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EFFECT OF INHIBITED ENDOCYTOSIS IN SINUSOIDAL LIVER CELLS ON CATABOLISM OF EQUINE HAPTOGLOBIN AND HAEMOGLOBIN IN THE HEN*

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Catabolism of equine haptoglobin (Hp), haptoglobin - haemoglobin complex (Hp-Hb) and haemoglobin (Hb) in the hen was studied in the liver reticuloendothelial system (RES) in which endocytosis of protein was inhibited by aggregated denatured albumin (Agr-Alb).

Intravenous injection of Agr-Alb together with equine $[^{125}]$Hp, $[^{125}]$Hp-Hb or $[^{125}]$Hb partially inhibited elimination of these proteins from hen circulation. The decrease of clearance was less pronounced when the labelled proteins were introduced 30 min after Agr-Alb injection due to stimulation of phagocytosis in RES by Agr-Alb. Elimination of equine proteins (Hp, Hp-Hb and Hb) from hen circulation by RES is only one of the possible metabolic fates of these proteins.

In the blood of mammals and birds, haptoglobin$^1$ binds to haemoglobin to form a stoichiometric, practically irreversible complex. Avian Hp, unlike mammalian Hp, binds only with homologous Hb (Musquera et al., 1979). Formation of Hp-Hb complex and its clearance from circulation by liver cells is the first step of Hb catabolism (Wada et al., 1970; Müller-Eberhard & Liem, 1974). Dobrszycka et al. (1981) found that the half-life of mammalian Hp-Hb introduced to chicken did not differ from that of Hp, whereas duration of the complex in mammals is shorter than that of Hp by a factor of at least 5 (Engler et al., 1967; Dobrszycka et al., 1969, 1979).

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Abbreviations used: Hp, haptoglobin; Hb, haemoglobin; Hp-Hb, complex of haptoglobin with haemoglobin; Agr-Alb, aggregated denatured albumin; RES, reticuloendothelial system.
So far, the successive steps of Hp-Hb catabolism have not been fully elucidated. Engler et al. (1967) and Bocci (1976) believe that Kupffer cells are the site of binding and degradation of Hp-Hb, whereas Wada et al. (1970) are of the opinion that Hp-Hb is bound by Kupffer cells but is transported to hepatocytes where it undergoes proteolytic degradation. According to Kino et al. (1980) specific receptors for Hp-Hb in hepatocytes probably are able to recognize changes in conformation of Hp complexed with Hb. Studies of Higa et al. (1982) on Hp catabolism in rat liver indicated that the Hp-Hb complex and its degradation products are first bound by the Golgi subfraction, and then by the lysosomal fraction of hepatocytes.

In our attempt to elucidate the mechanism of Hp-Hb elimination in avian liver, we have examined binding capacity of the receptors for equine Hp, Hp-Hb and Hb in hen sinusoidal hepatic cells in which endocytosis of proteins was inhibited by denatured albumin.

**MATERIALS AND METHODS**

Electrophoretically pure haptoglobin, haemoglobin and Hp-Hb complex were prepared as described by Dobryszycka & Krawczyk (1979). Heat-denatured bovine albumin containing aggregates of proteins selectively binding to hepatic Kupffer and endothelial cells, were obtained according to Iio et al. (1963); the preparation was additionally filtered through the Millipore HA MK 02412 bacterial filter.

Labelling with $[^{125}I]$iodine was performed by the method of Mc Conahay & Dixon (1966) with chloramine T, using $^{125}$INa, carrier-free (Institute of Nuclear Research, Święk, Poland). The preparation obtained had a specific activity of 40 - 60 μCi/mg protein.

Adult White Rock hens, weighing 2 - 3 kg, were used for the experiments. Protein endocytosis in liver reticuloendothelial system was inhibited by administration of a mixture containing 110 mg of Agr-Alb and the appropriate labelled protein, i.e. 3.5 mg of Agr-$[^{125}I]$Alb, 0.5 mg of $[^{125}I]$Hp, 0.5 mg of $[^{125}I]$Hp-Hb or 0.2 mg of $[^{125}I]$Hb. In another series of experiments 110 mg of Agr-Alb was administered 30 min before administration of the labelled proteins. The preparations dissolved in sterile physiological salt solution were injected into the elbow vein. Blood samples were collected through a heparinized tube and centrifuged immediately. Radioactivity was measured in a MRG-603 counter (Tesla, Czechoslovakia) directly in plasma and in proteins precipitated with 10% trichloroacetic acid containing 2% phosphotungstic acid (1:1, v/v).

Clearance of labelled proteins from hen circulation was determined according to Attie et al. (1979) and Matthews (1957).
RESULTS

The time-course of clearance of $^{125}$I-labelled albumin aggregates from hen circulation is presented in Fig. 1. It was found that Agr-$[^{125}]$Alb disappears very rapidly with $t/2$ of 0.6 h. The use of Agr-Alb in a 30-fold excess decreased the clearance of Agr-$[^{125}]$Alb to 71.5%, of the total dose applied (Table 1).

On intravenous administration of 110 mg of Agr-Alb 30 min before a dose of 3.5 mg of Agr-$[^{125}]$Alb, clearance was lower than when the same amount was introduced simultaneously, and amounted up to 48.4%, of the total dose. Irrespective of the mode of administration, an excess of Agr-Alb reduced the half-life span of Agr-$[^{125}]$Alb by a half.

As compared with a control sample containing only $[^{125}]$Hp intravenous administration of $[^{125}]$Hp and Agr-Alb, independently of the time of Agr-Alb injection, resulted in initial inhibition, followed by stimulation of Hp clearance from circulation (Fig. 2). When $[^{125}]$Hp and Agr-Alb were administered simultaneously, the inhibition was more notable (the difference corresponded to about 30%, of the dose applied) but lasted shortly, whereas decrease of clearance was weaker and lasted longer on administration of Agr-Alb 30 min before $[^{125}]$Hp (17%, of the dose applied). In both cases the half-life span of Hp was shortened from 6.5 to about 4 h.

The time-course of Hp-Hb clearance was similar as with Hp; both the decrease and subsequent stimulation of clearance were observed (Fig. 3). Simultaneous intravenous administration of the mixture of $[^{125}]$Hp-Hb and Agr-Alb decreased clearance down to 27%, of the dose applied, and administration of $[^{125}]$Hp-Hb 30 min after Agr-Alb reduced clearance drastically (to about 5%, of the dose applied). At the same time the half-life of Hp-Hb was shortened slightly from 5.9 to 5.2 h in the control sample and to 4.8 h in blood samples collected after administration of the $[^{125}]$Hp-Hb complex together with Agr-Alb.

After intravenous injection of equine Hb and Agr-Alb the clearance of Hb from hen circulation was decreased by not more than 25%, of the dose, and injection of $[^{125}]$Hb 30 min after Agr-Alb resulted in some stimulation of the clearance (8%, of the dose applied). The $t/2$ values were slightly higher in both cases, i.e. 0.92 h as compared with 0.83 h for the control sample.

DISCUSSION

Cells of the reticuloendothelial system of liver consist mainly of sinusoidal cells and are able to bind selectively numerous substances, e.g. molecules of colloidal carbon (du Souich et al., 1981) or molecules of heat- or
Fig. 1. Time-course of clearance of aggregates of denatured $[^{125}\text{I}]$albumin (Agr-$[^{125}\text{I}]$Alb) in the hen.

A. Clearance curves following administration of Agr-Alb given in excess: a, 3.5 mg of Agr-$[^{125}\text{I}]$Alb simultaneously with 110 mg of Agr-Alb; b, 110 mg of Agr-Alb followed after 30 min by 3.5 mg of Agr-$[^{125}\text{I}]$Alb; C, control, 3.5 mg of Agr-$[^{125}\text{I}]$Alb.

B. Exponential expression of the radioactivity elimination: double-exponential distribution (CD) and elimination (CE) curves calculated from the control (C); $a' = a - CD$; $b' = b - CD$.

Fig. 2. Effect of aggregates of denatured albumin (Agr-Alb) on clearance of equine haptoglobulin (Hp) in the hen.

A. Clearance curves following administration of Agr-Alb given in excess: a, 0.5 mg of $[^{125}\text{I}]$Hp simultaneously with 110 mg of Agr-Alb; b, 110 mg of Agr-Alb followed after 30 min by 0.5 mg of $[^{125}\text{I}]$Hp; C, control, 0.5 mg of $[^{125}\text{I}]$Hp.

B. Exponential expression of the radioactivity elimination. For designations see Fig. 1B.
Fig. 3. Effect of aggregates of denatured albumin (Agr-Alb) on clearance of the equine haptoglobin-haemoglobin complex (Hp-Hb) in the hen.

A. Clearance curves following administration of Agr-Alb given in excess: a, 0.5 mg of $[^{125}\text{I}]	ext{Hp-Hb}$ simultaneously with 110 mg of Agr-Alb; b, 110 mg of Agr-Alb followed after 30 min by 0.5 mg of $[^{125}\text{I}]	ext{Hp-Hb}$; C, control, 0.5 mg of $[^{125}\text{I}]	ext{Hp-Hb}$.

B. Exponential expression of the radioactivity elimination. For designations see Fig. 1B.

Fig. 4. Effect of aggregates of denatured albumin (Agr-Alb) on clearance of equine haemoglobin in the hen.

A. Clearance curves following administration of Agr-Alb given in excess: a, 0.2 mg of $[^{125}\text{I}]	ext{Hb}$ simultaneously with 110 mg of Agr-Alb; b, 110 mg of Agr-Alb followed after 30 min by 0.2 mg of $[^{125}\text{I}]	ext{Hb}$; C, control, 0.2 mg of $[^{125}\text{I}]	ext{Hb}$.

B. Exponential expression of the radioactivity elimination. For designations see Fig. 1B.
Table 1

<table>
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<th>Exp. no.</th>
<th>125I-labelled protein preparation (mg)</th>
<th>Time of binding of the dose (h):</th>
<th>Half-life of the dose (h):</th>
<th>Slope***</th>
<th>Intercepts***</th>
<th>Maximum difference between the control and experimental clearance curves (% of total dose)***</th>
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<td>z</td>
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* Control: 125I-labelled protein alone, a mixture of the labelled protein and 110 mg of Agr-Alb b 110 mg of Agr-Alb and after 30 min, the labelled protein.

** Calculated from the equation (du Souich et al., 1981): 

\[ C_t = A e^{-t} + B e^{-t} \]

*** Indicates increased clearance of the labelled protein.
chemically modified albumin (Iio et al., 1963; Buys et al., 1975; Praaning-van Dalen & Knook, 1982). Heat-denatured bovine albumin was frequently used to inhibit protein endocytosis by rat liver RES cells. Intravenous administration of this albumin preparation had no effect on clearance of asialothyroglobulin (Tatumi et al., 1979), asialocoeuruloplasmin (Gregoriadis et al., 1970), asialo-α1-antitrypsin (Gan, 1979) and rat Hp-Hb complex (Tsunoo et al., 1977), however endocytosis of α1-antitrypsin-trypsin and asialo-α1-antitrypsin-trypsin complexes was inhibited (Gan, 1979).

Regoeczi et al. (1975) found that the half-life span of chicken and human orosomucoid in the circulation of chicken are the same, whereas chicken orosomucoid in the rabbit is cleared much more rapidly than human orosomucoid. This points to significant differences in catabolism of glycoproteins in liver of birds and mammals.

Previously, we have found that the half-life of mammalian Hp and Hp-Hb in chicken do not differ (Dobrzszycka et al., 1981), whereas in mammals clearance of both homologous and heterologous Hp-Hb complexes is more rapid than of Hp (Dobrzszycka et al., 1979). Catabolism of haptoglobin is started in chicken by enzymatic splitting of sialic acid and galactose, and binding of agalacto-Hp by specific receptor of hepatocytes (Lunney & Ashwell, 1976). The homologous Hp-Hb complex is rapidly cleared from chicken circulation (unpublished), probably by binding with a specific liver receptor. The occurrence of specific receptors for Hp-Hb in rat liver was reported by Kino et al. (1980) and Lowe & Ashwell (1982). In our opinion, the mammalian Hp-Hb complex is eliminated from circulation by liver RES cells of chicken because of its heterology, and this process is slower than interaction of the Hp-Hb complex with a homologous receptor.

Presently we have found that inhibition of protein endocytosis in hen liver Kupffer cells and endothelial cells by denatured albumin resulted in a partial inhibition of the clearance of mammalian Hp and Hp-Hb complex (Figs. 2 and 3); with both proteins the decrease was similar and did not exceed 30% of the dose applied. This indicates that RES cells are involved in catabolism of heterologous equine Hp and Hp-Hb in the hen. Acceleration of the clearance of Hp and Hp-Hb in the hen by injection of denatured albumin before glycoprotein injection supports the involvement of RES in catabolism of mammalian Hp and Hp-Hb in chicken. It is known (Iio et al., 1963; du Souich et al., 1981) that administration of high doses of the substances phagocytized by RES stimulates capacity of this system for phagocytosis. Low decrease in the clearance of Hp and its complex with Hb suggests the existence in hen liver of some other alternative routes of metabolism of heterologous glycoproteins, i.e. catabolism of agalactoglycoproteins, endocytosis in RES, or binding by specific receptors. Block of one metabolic pathway results in enhanced catabolic transformations of Hp or Hp-Hb complex by an alternative pathway, similarly as in the case of asialotrans-
ferrin 3 or glucose-albumin, a neoglycoprotein, in rat (Regoeczi et al., 1982; Schlesinger et al., 1980).

The homologous Hp-Hb complex in rat is bound to specific receptors and the clearance rate is dose-independent when receptors are in excess, in this case therefore RES does not participate in glycoprotein catabolism. In mammals, the Hp-Hb complex bound by the receptor is probably degraded in the Golgi fraction of hepatocytes without prior separation of haem (Oshiro & Nakajima, 1981). Under these conditions, the excess of free Hb not complexed with Hp is taken up by Kupffer cells or excreted by kidney proximal cells (Seki et al., 1979).

Clearance of mammalian Hb from hen circulation was inhibited in part by denatured albumin, similarly as clearance of Hp and Hp-Hb. This also confirms participation of RES in catabolism of Hb. Only partial inhibition of Hb clearance might be explained by the involvement of an alternative pathway of haemoglobin clearance, i.e. its excretion through kidney.

REFERENCES


Wpływ blokady komórek sinusoidalnych wątroby na katabolizm haptoglobinu i hemoglobiny ssakowej u kur

Streszczenie

Katabolizm ssakowej haptoglobininy (Hp), kompleksu haptoglobininy z hemoglobiną (Hp-Hb) i hemoglobiny (Hb) u kur był badany za pomocą blokowania endocytozy białka w układzie retikuloendothelialnym (RES) wątroby przez agregaty zdenaturowanej albuminy (Agr-Alb).
Określano kinetykę eliminacji wyżej wymienionych preparatów znakowanych $^{125}\text{I}$, podawanych w kombinacjach z Agr-Alb i wyznaczano ich okresy półtrwania. Stwierdzono, że dożynne podanie Agr-Alb w mieszaninie z $^{125}\text{I}-\text{Hp}$, $^{125}\text{I}-\text{Hp-Hb}$ lub $^{125}\text{I}-\text{Hb}$ powodowało częściowe zahamowanie ich eliminacji z krwiobiegu kury. Podanie znakowanych białek w 30 min po Agr-Alb zmniejszało hamowanie na skutek stymulacji RES przez Agr-Alb. Eliminacja obco-gatunkowych białek (Hp, Hp-Hb, i Hb) z krwiobiegu kury przez RES jest jedną z kilku możliwych dróg ich katabolizmu.

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