during the first 4 weeks of experiments lasting 8 weeks. During the 4-8 week or post injection period, the immunized chicks gained 905 g *vs.* 777 g for their controls and were more efficient in converting feed to weight gain. In 2 successive experiments immunized rats grew slightly faster and showed a difference in feed efficiency which was statistically significant. In both chicks and rats there were no differences in body weight during the 0-4 week injection period. Antibodies to urease were demonstrable in sera of immunized rats by *in vivo* methods with C-14 urea and by *in vitro* tests. Soybean diets were fed.

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Haptoglobin Hemoglobin Metabolism in Rabbits Studied with I^{131} and Fe^{59} Labelled Complexes.* (26047)

EDWARD C. FRANKLIN, MURRAY ORATZ, MARCUS A. ROTHSCHILD AND DOROTHEA ZUCKER-FRANKLIN

Department of Medicine and Rheumatic Disease Study Group, N.Y.U. School of Medicine, Radioisotope Service, VA Hospital, New York, and Department of Hematology, Montefiore Hospital, Bronx, New York

Several studies(1-3) attempting to trace the fate of haptoglobin-hemoglobin (HpHb) in man have pointed to the complexity of the problem. They have shown that the complex disappears from the blood either in a linear or exponential manner with a half life of about 4 hours, and it has been suggested that the complex may be removed *in toto* by the reticuloendothelial system(1). Studies in man have been restricted to short periods of observation because of the small amounts of hemoglobin that can be injected safely. The present experiments with I^{131}-labelled haptoglobin-hemoglobin (I^{131}HpHb) and haptoglobin Fe^{59} labelled hemoglobin (HpHbFe^{59}) complexes were undertaken in rabbits to follow the metabolism of the complex over longer periods of time. Attempts were also made to evaluate more directly the role of the reticuloendothelial system in the metabolism of the complex.

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Materials and methods. Concentration of haptoglobin in serum was determined by starch zone electrophoresis on blocks, 45 cm x 33 cm, using potato starch and barbital buffer pH 8.6 μ 0.05 at 400 volts for 15-18 hours(4). Increasing amounts of hemoglobin were added to 0.1 ml aliquots of serum, and the mixtures were introduced into 3 parallel rows of 15 slits. After electrophoresis, control sera containing hemoglobin in 3-fold excess of the combining capacity demonstrated

2 red spots. Corresponding test segments were eluted with 2-3 ml of 0.15 N NaCl and tested with benzidine. Forty-five specimens were studied simultaneously and as little as 1 mg haptoglobin/100 ml serum could be detected. Hemoglobin binding capacity was expressed as the largest amount of hemoglobin bound by the more rapidly migrating fraction. α_2 globulins were isolated from non-hemolized type 1-1 human serum by starch zone electrophoresis(4). Iodination of α_2 globulins and rabbit serum albumin at levels of less than 1 atom of iodine/molecule of protein was performed by standard methods(5). Unbound iodide was removed by passage through an ion exchange resin. Following addition of hemoglobin C, the iodinated α_2 globulins were re-electrophoresed to effect separation of I^{131} HpHb from the remainder of the I^{131} α_2 globulins(4). While separation was relatively complete, it seems likely that the haptoglobin was contaminated with about 10% slowly migrating α_2 globulin.

Hemoglobin was prepared by the method of Drabkin(6) from thoroughly washed erythrocytes from mice, and humans homozygous for hemoglobin A or C. Fe^{59} labelled hemoglobin was prepared in a similar manner from erythrocytes of mice 48 hours after intraperitoneal injection of mouse plasma containing 100 to 130 μc Fe^{59} buffered to pH 5.6. Five to 20 mg of hemoglobin containing 2 to 15 μc of Fe^{59} were mixed with haptoglobin prior to injection into the rabbits. I^{131} labelled proteins were assayed in a well type scintillation counter with a sensitivity for I^{131} of 9.05 x 10^5 c/min/μc above a background of 180 c/min. I^{131} and Fe^{59} combinations were assayed in a pulse height analyser. The base line and channel width were adjusted to settings such that Fe^{59} was counted free of I^{131} and a 15% correction for Fe^{59} was made at the I^{131} peak.

Study design. Tracer studies were performed in 13 rabbits weighing 2-5 kg. Since concentration of plasma haptoglobin was low (ranging from 5-30 mg/100 ml in 20 animals), non-radioactive haptoglobin was injected in the form of human serum, or whole α_2 globulins, to raise plasma concentration of the complex to approximately 25-50 mg/100 ml. Two to 25 mg HpHb labelled with either 30-100 μc of I^{131} (8 studies) or 5-10 μc Fe^{59} (5 studies) were injected into an ear vein with sufficient hemoglobin to saturate the plasma haptoglobin. Heparinized blood was obtained at 2-5 minute intervals from the ear opposite to the side of injection for 30 minutes and at greater intervals for 4-6 days. Radioactivity was determined in plasma and following precipitation with cold 20% trichloracetic acid (TCA). In specimens containing Fe^{59}, precipitation with TCA was performed after hydrolysis with 2 N HCl to release Fe^{59} bound to transferrin(7).

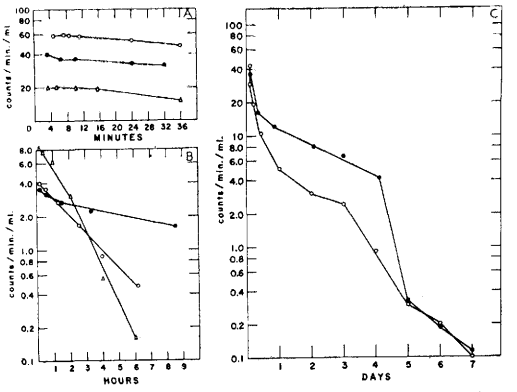

FIG. 1. Typical plasma disappearance curves for I^{131} HpHb (○), I^{131} α_2 globulins (●) and HpHb-Fe^{59} (△). While an initial rapid decrease in plasma concentrations was not observed (1A) plasma concentrations of I^{131} HpHb and HpHb-Fe^{59} decreased exponentially for 6-8 hr following inj. (1B). Three to 4 days following inj. plasma concentration of iodinated proteins fell sharply (1C, see text). I^{131} HpHb and I^{131} α_2 globulin plasma concentrations are expressed as counts/min./ml × 10^{-3} and HpHb-Fe^{59} as counts/min./ml × 10^{-2}.

The effect of HpHb on rate of clearance of carbon was studied in 200-300 g rats according to the method of Biozzi, Benacerraf and co-workers(8). Ten to 12 mg carbon particles per 100 g body weight were injected intravenously. Following the appearance of a steady rate of removal of carbon, 15-35 mg of HpHb were injected intravenously, and the effect on rate of carbon clearance was noted. The role of the liver in removing HpHb from the plasma was studied in 2 rabbits, by injecting a mixture of albumin I^{131} and HpHb Fe^{59} into the portal vein and drawing blood immediately from the hepatic vein. The ratio Fe^{59}/I^{131} was determined in the injectate and hepatic vein samples.

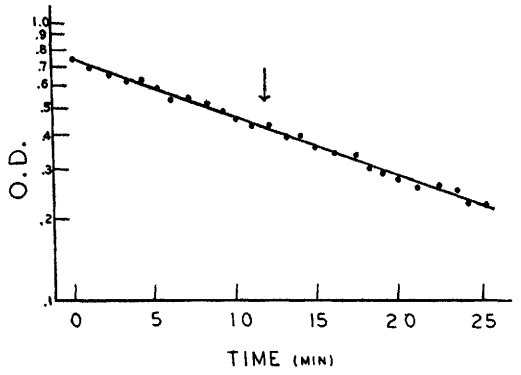

FIG. 2. Typical carbon clearance study. No interference with clearance of carbon particles from the blood occurred following inj. (↓) of haptoglobin hemoglobin complex.

Results. During the first 20 minutes following injection, concentration of the labelled haptoglobin complexes and α_2 globulins did not decrease sharply (Fig. 1a), and the Fe^{59} Hb label disappeared from the plasma exponentially with a mean half time of 110 ± 37 minutes (SD) (Fig. 1b). Studies with HpHb Fe^{59} were limited to an 8 hour period due to the rapid labelling of erythrocytes with Fe^{59} released from the complex. Following injection, plasma concentration of I^{131} HpHb decreased more or less exponentially with a mean half time of 78 ± 26 minutes (SD) reaching levels of 5-20% of initial plasma concentration after 6-8 hours (Fig. 1 b).† Thereafter the rate of fall of plasma I^{131} decreased and the plasma decay curve demonstrated a slower component lasting 1 to 3 days (Fig. 1c). A similar slow component was observed in 3 rabbits injected with I^{131} labelled α_2 globulins (Fig. 1 c). Three to 4 days after injection, the residual plasma radioactivity from both I^{131} HpHb and I^{131}-labelled α_2 globulins rapidly disappeared (Fig. 1c), suggesting development in the rabbit of antibodies against the human proteins. No TCA precipitable radioactivity was recovered in the urine.

Role of liver and reticuloendothelial system in metabolism of haptoglobin hemoglobin complex. Injection of haptoglobin hemoglobin complex into 3 rats during the steady state of carbon clearance had no effect on the latter (Fig. 2). Thus, in the amounts used, there was no evidence that the complex significantly influenced clearance of carbon particles by the reticuloendothelial system. In 2 studies in rabbits in which I^{131} rabbit serum albumin and HpHb Fe^{59} were injected into the portal vein, the ratio of Fe^{59} to I^{131} in the injectate and hepatic vein blood were 1.01 and 1.02 respectively. Thus no evidence for preferential removal of the Fe^{59} labeled complex was obtained in one circulation through the liver. Similar studies with albumin-I^{131} and P^{32} labelled erythrocytes had demonstrated no selective removal of albumin during a single passage through the liver(9).

Discussion. These results indicate that haptoglobin combined with Fe^{59} hemoglobin is removed from the plasma of the rabbit with a half time of about 110 minutes. While the results with I^{131}HpHb are more difficult to interpret because of the presence of small amounts of α_2 globulins, they are sufficiently similar to suggest that the complex is cleared as a whole. The slightly faster plasma disappearance of I^{131}HpHb may reflect some denaturation of the protein during iodination and electrophoresis.

While there may be some reservation in accepting the data obtained with heterologous I^{131} HpHb for these reasons, the close similarity in the fate of both labels suggests that the iodinated protein may also be used to measure endogenous haptoglobin metabolism. Furthermore, recent studies by Murray and associates(10) employing rabbits whose haptoglobin had been raised by injection of turpentine have also yielded comparable values. While the present studies suggest that the complex is removed *in toto,* they fail to characterize its sites of removal or degradation. If the reticuloendothelial system should be shown to play a role, its low affinity for the

† The exact interpretation of the plasma decay curves of I^{131} HpHb would necessitate measurement of the space of distribution of the contaminating I^{131} α_2 globulins. While this is not known, an approximation may be obtained by assuming the same distribution as for albumin, namely an eventual intravascular-extravascular ratio of 40-60. This correction was applied in 4 of the 8 I^{131} HpHb curves, where there were sufficient data. Graphic analyses of the curves resulted in a decrease in mean half time of about 10 minutes.

haptoglobin hemoglobin complex, when small amounts are administered, suggests that a minor component, possibly the bone marrow, may be primarily involved. A final answer to these questions will have to await studies by which the complex can be localized in tissues.

Summary. 1. Haptoglobin hemoglobin complex labelled with Fe^{59} or I^{131} injected into 13 rabbits was removed from the blood with half times of 110 minutes and 78 minutes respectively. 2. The complex did not significantly interfere with phagocytosis of carbon particles by the reticuloendothelial system and was not selectively removed by the liver during a single passage.

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Incorporation of Selenium-75 into Dog Hair. (26048)

KENNETH P. MCCONNELL AND A. E. KREAMER
Radioisotope Service, Veterans Administration Hospital, and School of Medicine, University of Louisville, Louisville, Ky.

With the proposal that selenium be included among the essential dietary elements (1), interest has been focused on many unanswered questions about the metabolism and fate of selenium in the mammalian organism, especially the chemical nature of selenium compounds in animal tissues. The apparent incorporation of trace amounts of selenium in various tissue proteins has been discussed(2, 3). Continuing these studies, hair which is high in cystine content was examined for selenium incorporation. Hair from Se^{75}-injected dogs was fractionated and assayed for Se^{75}, which was found when cystine-rich protein keratin was isolated as the thiosulfate, S-sulfokerateine. The cystine fraction isolated from the hair by conventional methods contained Se^{75}.

Methods and procedures. The method of Swan(4) was used for the specific and symmetrical fission of disulfide bonds in keratinous tissue. The cystine disulfide bonds in dog hair containing Se^{75} were broken by the reaction with cupric-ammonium sulfite, as illustrated in the equation:

$$RSSR + 2\,Cu^{++} + 2\,SO_3^{=} \rightarrow 2\,RSSO_3^{-} + 2\,Cu^{+}$$

The protein S-sulfokerateine was precipitated from the copper reagent extracts of hair containing Se^{75} by acidification and dilution(4). The protein was then washed repeatedly with water, lyophilized, weighed, and assayed for Se^{75}. A small fraction of the hair that was insoluble in the copper reagent was separated by centrifugation, washed thoroughly with water, lyophilized and counted. The uniformity of the S-sulfokerateine preparations was established by paper electrophoresis analysis. Samples of S-sulfokerateine were dissolved in Veronal buffer (pH 8.6; μ 0.075) and applied to Schleicher and Schuell 2043-A mgl. electrophoresis paper. A constant current of 7 ma was applied for 6 hours. The protein fractions were developed using the standard alcoholic bromphenol blue method. In another experiment, cystine from hair containing Se^{75} was isolated by the method of Gortner and