

BSP including 20  $\mu$ Ci of  $^{35}\text{S}$  BSP was administered and blood sampling was also performed 5, 10, 15 and 20 min. after injection.

Counts of each sample were plotted in a semilogarithmic paper. From these curves  $t_{1/2}$  and fractional clearance constant ( $k$ ) were calculated. One half time of disappearance curve ( $t_{1/2}$ ) was very short in tracer dose administration and its clearance seemed to be completely dependent on hepatic blood flow in normal subjects. But in one hepatitis

disappearance rate of tracer dose BSP was very low ( $k$  0.063) compared with reduction of hepatic blood flow.

Clearance of BSP could be fitted with Michaelis-Menten equation or Lineweaver-Burk method as well as  $^{131}\text{I}$  AA reported previously and ICG by others. Maximum removal rate of BSP obtained in this method was 0.75 mg./min./kg. in normal subjects. In hepatic V max. was decreased.

## IV. Blood

### A Simple Method for Determining the Amount of Iron Absorption

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It has been generally accepted that iron absorption can be estimated exactly by the following three methods:

1) double isotope method, 2) isotope balance method and 3) whole body counting. However, these methods are not suitable for routine clinical use because of complicated techniques. The present investigations were undertaken to devise a simpler method for estimating iron absorption.

1. The simplest method for estimating iron absorption is to measure the amount of  $^{59}\text{Fe}$  which appears in the circulating erythrocytes 14 days after the oral administration of the radioiron. With circulation blood volume estimated as 70 ml. per kg. body weight and the per cent red cell utilization of absorbed iron as 90 per cent, the results of this method was in good accord with those of the double isotope method in subjects with various diseases, the correlation coefficient being +0.98 in 36 cases. Even in case of aplastic anemia, in which the red cell utilization of absorbed iron was greatly depressed, these two methods resulted in little difference. The greatly decreased absorption in such cases minimized the difference of the

absorption value caused by using the utilization value of 90 per cent instead of the decreased utilization.

2. The chief disadvantage of the isotope balance method lies in prolonged fecal collections. To solve this problem,  $^{51}\text{Cr}_2\text{O}_3$  was used as a nonabsorbable marker simultaneously. In the preliminary experiment the following observations were made.

i) The fecal excretion of  $^{51}\text{Cr}_2\text{O}_3$  completed (90~96 per cent) within five days after the oral administration of  $^{51}\text{Cr}_2\text{O}_3$  in all subjects studied, and the maximum excretion was observed on the second day in most subjects.

ii) The fecal excretion of oral  $^{59}\text{Fe}$  required ten days or more in all except iron-deficient subjects, (where it required no more than five days,) as far as a smaller dose (0.05 or 1.0 mg.) is given. With a dose of 40 mg., however, it did not require more than five days in all subjects, and the ratio of  $^{59}\text{Fe}$ -amount to  $^{51}\text{Cr}$ -amount, both expressed as a percentage of the oral dose, was almost constant in each stool specimen.

iii) There was no significant difference of the  $^{59}\text{Fe}$ — $^{51}\text{Cr}$  ratio between samples with

and without mixing, and the ratio corresponded well with the total fecal excretion of  $^{59}\text{Fe}$  expressed as a percentage of the oral dose. From these observation, we proposed the simple method as a standard test: 40 mg. of  $^{59}\text{Fe}$ -labeled iron solution and 1 mg. of  $^{51}\text{Cr}$  with 100 mg. of ascorbic acid are simultaneously given orally and a piece of ascorbic

acid are simultaneously given orally and a piece of stool (1 to 2 g.) is subjected to assay for  $^{59}\text{Fe}$  and  $^{51}\text{Cr}$  two days later. Then the iron absorption is calculated from a formula;  $1 - \frac{^{59}\text{Fe}}{^{51}\text{Cr}}$ . When available, the value thus obtained is checked with the amount of radioactivity appearing in the circulating erythrocytes two weeks later.

## Some Studies on Proliferation and Differentiation of Leukemic Cells By Means of Radioautography

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The proliferation and differentiation of human leukemic cells were investigated both in vitro and in vivo using radioautography.

In vitro study with  $^3\text{H}$ -thymidine revealed a marked decrease in labeling percent of acute leukemic blast cells as compared with CML and normal myeloblasts.

However, as far as mean grain counts are concerned, these cells did not show any significant difference from each other, and also mean grain counts of acute leukemic blast cells were not less than that of basophilic erythroblast on the same sample.

The blast cells of acute leukemia were further divided into different groups according to their size. It was found that the larger the cells, the higher and more were labeling percent and mean grain counts, and it was also noted that the smaller cells that usually occupy the majority of the blast cells in acute leukemia did show only a few labeling percent.

As regard to mean grain counts, the similar results were obtained in the study with  $^3\text{H}$ -uridine and  $^3\text{H}$ -leucine.

For in vivo study, bone marrow and blood samples were taken at intervals following a single injection of 5 cm  $^3\text{H}$ -thymidine to a patient with acute leukemia, and examined for half time of the grain counts and labeling percent of mitotic figures. The estimate of TG was about 84 hrs,  $G_1$  60 hrs, S 20 hrs,  $G_2$  3 hrs and M 1 hr.

In the bone marrow, the initial labeling percent of leukemic blast cells was 2.5 per-

cent, while in the blood that was 0 percent and the labeled cells appeared 12 hours later, possibly being derived from bone marrow and other tissues.

Labeling percent of the larger blast cells in the bone marrow was initially fairly high and decreased with time, whereas that of the smaller cells was very low and increased gradually.

The finding that S/TG was nearly 24 percent, while the initial labeling percent was 2.5 percent strongly suggests that blast cells of acute leukemia may be composed mainly of nondividing compartment ie; smaller cells.

It is also suggested that the dividing compartment ie; larger cells may represent the major part of cells, which are actively participating in DNA, RNA and protein synthesis, and some of them may become smaller after division to join non-dividing compartment.

Similar study was made on a patient with chronic myelocytic leukemia who was also injected within 5mCi  $^3\text{H}$ -thymidine.

The estimate of TG of myeloblast was 108 hrs,  $G_1$  84 hrs, S 20 hrs,  $G_2$  3 hrs, and M 1 hr.

S/TG was about 19 percent almost corresponding to the initial labeling of 22 percent of myeloblast in the bone marrow.

The initial labeling percent was found highest in the promyelocytes, lowest in the myeloblasts, and the myelocytes came in between these two.

In the myeloblasts and myelocytes, the labeling percent increased during the first 24