

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}C in the presence or absence of CA at 37°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- ^3H 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°C for 10 min. However, incorporation of uridine- ^3H 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}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}C -Serotonin Release Index in Various Diseases

T. HASHIBA, A. HANZAWA and E. KUYAMA

*The Second Department of Internal Medicine, Okayama University School
of Medicine, Okayama*

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

by the technique of Caspary and Yasunaga.

Results:

1) A high serotonin release index was obtained in 6 of 7 cases of A.M.L. (86%), but in all four cases of A.L.L. it was within normal limits. In C.M.L. and C.L.L. there were too few cases to reach a conclusion. Seven out of 11 cases (64%) of hypoplastic anemia showed a high serotonin R.I. One case of Coombs-positive autoimmune hemolytic anemia also showed a high R.I. and Imuran was effective to reduce its value to a normal range. In one case of I.T.P. treatment with steroid hormone and ACTH increased platelets counts and serotonin R.I. returned to a normal range. In other diseases, we observed a high

R.I. in one of 7 cases of bronchial asthma, in all 3 cases of lung cancer and two cases of diabetic mellitus, SLE and dermatomyositis respectively.

2) Serotonin R.I. had little correlation with immunoglobulin levels and plasmin activities.

3) There were no significant differences among the groups of people receiving over 2000cc blood transfusion, less than 2000cc blood transfusion and no blood transfusion.

4) In a patient with pure red cell aplasia who went into shock by γ -globulin injection, the R.I. was very high soon after shock and returned to normal gradually.

Studies on the Measurement of Serum Unsaturated Iron-Binding Capacity with ^{59}Fe Irosorb Kit

K. MATOBA and T. HATTA

Department of Internal Medicine, Okayama University Medical School, Okayama

Recently Abbott Laboratories offered the Irosorb-59 test for a quick, easy, and accurate means of measuring unsaturated iron-binding capacity of the serum. One milliliter of the patients serum is incubated with resin-sponge for one hour at 37°C, and counted its radioactivity of the tube in a well-type scintillation counter. After an hour of incubation, the sponge is washed with distilled water. The radioactivity remaining in the resin-sponge is counted in the same manner. Results are expressed as unsaturated iron-binding capacity;

$$\frac{(\text{Net original cpm}) - (1.05 \times \text{Net sponge cpm})}{\text{Net original cpm}}$$

$$\times \text{mcg. Fe added} \times 100 = \text{mcg. Fe/100 ml}$$

The results obtained from our experiments regarding the conditioning of this method are summarized as follows:

1) Reproducibility; Nine milliliters of a normal serum was examined nine times with a same condition one milliliter each. The mean value was 156.8 mcg per 100 ml and the standard deviation was 14.8 mcg per 100 ml. The ratio of the standard deviation to the mean value was 9.4 per cent.

2) Dilution effect of the serum; The serum

was diluted to 1,4/3,2 and 4 times. Values of unsaturated iron-binding capacity of diluted serum made an adverse correlation:

$$y = -0.84X + 30$$

3) The influence of hemolysis; A small amount of hemolysis up to 30% is negligible for measuring the unsaturated iron-binding capacity.

4) Preincubation time; Preincubation time was permitted to have a range of 0 to 60 minutes.

5) Incubation time; The level of unsaturated iron-binding capacity was higher with 30 minutes than over 45 minutes incubation period. Incubation time of 105 minutes and 660 minutes were tried. The constant levels were observed during 45 minutes to 90 minutes.

6) Incubation temperature; The constant levels were obtained between 10°C and 40°C.

7) Washing of the sponge; Two times washing of the sponge was not enough to remove non-absorbed radioactivity. Over three times washing was necessary, however, even ten times washing did not change the level of unsaturated iron-binding capacity.

8) Heparin; Heparinized plasma showed