RAPID HEPATIC TURNOVER OF RADIOACTIVE HUMAN SERUM ALBUMIN IN SENSITIZED DOGS

Kenichi Kitani* and George V. Taplin

Los Angeles Harbor General Hospital, Torrance, California and the University of California, Los Angeles, California

Technetium-99m-HSA solutions are cleared from the plasma at slightly faster rates than ^{131}I-IHSA in normal man and in dogs. HSA solutions labeled with either ^{99m}Tc or with ^{131}I are cleared much more quickly from the plasma of dogs previously sensitized to HSA. Rapid hepatic accumulation of ^{99m}Tc-labeled HSA is demonstrable by sequential gamma camera imaging in sensitized dogs.

The catabolic fate and biologic effect of intravenously administered heterologous protein has been of keen interest to immunologists (*1*). Pathologists have also studied this phenomenon with respect to experimental liver injury produced by foreign protein challenges (*2,3*).

Recent successful trials to treat patients in hepatic coma with heterologous liver perfusion (*4,5*) or cross circulation (*6*) have introduced this problem to the field of clinical medicine. The immunological effects of injecting foreign proteins repeatedly in the same animal species have been reported (*7*).

Some discrepancy exists, however, concerning the organ distribution and blood clearance of injected foreign protein in sensitized animals. It has been assumed that the reticuloendothelial system plays a major role.

This communication provides objective evidence of the rapid hepatic turnover of radioactive human serum albumin in sensitized dogs as demonstrated by sequential liver–abdominal imaging with a gamma camera.

MATERIALS AND METHODS

Radioalbumin preparations. Three different radioalbumins were used in this study: ^{131}I-human serum albumin (IHSA) obtained commercially (Abbott Laboratories, North Chicago, Ill.), ^{99m}Tc-human serum albumin as prepared in the laboratory (*8*), and canine serum albumin (CSA) (Miles Laboratories, Elkhart, Ind.) labeled with ^{131}I in the laboratory and dialyzed overnight for chemical purification according to a method previously reported (*9*).

Experimental procedures. Human serum albumin was administered to seven mongrel dogs of both sexes. Two dogs were injected with 10 mg/kg of body weight and a third with 20/mg/kg of human serum albumin and challenged after 2 weeks. Another three dogs were given 5 mg/kg doses of human serum albumin in particulate form (microaggregated albumin) (*10*) three to five times at 2-week intervals. One dog which had received multiple inhalations of aerosolized albumin was also challenged.

Test procedures. Both ^{131}I- and ^{99m}Tc-albumins were injected simultaneously but in different peripheral veins. The test doses were 50 μCi of ^{131}I and 1 mCi of ^{99m}Tc-albumin. The sequential changes in the organ distribution and plasma disappearance rates were examined by the following procedures.

1. Plasma clearance curves: Blood samples were taken through a peripheral vein at 2, 5, 10, 15, and 30 min after injection and then every hour for 3 hr. Two 1-ml aliquots of plasma were prepared from each blood sample. A 5% tannic acid solution was added to one sample to precipitate the protein after which it was centrifuged and the supernatant was discarded. This procedure was repeated three times. Both plasma samples were then measured in a well scintillation counter. Iodine-131 and ^{99m}Tc were counted simultaneously by using the different photon energy peaks of the two radionuclides. Total and protein-bound plasma ac-

Received May 2, 1974; original accepted May 22, 1974.

For reprints contact: George V. Taplin, Laboratory of Nuclear Medicine and Radiation Biology, 900 Veteran Ave., Los Angeles, Calif. 90024.

* Present address: First Laboratory of Clinical Physiology, Tokyo Metropolitan Institute of Gerontology, 35-2 Itabashi, Tokyo, Japan.

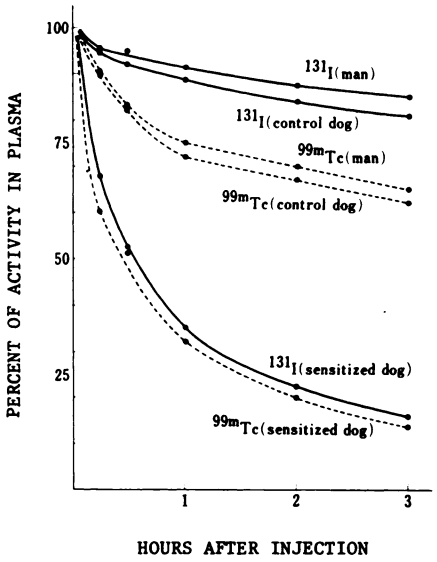

FIG. 1. Plasma clearance rates of HSA. Clearance rates of both ^{131}I- and ^{99m}Tc-albumins in sensitized dog are definitely faster than those in controls.

tivities were expressed in percent of the activity at 2 min which was assumed to be 100%.

2. Continuous recording of ^{99m}Tc activity over the heart and liver areas was performed for 15 min.
3. Sequential images of the chest and abdominal areas were made for 5 hr at timed intervals.

In each experiment, dogs with and without protein sensitization were paired and tested using the same procedures and the same batch of radioindicator. One healthy man was also tested for control purposes.

RESULTS

Figure 1 shows examples of the plasma clearance rates of ^{131}I and ^{99m}Tc-labeled albumins in a normal man, in a control dog, and in one dog previously sensitized to human serum albumin. Figure 2 compares the plasma clearance curves of ^{131}I canine versus human serum albumins in HSA-sensitized dogs. Protein-bound plasma radioactivity values at 1 and 3 hr are listed for each group in percent of the 2-min values (Table 1). The difference in radioactivity values between the total and the protein-bound fraction (the protein-free fraction) was less than 3% for ^{131}I-labeled albumin, whereas the total ^{99m}Tc activity in the plasma exceeded that of the protein-bound fraction by 5–10% during the first 15 min. Thereafter the differences were similar to those with ^{131}I-labeled albumin. The normal plasma clearance of protein-bound ^{99m}Tc was always faster than that of ^{131}I, particularly during the first 30 min (Fig. 1). In sensitized dogs the ^{99m}Tc- and ^{131}I-labeled albumins were both cleared from the blood very rapidly (Fig. 1). By 3 hr, protein-bound plasma activity levels fell to about 15% of the 2-min values. Canine albumin in an HSA-sensitized dog (Fig. 2) is cleared from the blood very slowly, the same as it is in control dogs or as human serum albumin is cleared in normal man.

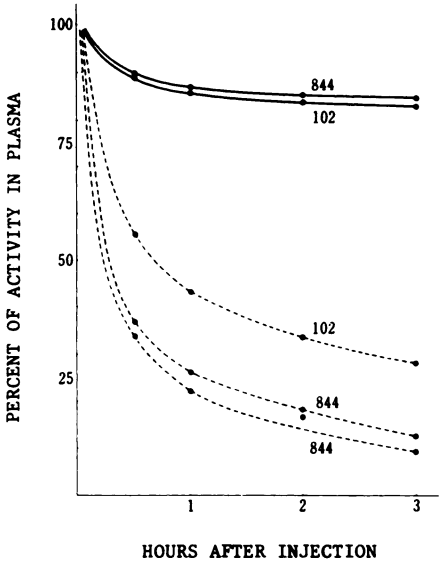

FIG. 2. Comparison of plasma clearance rates of canine versus human serum ^{131}I-albumins in dogs sensitized to HSA. (Numbers near curves indicate dog number under study.) Same dogs were studied at 2-week intervals. HSA-sensitized dogs show rapid clearance rates for HSA (- - -) but not for CSA (——).

TABLE 1. PROTEIN-BOUND PLASMA RADIOACTIVITY

Agent	Number tested	1-hr values (%) mean s.d.	3-hr values (%)* mean s.d.
^{131}I-IHSA† in man	5	85.30 ± 1.50	80.00 ± 2.10
^{99m}Tc-HSA in man	5	74.22 ± 1.67	67.20 ± 1.29
^{131}I-IHSA in control dog	7	86.84 ± 1.59	79.50 ± 2.58
^{99m}Tc-HSA in control dog	7	78.37 ± 2.09	66.42 ± 2.39
^{131}I-CSA‡ in sensitized dog	4	84.13 ± 1.55	79.50 ± 1.80
^{131}I-IHSA in sensitized dog	7	41.50 ± 13.12	23.91 ± 13.26
^{99m}Tc-HSA in sensitized dog	10	33.05 ± 12.44	15.28 ± 14.00

* Radioactivity values of 2-min plasma sample assumed to be 100%.
† Human serum albumin, HSA.
‡ Canine serum albumin, CSA.

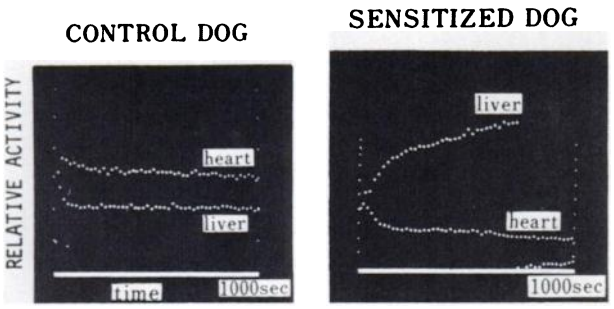

FIG. 3. Changing ^{99m}Tc activity levels over heart and liver area. In control dog (left), heart and liver curves fall rapidly to plateau indicating only initial mixing and dilution in blood pools. In sensitized dog (right), liver curve ascends rapidly while heart blood pool levels decrease in reciprocal relation.

The effect of sensitization on altering the plasma clearance rates was observed in three dogs which had been injected repeatedly with colloidal albumin and in one dog given a single dose of 20 mg/kg of HSA. One other dog that had been given multiple inhalations of HSA and a subsequent challenging dose of macroaggregated albumin also exhibited an accelerated clearance rate of HSA but to a lesser degree than the other sensitized dogs. The plasma clearance rates in two dogs given a single 10 mg/kg-dose of HSA by injection did not differ significantly from the normal control values indicating that sensitization had not developed.

Figure 3 shows changing ^{99m}Tc activity levels in the liver and heart areas. On the left (a control dog), the amounts of activity in the liver and heart fell rapidly to plateau levels indicating only mixing and dilution of labeled albumin in the blood. On the right, the heart and liver curves of a sensitized dog show rapid accumulation of activity in the liver and a reciprocal decrease from the heart.

Figure 4 displays sequential heart and liver scintiphotos in a control dog. Slight reductions in heart and liver activity levels are visible. However, after 3 hr, most of the activity still remains in the blood and the heart and liver images represent blood pools only.

Figure 5 shows sequential liver images of a dog given 20 mg/kg of HSA 2 weeks previously. Immediately after ^{99m}Tc-HSA injection, activity accumulated in the liver as if a 1–5 μm-size aggregated albumin suspension had been injected. The 10-min image shows that activity in the heart had nearly disappeared and the gallbladder fossa is visible as an area of reduced activity. Activity continued to accumulate in the liver in the 1- and 3-hr images whereas the gallbladder area became visible as a discrete region of relatively high activity during the same period.

Figure 6 shows liver–abdominal images at 3 hr in a sensitized dog. Collections of radioactivity are seen in the upper abdominal regions which have the configuration of the small intestine. Figure 7 displays sequential images of the liver and chest in another sensitized dog following the injection of ^{99m}Tc-albumin. The rapid accumulation of activity in the liver and reciprocal decrease from the heart is apparent in the 1, 3, 10, and 20-min images. However, less activity is seen in the heart area compared with that in the lungs at 10 and 20 min. Lung deposition was not visible in the 1-hr image which showed the liver only. Thus, lung deposition in sensitized dogs is transitory and suggests that small (9–15 μm) particles might be involved.

DISCUSSION

The plasma clearance curves in this study were plotted using the assumption that the activity of the 2-min plasma sample equals 100%. This value is acceptable in control studies where mixing is almost complete by this time and the blood levels remain nearly constant thereafter. In sensitized animals, however, the 100% 2-min value is not accurate because of the rapid blood clearance rate as demonstrated in the continuously recorded curves (Fig. 3).

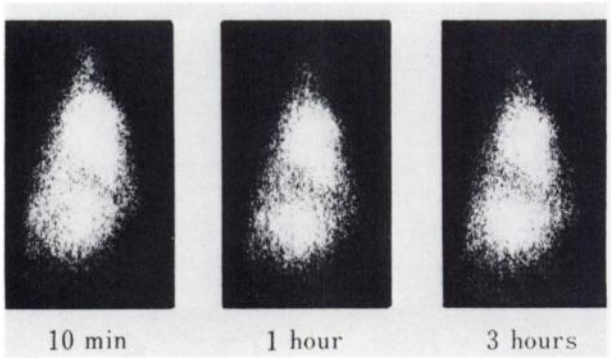

FIG. 4. Sequential images of liver and heart of control dog. Following ^{99m}Tc-HSA injection, both organ images reflect retention of activity in vascular compartments.

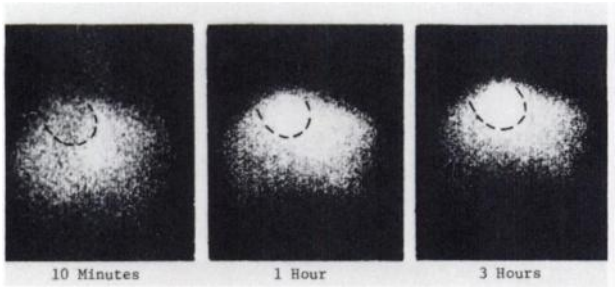

FIG. 5. Sequential images of liver of sensitized dog. Following ^{99m}Tc-HSA injection 10-min image shows discrete area of reduced activity (gallbladder fossa). Images at 1 and 3 hr show gallbladder region as area of increased activity.

Here the 2-min value represents about 50% clearance. Consequently, the actual plasma clearance rates in sensitized dogs are much more rapid than the plotted curves. Nevertheless, the differences in the clearance rates between control and sensitized dogs are highly significant.

Dixon (*1*) reported the abrupt disappearance of radioiodinated bovine albumin in rabbits beginning 9–10 days after injection. Law, et al (*11*) also reported the rapid blood disappearance of ^{131}I-bovine serum albumin in sensitized rabbits. In the present study, antibody titers of the dog sera were not measured. However, the slow clearance of ^{131}I canine serum albumin in HSA-sensitized dogs (Fig. 2 and Table 1) indicates that these animals were not sensitized to their own albumin. The rapid plasma disappearance rate of ^{99m}Tc-HSA in these dogs is an index of their degree of HSA sensitization.

The organ distribution of injected foreign proteins in sensitized animals has also been studied by several investigators. Haurowitz, et al (*12*) found that the deposition of ^{131}I-iodo-ovalbumin in the liver of a sensitized rabbit is almost the same as that in the spleen and kidney when expressed as activity per gram tissue weight. Garvey, et al (*13*) found only 20% of the injected activity in the liver 6 hr after the injection of ^{35}S-labeled hemocyanin-*p*-azo-phenylsulfonate in a rabbit sensitized to this material. Steiner, et al (*14*) stressed the deposition of heterologous albumin in the lung capillaries of sensitized animals.

Rapid accumulation of radioactive HSA in the liver is demonstrable by sequential imaging and external monitoring. Although such measurements are not quantitative, the amount of injected protein removed by the liver is probably greater than that reported in other studies. One possible cause of this difference may be related to the dose of challenging protein injected. The doses employed in our experiments of 1 mg/dog or less are smaller than those used in other studies. The most comparable doses are the few milligrams per rabbit used by Law, et al (*11*). The blood disappearance rates in our study are more rapid but similar to those of Law (*11*) who

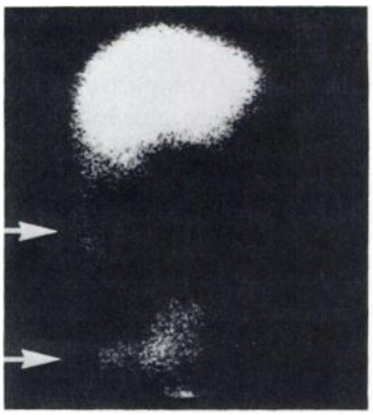

FIG. 6. Liver–abdominal images at 3 hr in sensitized dog. Traces of ^{99m}Tc activity are seen in regions of small intestine with most of activity remaining in liver.

found 5–12% of the initial activity 5 hr after injection.

Another possible factor which could influence the distribution of the injected protein might be the serum antibody titers. Garvey and Campbell (*15*) found greater localization of the injected protein in the immune liver and lung (particularly the latter) as the serum antibody titer of the test animal was increased. Our findings do not add new information concerning the relation between antibody titers and tissue distribution. However, the slight and transient lung retention seen soon after injection (Fig. 7) suggests that organ distribution should be measured dynamically and especially during the first 20 min after injection. The imaging procedures employed in our studies are valuable because they clearly depict changes in organ distribution by a technique which creates no functional impairment.

Although the exact mechanisms of biliary excretion of ^{99m}Tc-HSA remain unknown, a likely hypothesis is that particulate antigen–antibody complexes are formed intravascularly and then are engulfed by the liver's Kupffer cells in a manner similar to other particulates such as bacteria (*16*), red blood cells (*17*), microaggregates of HSA, and immune complexes of foreign proteins (*18*).

The transient deposition of ^{99m}Tc-albumin in the

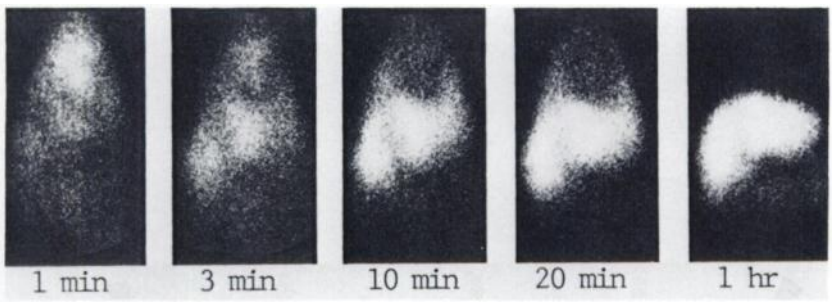

FIG. 7. Sequential heart–lung–liver images of sensitized dog. ^{99m}Tc activity in heart disappears by 10 min but is transiently retained in lungs for at least 20 min, and almost totally deposited in liver by 1 hr.

lung may be caused by one of two main mechanisms. First, the RE cells of the lung may phagocytize and metabolize the immune complexes of albumin. The second and more likely mechanism is that the lung capillaries temporarily trap particulate albumin–globulin complexes if they are slightly larger (9–12 μm) than the 6–9 μm-size diameters of the capillary lumena.

The rapid decrease in lung activity also may involve a mechanical mechanism, namely fragmentation of these small, fragile aggregates which then reenter the general circulation. In animal experiments wherein radioactive 10–25 μm-size albumin suspensions are injected intravenously, the aggregates are first trapped in the arteriolar–capillary bed of the lungs. They are then released from the lung during the first hour and subsequently localize in the liver and spleen (*19*). With slightly smaller (9–15 μm) size albumin–globulin complexes, a similar mechanical mechanism could account for the faster lung removal rates (*20*).

CONCLUSION

Immediately following intravenous injection of ^{99m}Tc-HSA solutions in HSA-sensitized dogs: (A) Particulate albumin-immune globulin complexes are presumed to be formed intravascularly and phagocytized by the liver's Kupffer cells. (B) They are then believed to be catabolized by proteolytic digestion to smaller molecular-size protein degradation products which could possibly be transferred to the polygonal cells. (C) The ^{99m}Tc label is secreted in the bile still attached to some unidentified albumin–globulin degradation product(s). (D) Further investigation is needed to verify these hypotheses or to establish the exact mechanisms involved in the rapid hepatobiliary turnover of HSA solutions in HSA-sensitized dogs.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Gordon Lindenblad of Mallinckrodt/Nuclear, St. Louis, Mo.; to H. Maroon and G. Bruno of E. R. Squibb and Sons, New York, N.Y., for donating supplies of radionuclides used in these studies; to Michael D. Cragin and Winona Victery who kindly labeled canine albumin and to Mary Lee Griswold for preparing the illustrations; and to the GEN-12 Contract between the United States Atomic Energy Commission and the University of California for major support of this project. Part of this work was presented at the 19th Annual Meeting of the Society of Nuclear Medicine, in Boston, Massachusetts, June 1972.

REFERENCES

1. DIXON FJ: The use of I^{131} in immunologic investigation. *J Allergy Clin Immunol* 24: 547–555, 1953

2. BLACKWELL JB: Cirrhosis resulting from repeated injection of antigen. *J Pathol Bacteriol* 90: 245–257, 1965

3. PARONETTO F, POPPER H: Aggravation of hepatic lesions in mice by in-vivo localization of immune complexes (Auer Hepatitis). *Am J Pathol* 47: 549–563, 1965

4. ABOUNA GM, FISHER LM, STILL WJ, et al: Acute hepatic coma successfully treated by extracorporeal baboon liver perfusions. *Br Med J* 1: 23–25, 1972

5. ABOUNA GM, COOK JS, FISHER LM: Treatment of acute hepatic coma by ex-vivo baboon and human liver perfusions. *Surgery* 71: 537–546, 1972

6. HUME DM, GAYLE WE, WILLIAMS GM: Cross circulation of patients in hepatic coma with baboon partners having human blood. *Surg Gynecol Obstet* 128: 495–517, 1969

7. ABOUNA GM, BOEHMIG J, AMEMIYA H, et al: Langzeitige Leberunterstützung durch Multispezies ex-vivo Leberperfusionen. *Acta Hepatosplenol (Stuttg)* 18: 336–337, 1971

8. YAMADA H, MACDONALD NS, TAPLIN GV: Factors in improving yield and quality of ^{99m}Tc-labeled serum albumin. *J Nucl Med* 10: 454, 1969

9. BALE WF, HELMKAMP RW, DAVIS TP, et al: High specific activity labeling of protein with I-131 by the iodine monochloride method. *Proc Soc Exp Biol Med* 122: 407–414, 1966

10. TAPLIN GV, DORE EK, JOHNSON DE: Clinical studies of reticuloendothelial functions with colloidal suspensions of human serum albumin ^{131}I. *AEC Report, Biology and Medicine UCLA*, 1961, p 489

11. LAWS JO, WRIGHT GP: The disposal and organ-distribution of radioiodinated bovine serum proteins in control and specifically-sensitized rabbits. *Br J Exp Pathol* 33: 343–353, 1952

12. HAUROWITZ F, CRAMPTON CF: The fate in rabbits of intravenously injected 131-I-iodoovalbumin. *J Immunol* 68: 73–85, 1952

13. GARVEY JS, CAMPBELL DH: Studies of the retention and properties of 35-S labeled antigen in livers of immunized rabbits. *J Immunol* 76: 36–45, 1956

14. STEINER JW: Investigation of allergic liver injury I. Light, fluorescent, and electron microscopic study of the effects of soluble immune aggregates. *Am J Pathol* 38: 411–436, 1961

15. GARVEY JS, CAMPBELL DH: The relation of circulating antibody concentrations to localization of labeled (S-35) antigen. *J Immunol* 72: 131–138, 1954

16. JEUNET FS, CAIN WA, GOOD RA: Reticuloendothelial function in the isolated perfused liver. 3. Phagocytosis of Salmonella typhosa and Brucella melitensis and the blockade of the reticuloendothelial system. *J Reticuloendothel Soc* 6: 391–410, 1969

17. HALPERN BN, BIOZZI G, BENACERRAF B, et al: Phagocytosis of foreign red blood cells by the reticuloendothelial system. *Am J Physiol* 189: 520–526, 1957

18. WEISER RS, LAXSON C: The fate of fluorescein-labeled soluble antigen-antibody complex in the mouse. *J Infect Dis* 111: 55–58, 1962

19. KITANI K, TAPLIN GV: Biliary excretion of ^{99m}Tc albumin microaggregate degradation products (A method for measuring Kupffer cell digestive function?). *J Nucl Med* 13: 260–265, 1972

20. KITANI K, TAPLIN GV: Catabolic pathway differences between ^{131}I and ^{99m}Tc labeled albumin colloids and microaggregates. *J Nucl Med* 13: 313–317, 1972