The Early Disappearance of Extracellular Tracers from Plasma After Intravenous Injection (36906)

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The experiments presented here were designed to test the hypothesis that the initial loss of extracellular tracers from plasma during the first 30 to 60 sec after intravenous (iv) injection is due to early distribution to extracellular space (ECS) and that even complete renal clearance does not measurably affect this early loss from blood. Renal clearance becomes evident only after equilibration with ECS is nearly complete.

The radiolabeled tracers used were ^{3}H-mannitol, and ^{14}C-para-aminohippurate (PAH). Mannitol was used as a tracer excreted from the body predominantly by glomerular filtration and PAH as a substance removed even more rapidly from blood due to its nearly total renal tubular excretion (1–4).

Method. Wistar rats weighing approximately 300 g, of either sex, were rendered unresponsive with intraperitoneal pentobarbital. Both femoral veins were surgically exposed. One was cannulated with a 20 gauge (0.8 mm) catheter for collecting blood samples. The other vein was used for injection of radiotracers.

Experimental animals were divided into nonnephrectomized and nephrectomized groups. The latter group was functionally nephrectomized by ligation of both renal pedicles through bilateral flank incisions.

Approximately 1 μCi of ^{14}C-PAH, 6 μCi ^{3}H-mannitol and 50 μCi ^{113m}indium-transferrin (5) were injected.[1] The latter was prepared by mixing 0.04 *N* HCl from a commercial ^{113}Sn generator with an equal volume of rat serum. A total fluid volume of 0.5 ml was injected into each rat.

At 0.5, 1, 2, 4, 8, 16, and 32 min after injection, 0.3 ml of whole blood was withdrawn into heparinized syringes. The blood was centrifuged and 0.1 g of plasma was prepared for routine counting by liquid scintillation spectrometry of the beta emissions of ^{3}H and ^{14}C and of the conversion electrons of ^{113m}In (6). The plasma specimens were solubilized by mixing with 1.5 ml of Soluene (Packard Instruments) and shaken for 60 min at 37°. After cooling to room temperature, 10 ml of a liquid scintillator, Instagel (Packard Instruments), and 3 ml of distilled water were added and were briefly mixed by hand shaking. The specimens were then counted in a liquid scintillation spectrometer (Tri-Carb 3320, Packard Instruments).

Plasma levels of both isotopes were determined with reference to the transferrin-bound ^{131m}In in the original mixture. An aliquot of the original triple isotope mixture was counted in the same manner as the plasma specimens. Calculation was made according to the following formula and the results expressed as a percentage of dose remaining in plasma.

$$\% = \frac{\text{cpm } (^{14}\text{C or } ^{3}\text{H})/\text{cpm } (^{113m}\text{In}) \text{ (plasma specimen)}}{\text{cpm } (^{14}\text{C or } ^{3}\text{H})/\text{cpm } (^{113m}\text{In}) \text{ (original mixture)}} \times 100.$$

Results. The plasma concentrations of ^{3}H-mannitol and ^{14}C-PAH, expressed as percentage of dose remaining in plasma during the first 32 min after injection, are shown in Fig. 1, and Table I.

[1] The D-mannitol-1-^{3}H (N) 2.65 Ci/mmole and ^{113}Sn/^{113m}In generator were from New England Nuclear, Billerica, MA and the para-aminohippuric (2-^{14}C) acid 23.5 mCi/mmole was from Dhom Products, North Hollywood, CA.

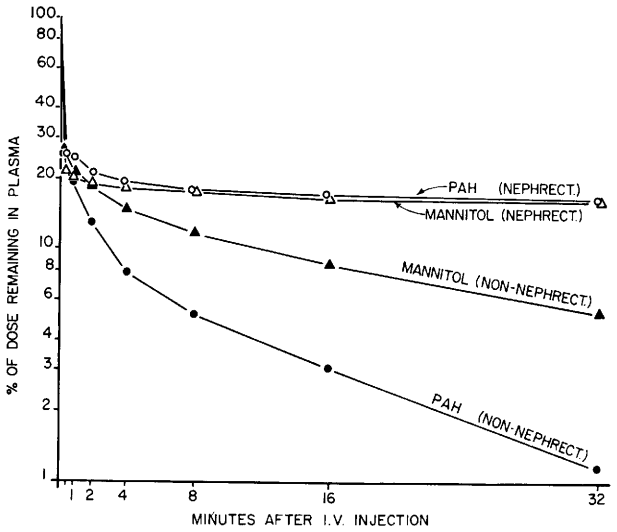

FIG. 1. Indicating the percentage of ^{3}H-mannitol and ^{14}C-PAH remaining in plasma after iv injection in rat. The initial drop of both substances represents equilibration between plasma and extracellular fluid. This is nearly complete by 30 sec. Subsequently, renal excretion becomes the dominant mechanism of loss from plasma. Even with PAH, which undergoes essentially complete renal clearance, the major mechanism of early loss from plasma is trans-capillary extracellular equilibration, since nephrectomy produces only a trivial effect until after 1 min. For each mean value, $n = 3$.

Non-nephrectomized animals. The plasma levels of ^{3}H-mannitol and ^{14}C-PAH fell rapidly to about 20% of the original level during the first minute. After this initial, rapid phase, a slower rate of disappearance continued during the remainder of the experimental period. The PAH during this second phase disappeared considerably more rapidly than mannitol.

Nephrectomized animals. The rapid disappearance of both tracers during the first minute was indistinguishable from that seen in the nonnephrectomized group. The subsequent, slower loss due to renal excretion was absent in this group.

Discussion. The use of a macromolecular internal standard, ^{113m}indium-transferrin, allows a calculation independent, within a wide range, of injected dose, animal weight, and specimen weight. In these studies the plasma concentration of ^{3}H-mannitol and ^{14}C-PAH are expressed as a percentage of the ^{113m}indium-transferrin. The ^{113m}indium used in this study as a plasma label binds firmly to plasma transferrin (5). The distribution of ^{113m}indium-transferrin following iv injection is approximately the same as that of radioiodinated serum albumin (7, 8). The ^{113m}indium-transferrin thus remains essentially completely confined to the plasma space during the first 32 min after iv injection and serves as an internal standard correcting for plasma volume and initial incomplete mixing with blood plasma (9, 10).

A suitable extracellular tracer is not ionized, is metabolically inert, and has negligible affinity for plasma protein binding sites and cellular transport systems. It should be sufficiently small to readily penetrate the general capillary wall and equilibrate rapidly between plasma and general ECS. The ^{3}H-mannitol used in the present experiment fulfills these criteria and exhibits a distribution space approximately the same as that measured by inulin and sucrose (11).

The initial plasma disappearance curve of the ECS tracers used here can be divided into two major exponential components.

Initial disappearance. This initial phase exhibits a $T_{1/2}$ less than 30 sec. About 80% of both mannitol and PAH has left the blood by 1 min whether or not the animal has been

TABLE I. Percentage of ^{3}H-Mannitol and ^{14}C-PAH Remaining in Plasma after iv Injection Expressed with Reference to a Simultaneously Injected Macromolecular Plasma Marker (^{113m}In-transferrin).

	Not nephrectomized	Nephrectomized	Not nephrectomized[a] vs nephrectomized
	Mean SD	Mean SD	p
	^{3}H-Mannitol[b]		
0 sec	100% (assumed)		
30 sec	24.6 ± 2.6	21.7 ± 3.7	<.4
1 min	21.4 ± 0.7	20.4 ± 2.1	<.5
2 min	18.5 ± 0.6	18.9 ± 1.5	<.8
4 min	14.9 ± 0.7	18.1 ± 1.0	<.02
8 min	11.7 ± 1.0	17.5 ± 1.1	<.01
16 min	8.6 ± 0.7	16.4 ± 0.3	<.001
32 min	5.3 ± 0.9	16.2 ± 0.8	<.001
	^{14}C-*p*-Aminohippurate[b]		
0 sec	100% (assumed)		
30 sec	24.2 ± 1.4	25.4 ± 5.2	<.8
1 min	19.3 ± 0.8	24.9 ± 3.2	<.05
2 min	12.9 ± 0.2	21.6 ± 1.3	<.001
4 min	7.9 ± 0.5	19.6 ± 0.7	<.001
8 min	5.3 ± 0.3	18.1 ± 0.7	<.001
16 min	3.1 ± 0.3	17.4 ± 0.3	<.001
32 min	1.2 ± 0.3	16.6 ± 0.4	<.001

[a] The difference between nephrectomized and nonnephrectomized animals becomes significant earlier with PAH than with mannitol because of its more rapid renal excretion.

[b] For all mean values, $n = 3$. The mean values are plotted in Fig. 1.

nephrectomized. This presumably represents equilibration of the tracers with the general ECS and is probably representative of the disappearance from plasma of most polar solutes remaining unbound to plasma protein and having little affinity for entrapment mechanisms on general cell surfaces.

Subsequent slower disappearance. With mannitol the $T_{½}$ of this second major exponential fall is approximately 15 min and, with PAH, 10–12 min. In the nephrectomized animals a $T_{½}$ of several hours is seen because of the absence of renal excretion.

These studies indicate that even solutes which undergo virtually complete clearance by a major organ, such a clearance by kidney described here, largely leave the plasma during the first minute after iv injection due to equilibration with the general ECS. This initial rapid loss from plasma is not significantly affected by interruption of specific organ entrapment (Table I). Such specific organ entrapment becomes evident only after the first few minutes, by which time most of the solute has left the blood plasma.

Summary. The early disappearance from plasma of extracellular tracers ^{3}H-mannitol and ^{14}C-para-aminohippurate was studied after intravenous injection in nonnephrectomized and nephrectomized rats. The amount of each tracer remaining in the plasma was studied between 30 sec and 32 min after injection and was expressed as a percentage of the dose remaining in plasma relative to a simultaneously injected ^{113m}indium-transferrin plasma tracer internal standard. Approximately 80% of the ^{3}H-mannitol and ^{14}C-para-aminohippurate disappeared from plasma in the first minute in both nonnephrectomized and nephrectomized groups. The effects of nephrectomy became clearly evident only after the first minute when blood plasma and extracellular fluid equilibration was nearly complete. This initial disappearance from plasma of both tracers with a $T_{½}$ less than 30 sec is probably represen-

tative of many polar solutes not appreciably bound to plasma proteins and having minimal affinity for general cell surfaces.

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