

incubated in 3.0 cc of phosphate buffer. After incubation the radioactivity in the bag and dialysate were counted.

And then the blood plasma in dialysis bag was analysed by Sephadex G-25 filtration.

As in vitro experiment for analysis of blood plasma, 3.0 cc of normal blood plasma was incubated with 0.1  $\mu$ Ci of  $^{131}\text{I}$  in 3.0 cc of phosphate buffer. After incubation, the radioactivity in blood plasma was analysed by the above mentioned method.

Sephadex G-25 filtration

After incubation,  $^{131}\text{I}$  labeled compounds in the blood plasma in dialysis bag were analysed

by Sephadex G-25 column (length 8 cm, diameter 0.6 cm).

Result:

As compared with in vitro experiment used  $^{131}\text{I}$  only, it was found that  $^{131}\text{I}$  labeled compounds in red blood cell after  $^{131}\text{I}$  administration were only  $^{131}\text{I}$ , on the other hand,  $^{131}\text{I}$  labeled compounds in blood plasma were  $^{131}\text{I}$  and hormonal  $^{131}\text{I}$ .

It was proved that the proportion of  $^{131}\text{I}$  in red blood cell and blood plasma is constant irrespective of the thyroid state and  $^{131}\text{I}$  red cell-plasma ratio is dominated by the radioactivity of hormonal  $^{131}\text{I}$  in blood plasma.

## Studies on Bone Marrow Nucleated Cells in Various Blood Diseases

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We have studied on the metabolism of DNA and proliferation activities of bone marrow cells in various blood diseases (AML 5, Megaloblastic anemia 2, Iron deficiency anemia 5, Polycythemia vera 2, ITP1) by means of the microautoradiographic technique with  $^3\text{H}$ -thymidine.

The results were summarized as follows:

1) Remarkably lower labeled % of  $^3\text{H}$ -thymidine was observed in the acute myeloblastic leukemia in both myeloid and erythroid series. It was indicated that the proliferative activities of the acute leukemic cells were very weak, and it was suspected that the DNA synthetic time of these cells was more prolonged than the normal.

2) In the megaloblastic anemia labeled %

of myeloid cells was similar to normal, but incorporation into metamyelocytes was moderately elevated. Remarkably higher labeled % was observed in basophilic erythroblasts, but it was low in polychromatic erythroblasts.

3) Remarkably higher labeled % of  $^3\text{H}$ -thymidine was observed in the iron deficiency anemia both in myeloid and erythroid series, especially in the erythroblasts.

4) In the polycythemia vera incorporation into myeloid series was depressed slightly, but in the erythroid series the incorporation was more than normals.

5) In the ITP labeled % of  $^3\text{H}$ -thymidine was similar to normal in both myeloid and erythroid series.

## Inhibition of DNA Biosynthesis in Human Leukemic Leukocytes by Cytosine Arabinoside and Clinical Effects of the Agent

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Effects of cytosine arabinoside (CA) on nucleic acid metabolism of human leukemia leukocytes were investigated with  $^{14}\text{C}$ -labeled

precursors of nucleic acid in leukocyte suspension prepared from peripheral blood of leukemia patients. Rate of inhibition of DNA-

biosynthesis in the leukocytes by CA was correlated with hematological effects of the agent.

The leukocyte suspensions were prepared from venous blood of patients with various types of leukemia by the method of Skoog and Beck. The suspensions were incubated with adenine- $^{14}\text{C}$  in the presence or absence of CA at  $37^\circ\text{C}$  for 3 hours. DNA and RNA of the leukocytes were extracted by the method of Hecht and Potter. Radioactivities of the DNA and RNA fractions were determined in a gas flow counter.

In a concentration of  $5\text{ }\mu\text{g/ml}$  of CA, specific activities of DNA fraction were inhibited by 10 to 30 percent of the control, whereas no significant inhibition could be found in the specific activities of the RNA fraction. Nucleotide analysis with Dowex I column chromatography by the method of Reichard revealed that incorporation of uridine- $^3\text{H}$  into both CMP and d-CMP of acid soluble fraction of the leukocytes was not inhibited in the presence of CA after conversion of diphosphate and triphosphate of cytidylic or deoxycytidylic acids to their monophosphates by heating at  $100^\circ\text{C}$  for 10 min. However, incorporation of uridine- $^3\text{H}$  into the DNA fraction was significantly inhibited in the presence of CA. These findings suggest that CA, probably after converted to its phosphoric compounds, inhibit DNA polymerase or deoxy-CDP kinase. In human leukemia leukocytes, inhibition of ribotide diphosphate reductase by CA phosphate as suggested by Chu and Fisher is unlikely.

Rate of inhibition of DNA biosynthesis by CA in the concentration of  $5\text{ }\mu\text{g/ml}$  was determined in leukocyte suspensions obtained from 14 cases of leukemia by means of the

adenine- $^{14}\text{C}$  incorporation into DNA fraction of leukocytes. The rate of inhibition was higher in cases hematologically sensitive to CA treatment than those resistant to the agent. Leukocytes suspension obtained from 4 out of 5 cases of chronic myelocytic leukemia (CML) had low inhibition rate of DNA biosynthesis by CA. These findings suggest that large parts of CML cases might not be sensitive to the CA treatment, which agreed with observations on clinical effects of CA by other hematologists. However, one of 5 cases of CML had a higher inhibition rate of DNA synthesis in the same concentration of CA which indicated that a small part of CML cases might be sensitive to the CA treatment. In the clinical course of patients with erythroleukemia who were sensitive to the CA treatment, the inhibition by CA was kept in high rates during the CA treatment, whereas the rate of inhibition decreased during the treatment in a resistant case to the agent.

In a case of erythroleukemia who has been sensitive and showed good response to the CA treatment for 9 months but was resistant at 4th treatment by CA, the rate of DNA inhibition in vitro was kept in a high rate in the stage hematologically resistant to the CA treatment but the rate of inhibition in vivo decreased. These findings suggest that rapid inactivation of CA by host such as deamination might be a cause of the resistance.

In conclusion, CA inhibited DNA biosynthesis in human leukemic leukocytes. The rate of the inhibition was closely correlated with the clinical effects of the agent. Therefore, determination of the rate of inhibition is valuable for estimating clinical effects of CA before the treatment.

## Studies of $^{14}\text{C}$ -Serotonin Release Index in Various Diseases

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The liberation of serotonin as well as histamine from platelets by antigen-antibody reaction was described in details by Humphry and Jaques using platelets from several spe-

cies including man.

We have used the release of serotonin labelled with carbon-14 from platelets as an indication of the presence of human serum