

## VII. Blood and Spleen

### Study on the MHP Penetration Through RBC Membrane

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In order to evaluate the possibility of MHP penetration through RBC membrane, following results were obtained.

1) Effect on the intra RBC reduced glutathione (GSH). GSH was measured by "Alloxan 305" method of Patterson et al. The unblocked amount of GSH after various concentrations of BMHP administration is expressed as % of the original amount (100%). a) NEM and BMHP caused complete inhibition of 2  $\mu$ M GSH solution by using more than 1.6  $\mu$ M. (2  $\mu$ M of GSH is contained in the 1 ml RBC.) b) GSH in 1 ml RBC (intact) was inhibited by over 1.6  $\mu$ M NEM, however, only 70% is inhibited by 10  $\mu$ M of BMHP. c) Seventy % of GSH in hemolysate from 1 ml RBC was inhibited by 10  $\mu$ M of BMHP.

From these studies (B)MHP has inhibitory effect on the GSH solution, however, no inhibitory effect was found on the GSH in intact RBC as well as GSH in hemolysate.

This shows (B)MHP neither penetrate into RBC nor combined with SH-radical compounds except GSH inside RBC after penetration. Following study, therefore, is performed.

2) After labeling RBC by  $^{203}\text{Hg}$ -MHP (10  $\mu$ M/1 ml RBC), RBC was separated into stroma and endosoma. Stroma contained 4.6% of activity whereas endosoma contained 8.68% of total activity administered. By the additional experiment using isotonic cold endosoma it was found that there is no possibility of transfer of MHP into endosoma from the stroma.

It was concluded that MHP at the concentration of 10  $\mu$ M/1 ml RBC mostly penetrated red cell membrane and was combined by SH radical compounds inside RBC (probably cysteine radical of hemoglobin). This caused no inhibition on the GSH in RBC. Only small amount (4.6%) was combined by SH radical in the RBC membrane.

### Study of Splenic Sequestration Function with $^{203}\text{Hg}$ -MHP Method-Leukemia & Banti's Syndrome

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As we have previously reported splenic clearance of hypoplastic anemia by  $^{51}\text{Cr}$  labeled red cell and  $^{203}\text{Hg}$  MPH method, so now studied splenic clearance of all sorts of leukemia and Banti's syndrome especially about the relation between splenic clearance and a degree of splenomegalia, and so tried to find splenic sequestration function.

Method: Following intravenous injection of mixed blood with  $^{203}\text{Mg}$  MHP (100  $\mu$ Ci),

serial blood samples were taken and half time clearance was calculated. After one hour of injection external counting over the spleen, liver and heart was done and then scintiscanning.

Results:

(1) In most of acute myeloid leukemia clearance is more prolonged than in normal controls (normal average; 56 minutes).

(2) In one case of chronic myeloid leuke-

mia, clearance is prolonged regardless of marked splenomegalia, and so it is suggested splenic sequestration functions is decreased.

(3) In one case of chronic lymphatic leukemia clearance is shortened with marked splenomegalia, and its red survival time is also shortened.

(4) In all cases of Banti's syndrome clea-

ance is more shortened than normal subjects, but it is suggested there is no relation between clearance and a degree of splenomegalia.

(5) As regards of relation between clearance and white blood cell count it is suggested that the shorter clearance is, the less white blood cell count is.

## Measurement of Splenic Blood flow Using $^{133}\text{Xe}$ , $^{131}\text{I}$ HSA and $^{51}\text{Cr}$ RBC

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With washing out or dilution technique of radiotracers splenic blood flow was measured in order to investigate the relationship between its change and manifestation of portal hypertension of hypersplenism.

After the sudden injection of  $^{133}\text{Xe}$ ,  $^{131}\text{I}$ -HSA and  $^{51}\text{Cr}$  erythrocytes through a catheter selectively into the celiac artery, radioactivity over the spleen and the liver was continuously measured and recorded.

Radiosplenogram of  $^{133}\text{Xe}$  was analyzed on semilogarithmic scale into one or sum of two exponential components and the clearance rate was calculated.

In 7 normals this rate constant was between 0.890 and 1.647  $\text{min}^{-1}$ .

In 8 cases with hepatic cirrhosis this value was from 0.502 to 1.520 and in 4 cases with so called Baniti's syndrome this was from 1.38 to 2.16.

This value is generally thought to reflect blood flow rate through the organ expressed as ml. per minutes per unit weight of splenic tissue. The difference among these three groups,

which had been previously suspected, was not statistically significant.

In hepatic cirrhosis this value appeared to be inversely correlated with splenic volume estimated by scintigram, although this correlation did not stand the statistical analysis. Considering much expanded volume of the spleen in cases with chronic congestive splenomegaly, total blood flow must be taken significantly increased, the result which coincides with the previously reported findings.

In analysis of radiosplenogram of  $^{131}\text{I}$  HSA and  $^{51}\text{Cr}$  erythrocytes radioactivity of the tracers in recirculation was eliminated in following ways.

Assuming the initial circulation character to be expressed in one or sum of two exponential components and recirculation character to become constant level 60 seconds later concerning  $^{131}\text{I}$  HSA, the former was determined and from the difference the latter was then obtained.

With the same recirculation character initial circulation character of  $^{51}\text{Cr}$  erythrocytes