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Van Dyke

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PROGRESS REPORT

Period: 1965-1975

SUMMARY

Hematopoietic bone marrow was the organ under investigation throughout the 10 years covered by this report. At the beginning (1965), three methods applicable to the study of marrow function had recently been developed in this laboratory, and were being applied to a variety of hematologic disease syndromes. These were: (1) a method of assay of erythropoietic hormone in human urine; (2) a method of imaging the erythropoietic marrow with ^{52}Fe and the positron scintillation camera; and (3) a method of seeing the relative distribution of blood to the skeleton, using ^{18}F and the positron camera.

The results of 10 years investigation in this and other laboratories can be summarized as follows: The role of erythropoietin in the regulation of erythropoiesis has been well established. High levels of erythropoietin are associated with anemia and hypoxia, and low levels of erythropoietin are found in renal insufficiency, cancer, malutrition, etc. Polycythemia vera is independent of normal control, and unregulated secretion of erythropoietin by neoplastic tissue occurs. The use of erythropoietin in treatment of human disease has not been achieved.

Erythropoietic marrow imaging has been shown to be indicated in clinical hematology when marrow biopsies disagree with peripheral blood findings; to determine whether significant medullary erythropoiesis exists before removing the spleen; to determine whether marrow failure is due to tumor infiltration (patchy), radiation (failure restricted to radiation field), or suppression by chemotherapy.

Imaging of the relative distribution of blood to the skeleton has demonstrated a close relationship between bone blood perfusion rate and marrow growth, and indicates that marrow proliferates best in those parts of the skeleton most richly supplied with blood.

Detailed Report: Erythropoietin

Initial work in this laboratory showed that a standard method of concentrating urinary erythropoietin, and a standard assay procedure could be used to demonstrate the hormone in the urine of normal human beings. The erythropoietically active material recovered produced increasing response to increasing dose. It was completely neutralized by rabbit serum containing antibodies to human urinary erythropoietin. The average normal man was found to excrete approximately 1 standard A unit of erythropoietin per day, and the average normal woman excreted approximately 0.4 units per day. Other investigators subsequently found higher values (3 to 4 units per day), with women consistently 50% lower. A 20-fold increase in excretion of erythropoietin was found in permanent residents at high altitude (Chacaltoya, Bolivia, 17,000 ft.). These studies were extended to include as many hematologic disease syndromes as possible in order to clarify the role of erythropoietin in human pathology. A less-than-normal level of erythropoietin was found in patients with polycythemia vera, and red cell production in this illness appears to be independent of the usual control system. Studies in this and other laboratories indicated that inability to produce adequate amounts of erythropoietin contributed to the anemia which accompanies severe renal disease. However, several studies have shown that, although erythropoietin will stimulate erythropoiesis in nephrectomized animals, the response is approximately one-tenth that obtained

in normal controls. Such results suggested that the inability of the uremic patient to compensate for this anemia resulted primarily from a decreased responsiveness of the marrow to erythropoietin, rather than inability to increase production. Preliminary clinical trials indicated that the uremic patient, like nephrectomized rats and dogs, may require in the order of 10 times the normal amount of erythropoietin. It was predicted that successful treatment of the anemia, which accompanies severe uremia in man, may require a dose of erythropoietin in the range of 200 IRP units/Kg/day.

These results have contributed to the present day understanding of erythropoietin levels in health and disease. The presence of erythropoietin in the urine and plasma of normal persons indicates that the hormone is required for normal erythropoiesis. Additional evidence comes from the demonstration that administration of antibodies to erythropoietin depresses normal erythropoiesis, and that hypertransfusion of normal subjects decreases urinary erythropoietin levels. When anemia is induced in normal persons by phlebotany, urinary erythropoietin levels increase. There are several conditions in which the erythropoietin level is lower than would be predicted. These include the anemias of renal insufficiency, cancer, chronic infection, and starvation. Erythropoietin levels increase in the presence of acute hypoxia, and increased levels are found in permanent residents of high altitude. In polycythemia vera, erythropoietin levels are low unless the patient has been phlebotomized, and red cell production is apparently autonomous in that disease. A number of tumors such as hypernephroma and cerebellar hemangioma are capable of unregulated erythropoietin secretion.

Radioisotope imaging procedures in evaluating patients with hematologic disorders:

Development of radionuclide imaging devices and tracers, such as radioactive iron (^{52}Fe) and radioactive technetium (^{99m}Tc) sulfur colloid, have made it possible for the physician to take pictures that show the distribution of erythropoietic and reticuloendothelial marrow throughout the body. In addition, bone blood flow, which is often abnormal in hematologic disease, can be assessed using ^{18}F. In the normal individual the various components of hematopoietic marrow (granulopoietic, megakaryocytic, erythropoietic, and reticuloendothetical) are invariably linked together, and therefore have the same overall distribution. But in the presence of disease, erythropoietic functions may drop out or migrate to the spleen so that the two types of marrow labeling currently available (radioactive colloid and radioactive iron) can give different information. Examples of dissociation of erythropoietin and reticuloendothelial marrow became apparent in experimental, as well as clinical, situations. When the marrow cavity of rabbits was mechanically evacuated, uptake of ^{99m}Tc-sulfur colloid occurred in the area soon after operation, and weeks before hematopoietic function returned. Since phagocytic activity can persist in the presence of partial or complete failure of erythropoiesis, the colloid method of marrow imaging may be misleading for localization of erythropoiesis in patients with red cell aplasia. Comparisons in patients in various stages of polycythemia vera showed that both cell types are similarly affected, and that the use of iron or colloid will give essentially the same skeletal marrow distribution.

~~One-fourth of all~~ an appreciable percentage of patients with polycythemia vera die from marrow failure. Frequently the peripheral blood picture shows a sudden rapid

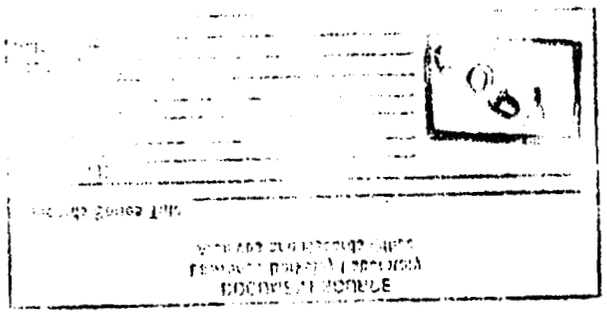
deterioration, evidence of far-advanced marrow deterioration which has been progressing for some time. A method for predicting which patients were starting the process, which would eventually lead to acute marrow failure, would be helpful in management of the disease. Total marrow distribution studies on a series of patients with polycythemia vera have shown that long before the onset of overall marrow failure there is regional failure in the usual marrow sites with compensating peripheral extension. Marrow distribution studies may be of important prognostic value in the management of polycythemia vera.

Bone blood flow shown with ^{18}F:

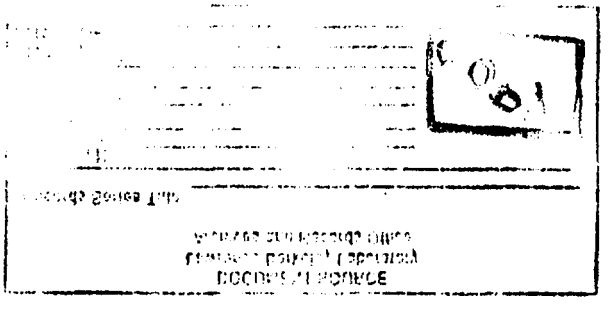
The same developments in instrumentation, which have made possible the imaging of the positron-emitting isotope of iron, ^{52}Fe, are suited for imaging of the short-lived positron emitting isotope of fluorine, ^{18}F. Fluoride ion taken into the body is promptly deposited in the skeleton or excreted via the kidneys. The distribution of ^{18}F within the skeleton depends on the blood supply to bone.

A similarity between the distribution of blood flow and the distribution of marrow in the skeleton became apparent from studies with ^{52}Fe and ^{18}F. This was true in normal man and animals, and in a variety of pathologic conditions. These results demonstrated a close relationship between bone blood perfusion rate and marrow growth, and indicated the marrow proliferates best in those parts of the skeleton most richly supplied with blood.

Whether the marrow cavity of a bone is filled with fat or red hematopoietic marrow depends on local environmental factors, important among which would appear to be whether the surrounding bone has a rich blood supply or is relatively poorly perfused with blood. With the development of the positron scintillation camera came methods for in vivo



A. Relative washout kinetics of paired substances injected into red bone marrow. Following a direct intramedullary injection into tl bone marrow, we compared the rate of washout of pairs of radioactive tracers administered mixed in the same solution. The detection syst used was an Anger scintillation camera with fast digital recording. With this system, we could follow the washout from the instant of injection because the injection syringe and the injection site are easily separable with the Anger camera. The initial portion of the washout pattern from the distal femoral marrow of the dog's leg was v rapid, with half times of a few seconds. Iodide ion, pertechnetate i sodium ion, and labeled albumin in various pairs followed essentially identical multiexponential washout patterns. Iodoantipyrine, however washed out somewhat more slowly. Retention of a sizeable fraction of injected colloidal particles (microaggregated albumin and Tc-sulphur colloid), as well as fluoride ion, verified that these were not simply intravenous injections. The similarity in pattern between



with blood, the clotted blood is rapidly and completely converted to hematopoietic marrow. Evacuation of the medullary cavity institutes something akin to re-enactment of the embryological development of the bone marrow, the series of events following closely the pattern of fetal development.