Iodohippurate Sodium ^{131}I (OIH) Clearance in Mice Bioassay of Radiopharmaceuticals (35785)

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We have previously utilized mice for renal clearance studies using inulin-^{14}C-carboxyl as a standard of reference for measuring renal blood flow (RBF) (1). There is evidence that inulin fulfills all specifications for measuring glomerular filtration in all vertebrates (2), and indeed, our values in mice are in close agreement with those reported by Smith (2) for other species. In this report, we are extending our efforts to improve and utilize a technique which will give a satisfactory estimate of renal plasma clearance in mice and will serve as a means of evaluating and comparing radiopharmaceuticals. We are reporting below, renal clearance values in terms of renal plasma flow (RPF).

For this purpose, we have selected *o*-iodohippurate ^{131}I (OIH) as the standard for renal plasma clearance or renal plasma flow at low and high plasma concentration. Radioactive OIH is frequently used for estimating RPF as a substitute for diodrast, *p*-aminohippuric acid (PAH), or phenol red. All of these substances are known to be removed in part from the plasma by glomerular filtration and in part by tubular excretion from postglomerular blood. Generally, many substances thus excreted are not metabolized and are quantitatively excreted in the urine (3).

Assuming that other factors do not alter the biological pathway, the fraction of any substances removed from the blood by the kidney may suggest that the substance is filtered solely through the glomeruli; filtered, and partly excreted through the tubules; or filtered and partly reabsorbed by the tubules. The very fact that the clearances of various substances differ shows that pathways through the kidney must differ. This study deals with the magnitude of the processes of filtration and tubular excretion of *o*-iodohippurate ^{131}I, and its application as a gamma-emitting standard for the evaluation of radiopharmaceuticals. Its renal pathway and plasma clearance value in mice are reported herein. Clearance data on three of the newer radiopharmaceuticals, ytterbium-169-DTPA, indium-113m-DTPA and technetium-99m-DTPA, are also compared to it.

Materials. 1. Iodohippurate sodium ^{131}I [(OIH), Hipputope (^{131}I), E. R. Squibb and Sons, Inc.; sp act 0.413 mCi/mg as of date of assay]. Contains less than 1% free iodide. The injected solution contained 0.413 μCi/μg/0.01 ml.

2. Ytterbium-169-DTPA (diethylenetriaminepentaacetic acid) chelate (Minnesota Mining and Manufacturing Company; sp act 0.2 mCi/ml (0.255 mg of DTPA) as of date of assay). The injected solution contained 0.4 μCi/0.51 μg of DTPA/0.01 ml.

3. Indium-113m-DTPA chelate (Indium-113m Generator, New England Nuclear Pharmaceutical Division). Chelate prepared by the method of Clements *et al.* (4). The injected solution per 0.01 ml contained 0.25 μg of Fe, 2 μg of DTPA, and an average of 1.3 μCi of ^{113m}In activity as of 1400 the day of use.

4. Technetium-99m–iron-ascorbic acid–DTPA complex (Pertechtin, Hastings Radiopharmaceutical Works, and Renotec Kit, E. R. Squibb and Sons, Inc.). The injected solution per 0.01 ml contained 0.5 μCi of ^{99m}Tc, 14.3 μg of ferric chloride, 14.3 μg of ascorbic acid, and 7.1 μg of DTPA.

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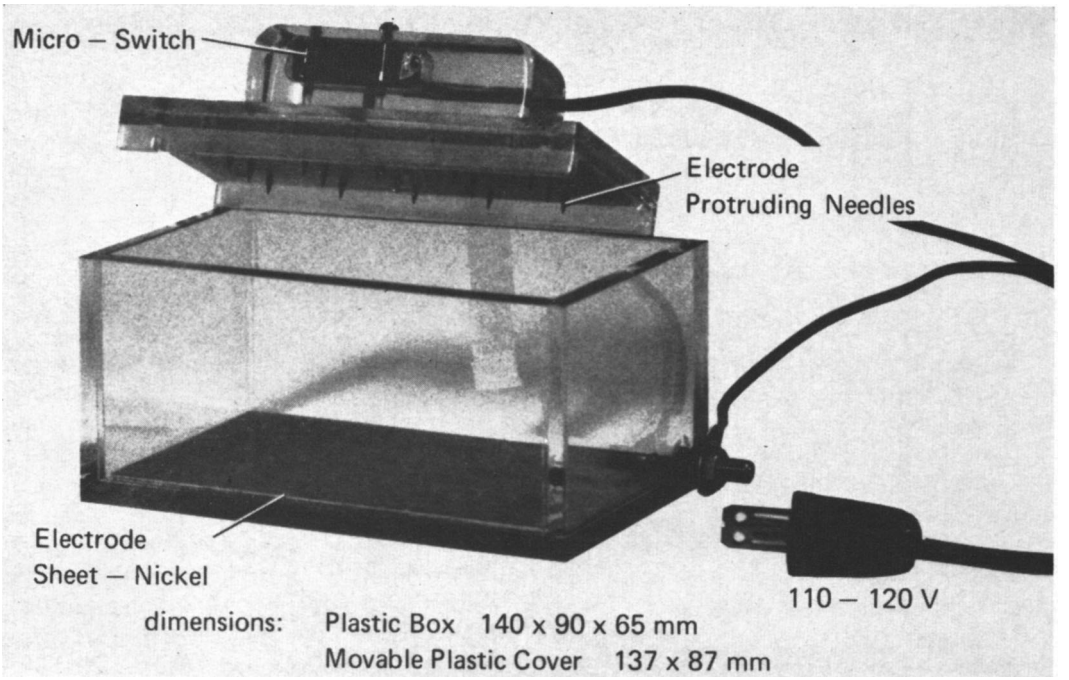

FIG. 1. Mouse sacrifice unit.

5. Scintillation well detector-analyzer scaler (Nuclear-Chicago).

6. Yale-Swiss mice, male, 5-week-old, weight averaging 20 g and ranging from 19 to 21 g.

7. Mouse sacrifice unit, described in detail in Fig. 1.

Procedure. The method for renal clearance in mice of Konikowski *et al.* (1) was employed in this study.

In classical clearance studies, drugs are administered using constant infusion techniques. In this study, a single intravenous injection technique was used. This permits observation of high and low plasma concentrations in a single study. A low plasma concentration is arbitrarily defined as less than 1% of the dose per ml of plasma, and a high plasma concentration as greater than 1%.

For each experimental point at 5, 10, 20, 30, 40, 60, 90, and 120 min, the average value from 6 mice was taken for estimation of clearance. To allow for necessary "mixing time" within the mouse, clearance data from 0 to 5 min was not taken for "rapidly" excreted substances, and from 0 to 10 min for more "slowly" excreted substances.

For excretion time intervals up to 60 min, the mouse was induced to urinate before injection of the test solution, and the penis was then ligated with surgical suture. For excretion periods of more than 60 min, the mouse was injected first and placed in a beaker on a wire screen, left undisturbed with food and water present until about 45 min before sacrifice, and forced to urinate into the beaker; the penis then was ligated.

Injection was made into the tail vein in the amount of 0.01 ml/g body weight at the rate of approximately 0.02 ml/sec. At the appropriate time, the animal was killed by electrocution. The heart blood was collected in a heparinized vial. The intact bladder was dissected and its contents were washed quantitatively into a volumetric flask and made up to volume with water. In the case of extended time intervals, the initial beaker containing the voided urine was rinsed several times and the washings were transferred to the flask containing the bladder and its contents and made up to volume with water. A 1-ml aliquot was pipetted into a vial and counted in the scintillation counter.

A 0.1-ml sample of blood diluted with 1.0

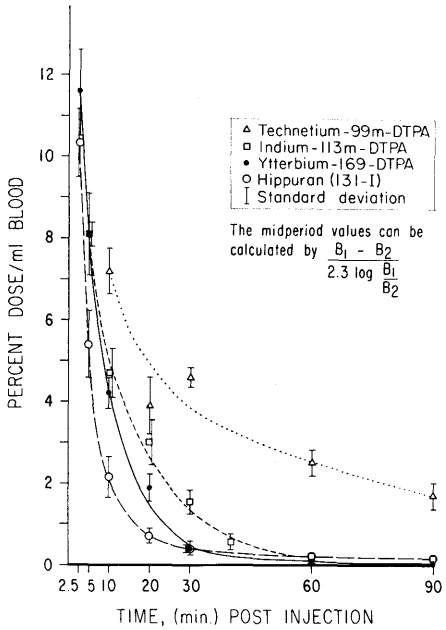

FIG. 2. Blood concentration disappearance curves in mice after single iv injection of *o*-iodohippurate sodium ^{131}I (○--); ytterbium-169 DTPA (●—); indium-113m DTPA (□- - -); and technetium-99m DTPA (△- - -). The discrepancy in values at the 20- and 30-min points in the technetium-99m-DTPA curve was caused by change in manufacturing lot of the kit used. Additional data at the 120-min point were used to develop a more accurate curve.

ml of nitric acid was counted in the scintillation counter for the determination of total blood activity. The remaining blood was centrifuged, and 0.1 ml of plasma diluted with 1.0 ml of nitric acid was counted for the determination of the red blood cell:plasma ratio.

The standard for estimating total body dose was based on the amount of solute injected per gram of body weight. It was prepared by pipetting 0.25 ml of injection solution into a 25-ml volumetric flask and then making the solution up to volume with water. One ml of this solution was then pipetted into a counting vial for counting.

Calculations. Specific details relating to the calculations used are given in a previous publication (1). Total body dose was determined by multiplying the weight of the animal by the activity (cpm) of the standard. This value was then used to estimate the percentage dose per milliliter of blood and the percentage of dose excreted in urine.

The RPF is defined as the amount of plasma (ml/min) that circulates through functional renal tissue (5). RPF measurements with a compound are usually done at low plasma concentrations. The substance is excreted by the tubules as well as filtered by the glomeruli. In this study, renal clearance was calculated by the standard formula UV/P where UV is the urine concentration per minute and P is the plasma concentration per milliliter. The clearance (ml/min) is obtained by dividing the estimated urine excretion (cpm% dose/min) by the midpoint plasma concentration of the period (cpm% dose/ml). These values were obtained by constructing a linear graph for urine excretion from the data in Table I, and by using the blood concentration curve. The blood values for each urine period were taken from a semilogarithmic graph, plotting the blood concentration logarithmically against time, and then interpolating to the midperiod. More accurately, the midperiod values can also be determined by plotting the data on linear graph paper (Fig. 2) and calculating the blood values from the formula (6):

$$\frac{B_1 - B_2}{2.3 \log (B_1/B_2)}$$

Blood values were thus determined and converted to plasma values by the equation

$$P = \frac{B}{1 + (\mathrm{RBC}/P)} \times 1.805,$$

where P = plasma value for 1.0 ml, B = blood value for 1 ml of blood, RBC/P = red blood cell to plasma ratio, estimated by interpolation from the 5- and 60-min ratios, and 1.805 is the factor which converts the plasma in 1.0 ml of blood to 1.0 ml of plasma. It is based on a mouse hematocrit of 44.6%.

These data were then standardized to a surface area of 1.73 m^2 by the equation $UV/P \times 1.73/0.114\ W^{2/3}$. For the average 20-g mouse, the surface area was calculated to be 0.0084 m^2 and the factor for extrapolation to the surface area of 1.73 m^2 was 206.0.

In the construction of the graphs, points at 90 and 120 min were included to make more accurate curves for better interpolation.

The RBC/P was calculated by the equation

$$\frac{\text{cpm \% dose per ml blood} - (\text{cpm \% dose per ml plasma} \times 0.554)}{\text{cpm \% dose per ml plasma} \times 0.554}$$

where 0.554 represents 55.4% plasma per ml

TABLE I. Values of Urinary Excretion in Individual Mice, Percentage of Injected Dose.[a]

	Time (min) post-single intravenous injection						
	0–5	0–10	0–20	0–30	0–60	0–90	0–120
Hippuran (^{131}I)	47.13	64.11	78.83	78.24	94.28	95.74	97.70
	56.10	71.22	90.60	79.57	92.94	91.38	97.39
	51.30	67.18	91.52	89.28	90.30	93.47	97.40
	47.60	66.74	66.73	78.31	90.38	90.30	99.45
	50.43	53.65	87.85	81.82	94.39	96.56	100.07
	60.27	70.61	76.50	88.57	94.24	91.09	100.97
Mean	52.14	65.58	82.01	82.63	92.76	93.09	98.83
SD	±5.12	6.41	9.72	5.05	1.94	2.60	1.54
SEM	±2.09	2.62	3.97	2.06	0.79	1.06	0.63
50% excretion,[b] 4.5 min							
Ytterbium-169-DTPA	31.5	57.6	77.7	92.1	94.6	95.8	99.8
	28.4	54.9	52.6	75.9	99.6	92.2	100.2
	31.2	64.3	67.4	72.5	97.2	96.2	100.7
	37.1	36.1	69.8	72.8	72.0	98.4	100.3
	21.2	48.8	83.9	85.1	97.0	97.0	99.8
	39.9	50.7	67.1	80.0	94.0	96.4	98.1
Mean	31.6	52.1	69.8	83.2	92.4	96.0	99.8
SD	±6.60	9.95	10.68	8.35	10.19	2.07	0.91
SEM	±2.69	3.40	4.36	3.41	4.16	0.85	0.37
50% excretion,[c] 9.5 min							
Indium-113m-DTPA	26.0	40.2	67.5	65.1	84.2	92.8	95.5
	20.3	45.8	59.0	80.3	88.8	92.0	96.4
	32.4	44.3	68.8	76.6	86.8	90.8	97.3
	25.9	53.2	70.5	87.9	93.2	88.9	97.8
	37.1	43.5	67.6	77.6	94.6	95.0	95.2
	27.3	54.4	64.5	75.5	88.4	97.9	96.9
Mean	28.2	46.9	66.3	77.2	89.3	92.9	96.5
SD	±5.83	5.66	4.09	7.40	3.91	3.18	1.02
SEM	±2.34	2.31	1.67	3.02	1.60	1.30	0.42
50% excretion,[c] 11.5 min							
Technetium-99m-DTPA		24.0	51.7	47.7	59.1	77.6	81.8
		33.8	60.3	65.3	69.6	84.0	80.2
		30.1	47.5	60.1	61.4	74.9	90.9
		32.1	48.1	53.0	64.4	81.2	89.4
		34.9	44.6	47.5	69.5	75.5	77.4
		38.3	35.8	54.4	63.1	83.8	84.8
Mean		32.2	48.0	54.7	64.5	79.5	84.1
SD		±4.87	8.07	7.00	4.28	4.06	5.30
SEM		±1.99	3.29	2.86	1.75	1.66	2.16
50% excretion,[c] 25 min							

[a] SD, standard deviation; SEM, standard error of the mean.

[b] The 50% excretion is at the time given, determined by: extrapolation; [c] interpolation.

blood of a 20-g, 5-week-old male Yale-Swiss mouse.

A substance undergoes true glomerular filtration if it is cleared by the glomerulus only, with no tubular reabsorption or excretion, at the same rate from the blood at any concentration. Inulin has this property. The inulin blood clearance in mice corrected to 1.73 m^2 surface area was determined in our previous study to be 101.2 ml/min; however, when calculated to renal plasma clearance, the glomerular filtration (GFR) is 56.1 ml/min. This plasma value will be used as a standard of reference. The inulin clearance ratio (Cr_{In}) of 1.0 will serve as the standard in the estimation of the behavior of other substances in relation to inulin, making possible the determination of their mode and rate of excretion.

The fraction of substance being reabsorbed by the tubules is known as the tubular reabsorption (TR) fraction. The clearance ratio of the substance to inulin is less than 1.0 (inulin). TR is calculated from the equation $(Cr_{In} - Cr_X) \times 100$.

The tubular excretion (TE) fraction is the fraction of substance being excreted by the tubules in addition to that undergoing glomerular filtration. Its clearance ratio Cr_X is greater than that of inulin (1.0). It is calculated by the equation $(Cr_X - Cr_{In})/Cr_X \times 100$.

The use of these radiopharmaceutical indices (RPF, TE and TR) at low and high plasma concentrations is based on a simplified view of the complex clearance function of the kidney. This view does not take into consideration such complicating factors as plasma protein binding, bidirectional tubular transport, simultaneous secretion and reabsorption, and others. These complicating factors may be necessary in detailed clearance studies, but are not necessary in the use of clearance as a bioassay for evaluating and comparing radiopharmaceuticals. Our interest is in overall clearance differences, not in the precise reasons how. If a detailed understanding of clearance is desired, considerably more involved experimentation is required.

For ready comparisons of renal clearances of different compounds, a clearance ratio graph was constructed (Fig. 3). The ratio of the average clearance of the substance at high and low concentrations to inulin was plotted against percentage dose per milliliter in the plasma. Inulin clearance (ratio 1.0) was used as a standard. Substances with an average clearance value appearing above the inulin line are cleared by glomular filtration and tubular excretion. Those with values which appear below the inulin standard line are assumed to be reabsorbed by the tubules. Sample calculations for TE and TR are given on the graph.

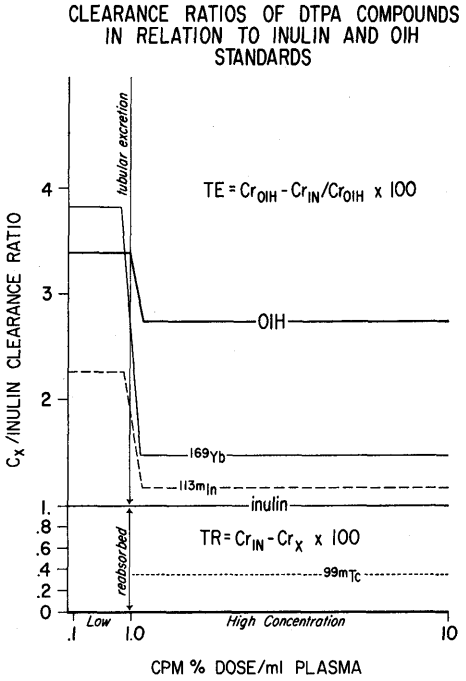

FIG. 3. Average clearance ratio graphs of orthoiodohippurate sodium ^{131}I, ^{169}Yb-DTPA, ^{113m}In-DTPA, and ^{99m}Tc-DTPA to inulin at low and high plasma concentration. This graph gives examples of how the clearance ratio of a substance is evaluated against standard inulin.

Results. Experimental data are given in Tables I, II, and III, and in Figs. 2 and 3. The average plasma clearance values of purified OIH in the 20-g mouse were found to be 190.6 ml/min at low plasma concentration, and 153.3 ml/min at high plasma concentration, or 3.36 and 2.73 times as fast as inulin, respectively. According to our simplified indices, it was cleared 70.2% by TE at

TABLE II. Clearance Calculations in Mice.

Collection period (min after inj)	Urinary excretion interval		P (plasma)	UV/P clearance	UV/P^a clearance (ml/min) (plasma level)		C_X/C_{IN}[b]	
	%/period	UV (%/min)	(%/ml)	(ml/min)	Low	High	L	H
Hippuran (^{131}I)								
5–10	13.5	2.70	4.12	0.655		134.9		2.40
10–15	7.5	1.50	1.80	0.833		171.6		3.06
15–20	5.4	1.08	1.00	1.080	222.5		3.97	
20–25	3.7	0.74	0.71	1.042	214.7		3.83	
25–30	2.8	0.56	0.53	1.057	217.7		3.88	
30–35	2.0	0.40	0.43	0.930	191.6		3.42	
35–40	1.8	0.36	0.35	1.029	212.0		3.78	
40–45	1.3	0.26	0.31	0.839	172.8		3.08	
45–50	1.1	0.22	0.27	0.815	167.9		2.99	
50–55	0.9	0.18	0.25	0.720	148.3		2.64	
55–60	0.8	0.16	0.22	0.727	149.8		2.67	
Av			0.91	0.883	190.6	153.3	3.36	2.73
Ytterbium-169-DTPA								
5–10	20.5	4.10	12.04	0.341		70.2		1.25
10–15	12.0	2.40	7.22	0.332		68.4		1.22
15–20	8.0	1.60	4.33	0.370		76.2		1.36
20–25	5.2	1.04	2.56	0.406		83.6		1.49
25–30	4.0	0.80	1.57	0.510		105.1		1.87
30–35	2.9	0.58	0.96	0.604	124.4		2.22	
35–40	2.1	0.42	0.63	0.667	137.4		2.45	
40–45	1.8	0.36	0.42	0.857	176.5		3.15	
45–50	1.8	0.36	0.27	1.333	274.6		4.89	
50–55	1.4	0.28	0.19	1.474	303.6		5.41	
55–60	0.8	0.16	0.13	1.231	253.6		4.52	
Av			2.76	0.739	211.7	80.7	3.77	1.44
Indium-113m-DTPA								
5–10	18.7	3.74	11.35	0.330		68.0		1.21
10–15	12.0	2.40	7.65	0.314		64.7		1.15
15–20	7.4	1.48	5.48	0.270		55.6		0.99
20–25	5.7	1.14	3.85	0.296		61.0		1.09
25–30	4.5	0.90	2.74	0.328		67.6		1.20
30–35	3.2	0.64	1.90	0.337		69.4		1.24
35–40	2.6	0.52	1.33	0.391		80.5		1.43
40–45	2.2	0.44	0.94	0.468	96.4		1.72	
45–50	1.8	0.36	0.65	0.554	114.1		2.03	
50–55	1.7	0.34	0.48	0.708	145.8		2.60	
55–60	1.3	0.26	0.36	0.722	148.7		2.65	
Av			3.34	0.429	126.3	66.7	2.25	1.19
Technetium-99m-DTPA								
10–15	7.8	1.56	11.28	0.138		28.4		0.51
15–20	6.0	1.20	9.12	0.132		27.2		0.48
20–25	4.2	0.84	8.16	0.103		21.2		0.38
25–30	4.0	0.80	7.44	0.108		22.2		0.40
30–35	3.3	0.66	6.95	0.095		19.6		0.35
35–40	2.8	0.56	6.50	0.086		17.7		0.32
40–45	2.6	0.52	6.08	0.086		17.7		0.32
45–50	2.2	0.44	5.78	0.076		15.7		0.28
50–55	2.0	0.40	5.45	0.073		15.0		0.27
55–60	1.9	0.38	5.14	0.074		15.2		0.27
Av			6.54	0.088		20.0		0.36

[a] Extrapolated to 1.73 m² surface area.

[b] Inulin clearance = 56.1 ml/min extrapolated to 1.73 m².

TABLE III. Radiopharmaceutical Evaluation Indices.

Compound	Labeled Compound or Carrier †		Average Plasma Concentration and Range/ml		RBC/P		*UV/B Clearance ml/min		*UV/P Clearance ml/min		Ratio (UV/P) $\frac{C_x}{C_{IN}}$		TR (−) TE (+) %		UV/P Deviation From OIH Standard %	
	Body Dose µg	Dose µg/g BW	cpm%	µg%	5 min	60 min	L	H	L	H	L	H	L	H	L	H
^{14}C-Inulin †	*254.0*	*12.7*	2.27 *0.74 - 5.88*	5.77 *1.88 - 14.94*	0.00	0.00	101.2		56.1		1.00		0.00		----	
^{131}I-Hippuran †	*20.0*	*1.0*	L 0.45 *0.22 - 1.00* H 2.96 *1.80 - 4.12*	0.09 *0.04 - 0.20* 0.59 *0.36 - 0.82*	0.44	0.63	221.1	190.0	190.6	153.3	3.36	2.73	+70.2	+63.4	Standard	
^{169}Ytterbium - DTPA †	*10.2*	*0.51*	L 0.43 *0.13 - 0.96* H 5.54 *1.57 - 12.04*	0.04 *0.01 - 0.10* 0.57 *0.16 - 1.23*	0.05	0.00	356.6	134.6	211.7	80.7	3.77	1.44	+73.5	+30.6	+11.0	-47.4
^{113m}Indium - DTPA †	*40.0*	*2.0*	L 0.61 *0.36 - 0.94* H 4.90 *1.33 - 11.35*	0.24 *0.14 - 0.38* 1.96 *0.53 - 4.54*	0.03	0.40	169.8	109.0	126.3	66.7	2.25	1.19	+55.6	+16.0	- 37.7	-56.5
^{99m}Technetium - DTPA †	*142.0*	*7.1*	7.18 *5.14 - 11.28*	10.20 *7.30 - 16.02*	0.09	0.00	----	36.1	----	20.0	----	0.36	----	-64.0	----	-87.0

† The substance calculated.
L Low concentration of substance in blood or plasma/ml :: 0.1 - 1.0% dose.
H High concentration of substance in blood or plasma/ml :: 1.0 and over % dose.
RBC/P Red blood cells to plasma ratio.
* Extrapolated to a 1.73 m^2 surface area UV/B or UV/P · 1.73/0.114 $W^{2/3}$ F = 206.0 for a 20g mouse.
C_x Renal Clearance of substance under investigation.
C_{IN} Renal clearance of inulin (GFR).
TR (Tubular reabsorption) ratios of $C_{IN} - C_x \times 100$.
TE (Tubular excretion) ratios of $C_x - C_{IN}/C_x \times 100$.

The values of the labeled compounds in the plasma can be calculated from the cpm% multiplied by the body dose injected. For example, if the cpm% dose per ml plasma was 2.27 and the body dose was 254.0 µg, the average plasma concentration would be 0.0227 x 254.0 = 5.77 µg%.

low plasma concentration and 63.4% by *TE* at high plasma concentration. These values are standardized to a body surface area of 1.73 m^2. The point of 50% urinary excretion occurred at 4.5 min. Our values for the RBC/*P* ratios in the mouse for OIH are 0.44 at 5, and 0.63 at 60 min postinjection. The RBC/*P* ratios for OIH reported by Smith and associates (7) was 0.66 in dogs and 0.49 in man. The ratio estimated by Burbank and associates (8) in man was 0.29.

The plasma clearance of OIH has thus been standardized against the primary standard inulin-^{14}C-carboxyl, and is now used as the standard for other rapidly excreted substances.

The average plasma clearance values for ytterbium-169-DTPA were found to be 211.7 ml/min at low plasma concentration and 80.7 ml/min at high plasma concentration per 1.73 m^2 surface area. These values are 11% faster than OIH at low plasma concentration, and 47% slower than OIH at high plasma concentration. At low plasma concentrations, 73.5% was cleared by *TE,* and at high plasma concentration 30.6% was cleared by *TE.* This marked decrease in clearance would suggest an overloading of the tubules at high plasma concentration. The point of 50% urinary excretion occurred at 9.5 min.

For indium-113m-DTPA the average clearances in a 20-g mouse were found to be 126.3 ml/min at low plasma concentration and 66.7 ml/min at high plasma concentration per 1.73 m^2 surface area. These values are 38 and 56% slower than the corresponding OIH values. At low plasma concentrations, 55.6%, and at high plasma concentrations, 16% were cleared by *TE.* The point of 50% urinary excretion occurred at 11.5 min.

Technetium-99m-DTPA was cleared so slowly that only high plasma concentrations were observed during the period of study. The average plasma clearance for a 20-g mouse was found to be 20.0 ml/min/1.73 m^2 surface area, or 87% slower than the OIH standard. About 64% of the substance was reabsorbed by the tubules. The point of 50% urinary excretion occurred at 25 min.

Discussion. The GFR for inulin in 20-g male Yale-Swiss mice is remarkably constant and may be used as a standard to compare renal clearance of other compounds in mice (1). A gamma-emitting standard for clearance studies was developed illustrating both glomerular filtration and tubular function. This permits the rate and mode of renal excretion of radiopharmaceuticals to be readily compared to that of other suitably labeled compounds, using gamma counting techniques.

Meschan *et al.* (9) have reported on the clearance of labeled Hippuran in dogs and have shown that the clearance is greatly depressed by the presence of free iodide. A similar observation was made by Burbank *et al.* (8) in man. For this reason, specially prepared iodohippurate sodium ^{131}I containing less than 1% free iodide was used in this study. Material of this degree of purity is required if it is to be used as a standard for renal clearance studies. The renal clearance values reported here closely correlate with those reported by other investigators for labeled Hippuran with low free iodide concentration (8, 9).

Three other radiopharmaceuticals were evaluated by comparing their renal clearance in mice with those of inulin and iodohippurate sodium ^{131}I. These compounds, ytterbium ^{169}Yb-DTPA, indium ^{113m}In-DTPA, and technetium ^{99m}Tc-DTPA, are nuclides chelated or complexed with diethylenetriaminepentaacetic acid. The nuclides ^{169}Yb and ^{113m}In are cationic in nature and probably form true chelates; ^{99m}Tc is in the form of pertechnetate, which is anionic in nature, and is probably only complexed by DTPA. It is clearly evident that these nuclides, though all are chelated or complexed with DTPA, are dealt with very differently by the mouse kidney when compared to both inulin and labeled Hippuran. The ytterbium and indium chelates are cleared in mice by filtration and *TE.* The ytterbium clearance is in some variance with the work of Hosain *et al.* (10), who reported that ^{169}Yb-DTPA is cleared in dogs and man by glomerular filtration only. The technetium complex is filtered and reabsorbed, and thus is cleared relatively slowly. The slow clearance in humans for this compound has just been reported (11). The clearances of the

compounds reported here are examples of this bioassay technique as a method of evaluating and comparing radiopharmaceuticals of the same general structure. The method has also been successful in intercomparing OIH from four different sources and the difference in renal clearances in mice of the four samples have been related to iodide impurity, differing formulations, and to nephrotoxicity. Clearance studies and isotope distribution studies in tumor-bearing mice have shown that there is a striking relationship between clearance and tumor uptake. These findings will be reported in subsequent publications. Studies with other radiopharmaceuticals in regard to source, stability, and toxicity are continuing.

Summary. The renal plasma rates and modes of clearance of iodohippurate sodium ^{131}I, ^{169}Yb-DTPA, ^{113m}In-DTPA, and ^{99m}Tc-DTPA have been determined in mice and compared with those of inulin and OIH. The DTPA chelates or complexes of different radionuclides were found to be cleared by the mouse kidney by different pathways at surprisingly different rates. Clearance values were found to be closely reproducible, but should not be compared with values in man or other species. Iodohippurate sodium ^{131}I can be used as a satisfactory clearance standard if it contains less than 1% free iodide in a formulation and concentration not toxic to renal function. The method reported has been shown to be a satisfactory bioassay for evaluating and comparing the clearance of radiopharmaceuticals of similar structure.

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