Assessing Human Brain Characteristics Using Radioisotopes

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Many active physical and chemical processes occur continuously in the brain. We know very little about these vitally important processes because the human brain is so inaccessible to ordinary methods of assessment, encased as it is, in a tight, hard skull. Furthermore, brain functions are incredibly fragile and any loss of function is likely to be permanent. The only safe means of continous, objective methods of assessment of brain function now widely available are electrical monitoring and ultrasound echoing. These, however, define extremely restricted aspects of brain function.

Radioisotopes offer a versatile tool for measuring foreign atoms in living systems. Nowhere is this capability more applicable than in brain studies since such techniques are completely atraumatic. A method is described here which should open a new window into brain function by making accessible to simple measurement a number of previously inaccessible brain characteristics.

Much information about dynamic processes in brain (or any other organ) could be obftained if the following steps could be performed:

1. A radio-labeled tracer is injected into the blood at time zero.
2. At a subsequent time when the brain conten of this tracer is of interest the entire brain is removed from the skull and placed in a well-counter.
3. The isotope content is measured.
4. The brain is replaced in the head in the same condition in which it was removed.
5. The steps are repeated at intervals.

Since the living human brain is not removable, much less replaceable, such studies have been carried out largely in expendable animals, with the postmortem brain isotope content of each animal supplying one point on a curve. Techniques such as this could be applied to humans if the total isotope content of brain could continuously be measured while functioning normally in place in the head.

Measuring the absolute isotope content of living human organs has been the object of considerable research but only the thyroid content of radioiodine has been notably successful. In general, the counting of total organ isotope content has been attempted by directing a collimated gamma-detector at the organ and measuring the isotope seen; comparing this measurement with that of a phantom organ containing a known amount of the same isotope present in the real organ.

To calibrate such an externally positioned detector is difficult, because some unwanted isotope in adjacent and overlying tissues is also seen and the changing counting efficiences for isotope located at various depths of tissue and at varying distances from the detector introduce significant errors difficult to correct.

Absolute measurement of thyroid iodine is relatively easy. A few hours following injection virtually all of the radioiodine in the region of the neck is in the thyroid. The location of this gland is quite predictable. In contrast to this desirable situation the absolute isotope content of other organs, such as heart, lungs, liver, kidney, spleen, etc. is difficult to measure either because it is difficult to incorporate the entire organ in the detector field at a known distance or isotope in nearby organs is seen superimposed upon the isotope in the organ of interest. Detectors looking at kidney, as an example, will also see considerable overlying muscle and

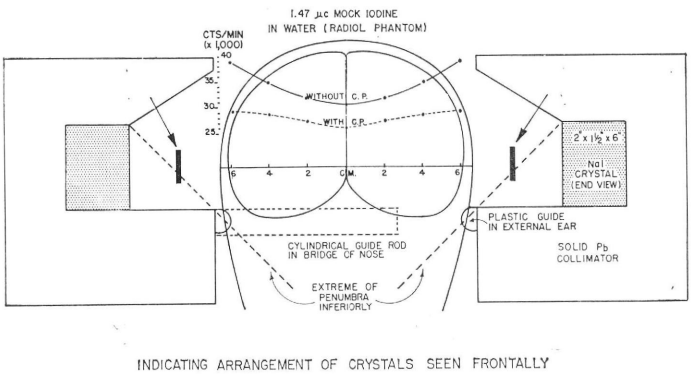
in the detection field there will also be unknown amounts of isotope in liver, pancreas and blood in great vessels. The exact depth and location of the kidney is difficult to predict.

The human brain lends itself to total organ counting since it fills the upper half of the head and is conveniently located well away from other large organs which might contribute significantly to external gamma-counting. This latter point is particularly true if the detectors are placed lateral to the head and shielded from the tissues below the floor of the cranial cavity, and compton scatter from radiation originating elsewhere in the body is rejected by suitable pulse-height discrimination. Brains of other animals are less suitable to such external measurements because they occupy relatively small proportions of the head and most animals have relatively large volumes of bone and muscle overlying brain.

The three pound adult human brain occupies virtualy the entire head above the plane extending approximately through the eyebrows and ne external ear canals. The outer half inch of this cranial portion of the head is scalp and skull and the remainder is brain tissue.

The ease with which high-energy gamma-rays penetrate bone allow their utilization to deduce the presence of isotope in a large structure such as the human brain, even though it is surrounded by a calcium and phosphorus shield (skull). The localization by external detectors of isotopes within brain has been applied in clinical medicine largely in the detection of the brain toumors. Another class of studies, to be described here, does not attempt to localize the isotope within the brain but rather seeks to determine the total brain content of isotope. This approach is analogous to measurement of total uptake of iodine by the thyroid gland.

To do this requires a detection system which, ideally, would have a distribution of counting efficiency confined to the brain. A small amount of isotope in the scalp and skull will, of course, be superimposed on the brain content. This efficiency should be uniform for all regions and depths of the brain so that the distribution of isotope in various regions of the brain will not alter the total brain count. In addition, the counting efficiency should be as high as possible so that accurate counts can be obtained with a minimum dose of isotope administered to the patient. By sacrificing localization by using open rather than focal collimation a considerable increase in counting efficiency can be achieved. One form of such a counting system is shown in Figure 1.



INDICATING ARRANGEMENT OF CRYSTALS SEEN FRONTALLY

Fig. 1

In this detection system, two large (3.5 × 5 × 15 cm) thallium activated sodium iodide crystals are bilaterally arranged about 5 cm lateral to the head with their long axes approximately paralleling the main mass of the brain (Figure 2). A photo-multiplier is coupled to each crystal. When the pulse outputs of these crystals are summed and the

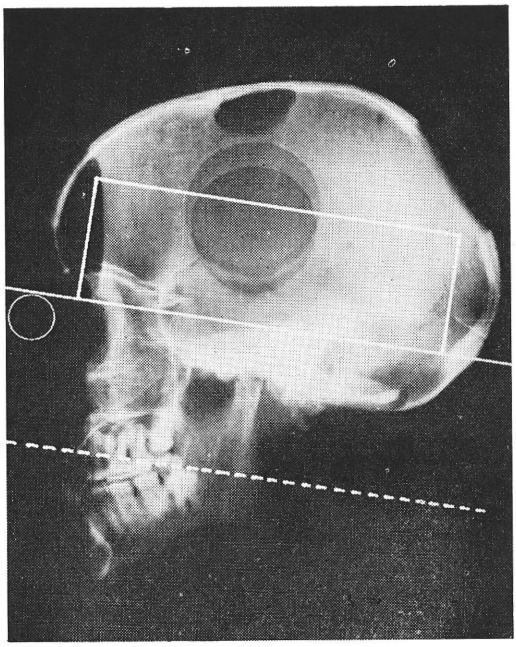


Fig. 2

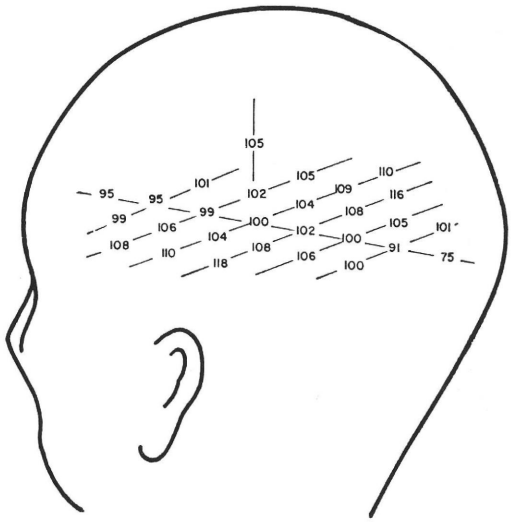


Fig. 3

distribution of counting efficiency plotted using a water-filled radiological phantom, a reasonably uniform efficiency is achieved. This can be improved upon by pulling the crystals farther from the side of the head but with the loss of counting efficiency.

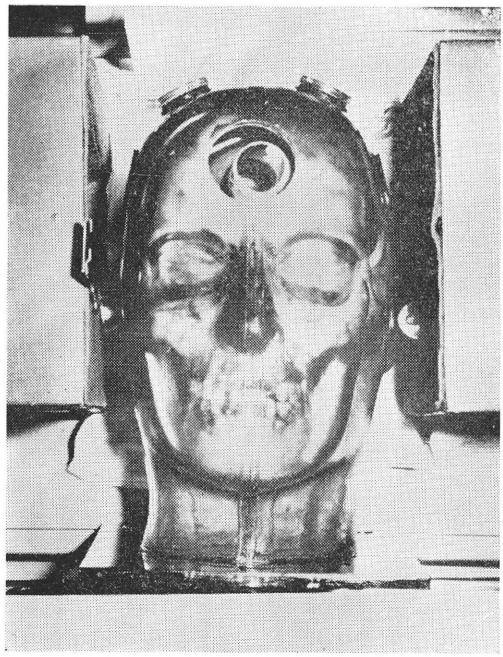


Fig. 4

We have sought to flatten the field of efficiency of the dual detector without excessive loss of count by introducing a flat lead shield between the head and each crystal (Figure 1), to partially absorb superificially originating gamma-radiation. The quantum efficiency of the present system is about 1%, with most common medical isotopes, allowing studies to be carried out with only 5-10 uc dosage. The distribution of counting efficiency within a phantom head is shown in Figure 3. A mock iodine source was used in a water-filled phantom. The quantum efficiency for any given isotope in a real head can easily be determined as indicated in Figure 4. A more advanced system using six 5×5 cm sodium iodide crystals is now in use in our laboratory and is shown in Figure 5A,B.

Given such a system for monitoring the total isotope content of the cranial portion of the head, to what use might it be put? Two examples of informaion derived from application of this apparatus, and probably unobtainable in any other way, are presented below.

Red Blood Cell and Plasma Content of Brain

Human blood in large blood vessels con-

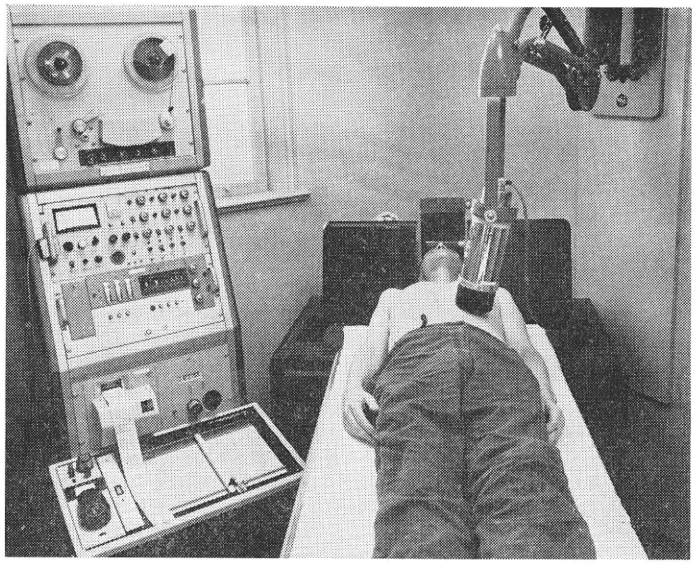


Fig. 5(A)

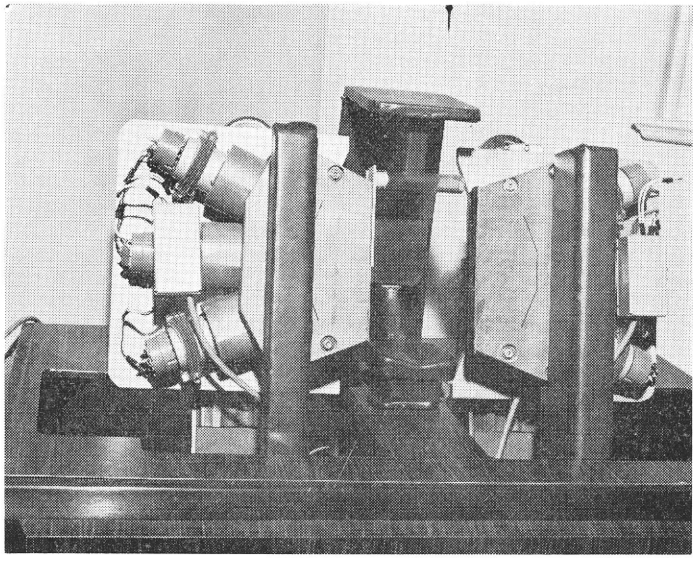


Fig. 5(B)

sists of about 45% red cells and 55% plasma (fluid part of the blood). It has been shown that this percentage of blood which is red cells (the hematocrit) is, when the total body red cell and plasma volumes are measured, about 9% less than the 45% found in large blood vessels.[1-3] Thi sis explainable based upon the differential rates of passage of red cells and plasma through the smaller blood vessels.[3-5] In smaller vessels (such as capillaries) red cells move substantially faster than the plasma (Figure 6). There are, accordingly, less red cells in proportion to plasma ih these small vessels[3] than in large vessels.

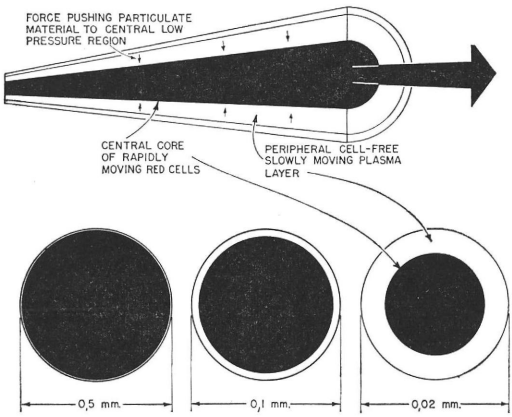


Fig. 6

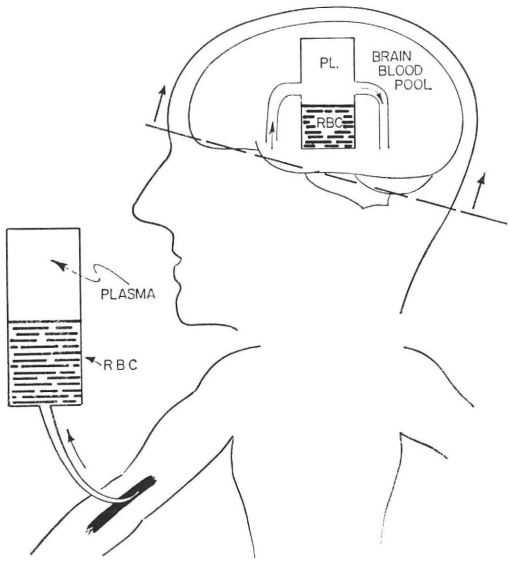


Fig. 7

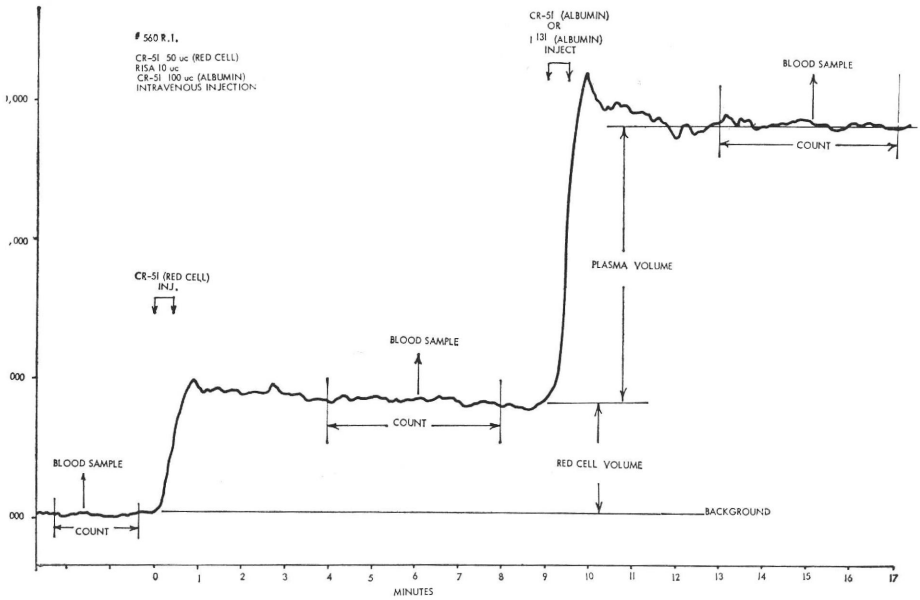


Fig. 8

In view of this factor, very vascular organs such as brain and kidney, containing many capillaries having high flow rates, would be expected to contain less red cells than might be anticipated, knowing the large blood vessel hematocrit. The hematocrit relationships are shown diagrammatically in Figure 6.

If, in view of these considerations, direct measurements of the total amount of red cells and of plasma in brain were made, one might expect to find a lower hematocrit than in blood drawn from an arm vein (Figure 7). By using the brain well-counter to measure the amounts of red cell and plasma labels in the total living brain, we determined the percentage of red cells in the blood of the brain to be 16% lower than in a large arm vein.[6]

To make these measurements, 50 microcuries of ^{51}Cr labeled red cells were injected into an arm vein and four minutes allowed for mixing with the blood (Figure 8). Other than the slight pain of venipuncture the subjects were comfortable and were studied in a healthy state. The brain ^{51}Cr content was then counted for four minutes. In the middle of this counting period, a venous blood specimen was withdrawn from an arm vein and counted. Approximately 100 microcuries of ^{51}Gr labeled albumin (for plasma labeling) were then injected and four minutes allowed for mixing. Brain and blood plasma isotopes were then counted as for the red cells. The net red cell and plasma counts in the brain were then compared with the net red cell and plasma counts in the venous blood samples counted in a standar dwell counter.

The net count derived from the labeled red cells in the head will be directly proportional to the volume of red cells within the detector field. The net count derived from the labeled albumin in the head will be directly proportional to the volume of plasma within the detector field. Approximately 20,000 net red cell counts and approximately 40,000 net plasma counts were obtained.

If the hematocrit of the total blood in brain were the same as in the arm vein, the ratio of the red cells to plasma volumes for brain would be the same as ratio for the arm vein.

What we have actually done is to measure separately the red cell and plasma volumes in two pools by placing the labeled compartments in each pool in separate well-counters. In the case of the cranial pool we used our cranial well-counter. For the venous blood pool we used an external specimen well-counter.

n 32 subjects so studied the cranial well-counter indicated substantially more plasma volume present relative to red cell volume when compared with venous blood studied in the external counter.

Brain Copper Uptake in Wilson's Disease

Humans normally ingest 2-5 mgm of copper daily. This copper, if absorbed and stored in tissues, would be highly toxic. Fortunately, most humans have an excellent biochemical mechanism for causing this copper to be excreted with very little uptake by body tissues.

In an occasional person this mechanism for handling dietary copper is defective. As a result, the body tissues accumulate a toxic amount of copper. This biochemical abnormality results in Wilson's disease. In this disease the brain (and all other tissues) contain from two to twenty times the normal amount of copper at the time of death.[7] The liver and lenticular nucleus of the brain are particularly affected by the deposition of copper and for this reason, the disease is commonly termed hepatolenticular degenration. The increased tissue content of copper implies that tissues will pick up an excessive

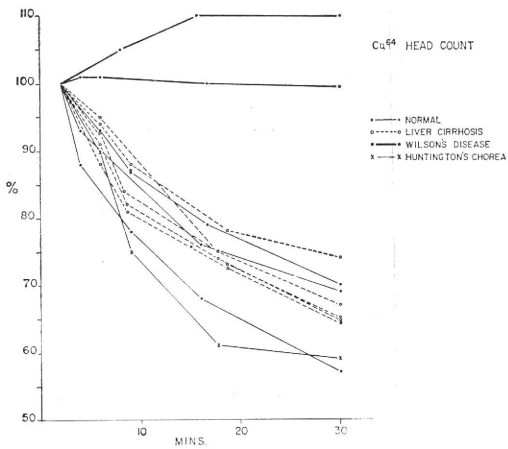


Fig. 9

amount of any copper introduced into blood. The amount of this uptake by brain can be demonstrated by plotting the total brain isotope after an itnravenous dose of ^{64}Cu as Cu-acetate.

Figure 9 illustrates the increased brain uptake of copper in two cases of Wilson's disease compared with normal subjects. By correcting for the isotope content of the blood in brain, actual tissue uptake can be estimated. The precise mechanism which results in this increased brain tissue uptake has been studied in greater detail by the method described here.[8]

In the performance of these studies approximately 50 microcuries of ^{64}Cu as the acetate were injected intravenously in two ways. Some studies used a rapid single injection in which all of the ^{64}Cu entered the blood within one minute. This resulted in an initially high blood level with a rapid fall due to removal of the Cu from blood by liver, and to a lesser extent, by all other tissues. In some studies the ^{64}Cu was pumped in at an initially rapid rate but this rate of injection was reduced periodically attempting to maintain a constant blood level.

With rapid injection the ^{64}Cu blood level fell at a slower than normal rate in Wilson's disease. This has been shown by others to be due to a failure of the liver to remove the Cu at the normal rate. This left the Cu level in the blood higher-than-normal during the first hour. This would allow more opportunity for the copper to leak out of the blood into other tissues thus explaining the high tissue copper levels.

Another explanation of the increased tissue uptake was that the copper, which is loosely bound to plasma albumin just after injection, was either abnormally loosely bound and thus more readily available for uptake from blood or that the tissues in Wilson's disease had an abnormally high affinity for the copper. These factors could not be resolved in those studies in which the blood level was falling rapidly because there were too many variables that could not be precisely defined. To remove the variability of the rapidly falling blood level a constant blood level was maintained in some studies. If the brain in Wilson's disease took up Cu at a faster-than-normal rate under

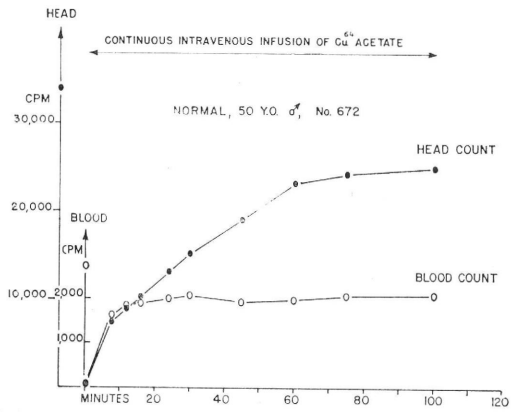


Fig. 10

these conditions it would indicate either an abnormally loose Cu binding by plasma albumin in an increased brain tissue affinity for Cu.

In the presence of a constant blood Cu level the rate of brain uptake was the same in Wilson's disease and the normal (Figure 10). This indicated the increased brain uptake was due to the impaired ability of the liver to remove Cu from blood shortly after it has appeared in blood from the intestine.

Brain Blood Flow

The continuous supply of blood to human brain is of great importance. This is very difficult to measure precisely because it is quite variable in different parts of brain. Its measurement is complicated by the inaccessibility of vessels leading to the brain, and the rigid skull prevents direct access to brain tissue.

A semi-quantitative indication of the total amount of blood passing through the brain can be obtained by measuring the time of passage of blood through the brain. There is about 130 cc of blood in the brain vevssels at any one time and the time of passage of the blood through this blood pool will be equal to the replacement time of the pool.

By releasing a bolus of ^{131}I of hippurate (^{99m}Tc pertechnetate is more desirable) into the right heart by intravenous injection, this turnover time can be determined..[9-11] If the cranial portion of the head is counted by the cranial detectors it will be seen that the

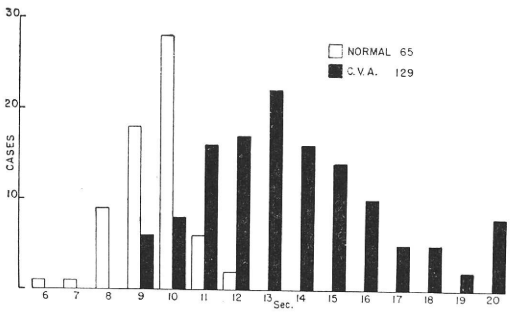


Fig. 11

isotope content of brain begins to rise about 7-8 seconds after vein injection. The maximum rate of rise of this head count will occur at the time of entrance into the brain of the most concentrated segment of the entering bolus (Figure 11). When this same most concentrated segment is leaving the brain (7-8 seconds later), the count rate is falling at a maximum rate. The time interval between the maximum rate of rise and the later maximum rate of fall is the most common (mode) transit time of the brain blood pool. These times of maximum rise and fall can most con-

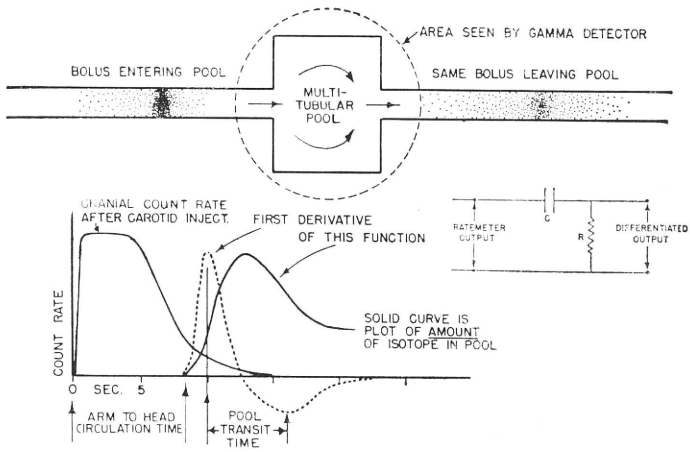


Fig. 12

veniently be determined by simple capacitance differentiation of the rate-meter output (Figure 11). The differentiated curve has an initial positive peak and a subsequent negative peak representing enterance and exit of the most concentrated segment of the radioactive bolus. The interval between positive and negative peaks is the most common (mode) passage time of blood through brain. There is no single time which designates the passage time through brain since there is a range of times through any one brain from about 5 to 15 seconds in normal adults. If one is to give a single passage time it must be designated as some statistical point on the distribution curve of these times.[10]

In a clinical application of this method, 135 patients with cerebrovascular occlusive disease scalp to brain concentrations were calculated, indicate the scalp contains perhaps 5-10 times the concentration albumin as brain. This high

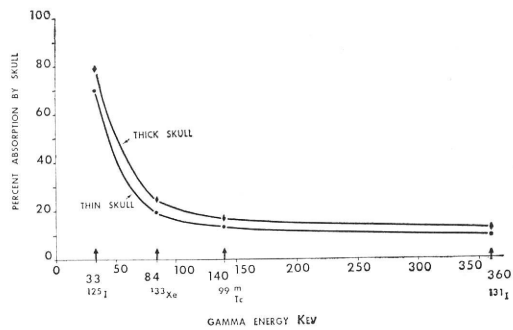


Fig. 13

proximately 15% of the total cranial volume. The skull is 16% of the total cranial volume.

Preliminary data obtained from rabbits using ^{131}I serum albumin, where the ratio of were compared with 65 normal controls. The longer passage times of the diseased subjects is shown in Figure 12.

Shielding Effects of Scalp and Skull

Although the brain is in the best location of any organ to be counted externally, it is still covered by an electron-dense skull and a considerable volume of scalp. We have studied artifacts created by skull and scalp.

The skull acts as a shield for radiation originating in brain. This will be especially marked for gamma radiations less than about 100 Kev because of photoelectric absorption due largely to calcium in skull. From Figure 13 it is clear that gama-emitters with energies less than about 100 Kev introduce a progressive artifact due to this shielding effect.

The volume of scalp covering brain measured 10 adult males by a soft-tissue technique and determined to be 232 cc. with a standard deviation of ± 50 cc. This is apconcentration in scalp relative to brain, coupled with the large volume of human scalp, indicates that the scalp contributes more counts to a common RIHSA brain scan than does the brain.

These studies are examples of physical and biochemical processes that lend themselves to analysis by this method of external counting. Many aspects of living human brain function should be similarly accessible to this harmless approach.

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BIBLIOGRAPHY

1. J. G. Gibson, 2nd, W. C. Peacock, A. M. Seligman, and T. Sack. Circulating red cell volume measured simultaneously by radioactive iron and dye methods, J. Clin. Invest., 25, 838, 1946.
2. H. Chaplin, P. L. Mollison, and H. Vetter. The body hematocrit ratio: its constancy over a wide range, J. Clin. Invest., 32, 1309, 1953.
3. R. Fahraeus. The suspension stability of the blood, Physiol. Rev., 9, 241, 1929.
4. P. Dow, P. F. Hahn and W. F. Hamilton. The simultaneous transport of T-1824 and radioactive red cells through the heart and lungs. Am. J. Physiol., 147, 493, 1946.
5. E. D. Freis, and J. R. Stanton, and J. R. Emerson. Estimation of relative velocities of plasma and red cells in the circulation of man. Am. J. Physiol., 157, 153, 1949.
6. W. H. Oldendorf, M. Kitano, S. Shimizu, and S. Z. Oldendorf. The hematocrit of the human cranial blood pool. Circulation Res., 17, 523, 1965.
7. J. N. Cumings. The copper and iron content of bran and liver in the normal and in hepatolenticular degeneration. Brain, 71, 410, 1948.
8. W. H. Oldendorf, and M. Kitano. Increased brain radio-copper in Wilson's disease, AMA Arch. Neurol., 13, 533, 1965.
9. W. H. Oldendorf. Measurement of the mean transit time of cerebral circulation by external detection of an intravenuously injected radioisotope. J. Nucl. Med. 3: 382, 1962.
10. W. H. Oldendorf. Isotope study of brain blood turnover in vascular disease. AMA Arch. Neurol. 12: 30, 1965.
11. W. H. Oldendorf, and M. Kitano. Radioisotope measurement of brain blood turnover time as a clinical index of brain circulation. J. of Nucl. Med. 8: 570, 1967.

LEGENDS

Figure 1. To obtain an approximately uniform counting efficiency for the entire brain, two opposed crystals are used. The external ears and bridge of the nose are used as positional reference points. The lead correcting plates interposed midway between crystals and side of the head reduce the counting efficiency for isotope on the lateral aspect of the head near the crystal.

Figure 2. A radiographic view of the commercial phantom head to indicate the relationship of the crystals to the cranial cavity.

Figure 3. A mock ^{131}I point source was moved about within the water-filled phantom

and the central count rate taken as 100%. By making the counting efficiency reasonably independent of position, regional differences of distribution of isotope within the brain become unimportant allowing total brain counting.

Figure 4. A commercial plastic radiological phantom head containing a human skull and filled with water is a good physical substitute for a living human head. A hole has been bored in the forehead of this phantom so it can be placed face-up while filled with water as indicated here. To estimate counting efficiencies relative to a standard well-counter, radioisotope in a test tube can be inserted into the water and counted while submerged and again after removal to a standard well -counter.

Figure 5A. A newer cranial well-counter now in use. The subject's head is positioned within the detector field in a position which will bring the 2.5 cm thick lead shields lateral to head on a line between the external ear canals and the bridge of the nose. The position of the top of the head when in this position is determined by a movable flat plate which is moved into contact with the top of the head and its position noted so the head can subsequently be re-positioned for later counting. A wall-probe is available to look at liver, heart or elsewhere during the test. Three pulse-height channels handle pulses from the left three crystals, the right three crystals and the wall probe. Every tenth pulse is put on tape for later playback through an E-put meter or rate meter for digital print-out or display on a T-Y plotter.

Figure 5B. On each side of the head are three 5×5 cm NaI (thallium activated) crystal-photomultiplier units shown here with dust-covers removed. The positions of the crystal faces are indicated by the black lines on the lead plates shielding the crystals from the subject's body. The left three outputs are in parallel as are the right three. The small gray boxes on left and right house the preamplifiers.

There is approximately 4 cm of lead between the crystals and the subject's trunk. Over the top of the subject's head is a 1.3 cm lead shield to minimize background count. With 30% windowing the background is about 600 cpm from all six head crystals and counting efficiency for intracranial ^{131}I is about 0.4%.

Figure 6. In small vessels (the microcirculation) red cells tend to be carried rapidly by a central, fast moving axial stream of blood plasma. For this reason, small vessels contain blood which has a lower percentage of red cells. This effect is more pronounced in very small, high velocity vessels as indicated diagrammatically in the cross-sections of vessels shown here.

Figure 7. If one were to determine the percentage of red cells in a blood specimen taken from an arm vein and in the blood present in the entire brain at any instant (100-150 cc) the percentage would be found to be lower in the blood in the brain because of the difference in speeds of passage of red cells and plasma shown in Figure 6. In recent years the importance of the peculiar behavior of blood in the microcirculation has become recognized but studies have been hampered by the lack of means of assessing blood inside small vessels deep within living organs.

Figure 8. A continuous plot of total brain radioactivity during injection of labeled red cells and plasma. ^{51}Cr labeled red cells are injected and allowed to mix with all of the blood. The amounts of label in the brain and in a specimen of blood drawn from an arm vein are determined. The same procedure is carried out using ^{51}Cr albumin as a plasma label. From the vein to brain count ratios, the percentage of red cells in the blood in the brain is calculated.

Figure 9. When ^{64}Cu as Cu-acetate is injected intravenously and the amount of this isotope in the brain is continuously plotted, the normal individual shows a continuously falling curve, largely due to removal of the copper from the blood by the liver. In Wilson's disease the amount of copper in the brain rises for some time, probably because of the defective ability of liver to remove the injected copper. The sustained high blood copper level allows excessive diffusion of the copper out of the blood into brain tissue resulting in an excessive total brain copper content.

Figure 10. The increased tissue uptake could be explained by postulating an abnormally high diffusibility of ^{64}Cu shortly after appearance in blood or that the ^{64}Cu just

after injection is in a normally highly diffusible state but that it persists in blood for an abnormally long time allowing more time for the ^{64}Cu to diffuse out of blood. To clarify this, ^{64}Cu was injected intravenously by a continuous infusion pump to obtain a constant blood level. The rate at which the cranial content rose in the presence of this constant blood level was used as an indication of diffusibility. The rate of rise of the brain Cu in Wilson's disease was found to be the same as in the normal curve shown here suggesting the increased uptake of Figure 9 was due to the prolonged elevated Cu blood level after a single injection.

Figure 11. A diagram showing the somewhat dispersed bolus of radioactive tracer approaching the cranial blood pool and the same, more dispersed bolus as it would appear leaving this pool and the field seen by the detection system. The times of maximum rate of increase and decrease mark the entrance and exit of the most concentrated part of the bolus from the cranial blood pool. This differentiation is easily achieved by a simple circuit which will provide a useful output voltage which closely defines the rate of change or first derivative of the count rate meter output voltage. A capacitor C is placed in series with the rate meter output voltage and the right plate of the capacitor is shunted to ground by resistance R. Current flow through C and R will closely approximate the rate of change of the rate meter output provided the time constant C and R is short relative to rates of change in rate meter output voltage. For a varying signal such as found in the present work, a differentiating time constant of about 0.1 second is adequate. This might be obtained with a value for C of 1 mfd and R of 0.1 megohms. Ideally, the output impedance of the rate meter will be substantially lower than the value of resistance R. A reserve of display sensitivity must be available since this additional circuitry results in a considerable attenuation of the input voltage. Because of an enhancement of noise inherent in the differentiation process, additional high frequency filtering may be required.

Figure 12. Indicating the distribution of mode circulation times in a group of normals contrasted with a group with known cerebrovascular disease.

Figure 13. The precentage of gamma ray absorption by thin and thick regions of wet skull. Each point represents an average value for ten skulls. Broad beam collimation was used with a pulse-height threshold set at approximately 75% of photopeak energy of the isotope being used.

^{99m}Technetium Labeled Compounds*

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Introduction

Advances in scintiphotographic instrumentation and the availability of an increasing number of radionuclides have been responsible for a rapid growth of nuclear medicine in recent years. The development and introduction of ^{99m}Tc has played an important role in this growth.

^{99m}Technetium was initially suggested for potential medical applications due to a com-

*This work was done under the auspices of the U.S. Atomic Energy Commission.