in ^{18}F uptake was unexplained. At the time of that report it was not realized that bone blood flow was abnormally high before surgical intervention and that a further increase in response to trauma was apparently not possible.

The possibility that changes in marrow function play an important part in the pathogenesis of age-related osteoporosis has been proposed (*16*). Agents which affect other bone diseases, e.g., calcitonin (*17,18*) and mithramycin (*19,20*) should be considered for therapeutic trial in myelofibrosis.

Fluorokinetic analysis. Initially we fitted blood data with a single exponential curve, which had proved a rather good approximation in the dog (*2*). As human data became available it was evident that the one-compartment model was inadequate, so an extensive examination was made of two-compartment models that could fit the data. The data obtainable to 3 hr, with doses that can be given to humans, have so far not suggested the necessity for fitting a three-component curve.

Particular attention was given to the two-compartment model in which the second major compartment beyond the initial mixing volume is a labile fluoride pool in bone. Such a model generally gives a value for bone blood flow about three times that of Fig. 1. The latter, however, provides a minimum value for flow, and a value the same as the extrarenal clearance, which requires fewer assumptions about the particular model used (see below).

It has become evident to us on a qualitative basis, from the scintiphotos, in vivo bone and soft-tissue counts, and difficulties with interpretation of two-compartment models, that a system with at least three compartments, an initial mixing volume, a "slowly exchangeable" ($T_{1/2}$ 10 min) extravascular fluoride pool in soft tissue, and a labile fluoride pool in bone, will probably be necessary in the future to fully interpret the human data. However, at present, without the ability to quantitate the fraction of the injected dose in bone and in extracellular fluid as a function of time, a complete solution of such a model is not possible in man without some arbitrary assumptions about relative volumes and rates of exchange. We have been making efforts toward such models with attempts to relate bone and soft tissue curves to blood and urine results, but without yet arriving at a single comprehensive mathematical treatment of the various data. It is interesting that the model of Fig. 1, arrived at here, resembles closely the one used for calcium turnover studies by Harris and others (*21*), in which a single large pool has proven an inadequate conception. In that case also, numerous elaborate models have been proposed, but they are as yet too cumbersome for routine application. The fluoride half-times are considerably faster than for calcium, and the dilution volumes obtained are entirely different. An interesting comparison of our fluoride data with human data from Harrison et al (*22*) indicates that during the first three hours, ^{18}F blood disappearance is much faster than for radioactive calcium and strontium, but very close to that of barium and radium.

It should be noted in considering the validity of the value for minimum bone blood flow that in obtaining the extrarenal fluoride clearance, it is not actually necessary to obtain a detailed solution of the model in Fig. 1. The overall clearance is an invariant of the data and can be most simply related to the total area under the (two-component) curve fitted to the blood data. It is independent of the relationships between the compartments of the model, and independent of the number of components fitted to the curve, so long as they approximate the area under the curve with acceptable accuracy.

Failure of the two-compartment model of Fig. 1 to provide for a labile pool in bone is undoubtedly one of its drawbacks. Application of the method of Costeas et al (*23*) to the data shown in Fig. 5 in the myelofibrotic patient suggested some degree of return of ^{18}F from bone since apparent bone uptake rate of ^{18}F decreased relative to the blood level, even though the bone ^{18}F content continued to increase steadily during the first three hours. This argues for the existence of a labile bone fluoride pool. However, the magnitude of tracer return must be small compared with deposition in the first three hours. The effect is too small to be clearly demonstrated in our normal subjects, and in the dog and

rat. It can be shown only with difficulty in the rabbit by Costeas et al (*23*) and in our myelofibrotic patients because the bone never reaches a peak value in the first few hours, but simply appears to accumulate at a decreasing rate relative to the blood level. This suggests that the measured extrarenal clearance may be a fairly close estimate of (as well as a minimum value for) the product of the bone blood flow and percent extraction.

The assumption that the extraction is high—nearly 100%—rests on other evidence (*2*). However, the results obtained here could only be explained on the basis of an increase in extraction efficiency if it were capable of an increase by a factor of 2.4 overall and a local increase of eight-fold. Extraction efficiency of the normal would have to be somewhere in the range of 12.5–42%. An explanation in terms of a pure increase in efficiency is thus incompatible with the previous data. It is difficult to avoid the conclusion that there has been some increase in bone blood flow in the myelofibrotics, and it appears quite probable that the effect on ^{18}F uptake is due principally to such an increased flow.

SUMMARY

The rate of appearance of an intravenously administered dose of ^{18}F in the skeleton is a function of the size of the skeletal blood supply. Fluorine-18 scintigraphic and kinetic data have shown a marked increase in skeletal blood flow in patients with myelofibrosis, both idiopathic and secondary to polycythemia vera. There was a clear-cut increase in the "minimum bone blood flow" from a mean value of 3.3% of cardiac output in the control group to 7.8% in patients with myelofibrosis. That the skeleton shows striking morphologic abnormalities in advanced cases of myelofibrosis is well known, but that the pathology is associated with an increase in bone blood perfusion comparable to that seen in generalized Paget's disease of bone has not previously been suspected.

The fact that three of the patients with myelofibrosis and markedly increased bone blood flow were not anemic (two with compensatory myeloid metaplasia and one in the active stage of polycythemia vera) rules out the possibility that the abnormality is secondary to anemia per se. The hypervascularity is then either primary or secondary to the poorly understood medullary disease.

ACKNOWLEDGMENT

This work was supported in part by the USAEC and in part by grant #5R0ICA08370 from the National Cancer Institute of the National Institutes of Health.

The authors wish to thank David G. Nathan, Chief, Division of Hematology, Children's Hospital Medical Center, Boston, Massachusetts, for referring two of the patients reported (including patient MM), the diagnoses of whom were established through training grant Tl AM 05581.

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