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Localization of a Polypeptide, Caseidin, in the Renal Cortex: A New Radioisotope Carrier for Renal Studies*

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Introduction

Previous investigations demonstrated concentration of certain polypeptides in the kidney (1,2,3,4,5). McAfee and co-workers suggested that certain of these agents might be useful in renal imaging (5).

Caseidin, a polypeptide obtained from controlled hydrolysis of casein, is stated to have low antigenicity and toxicity (6). 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 such localization and suggests its usefulness in renal studies in man.

Materials and Methods

Caseidin obtained from Dr. A. Khatchalsky-Kazir of the Weizman Institute, 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 unbound ^{51}Cr. ^{99m}Tc labeling was achieved by three methods, the Fe(III) plus ascorbate technique (7), and Fe(II) and the Sn(II) technique. The latter two methods were recently studied in our laboratory and will be the subject of a separate communication (8).

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DISCLAIMER

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 addition of 0.5 ml 6N HCl. 0.5 ml of saline solution containing 20 mgm of caseidin was added to the above mixture and the pH of mixture was titrated to 5.5 by addition of NaOH solution. The mixture was then passed through AG1-X8 anion exchange column (BioRad Labs., Richmond, Calif.) in the chloride phase to remove free pertechnetate. In the Sn(II) technique 0.5 ml of $SnCl_2$ in 0.2N HCl (total of 0.5 μM Sn(II)) was added to 2 ml of ^{99}Mo-^{99m}Tc generator eluate. 20 to 30 mgm of caseidin contained in 0.5 ml of saline solution was added and the mixture immediately passed through an AG1-X8 anion exchange column which had been previously washed with 0.1N HCl.

^{99m}Tc-Fe-Ascorbate was prepared in a fashion identical to that described by Harper et al (9). ^{203}Hg-Chlormerodrin was purchased from E.R. Squibb and Sons, Inc., New Brunswick, N. J.

Distribution of radioisotope 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 inches above the rat and the count rate determined at the photopeak with ~15% window. At laparotomy the urine and bladder were removed, the kidneys were removed and the liver and spleen removed and the count 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 matter was found not to differ significantly from that determined by direct assay of organ activity using homo-

genization and assay in a well-type NaI 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 (10). The animals were placed on a 1-meter arc and counted with a 9 3/8 in. x 4 in. NaI crystal. Counting at this distance reduced errors due to geometrical 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-type NaI 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 Figure I 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 radioisotope. In the middle of Figure I 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 Figure I an in vivo scintiphoto is shown of a rat's kidneys taken 15 minutes after I.V. administration of ^{99m}Tc labeled caseidin (labeled using Sn(II) method). The kidneys of this rat were ~1 cm in length, yet good detail of the kidney was obtained using the pinhole collimator (1/8" pinhole aperture).

Figure II shows the clearance of activity from the plasma (on the left) and the retention of activity in the body of dogs (on the right) given ^{51}Cr-Caseidin intravenously. The initial distribution volume of the ^{51}Cr-Caseidin was equal to the calculated plasma volume. ^{51}Cr-Caseidin was cleared from the plasma with an initial t 1/2 of 15' but within the first 30' 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 t 1/2 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 was collected for over 50 days the t 1/2 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 III shows the concentration of radioactivity in the urine and bladder, kidneys, liver and spleen in rats given ^{99m}Tc-Fe-ascor-

bate, ^{99m}Tc-Caseidin and ^{203}Hg-Chlormerodrin. Following administration of ^{99m}Tc-Fe-Ascorbate radioactivity rapidly appeared in the urine and such activity 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 minutes and 63% of the administered dose 1 hour 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 minutes after its I.V. administration 25% of the administered ^{99m}Tc-Caseidin activity was in the kidneys, 9% was in the urine and bladder and 8.7% was in the liver and spleen. There was some further accumulation of activity in the kidneys reaching a maximum of 32% by 15 minutes. In other experiments in mice, rats and dogs approximately 1/3 of the administered radioactivity was found in the kidneys 24 hours 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.

The polypeptides protamine, polymyxin, homocarnosine, polyhistidine, 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 which showed as great renal localization and as high a ratio of activity in kidney to liver and spleen as did caseidin.

Figure IV 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 this data and for the animals which had not been germ free prior to the study approximately 70% of the activity was cleared from the body with a t 1/2 of 4.7 days while approximately 30% of the activity was cleared from the body with a t 1/2 of 52.9 days. In the mice which were germ free prior to the study approximately half of the activity was cleared with a t 1/2 of 3 days and the other half with a t 1/2 of 48.5 days. The major differences 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 intermediate between the "non-germ free" and the "germ free" mice.

Discussion

The potential usefulness of polypeptides, such as caseidin, as radioisotope 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 radioisotopes 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.

Present opinion is that ^{51}Cr attached to various proteins does not undergo significant translocation within the body. However, there is no data which establishes that such translocation cannot occur. 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 (11).

The polypeptides caseidin (6) and homocarnosine (12), 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 prior 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 prolonged retention at this site and the "immunogenic" properties of certain of these polypeptides could be readily explained. That such 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 circulating 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" versus essentially identical mice which were not "germ free" prior to the study. If there are specific receptor sites in the kidney for certain polypeptides which are associated with renal control of lymphopoiesis then animals which 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.

The above noted discussion is an exercise of the authors' prerogative to interpret their work. The hypotheses which were proposed cannot be established by the present observations and require many

further independent observations to determine their validity or fallacy.

Summary

(1) The polypeptide caseidin labeled with ^{99m}Tc using the Fe(III) plus ascorbate method, the Fe(II) or the Sn(II) methods or when labeled with ^{51}Cr localizes in the renal cortex of mice, rats and dogs.

(2) 15 minutes after I.V. administration of ^{99m}Tc-Caseidin to mice, rats and dogs approximately 1/3 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.

(3) Retention of caseidin in the kidney is prolonged and in two dogs sacrificed 51 and 53 days after administration of ^{51}Cr-Caseidin activity in the kidney was 50 and 40% of the activity remaining in the body, respectively.

(4) The pattern of retention of activity in mice which were germ free prior to administration of ^{51}Cr-Caseidin was different from that in mice which were not germ free.

(5) 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 radioisotope carrier for renal studies.

(6) 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.

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

Percentage of Administered Activity in Various Organs and Tissues

Time Min.	^{99m}Tc-Caseidin			^{203}Hg-Chlormerodrin			^{99m}Tc-Ascorbate		
	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.

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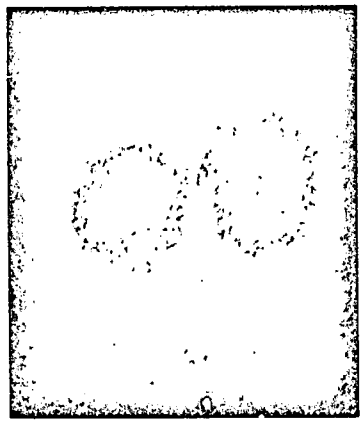
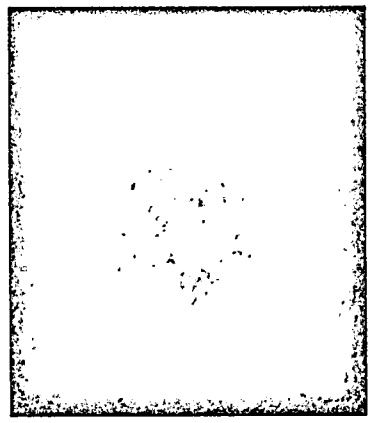
SCINTIPHOTOS USING PINHOLE COLLIMATOR FOLLOWING I.V. ADMINISTRATION OF ^{99m}Tc-CASEIDIN

DOG

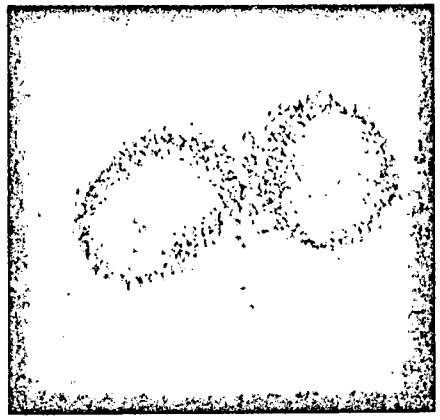
RAT



Posterior
view of kidneys
in vivo



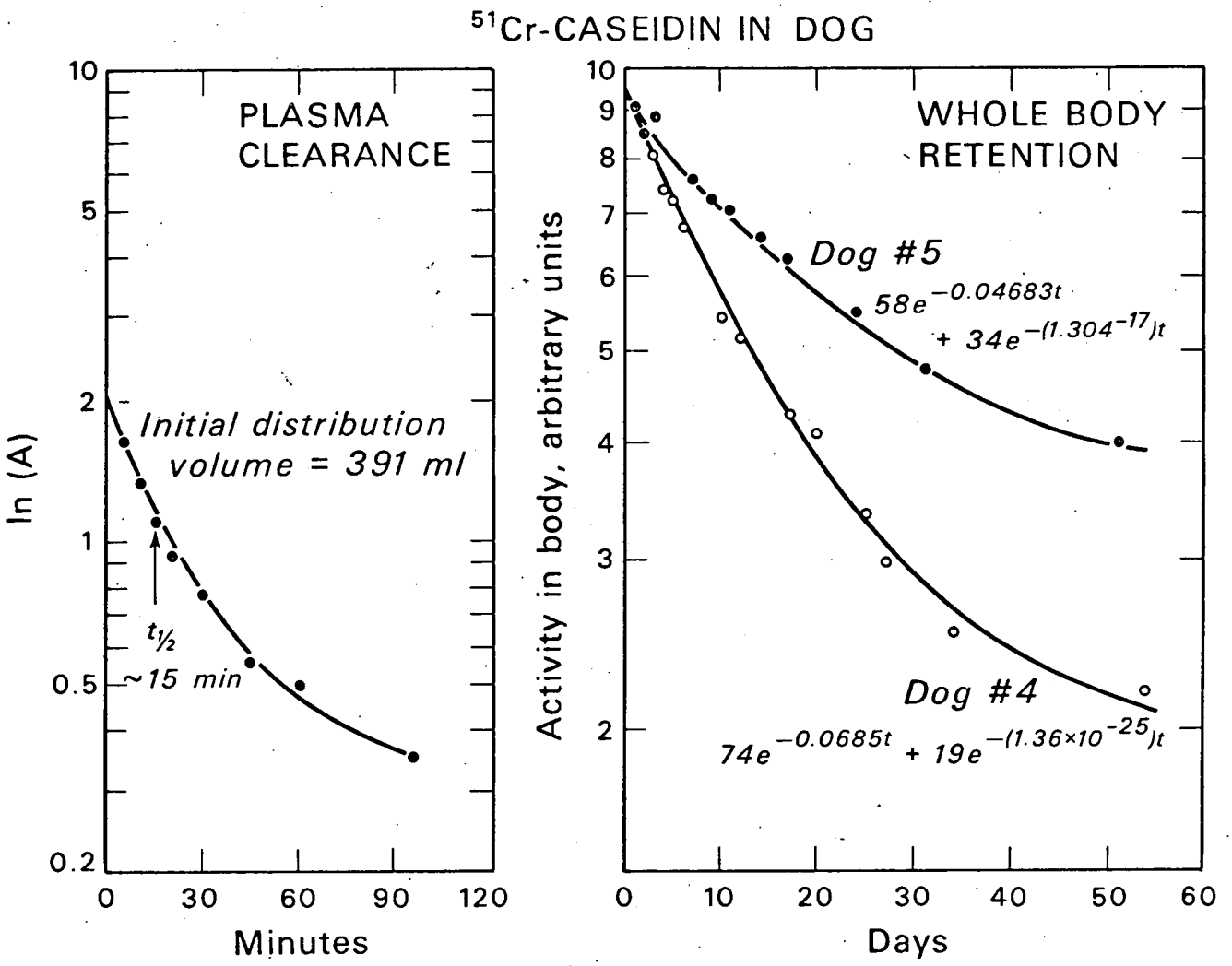
Midplane
slice of kidney
in vitro



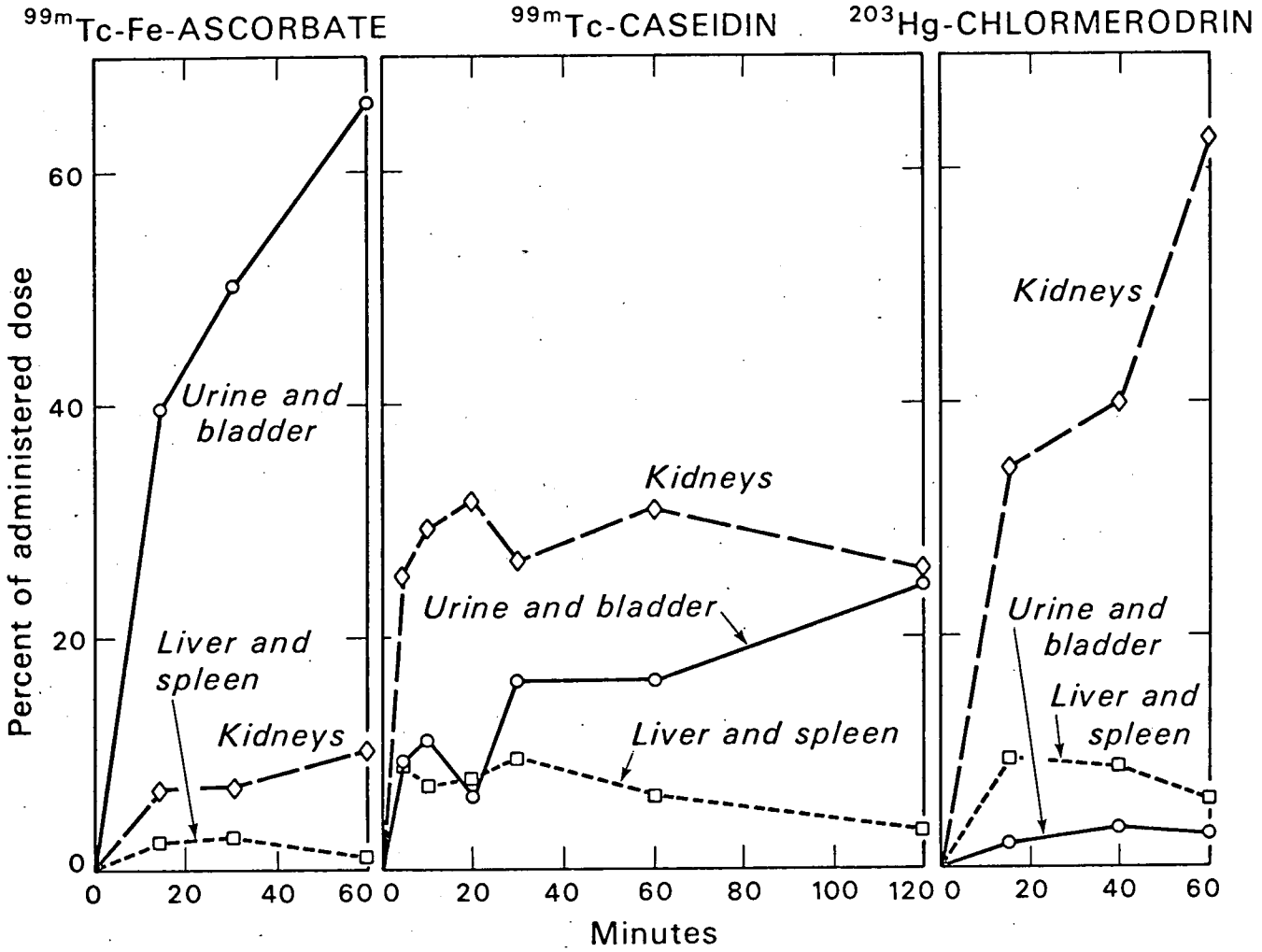
Posterior
view of kidneys
in vivo

15 min
after administration

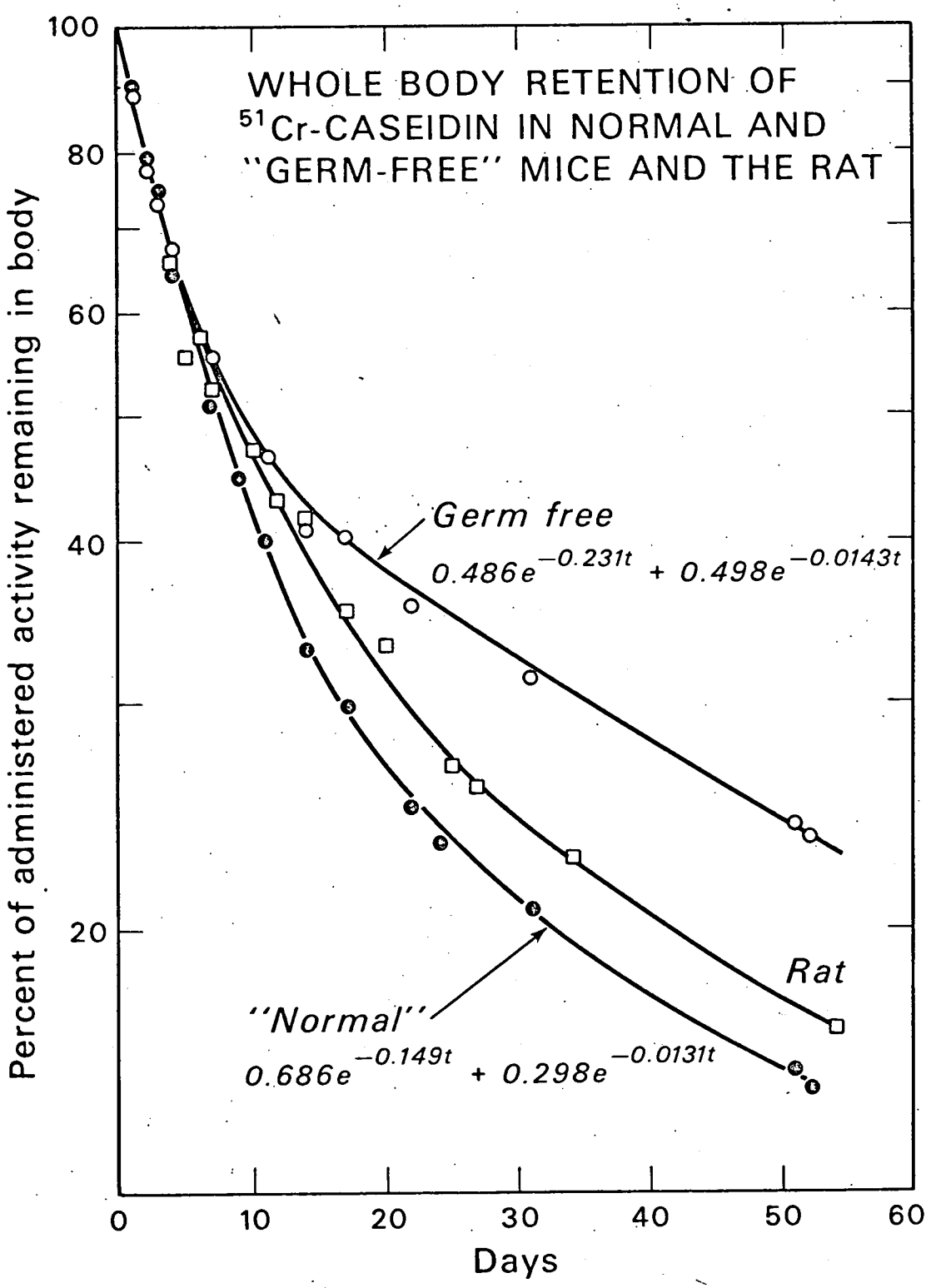
Fig 1


^{51}Cr-CASEIDIN IN DOG
PLASMA CLEARANCE
Initial distribution volume = 391 ml
$t_{1/2}$ ~15 min
ln (A)
Minutes
WHOLE BODY RETENTION
Dog #5
$58e^{-0.04683t} + 34e^{-(1.304^{-17})t}$
Dog #4
$74e^{-0.0685t} + 19e^{-(1.36\times10^{-25})t}$
Activity in body, arbitrary units
Days

DBL 709 5911


99mTc-Fe-ASCORBATE
99mTc-CASEIDIN
203Hg-CHLORMERODRIN
Percent of administered dose
0
20
40
60
Urine and bladder
Liver and spleen
Kidneys
Kidneys
Urine and bladder
Liver and spleen
Kidneys
Urine and bladder
Liver and spleen
0 20 40 60
0 20 40 60 80 100 120
0 20 40 60
Minutes

DBL 709 5913


WHOLE BODY RETENTION OF
^{51}Cr-CASEIDIN IN NORMAL AND
"GERM-FREE" MICE AND THE RAT
Percent of administered activity remaining in body
100
80
60
40
20
0
10
20
30
40
50
60
Days
Germ free
$0.486e^{-0.231t} + 0.498e^{-0.0143t}$
Rat
"Normal"
$0.686e^{-0.149t} + 0.298e^{-0.0131t}$

DBL 709 5910