CATABOLIC PATHWAY DIFFERENCES BETWEEN ^{131}I- AND ^{99m}Tc-LABELED ALBUMIN COLLOIDS AND MICROAGGREGATES

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Radioiodinated albumin suspensions have been used for 10 years for liver-spleen-bone marrow scanning and longer for studying reticuloendothelial system (RES) functions. The colloidal (0.01–0.02 micron) size preparation has been most extensively studied regarding its catabolism (*1–5*). The release rate of radioactivity from the liver following intravenous administration of ^{131}I- (or ^{125}I-) albumin colloids has been used as an index of Kupffer cell digestive function (*1–5*). Little is known about the catabolic pathways of ^{99m}Tc-albumin suspensions. This paper describes several differences in the catabolism of ^{131}I- compared with ^{99m}Tc-labeled albumin suspensions as determined in anesthetized dogs.

MATERIALS

Four types of radioalbumin suspensions were used. Iodine-131-albumin suspensions were made from commercial ^{131}I-human serum albumin and prepared in our laboratory by a method described previously (*2,4*). Colloidal albumin suspensions were prepared by the following procedure. After adjusting the pH of the 0.1% radioalbumin to 9.0–10.0, the solution was heated in a boiling water bath for 10 min with gentle shaking. After cooling to room temperature, the pH was adjusted to 7.5. The material is slightly opalescent. Colloidal suspensions made by this method have an aggregate size of 0.01–0.02 microns. Microaggregate suspensions were prepared by the method of Yamada, et al (*6*). Briefly, 0.1% radioalbumin at pH 5.2 is heated in a boiling water bath for 3.5 min with shaking which produces aggregates in the 10–20-micron size range. After cooling to room temperature, the suspension is ultrasonicated for 5 min to reduce aggregate size to the 1–5-micron range. More than 90% of the radioactivity can be removed from the suspension by centrifugation at 3,000 rpm for 10 min.

METHODS

Fourteen mongrel dogs of both sexes were studied following an overnight fast. After intravenous injection of each one of the radioalbumin preparations ($<$1.0 mg albumin/dose), the following procedures were performed.

1. Blood radioactivity determinations: Heparinized blood samples were obtained at 2-min intervals for the first 10 min, then at 15, 20, 30, 45, 60 min, and thereafter each 60 min for 4 hr. Two 1.0-ml plasma samples were prepared from each blood sample by centrifugation. The protein fraction of plasma samples was separated by repeated (three times) precipitation with tannic acid (5%) and centrifugation. Plasma activity in the microaggregate study was determined from whole-blood samples and corrected for the hematocrit value of each sample. Samples were assayed in a well scintillation counter.

2. Liver monitoring: Continuous recording of radioactivity over the liver area was performed with a collimated scintillation detector or a scintillation camera. Care was taken to position the collimated detector in a manner to avoid the registration of activity in the stomach for the ^{131}I preparations and for gallbladder activity with the ^{99m}Tc preparations.

3. Sequential abdominal imaging: Imaging was performed intermittently for 5 hr after injection using a Dynacamera II.

These three procedures were performed separately on different occasions. Some studies were done simultaneously when technically possible: for example, blood sampling and continuous external monitoring.

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TABLE 1. BLOOD DISAPPEARANCE RATES FOR THE FOUR RADIOALBUMIN PARTICLE SUSPENSIONS

	$T_{1/2}$ (min ± s.d.)	No. of animals
^{131}I-albumin colloids	4.3 ± 0.5	6
^{131}I-albumin microaggregates	2.3 ± 0.4	5
^{99m}Tc-albumin colloids	4.5 ± 0.3	7
^{99m}Tc-albumin microaggregates	2.5 ± 0.3	8

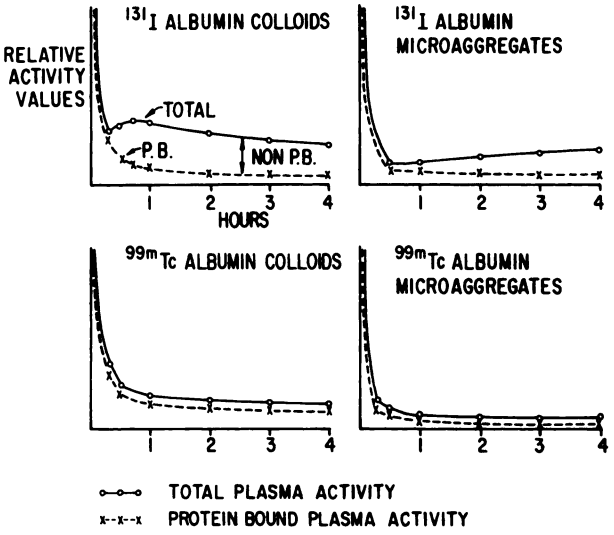

FIG. 1. Total and protein-bound plasma activity levels after i.v. injection of four different radioalbumin preparations.

4. Separate sequential imaging studies were performed during the first 5 hr using ^{99m}Tc-pertechnetate, ^{99m}Tc-albumin, and ^{99m}Tc-albumin macroaggregates for comparative purposes.

All dogs were pretreated with perchlorate for the technetium studies and with sodium iodide for those with ^{131}I. In three dogs, bile was collected via a common bile duct catheter between 3 and 4 hr following the injection of ^{99m}Tc-albumin colloid. The bile samples were examined by paper chromatography using an 85% methyl alcohol-15% water solvent. Radioactivities in the chromatograms were recorded by a chromatoscanner. The ^{99m}Tc-pertechnetate was mixed with dog bile samples obtained before radioactive material injection, and similar chromatographic studies were performed.

RESULTS

Blood disappearance rate differences. The half-time values ($t_{1/2}$) of the initial plasma radioactivity disappearance curves are summarized in Table 1. Both albumin microaggregate preparations had shorter $t_{1/2}$ values than those of colloidal size. The differences in half-time values for albumin suspensions of the same size were insignificant regardless of the label.

Figure 1 shows the sequential changes in the plasma activity. In the case of the ^{131}I-colloid, non-protein-bound or "free" iodide activity appeared in the blood 15–20 min after injection and was the main cause of the secondary rise of total plasma activity, which remains elevated for several hours. The ^{131}I-albumin microaggregate preparations showed a similar increase of free ^{131}I in plasma but at much slower rates. With both ^{99m}Tc suspensions, a secondary rise of plasma activity *was not observed,* and both the total and protein-bound activity levels continued to fall.

Liver release rate differences. Figure 2 shows the liver release rates of radioactivity for the four different albumin preparations. Counting rates for ^{99m}Tc preparations were corrected for physical decay. The ^{131}I-albumin colloid curve shows a rapid decline, beginning 15–20 min after injection, and by 3 hr it decreased to 40% of the peak level. The ^{131}I-microaggregate curve, on the other hand, shows a much slower decrease. By 3 hr only 15% of the maximum amount had disappeared from the liver. The ^{99m}Tc-albumin colloid curve shows a surprisingly slow liver rate, which is nearly the same as those of the two microaggregate preparations. The ^{99m}Tc-microaggregate preparation has the slowest liver release rate.

Sequential abdominal imaging. Figures 3 and 4 show the abdominal images obtained with four different preparations. With the ^{131}I-colloid, the liver image becomes indistinct within 1 hr and by 2–3 hr activity in the stomach region becomes well visual-

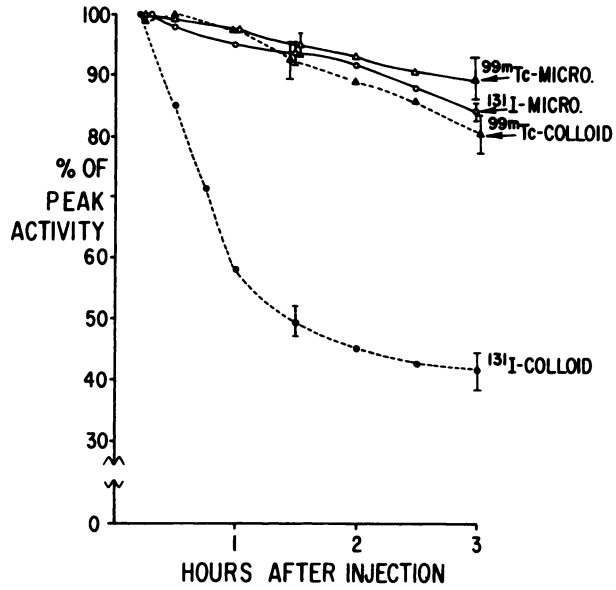

FIG. 2. Liver activity release rates of four different radioalbumin preparations.

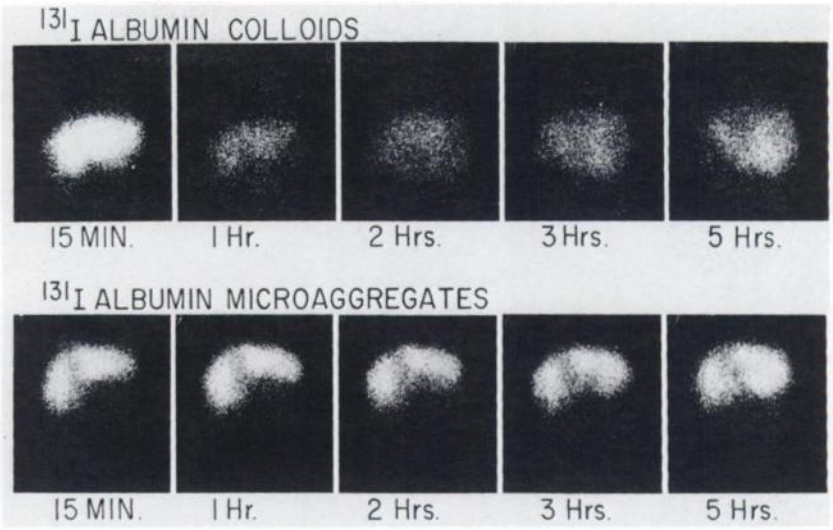

FIG. 3. Sequential abdominal images with ^{131}I-albumin colloids and microaggregates.

ized. Images following ^{131}I-microaggregate injection show similar patterns, but radioactivity in the stomach area is visualized much later (4–5 hr) and in smaller amounts (Fig. 3). The ^{99m}Tc suspensions show identical *liver* patterns. However, instead of revealing activity in the stomach, in 2–3 hr the gallbladder becomes visible (Fig. 4). The amounts of activity entering the gallbladder region are greater with the colloidal size preparations than with ^{99m}Tc-microaggregates. Also, the small intestine is visualized by 3–4 hr with colloidal technetium preparations and neither the stomach nor the large intestine is seen during the first 5 hr. Figure 5 shows delayed abdominal images (at 4 hr) with other ^{99m}Tc compounds. The gallbladder was *not* visualized with any of these.

Radiochromatography. Radiochromatograms of bile obtained from dogs given ^{99m}Tc-albumin colloids intravenously show a single peak of activity far from the origin. The R_f value is almost identical to that of ^{99m}Tc-pertechnetate. The activity in the bile could not be separated by centrifugation, nor could it be precipitated by tannic acid.

DISCUSSION

The observation of ^{131}I liver release, following albumin colloid and/or microaggregate injection and the re-entry of nonprotein iodide or "free iodide" into the plasma plus its subsequent concentration in the stomach confirm previous findings with these materials (*1–5,7*). The disparity in liver release rates between the *radioiodinated colloids and microaggregate suspensions* has been explained by gross differences in size and surface area of the aggregates in the two preparations (*6*). The small colloidal ^{131}I-albumin in trace doses is metabolized rapidly by the liver, whereas the 100-times-larger microaggregates in the same trace doses are handled much more

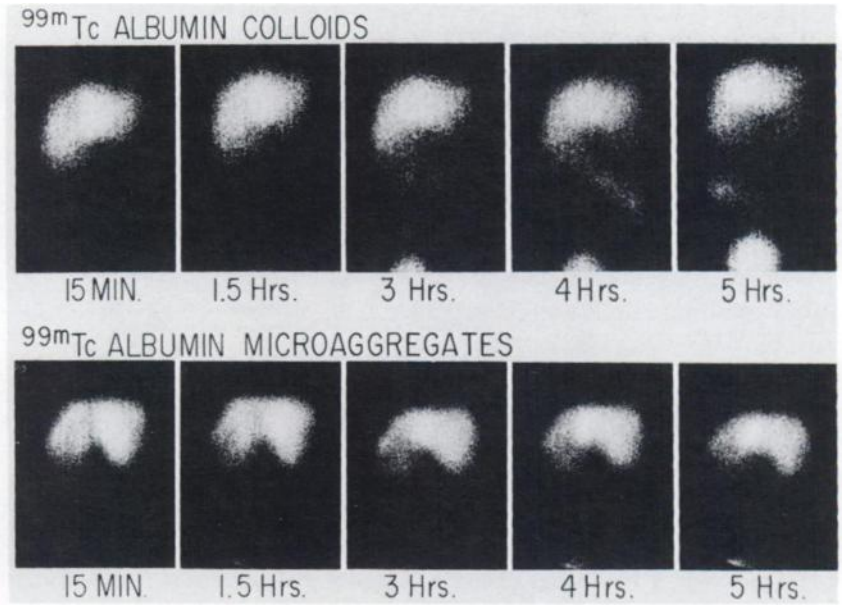

FIG. 4. Sequential abdominal images with ^{99m}Tc-albumin colloids and microaggregates.

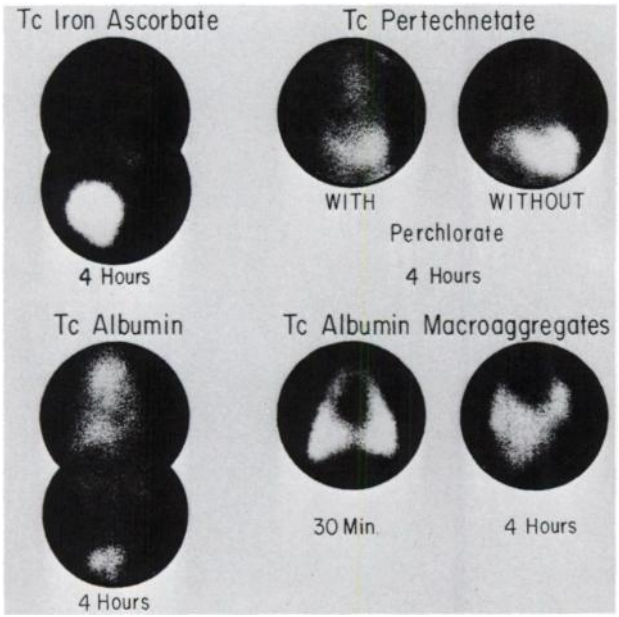

FIG. 5. Delayed images (4 hr) with ^{99m}Tc-pertechnetate, iron-ascorbic-acid complex, albumin solution, and albumin macroaggregates. None of preparations showed scan evidence of biliary excretion at 4 hr.

slowly as if a loading dose had been given. Therefore microaggregate preparations *may be suitable in trace doses* for the assessment of blood flow *and* proteolytic digestive function of the RES. Simultaneous blood flow and digestive function assessments are *not* valid with conventional small colloidal preparations given in trace doses; only blood flow estimation is possible with *trace doses* of this agent.

The slow liver release rates observed with the ^{99m}Tc-albumin suspensions cannot be explained by a gross surface area difference. If the first degradation process of albumin aggregates in the Kupffer cells is hydrolysis of the albumin molecule, as Mego (*8*) and others (*9*) have indicated, the hydrolysis rates of ^{131}I- *and* ^{99m}Tc-albumin colloids of the same size should be similar regardless of the radioactive label. The relatively slow liver release rates of ^{99m}Tc- compared with ^{131}I-albumin preparations may be due to differences in labeling sites on the albumin molecule and in catabolic processes.

With ^{131}I-albumin colloids, deiodination occurs quickly with rapid release of free iodide into the circulation (*1,5–10*). The longer retention of liver activity with *Tc-albumin* preparations may indicate that their degradation products are retained in the liver longer than those of *radioiodinated albumin* preparations.

Gallbladder visualization with ^{99m}Tc-albumin preparations supports a hepatobiliary pathway for a ^{99m}Tc-metabolite following albumin digestion in the Kupffer cells. Biliary excretion could explain the absence of a secondary rise in plasma nonprotein activity with both types of technetium preparations. The biliary excretion of ^{99m}Tc has not been described in previous animal studies. However, the authors have reported its biliary excretion in man via abdominal imaging after the administration of ^{99m}Tc-albumin microaggregate suspensions (*11*).

The similarity of the R_f value observed in radiochromatography between a possible Tc-albumin metabolic excreted in dog bile and Tc-pertechnetate, does not necessarily mean that the technetium in the bile is pertechnetate ion. As shown in sequential abdominal images, following ^{99m}Tc-pertechnetate injection, this ion *does not* have a significant biliary excretion pathway. Our current explanation is that the albumin aggregates are degraded in the Kupffer cells by proteolytic enzymes to smaller molecular entities which are then released into the sinusoids and then enter the hepatic cells for possible further catabolism and subsequent excretion in the bile. The chemical identity of the technetium compound(s) is unknown, but most probably it is a human serum albumin degradation product.

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