DISTRIBUTION OF VARIOUS CLASSES OF RADIO-LABELED TRACERS IN PLASMA, SCALP, AND BRAIN

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In clinical nuclear techniques such as routine brain scanning, brain scintillation angiography, and inert gas washout cerebral blood-flow studies, the distribution of various radiolabeled compounds in the head is mapped following introduction into the blood. The distribution of tracers in cranial tissues and plasma differs widely during the first half-hour after i.v. injection depending on molecular size and lipid solubility of the tracer. Since tissue concentrations are not readily measured quantitatively in humans, studies were undertaken to assess the concentrations achieved in rat serum, scalp, and brain after an i.v. injection of (A) a radiolabeled macromolecule, ^{113m}In-transferrin; (B) a highly diffusible small molecule, tritiated water (^{3}HOH); (C) a small, metabolically inert polar molecule not protein-bound, ^{3}H-mannitol; and (D) a partially plasma protein-bound tracer, $^{99m}TcO_4^-$. It was felt that these basic studies of tissue distribution in animals would aid in understanding radionuclide distributions in the human head during clinical studies.

METHODS

Approximately 300-gm Wistar rats of either sex on a routine diet were weighed and rendered unresponsive with intraperitoneal pentobarbital. The femoral vein was surgically exposed and cannulated with a 25-gauge needle. One to 10 μCi of ^{3}H-mannitol, ^{3}HOH, or $^{99m}TcO_4^-$ in 1-ml physiological saline were injected. Approximately 20 μCi of ^{113m}In-transferrin was prepared by adding a small volume (less than 0.02 ml) of 0.04 *N* HCl eluate from a New England Nuclear ^{113}Sn generator to 0.5 ml of rat serum, and this mixture was injected into the exposed femoral vein. The injection syringes were pre- and post-weighed to obtain the weight of injected solution, and a weighed aliquot of the injected solution was prepared for either liquid scintillation or routine sodium iodide crystal well counting.

At intervals of 1, 2, 4, 8, 16, or 32 min after injection the animals were decapitated and terminal arterial blood specimens collected in a heparinized tube. All of the test substances were not studied at all time intervals because of their unchanging concentrations. The blood was centrifuged and 0.2 gm of plasma prepared for liquid scintillation or routine sodium iodide crystal well counting. Three rats were examined for each time and test substance studied. In each animal two specimens, each about 0.25 gm of homogenized brain rostral to mid-brain, were counted. The choroid plexus was included and the area postrema was excluded from the specimens. About 0.25 gm of calvarial scalp, clipped free of hair, was also counted. All specimens were dissolved and prepared for routine liquid scintillation counting of the beta emissions of ^{3}H and conversion electrons of ^{113m}In (*1*) using Packard Soluene solubilizer and Packard Instagel liquid scintillator. The 140-keV gamma emissions of ^{99m}Tc were counted in a crystal well counter. At least 4,000 counts were accumulated for each measurement.

Tissue concentration was calculated as a percentage of the mean concentration for the animal based upon animal weight, dose injected, and counts per minute per gram of tissue according to the following:

$$\%\ \text{mean body concentration} = \frac{\text{cpm/gm tissue}}{\text{injected cpm/gm animal}} \times 100.$$

Brain and scalp concentrations were not corrected for residual blood content.

In the routine $^{99m}TcO_4^-$ brain scan the cranial cavity appears empty and is surrounded by a rim or "halo" of radionuclide. This could be due to blood

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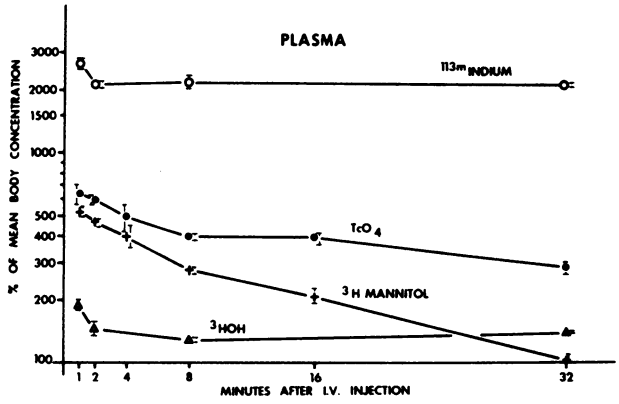

FIG. 1. Decreasing rat plasma concentrations of four classes of radiotracers used.

on the surface of the brain, in the dural sinus, and in skull and scalp. It could also be radionuclide in the extracellular fluid of scalp and, to some extent, in skull. If the "halo" originates in blood it should be most prominent in the first minute after injection but if it originates from the extracellular fluid of scalp and skull, it should appear later, after the blood level has fallen. To clarify this, frontal Anger camera views were taken during the first 12 min after i.v. injection of 12–14 mCi $^{99m}TcO_4^-$. The subject was an adult male whose scan was interpreted as normal. Blood concentrations of ^{99m}Tc were not determined.

RESULTS

The plasma, brain, and scalp radionuclide concentrations during the first 32 min after i.v. injection are shown in Figs. 1, 2, and 3, respectively. The ^{113m}In-transferrin remains confined to the plasma compartment, achieving 2,000–2,500% of the body mean value throughout the 32 min (Fig. 1). Essentially none appears in brain (Fig. 2) and very little in scalp (Fig. 3).

The ^3HOH distributes in 1 min to a much larger space than ^{113m}In-transferrin, and between 1 and 32 min, approximately equal, low concentrations are found in plasma, brain, and scalp.

The ^3H-mannitol distributes rapidly to a larger space than ^{113m}In-transferrin but not to as large a space as ^3HOH. Tritiated mannitol appears in scalp in considerable concentrations, but essentially none in brain tissue other than that contained in brain blood plasma.

The $^{99m}TcO_4^-$ distributes in a manner intermediate between ^3H-mannitol and ^{113m}In-transferrin. The serum level falls less rapidly than mannitol, probably because of a slower renal loss. More appears in rat brain but this probably is accounted for by the higher concentration in the residual blood.

The cranial distribution of $^{99m}TcO_4^-$ during the first 12 min after i.v. injection in man is shown in Fig. 4. The cranial "halo" representing scalp and skull radionuclide concentration did not develop until after the first few minutes, during which time the blood level presumably had decreased substantially.

DISCUSSION

The presentation of tissue concentration as a percentage of mean body concentration has the advantage of including injected dose, animal weight, and the specimen weight in the calculation. With this method, 100% tissue concentration represents that concentration which would be found if the tracer distributed uniformly throughout the entire animal. Relative tissue concentrations are thus easily compared within one animal and between different animals.

Macromolecule—^{113m}In-transferrin. The ^{113m}In used in this study as a plasma label (*2*) is firmly bound to plasma protein, predominantly to transferrin (*3*). The distribution space of intravenously injected ^{113m}In-transferrin is approximately the same as that of radioiodinated serum albumin (*4*). This observation is consistent with the findings of the present study in which the level achieved in blood plasma is approximately 2,100% of the body mean concentration after the first 2 min. The slightly higher concentration at 1 min is probably due to early incomplete mixing with the entire blood compartment.

In prolonged equilibration studies macromolecules slowly leave the blood plasma compartment, and the mechanism of this loss may be due largely to the pinocytosis of blood plasma evident on electron microscopy of general capillaries (*5*). In normal brain the capillary cytoplasm shows virtually no such pinocytotic vesicles (*6*), and this is strikingly less than seen in the general capillary (*7*). This virtual absence of pinocytosis may explain the unmeasurably

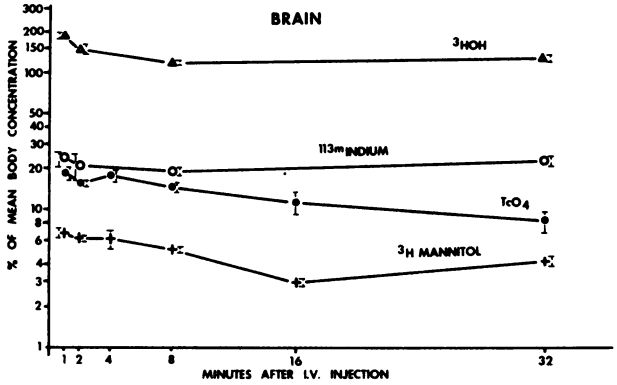

FIG. 2. Rat brain concentrations in first 32 min after i.v. injection.

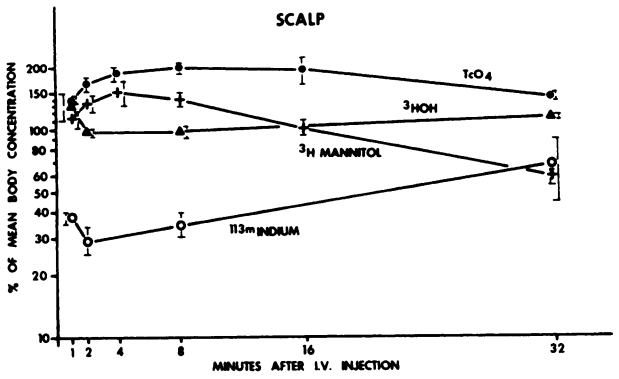

FIG. 3. Concentrations of various classes of radiotracers in rat scalp.

low blood brain barrier (BBB) permeability to macromolecules (*8*).

The ^{113m}In-transferrin appearing in brain almost completely represents residual blood plasma content.

Diffusible tracer—^{3}HOH. The ^{3}HOH studied here probably exchanges freely through all cell membranes (*9*). As a consequence of this characteristic, when injected i.v., it rapidly passes through capillary walls and distributes to the extracellular fluid and then into the intracellular compartments of all tissues. If the distribution space of ^{3}HOH is calculated, it is very large as evidenced by the low uniform concentration achieved in the three tissues studied here.

The BBB probably consists predominantly of the brain endothelial cell plasma membranes. There is a fusion of adjacent capillary cells with resulting tight junctions and obliteration of the intercellular clefts which normally create a low permeability of the general capillary wall to small molecules (*6,10*). The BBB behaves as though it is made up of a continuous plasma cell membrane (*11*). Exchanges of most solutes between blood and brain must, therefore, take place directly through the inner and outer cell walls of the capillary cell and through the interposed capillary cell cytoplasm rather than between capillary cells as found in most non-neural tissues.

Tritiated water passes freely through the BBB (*12*). The distribution of water is crudely representative of the distribution of diffusible tracers such as radioxenon and radiolabeled antipyrine which pass freely through all cell membranes by virtue of their lipid solubility. The distribution of these lipid soluble tracers in various tissues should differ somewhat from ^{3}HOH because their affinity for tissue fat commonly will be greater than that of labeled water for tissue fat. Thus, at equilibrium, more radioxenon would be expected to be found in brain than labeled water because of the greater affinity of xenon for brain lipid than water as expressed by the brain/blood partition coefficient of xenon (*13*).

Extracellular space marker—^{3}H-mannitol. The ^{3}H-mannitol distributes rapidly after i.v. injection to a space that is several times larger than the blood plasma as evidenced by a plasma concentration of 480% compared with an ^{113m}In-transferrin distribution space of 2,100% at 2 min. The mannitol distribution space demonstrated here approximates the mean extracellular space for the total rat as measured by the volume of distribution of inulin and sucrose (*14*). Although mannitol freely penetrates the general capillary wall and rapidly comes into equilibrium between plasma and extracellular fluid, it remains largely outside of cells and the fluid volume to which it distributes is less than for tritiated water. The early concentration of mannitol in blood plasma is approximately three times higher than for ^{3}HOH (Fig. 1).

Essentially no mannitol appears in brain, and the brain content indicated in Fig. 2 largely represents mannitol in the residual blood plasma in brain tissue (*8*). No correction was made in these studies for tissue blood plasma content, but such a correction could be made by subtracting the brain ^{113m}In-transferrin distribution space seen in Fig. 2. So that the present rat studies would more closely approximate the distribution in living patients, no blood correction for residual brain isotope was made. The blood content of rat scalp excised postmortem is almost nonexistent, but about 0.8% of brain weight is blood plasma in the decapitated rat. In vivo there probably is considerably more blood present in the rat

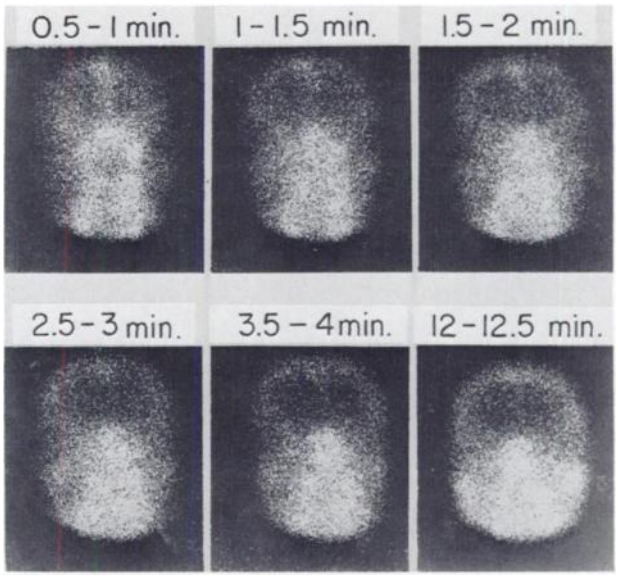

FIG. 4. Frontal Anger camera views during first 12 min after i.v. injection of $^{99m}TcO_4^-$. These studies suggest that "halo" seen about cranial cavity on routine brain scanning with this nuclide originates largely from radionuclide in extracellular fluid of tangentially viewed scalp and receives minor contribution from blood in vessels on surface of brain and in skull and scalp.

brain since some of the blood runs out of the organ after decapitation and during brain removal. In human clinical studies there probably is an even larger brain blood volume because of the elaborate system of venous sinuses and cerebral cortical veins present in humans. The human brain whole blood compartment in life may be 6–10% of the total brain volume (*15*).

To make these animal studies as relevant as possible to human studies, no nephrectomy was carried out. Since the limiting molecular weight for penetration through the rat renal glomerular membrane is approximately 62,000 (*16*) it may be assumed that mannitol (molecular weight 182) freely passes the renal glomerulus. As a result the blood level falls rapidly throughout the 32-min period examined here. The initial drop in plasma level of mannitol is primarily due to equilibration of the tracer with the general extracellular fluid which is approximately five times greater than the blood plasma volume (*14*). About 80% of the mannitol disappears from the blood in the rat in the first 1–2 min by virtue of this mechanism. This initial direct loss from blood after i.v. injection is probably representative of all lipid insoluble, small molecular radiotracers which are minimally bound to plasma protein and have no strong affinities for cell surfaces. Thus in clinical studies of any such small molecule, the major source of loss from blood in the first few minutes is distribution to the general extracellular fluid. In nephrectomized animals, the rate of mannitol loss from blood after the first few minutes is markedly decreased (*8*) because the long-term renal glomerular removal of mannitol from blood is absent. The rate of loss of mannitol during the first 1–2 min is not greatly different between nephrectomized and un-nephrectomized animals because the nephrectomy has no effect on the early distribution of the mannitol to the general extracellular fluid.

The ^{3}H-mannitol distributes to a large scalp space (*3*). This is representative of the large extracellular space in skin. The extracellular space in the skin of mammals is about 30% (*14*) and is considerably greater than the mean extracellular space of all tissues (*14*). The extracellular space of tissues is difficult to define since most measurements have been carried out by dilution indicators and the apparent distribution space of "extracellular" markers is quite variable, being quite strongly dependent on the specific indicator used and the time allowed for equilibration between plasma and extracellular fluid (*14*).

Combined extracellular fluid and blood plasma marker—$^{99m}TcO_4^-$. Many solutes in blood are partially bound to plasma protein with the unbound fraction in free solution. It is this tracer in free solution which is able to pass through the capillary intercellular cleft to equilibrate with the extracellular fluid. With tracers such as pertechnetate, which is 70–80% protein bound in plasma (*17,18*) there remains a substantial (20–30%) fraction which is in free solution and distributes as a small molecule. As this fraction in free solution is removed from the blood plasma, either by loss to the general extracellular fluid or by specific tissue entrapment, more of the bound $^{99m}TcO_4^-$ dissociates from the blood protein molecules maintaining about one-fourth of the total plasma TcO_4^- in free solution. As a result of this continuous process, most of the pertechnetate injected will be rapidly lost from the blood to extracellular fluid and to specific organ entrapment. The rate of loss of pertechnetate from blood thus is intermediate between mannitol and transferrin-bound indium.

The delayed appearance of the cranial "halo" after i.v. injection of $^{99m}TcO_4^-$ suggests it originates from radionuclide in tangentially viewed scalp and skull extracellular fluid rather than from blood.

SUMMARY

For interpretation of the distribution of the various nuclides and clinical brain dynamic studies a knowledge of the very different distribution of various classes of radionuclides employed in these studies is important. Animal studies were undertaken to provide basic data to further an understanding of clinical regional head mapping by various external nuclear scanning techniques.

The concentrations achieved in rat plasma, brain, and scalp tissues were studied during the first 32 min after i.v. injection of ^{113m}In-transferrin, ^{3}HOH, ^{3}H-mannitol, and $^{99m}TcO_4^-$. Serum, scalp, and brain concentrations are expressed as a percentage of mean body distribution of each tracer. The ^{3}HOH distributes to a large space and freely penetrates the BBB achieving, after the first minute, approximately equal concentrations in all three tissues studied. The ^{3}H-mannitol and $^{99m}TcO_4^-$ distribute to extracellular fluid, achieving high concentrations in scalp and very little in brain. The ^{113m}In-transferrin remains confined to the plasma. Serial gamma camera pictures during the first 12 min after i.v. injection of $^{99m}TcO_4^-$ show that the halo of isotope seen about the cranial cavity appears only after the first several minutes, suggesting that it is not due to radionuclide in blood in the superficial brain or extracranial tissues but rather to radionuclide in the extracellular fluid of scalp and skull.

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