Role of the Liver in the Catabolism of Serum Haptoglobin. (31314)

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Studies on the catabolism of serum haptoglobin (Hp) have been confined largely to the fate of its complex with hemoglobin (Hp-Hb). While all such experiments are in accord in documenting the rapid disappearance of the Hp-Hb complex from the circulation ($T\frac{1}{2}$ of 88 to 120 minutes) in man and experimental animals(1,2,3), the site of catabolism of the complex remains uncertain. It has been proposed that the reticuloendothelial (R-E) system plays a major role in removal of the Hp-Hb complex(1), but the importance of a major component of this system, the liver, in this process has been disputed(2). A recent study suggests that this organ is not of major importance in the catabolism of haptoglobin in its uncomplexed state(4). In an effort to define the role of the liver in the catabolism of both free and complexed haptoglobin, the clearance of I^{125}-labelled human haptoglobin (type 2-2) and I^{125} Hp-Hb was studied in the isolated perfused rat liver system, as well as in intact animals.

Methods and materials. Human Hp, type 2-2, was isolated from normal human plasma by the method of Smith *et al*(5) employing minor modifications. Purity of the product was confirmed by starch gel and immunoelectrophoresis. Radioiodination of human Hp was carried out as described by Yagi *et al*(6) and resulted in 1 atom of I per mole of Hp. The specific activity of the product was 100 μc/mg protein at time of preparation. Radioautography of the iodinated protein after starch-gel electrophoresis revealed only the multiple bands characteristic of Hp 2-2. The details of the rat liver perfusion apparatus and the perfusion technique have appeared previously(7). Livers and perfusing blood were obtained from normal fed Sprague-Dawley rats (250-300 g). Two perfusions were carried out: in the first, 2.1 μc of I^{125} Hp were added to the reservoir after 30 minutes of equilibration. In the second perfusion, 2 μc of I^{125} Hp in the form of its complex with human hemoglobin (I^{125} Hp-Hb) were added. Samples were obtained from the reservoir 10 minutes after introduction of the isotope and at 30-minute intervals thereafter. In addition, intake and outflow samples were obtained directly from the appropriate cannulae on the liver at hourly intervals. Plasma samples were counted for radioactivity in a well-type scintillation counter; then the proteins were precipitated with 10% trichloroacetic acid (TCA) and protein-bound (TCA-precipitable) radioactivity was determined.

In the experiments with intact animals, three 350 g Sprague-Dawley rats (Group 1) were injected *via* the dorsal vein of the penis with 1.4 μc of I^{125} Hp, and blood samples were obtained with capillary tubes from the retroorbital plexus. Samples were obtained 10 minutes after injection, 6 hours later, and thereafter at 24-hour intervals. These animals were sacrificed 4 days after injection, and portions of liver, spleen, thyroid, muscle, intestine, and pancreas were removed for counting. Group 2 consisted of 3 rats each injected intravenously with 2.2 μc of I^{125}-Hp-Hb; samples were obtained 10 minutes later, and then at 30-minute intervals. Because of the rapid disappearance of plasma radioactivity in these animals, they were sacrificed 5 hours after injection and portions of organs obtained as above.

Results. 1. Perfusions. There was no significant decrease in either total plasma or protein-bound radioactivity during 4 hours of perfusion with I^{125} Hp (Fig. 1). Because of the gross hemolysis which is evident in the perfusing plasma after 1-2 hours of perfusion, it is probable that the I^{125}-labelled Hp, although injected in the unbound state initially, later became complexed to liberated rat hemoglobin. Despite this likelihood, after

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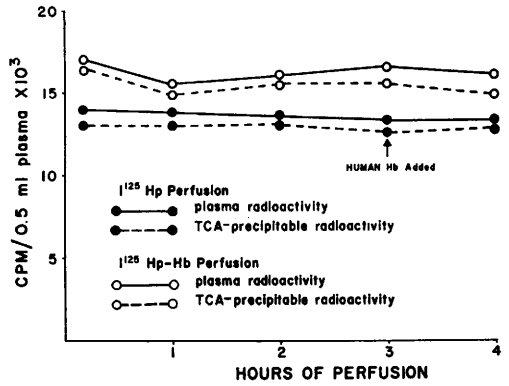

FIG. 1. Plasma radioactivity and trichloracetic acid-precipitable radioactivity during rat liver perfusion after addition of I^{125} Hp, and I^{125} Hp-Hb complex.

3 hours, sufficient human Hb was added to the perfusate to bind any free I^{125} Hp that might have remained. No change in plasma or protein-bound radioactivity was observed after this procedure. At the conclusion of this perfusion, the liver was weighed, and a weighed portion counted for radioactivity. About 2.5% of the injected radioactivity was calculated to be present in the liver and its retained blood. Negligible radioactivity (0.2% of the injected dose) was found in the 3.5 ml of bile produced during the perfusion. In the second perfusion where I^{125} Hp-Hb was injected, negligible clearance of the Hp-Hb complex was noted (Fig. 1). A portion of the liver was removed, weighed and counted, and the remainder of the organ was perfused with Ringer's solution until the effluent was clear; a portion of this tissue, considered to represent hepatic tissue free of capillary blood, was then weighed and counted. Only one-third of the counts in the organ, or 1% of the injected dose, remained after flushing with Ringer's solution.

2. In the intact rats, the disappearance curves of I^{125} Hp-Hb and I^{125} Hp are illustrated in Fig. 2A and 2B. The rapid *in vivo* clearance of the Hp-Hb complex ($T\frac{1}{2}$ 78-162 min; avg. 128 min) is in accord with previous studies in other species. When these animals were sacrificed, after 3 hours in one rat, and after 5 hours in the remaining animals, radioactivity appeared in the liver, spleen, kidney, and thyroid in order of decreasing magnitude. The rats had not received prior treatment with Lugol's solution, and bone marrow was not obtained. About 6% of the injected dose appeared in the liver. Data from the perfusion studies would indicate that about ⅔ of this radioactivity was in the blood retained in the organ. One-third, or 2%, represented true hepatic retention of the labelled protein. This figure is in good accord with that obtained in the perfusion studies and clearly suggests that the liver is not a major site of catabolism of Hp-Hb. When organ radioactivity was calculated per gram of tissue, the order of radioactivity was thyroid>spleen>liver>kidney.

In the animals injected with free I^{125}-labelled haptoglobin, clearance was much slower ($T\frac{1}{2}$ = 0.8 day). This is in accord with turnover studies performed with I^{131}- and I^{125}-labelled human haptoglobin(8,9). It is of interest that the half-life of this same batch of labelled Hp in humans with Hodgkin's disease was of the order 2.5 to 3 days (9); the faster turnover in the rat is consistent with the more rapid metabolic processes noted in smaller mammalian species (10). Most of the organ radioactivity in these animals, sacrificed after 4 days, was concentrated in the thyroid gland (about 25% of the injected dose). This would be expected, since blood radioactivity had fallen to low levels and much of the labelled protein would have been degraded. No iodine had been given to block thyroidal uptake. Kidney, liver, and spleen radioactivity was slightly higher in that order, per gram of tissue, than thymus, muscle, pancreas or intestine, but retained radioactivity did not exceed 0.01% in any of these organs.

Discussion. Studies on the site of degradation of serum haptoglobin have been concerned with the Hp-Hb complex, which is cleared rapidly from the circulation in man and laboratory animals. Murray *et al*(1) found that 4 hours after injection of the complex into rabbits, most of the radioactivity was found in the liver; the kidneys contained only traces. Franklin *et al*(2) studied the clearance of human I^{131} Hp-Hb, and Hp-Hb-Fe^{59} also in rabbits, and found plasma radioactivity half-times of 110 and 78 min-

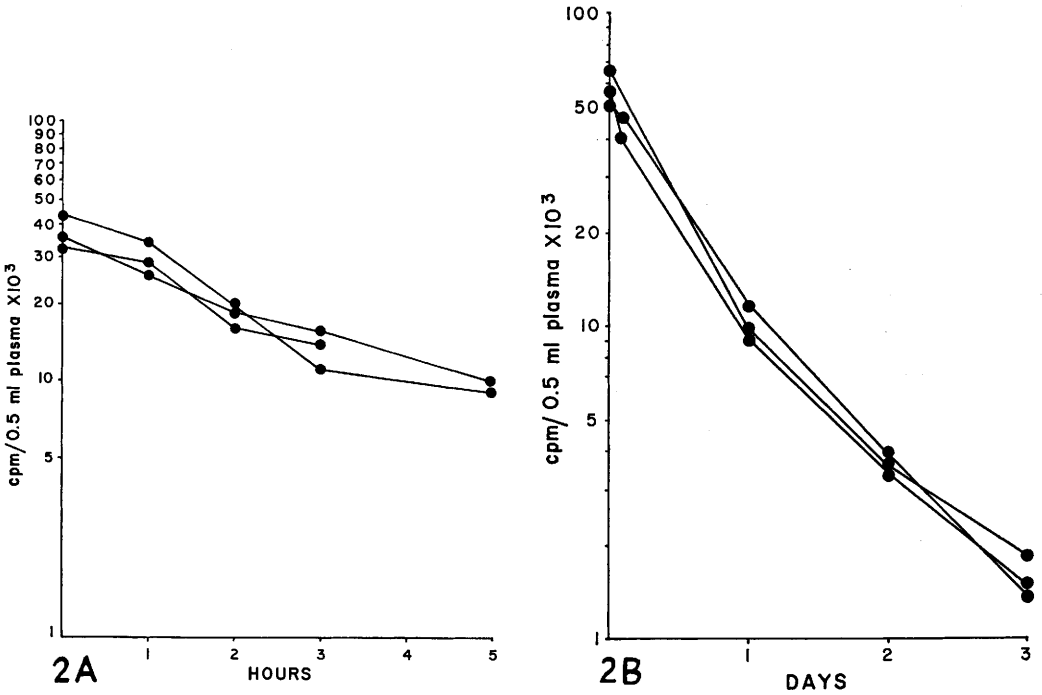

FIG. 2A. Disappearance of plasma radioactivity after intravenous injection of human I^{125} Hp-Hb complex into rats. Note time scale in hours.

FIG. 2B. Disappearance of plasma radioactivity after intravenous injection of human I^{125}-labelled Hp into rats. Note time scale in days.

utes, respectively, for the differently tagged Hp-Hb complexes. Injection of a mixture of I^{131}-albumin and Hp-Fe^{59}-Hb into the portal vein of rabbits with sampling of vena caval blood indicated that neither of these proteins was fixed by the liver; these data would appear to contradict the findings of Murray *et al* cited above. This apparent discrepancy is resolved by our data which indicate that I^{125} Hp-Hb is not taken up any appreciable degree by the liver, either in the perfusion system or *in vivo*. The apparent preponderance of uptake by the liver, compared with other organs, is due to the size of that organ and its large capillary bed; thus, at least ⅔ of the radioactivity can be attributed to retained blood.

Our *in vivo* studies also confirm the slower disappearance of uncomplexed haptoglobin noted in man and rabbits. The fate of this material remains unclear, since at the time of sacrifice, 4 days after injection, the retained protein had been largely degraded and most of the I^{125} was concentrated in the thyroid gland. The finding of Jayle and coworkers(4) that the half-life of I^{131}-labelled rabbit haptoglobin was not altered by carbon tetrachloride-induced liver damage in rabbits suggests that the liver does not play a major role in the degradation of uncomplexed haptoglobin.

Although these studies were performed with a heterologous protein, the period of observation in the intact animals was too short to expect immune reactions to play any role. The perfusion and *in vivo* studies lead to the conclusion that the liver is not a major site of catabolism of haptoglobin, either free or in the form of its complex with hemoglobin. While the reticuloendothelial system may participate in the degradation of this protein, R-E containing organs other than the liver, such as spleen or bone marrow, are probably of more significance in this regard.

Summary. 1. The fate of I^{125}-labelled human haptoglobin (Hp) and its hemoglobin complex (Hp-Hb) was studied in the isolated perfused rat liver as well as in intact ani-

mals. 2. No significant removal of either free or complexed Hp from the perfusate occurred during 4 hours of liver perfusion. 3. In intact animals, the T½ of I^{125} Hp-Hb was considerably shorter (2 hours) than that of free I^{125} Hp (19 hours); in both groups, hepatic uptake per gram of tissue was less than splenic uptake, and was minimal. 4. It is concluded that the liver, the major site of Hp synthesis, is of limited importance in the degradation of this protein and of its hemoglobin complex.

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Electron Microscopic Quantitation of Viral Particles in Tissues of Leukemic Mice.* (31315)

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There have been numerous reports in the literature concerning the presence of virus-like particles in the lymphatic tissue of leukemic mice(1). Investigators have made use of techniques to determine the frequency of particles in thin sections(2), which have proved to be of use when relatively large numbers of particles were present. However, when small numbers of cells and/or particles are inoculated, the number of viruses in the initial stages of leukemia may be quite small. While evaluating certain preliminary observations in mice, it became necessary to determine the presence and frequency of virus-like particles when few were present in the non-inoculated controls and inoculated mice. There was, therefore, a need to indicate an appropriate number of sections that should be observed before concluding the tissue "negative" of particles.

Description of inoculation. In the preliminary study one- to five-week-old axenic CFW_w mice, to date free of spontaneous leukemia, were inoculated intraperitoneally with a suspension of bone marrow from 4 human leukemic patients. Diagnosis of the leukemic patients was as follows: chronic myelocytic leukemia, acute reticulum cell leukemia, acute lymphoblastic leukemia, and acute leukemia. Serial passages of resultant leukemias were maintained by the intraperitoneal inoculation of 0.1 ml of a 20% homogenate of leukemic cells in 0.85% saline.

Electron microscopic techniques. Some of the tissues were fixed in 1% osmium tetroxide, pH 7.3, and buffered with a Palade's(3), or a phosphate buffer(4). Other tissues were fixed in a 4 or 6% phosphate buffered glutaraldehyde solution, pH 7.2(5), and post-fixed in osmium tetroxide. The tissues were

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