Role of Haptoglobin in the Organ Distribution of Hemoglobin in vivo

124. Studies on Hemoglobin Metabolism. I

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Site of heme uptake of intravenously administered $^{59}$Fe-hemoglobin (Hb) in small doses into rabbits has shown to be the liver parenchymal cells rather than reticuloendothelial system (RES) by Yamaoka and his associates$^{1,2}$ by means of autoradiography. It was the first indication to present the important role of liver cells in the Hb uptake contrary to the previous belief.$^{3}$ Concurrently, Nakajima et al.$^{4}$ have extracted an enzyme, heme $\alpha$-methenyl oxygenase, from guinea pig and beef liver which is capable to convert heme of hemoglobin-haptoglobin (Hb-Hp) complex to a possible precursor of biliverdin. Free Hb, however, has been shown to be quite inert as the substrate of the enzyme. These results have led us to determine the role of Hp in the recognition and incorporation of Hb by liver parenchymal cells.

The present study has been performed to clarify the role of Hp in the distribution of Hb in vivo, and the available evidence shows the selective incorporation of Hb-Hp into the liver while free Hb distributes into the kidney.

Materials and methods. Male Wistar rats weighing 170 to 300 g (average 200 g) were used. All operative procedures were done under ether anesthesia. Intravenous injections were performed into lateral tail veins in a rate of 0.2 ml/second. Preparation of Hb labeled with $^{59}$Fe was essentially the same as described by Ostrow et al.$^{5}$ Solutions of $^{59}$Fe-Hb were prepared after Werker et al.$^{6}$ with slight modification. Specific activity of the $^{59}$Fe-Hb was 140,000 cpm/ mg. At predetermined intervals, the animals were killed by exsanguination with a heparinized syringe followed by 5 times infusion with saline through abdominal vein and cervical dislocation. The liver and kidneys were removed, washed in cold saline, blotted dry, and weighed, after which samples were taken for counting. These samples, 1 ml specimen of blood, 1 g of liver, and two kidneys were each counted in a $\gamma$-well type scintillation counter, Aloka JDC-207.

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Blood volume was calculated as 6.5% of the body weight. Hp concentration was determined by radial immunodiffusion method using anti-rat haptoglobin antiserum from rabbit prepared in our laboratory. Induction of Hp was achieved by subcutaneous injection of 0.1 ml of turpentine per 100 g body weight with 2 ml of air, and after 48 hours of the injection, approximately 2 to 3 times of increase in Hp level was observed. According to the preliminary results, a group, where the animals were killed 40 minutes after injection, was chosen in the present investigation since within 40 minutes a linear incorporation of radioactivity into these organs was observed irrespective to the amount of Hb loaded.

**Results and discussion.** As shown in Fig. 1, when the different amounts of Hb were injected to the rats, the radioactivity incorporated in the liver was increased almost proportional to the injected dose below 1.6 mg Hb/100 g body weight after 40 minutes. Administration of Hb exceeding this limit, however, did not increase the radioactivity in the liver, but caused the distribution of the activity
in the kidney. This limit, 1.6 mg Hb/100 g body weight, was assumed to correspond to the serum Hb BC, approximately 70 mg Hp/100 ml serum: Hb administered in a dose below the limit could be expected to combine with Hp completely, while Hb exceeding the above limit remained in blood stream as free Hb. These results suggested that the factor which determines the organ distribution of Hb was Hp.

In order to explore the possibility further, the similar experiments have been performed using the rats with elevated Hp level by means of turpentine treatment. As has been clearly shown in Fig. 1, in the non-treated animals, the limit in the distribution of the radioactivity into kidneys was 1.6 mg Hb-100 g body weight, while in the turpentine-treated ones the limit was clearly elevated to 3.6 mg Hb/100 g body weight. Maximal hepatic incorporations of the radioactivity were observed also in these limits, and it is noteworthy that the amount of Hb was 1 mg per liver with no significant difference in both groups. This fact indicates that the amount of Hb incorporated was not proportional to the dose injected and there existed a certain limit in the liver in this process.

The discrepancies of the data between the present work and those of others\textsuperscript{10-14} are presumably arose by differences of the conditions used. Several authors\textsuperscript{10-12} investigated the behavior of free Hb in “Hp-depleted” animals, and presumed that free Hb was also incorporated in the liver. According to our data,\textsuperscript{15} however, it is practically impossible to deplete Hp completely, since even in the repeated administration of a large doses of Hb, active de novo Hp biosynthesis is observed shortly after the Hb administration. Thus, it is difficult to retain all of the Hb administered as free state. Another authors\textsuperscript{13,14} have reported organ distribution of Hb-Hp prepared \textit{in vitro}. In these cases, certain conformational change or aggregation of the molecules during the preparation would cause the change in the site of their distribution. Detailed discussions on these problems will be presented elsewhere.

**Summary.** Haptoglobin determines the site of Hb uptake when the Hb was administered intravenously: Hb-Hp and free Hb distribute into the liver and kidneys, respectively. There exists a certain limit in the liver to incorporate Hb-Hp suggesting the presence of some mechanism on liver cell membrane to recognize or incorporate the molecule.
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