LOCALIZATION OF POLYPEPTIDE CASEIDIN IN THE RENAL CORTEX: A NEW RADIOISOTOPE CARRIER FOR RENAL STUDIES

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During studies of the body distribution of polypeptides previous investigators have noted concentration of certain of these polypeptides in the kidney (*1–5*). McAfee and his coworkers suggested that polypeptides might be useful in renal imaging (*5*).

Caseidin, a polypeptide obtained from controlled hydrolysis of casein (*6*), is stated to have low antigenicity and toxicity (7). The present communication describes studies of the localization of ^{51}Cr- and ^{99m}Tc-labeled caseidin in the renal cortex of mice, rats, and dogs, discusses the implications of this localization, and suggests its usefulness in renal studies in man.

MATERIALS AND METHODS

Caseidin obtained from YEDA, Rehoveth, Israel, was labeled with ^{51}Cr or ^{99m}Tc. For ^{51}Cr labeling $^{51}CrCl_3$ was incubated with caseidin followed by overnight dialysis to remove the main portion of unbound ^{51}Cr. The ^{99m}Tc labeling was achieved by three methods: the Fe(III) plus ascorbate technique (*8*), the Fe(II) technique, and the Sn(II) technique. The latter two methods were recently studied in our laboratory and will be the subject of a separate communication (*9*).

Briefly, in the Fe(II) technique 0.5 ml of aqueous solution containing 10 μM of $FeSO_4$ was added to 2.0 ml of ^{99}Mo-^{99m}Tc-generator eluate acidified by adding 0.5 ml 6 *N* HCl. Then 0.5 ml of saline solution containing 20 mg of caseidin was added to the mixture, and the pH of the mixture was titrated to 5.5 by adding NaOH solution. The mixture was then passed through an Ag1-X8 anion exchange column (BioRad Labs., Richmond, Calif.) in the chloride form to remove free pertechnetate. In the Sn(II) technique 0.5 ml of $SnCl_2$ in 0.2 *N* HCl [total of 0.5 μM Sn(II)] was added to 2 ml of ^{99}Mo-^{99m}Tc-generator eluate. Twenty to 30 mg of caseidin contained in 0.5 ml of saline solution was added, and after mixing the mixture was immediately passed through an AG1-X8 anion exchange column which had been previously washed with 0.01 *N* HCl.

The ^{99m}Tc-Fe-ascorbate was prepared in a fashion identical to that described by Harper et al (*10*). The ^{203}Hg-chlormerodrin was purchased from E. R. Squibb and Sons, Inc., New Brunswick, N.J.

Distribution of the radionuclide in various organs and tissues was measured using the scintillation camera as a small-animal whole-body counter. The scintillation camera fitted with the ring collimator used with positron scintigraphy was placed 27 in. above the rat, and the counting rate was determined at the photopeak with a 15% window. At laparotomy the urine and bladder, the kidneys, and the liver and spleen were removed in order and the counting rate determined after each extirpation. The activity in each group of tissues or organs was determined by difference and expressed as a fraction of activity in the entire animal. The fraction of the administered dose determined in this manner did not differ significantly from that determined by direct assay of organ activity using homogenization and assay in a well NaI(Tl) crystal scintillation counter.

Retention of ^{51}Cr activity in the body of dogs, rats, and mice following intravenous administration of ^{51}Cr-caseidin was determined using the Donner Laboratory whole-body counter described previously (*11*). The animals were placed on a 1-meter arc and counted with a 9⅜ in. × 4 in. NaI(Tl) crystal. Counting at this distance reduced errors due to geo-

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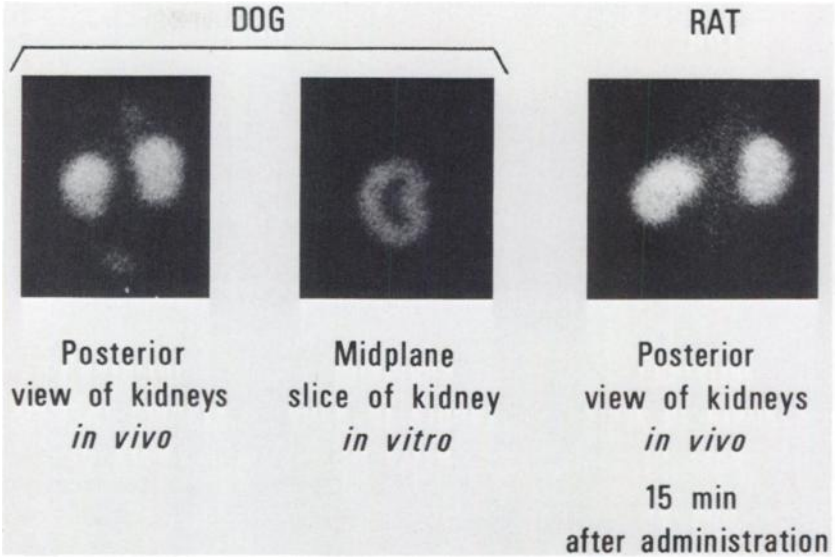

FIG. 1. Scintiphotos of ^{99m}Tc accumulation in dog and rat kidneys following i.v. administration of ^{99m}Tc-caseidin.

metrical variables. Using a 100-channel pulse-height analyzer, the counts in the photopeak from 280–380 keV were determined, and the counts on successive days were expressed as fractions of the count immediately after injection. The mice and rat each received 5–10 μCi of ^{51}Cr, the dogs approximately 30 μCi. The mice were counted in groups, the other animals individually.

Plasma clearance of ^{51}Cr activity following i.v. administration of ^{51}Cr-caseidin was performed in a routine fashion involving plasma separation following centrifugation and counting in a well NaI(Tl) crystal scintillation counter.

Buffalo rats were obtained from Simonsen Labs, Gilroy, Calif., and CD-1 germ free and CD-COBS mice were obtained from Charles River, Boston, Mass. Mice in the "germ-free" group were essentially identical to those in the "non-germ-free" group except for their lack of prior exposure to bacteria.

RESULTS

To the left in Fig. 1 are in vivo scintiphotos of dog kidneys obtained following the i.v. administration of ^{99m}Tc-labeled caseidin. Similar visualization of the kidneys of mice, rats, and dogs was achieved when the Fe(III) plus ascorbate, the Fe(II), or the Sn(II) method of labeling with ^{99m}Tc was employed and when ^{51}Cr was used as the labeling radionuclide. In the middle of Fig. 1 an in vitro scintiphoto of a longitudinal slice of dog kidney is shown (same dog as shown in left of figure). The ^{99m}Tc activity is confined to the renal cortex. To the right of Fig. 1 an in vivo scintiphoto is shown of a rat's kidneys taken 15 min after i.v. administration of ^{99m}Tc-labeled caseidin [labeled using Sn(II) method]. The kidneys of this rat were ~1 cm long, yet good detail of the kidney was obtained using the pinhole collimator (⅛-in. pinhole aperture).

Figure 2 shows the clearance of activity from the plasma (on the left) and the retention of activity (on the right) in the body of dogs given ^{51}Cr-caseidin intravenously. The initial distribution volume of the ^{51}Cr-caseidin was equal to the calculated plasma volume. The ^{51}Cr-caseidin was cleared from the plasma with an initial half-time of 15 min but within the first 30 min a second exponential component became evident. After the first day whole-body retention of activity could be described by a two-exponential function. In the two dogs studied, 63 and 80% of the activity was cleared from the body with a half-time of 14.8 days and 10.1 days, respectively. In both dogs the remaining activity had a very long retention rate and even though data were col-

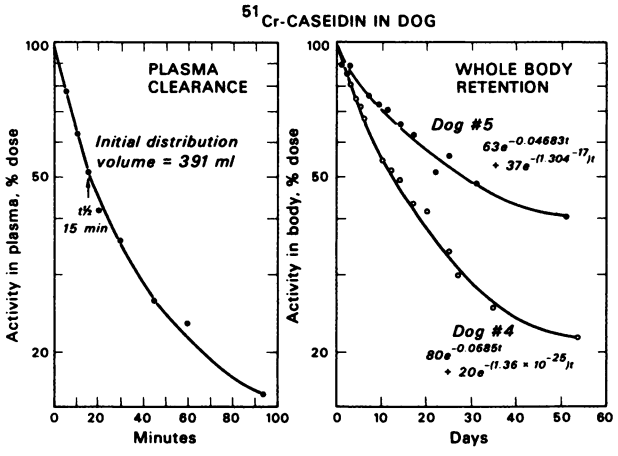

FIG. 2. Plasma clearance and whole-body retention of ^{51}Cr in dogs following i.v. administration of ^{51}Cr-caseidin.

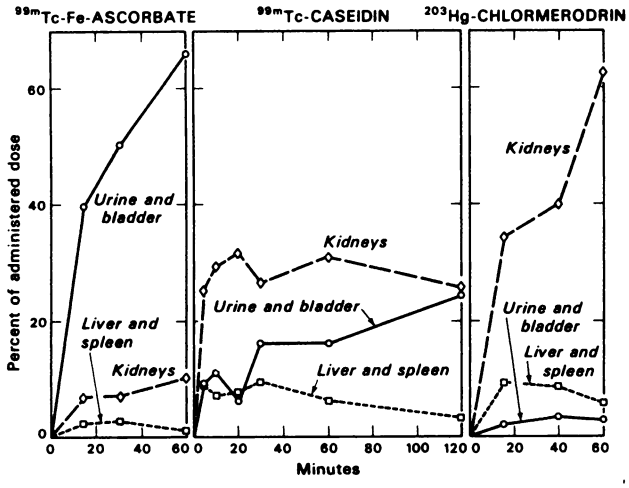

FIG. 3. Percent of administered radioactivity accumulating in liver and spleen, kidneys and urine and bladder of rats at various times after i.v. administration of ^{99m}Tc-Fe-ascorbate, ^{99m}Tc-caseidin, and ^{203}Hg-chlormerodrin.

lected for over 50 days the half-time of this slow component could not be well defined. When Dog 4 was sacrificed after 54 days, approximately 40% of the activity remaining in the body was localized in the kidneys. When sacrificed after 51 days Dog 5 had approximately 50% of the activity remaining in his body localized in his kidneys.

Figure 3 shows the concentration of radioactivity in the urine and bladder, kidneys, liver, and spleen in rats given ^{99m}Tc-Fe-ascorbate, ^{99m}Tc-caseidin, and ^{203}Hg-chlormerodrin. Following administration of ^{99m}Tc-Fe-ascorbate, radioactivity rapidly appeared in the urine and continued to accumulate in the urine and bladder, reaching 66% of the administered radioactivity by the end of the first hour. The kidney concentrated ^{99m}Tc slowly, reaching a level of 10% of the administered activity at the end of the first hour. Activity rapidly accumulated in the kidney following administration of ^{203}Hg-chlormerodrin, reaching 34% by 15 min and 63% of the administered dose 1 hr after administration. Fifteen minutes after administration of ^{203}Hg-chlormerodrin the ratio of activity in the kidneys to that in the liver was 3.6:1. Within 5 min after its i.v. administration 25% of the administered ^{99m}Tc-caseidin activity was in the kidneys, 9% in the urine and bladder, and 8.7% in the liver and spleen. There was some further accumulation of activity in the kidneys, reaching a maximum of 32% by 15 min. In other experiments in mice, rats, and dogs approximately one third of the administered radioactivity was found in the kidneys 24 hr after administration of ^{99m}Tc-caseidin. Fifteen minutes after administration of ^{99m}Tc-caseidin the ratio of activity in the kidney to that in the liver was 4.8:1 (Table 1).

The polypeptides protamine, polymyxin, homocarnosine, polyhistidine, and polyaspartic acid, were similarly labeled with ^{99m}Tc and were also found to accumulate in the kidneys and variably in the liver. None were found that showed as great renal localization and as high a ratio of activity in kidney to liver and spleen as did caseidin.

Figure 4 shows the whole-body retention of ^{51}Cr-caseidin in "germ-free" and "non-germ-free" mice, and in a rat. A two-exponential function was fit to these data. For the animals that had not been germ free before the study, approximately 70% of the activity was cleared from the body with a half-time of 4.7 days while approximately 30% of the activity was cleared from the body with a half-time of 52.9 days. In the mice that were germ free before the study, approximately half of the activity was cleared with a half-time of 3 days and the other half with a half-time of 48.5 days. The major difference between these two groups of mice, which were isogenic and identical in all respects other than in their prior exposure to bacteria, was that a larger fraction of the administered radioactivity was associated with the slower component of body clearance. The rat showed whole-body clearance of activity which was inter-

TABLE 1. PERCENTAGE OF ADMINISTERED ACTIVITY IN VARIOUS ORGANS AND TISSUES

	^{99m}Tc-caseidin			^{203}Hg-chlormerodrin			^{99m}Tc-ascorbate		
Time (min)	Urine and bladder	Kidneys	Liver and spleen	Urine and bladder	Kidneys	Liver and spleen	Urine and bladder	Kidneys	Liver and spleen
5	9.2	25.0	8.7						
10	11.1	28.5	7.1						
15	7.3*	34.9*	10.4*	2.1	34.2	9.5	40.1	6.8	2.8
20	6.1	31.9	7.4						
30	16.0	26.7	6.9	3.4	39.5	8.7	50.7	7.2	2.8
60	16.0	30.9	5.9	2.7	62.8	5.9	66.3	10.3	0.9
120	24.5	25.8	3.1						

* 15-min data for caseidin obtained using a different batch of caseidin and prepared on a different day than that for the remainder of the caseidin data.

mediate between the "non-germ-free" and the "germ-free" mice.

DISCUSSION

The potential usefulness of polypeptides, such as caseidin, as radionuclide carriers in the scintigraphic visualization of the kidneys is clearly demonstrated in the present report and is evident from previously published data. However, the rationale for understanding renal accumulation of radionuclides initially bound to polypeptides is not obvious. Proteins and various polypeptides in glomerular filtrate are known to be reabsorbed by the proximal convoluted tubule. It is assumed that they are subsequently degraded or returned to the circulation. If this is indeed the case, the prolonged retention of ^{51}Cr in the kidneys of animals given ^{51}Cr-caseidin in the present experiments requires further explanation. Either the ^{51}Cr label has been translocated from the caseidin to other materials within the kidney or a portion of or the entire caseidin molecule is retained within the kidney for very prolonged periods of time. If the former is the case, then ^{51}Cr-caseidin or similarly labeled polypeptides may be useful intermediates in labeling structures in the kidney. On the other hand, if portions of or entire polypeptides are retained in the kidney for prolonged periods of time, then it may be suggested that the kidney plays a role in the body's response to foreign protein.

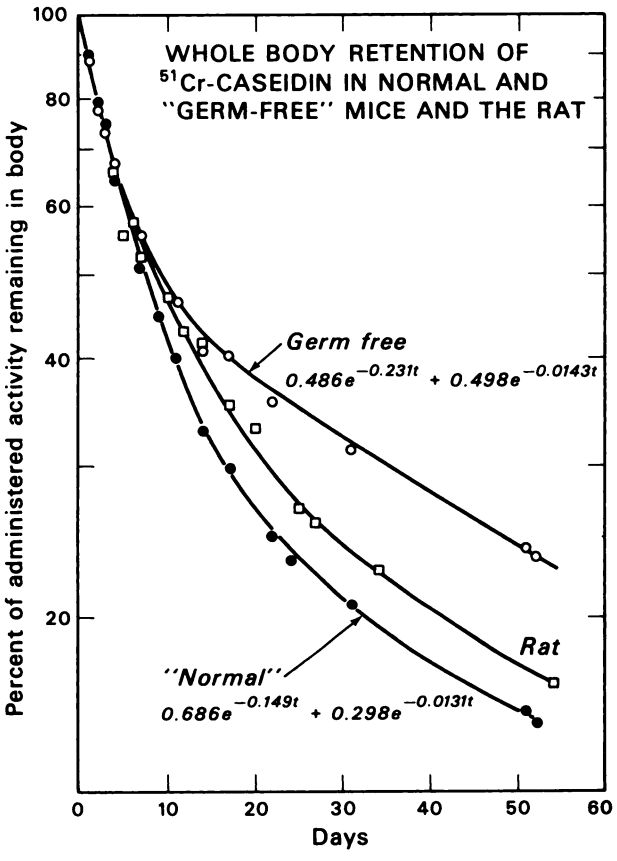

FIG. 4. Whole-body retention of ^{51}Cr in normal and "germ-free" mice and in the rat following i.v. administration of ^{51}Cr-caseidin.

Present opinion is that ^{51}Cr attached to various proteins does not undergo significant translocation within the body. However, there are no data which establish that such translocation cannot occur. Furthermore, ^{51}Cr administered as Cr(III) has a whole-body retention curve consisting of at least three components with half-times of 0.5 days (~45%), 5.9 days (~30%), and 83.4 days (~25%) (*12*). Should ^{51}Cr dissociate from ^{51}Cr-caseidin in the renal cortex as ^{51}Cr(III), its prolonged local retention in the renal cortex as a ^{51}Cr-complex with renal proteins is plausible. Also, one cannot exclude the possibility of prolonged retention of polypeptides within the kidney since at least D-amino acid polypeptides have been shown to do so (*13*).

The polypeptides caseidin (*6*) and homocarnosine (*14*), both of which show high concentration in the renal cortex, have been shown to be "immunogenic" in that subsequent to their administration to mice the mice show decreased lethality when given certain strains of staphylococci intravenously. The mechanism of such action is unknown, but the present observations and similar earlier observations demonstrating polypeptide localization in the renal cortex suggest that this "immunogenic" effect may be mediated by the kidney. The kidney is known to influence red blood cell production through production or control of the humoral agent erythropoietin. Control of lymphopoiesis, the tissue largely responsible for the immune response, may also be responsive to humoral control, and it is not inconceivable that such humoral control may reside in the kidney. If such is the case, one might ask what stimulus could direct the kidney to release lymphopoiesis-stimulating materials? It is thought that foreign proteins (e.g. bacteria, viruses, etc.) are converted to polypeptides in reticuloendothelial cells, and these polypeptides, possibly attached to a form of RNA, are transported to lymphoid cells where they stimulate antibody production. Is it possible that such polypeptides concentrate in the cells of the renal cortex and cause the production or release of lymphopoiesis-stimulating materials? If such were the case, the concentration of foreign polypeptides in the renal cortex, their possible prolonged retention at this site, and the "immunogenic" properties of certain of these polypeptides could be readily explained. That this may be the case is further suggested by the relative immunological tolerance of patients with advanced renal disease and the similarity between duration of time during which circu-

lating antibodies can be detected following antigenic stimulation and the turnover time of cells within the kidney.

This hypothesis may be used to explain our present results showing more prolonged retention of ^{51}Cr activity in the body of mice which were "germ free" compared with essentially identical mice which were not "germ free" before the study. If there are specific receptor sites in the kidney for certain polypeptides which are associated with renal control of lymphopoiesis, then animals that had no previous exposure to foreign protein might have a larger number of such receptor sites available for binding foreign polypeptides than an animal that had such previous exposure. Our results could equally be explained by proposing a varying capability to induce translocation of ^{51}Cr from caseidin to renal cell protein in germ-free versus nongerm-free animals.

The discussion noted above is an exercise of the authors' prerogative to interpret their work. The hypotheses which are proposed cannot be established by the present observations and require many further independent observations to determine their validity or fallacy.

SUMMARY

The polypeptide caseidin labeled with ^{99m}Tc using the Fe(III) plus ascorbate method, the Fe(II) method, the Sn(II) method, or labeled with ^{51}Cr localizes in the renal cortex of mice, rats, and dogs. Fifteen minutes after i.v. administration of ^{99m}Tc-caseidin to mice, rats, and dogs approximately one third of the administered activity is in the kidneys and the ratio of activities in the kidney to that in the liver is 4.8:1.

Retention of ^{51}Cr activity in the kidney is prolonged following administration of ^{51}Cr-caseidin since in two dogs sacrificed 51 and 53 days after administration of ^{51}Cr-caseidin radioactivity in the kidney was 50 and 40% of the activity remaining in the body, respectively. The pattern of retention of activity in mice which were germ free before administration of ^{51}Cr-caseidin was different from that in mice which were not germ free.

The rapid accumulation of a large fraction of activity in the renal cortex following administration of ^{99m}Tc- or ^{51}Cr-labeled caseidin suggests the usefulness of caseidin as a radionuclide carrier for renal studies.

The concentration and possible prolonged retention of labeled caseidin and other polypeptides in the renal cortex suggests a role of the kidney in the body's response to foreign protein. The possible nature of this role is discussed.

The initial intravascular distribution, subsequent rapid plasma clearance, and the molecular size of labeled caseidin indicate its possible suitability for imaging brain lesions.

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