

and without mixing, and the ratio corresponded well with the total fecal excretion of ^{59}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}Fe -labeled iron solution and 1 mg. of ^{51}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}Fe and ^{51}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 ^3H -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 ^3H -uridine and ^3H -leucine.

For in vivo study, bone marrow and blood samples were taken at intervals following a single injection of 5 cm ^3H -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 ^3H -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

hours, but in the promyelocytes, it once increased and decreased soon thereafter.

These phenomena are accounted for with

difficulty, but may possibly be related to the characteristics of proliferation and differentiation of the cells of CML.

RNA Metabolism of Human Leukemic Leukocytes

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In the study presented here, we demonstrate that, when human acute leukemic leukocytes were incubated by shaking at 37°C for a short time with ^{32}P orthophosphate and 0.2 mM each of four common nucleosides and subsequently were treated with water-saturated phenol at cold, rapidly labeled RNA fraction could be separated on the methylated albumin column.

The extraction medium consists of 0.14M NaCl 0.02M tris-buffer PH 7.8 0.5 mM EDTA with 0.5% SDS. The total nucleic acids resolved by the column into 4 components: acid soluble fraction, s-RNA, DNA, and r-RNA.

A chromatogram of the 60 min. labeled material obtained from a ALL patient show-

ed incorporation of ^{32}P into P_1 and P_2 . P_1 was a relatively small peak between DNA and r-RNA. P_2 was a high peak which was eluted later than r-RNA.

In a chromatogram of the 120 min. labeled material obtained from the same patient, both P_1 and P_2 peaks were still seen, but P_2 shifted toward the peak of r-RNA. The similar results were obtained in the patients with AML and monocytic leukemia.

These findings indicate that RNAs most actively synthesized in acute leukemic cells are high molecular weight RNAs distinct from s-RNA and r-RNA (P_2 and probably P_1). These RNAs are so called rapidly labeled RNAs and belong to nuclear RNAs.

Life Span of the Leukocytes Labelled with Diisopropylfluorophosphate (DF^{32}P)

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The life span of the leukocytes was measured by a method which leukocytes were labeled with radioactive diisopropylfluorophosphate (DF^{32}P) or radioactive chromium ($\text{Na}_2^{51}\text{CrO}_4$) in vivo or in vitro.

In vivo labelling method; DF^{32}P (100-200 μCi , 0.5-1.0 mg, and 20 ml of saline were injected intravenously for this study.

In vitro labelling method; 100-200 ml of blood was withdrawn from the subject. DF^{32}P of the same doses or $\text{Na}_2^{51}\text{CrO}_4$ (300-400 μCi) was added and the blood was remained at room temperature for one hour. Ten ml of the blood was obtained for a measure-

ment of blood volume, white blood cell count, its differential count and specific activity of the leukocytes in syringe. The remainder of the blood was injected intravenously, after adding 100 mg of ascorbic acid. Samples of the venous blood were withdrawn in determined interval. Leukocytes of each sample were separated by dextran sedimentation techniques, followed by 0.83 per cent NH_4Cl hemolysis or hypotonic lysis.

In DF^{32}P labelling method, the radioactivity was measured with gas-flow counter and in ^{51}Cr labelling method, it was measured with well-type scintillation counter. After count-