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Blood-brain barrier studies in man using the double-indicator method

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LASSEN, NIELS A., JENS TRAP-JENSEN, S. CRAIGHEAD ALEXANDER, JES OLESEN, AND OLAF B. PAULSON. *Blood-brain barrier studies in man using the double-indicator method.* Am. J. Physiol. 220(6): 1627–1633. 1971.—The permeability of the brain capillaries to small hydrophilic tracers was studied in man by the double-indicator method using T-1824 bound to albumin as intravascular tracer and ^{24}Na, EDTA-^{51}Cr, or inulin as test molecules. As calculated from the area under the cerebral venous indicator-dilution curves following slug intra-arterial injection, the recovery averaged 98.6, 99.1, and 99.8% of that of the T-1824 for the three test molecules, respectively. This demonstrates a very low permeability of the human blood-brain barrier even to so small a tracer as $^{24}Na^{+}$. Evidence pointing to a partial intravascular separation of the tracer molecules in their passage through the cerebral circulation was demonstrated. This separation could be explained on the basis of diffusion processes in laminarly flowing blood.

brain capillary permeability to ^{24}Na, EDTA-^{51}Cr, and inulin; indicator-diffusion technique; interlaminar diffusion; mean transit time; radioisotopes; relative volumes of distribution; Taylor diffusion; unidirectional extraction

THE BLOOD-BRAIN BARRIER in animals is characterized by a very low permeability to hydrophilic molecules which in other organs pass the capillary wall relatively freely. Most studies of the blood-brain barrier in man have been performed using semiquantitative techniques, such as blood-to-CSF uptake or tissue uptake (as, e.g., recorded by rectilinear scanning). So far the only clinically applicable technique giving a quantitative measure of the capillary permeability of the brain is the double-indicator method as developed by Crone (5). Crone used this method to study the blood-brain barrier in dogs. He found a very low permeability to hydrophilic tracers such as glycerol, fructose, sucrose, and inulin (6). Only glucose showed a marked permeability with the characteristics of facilitated diffusion (7). With the aim of studying the permeability of the blood-brain barrier in man, we have applied the double indicator method using ^{24}Na, EDTA-^{51}Cr, and inulin as test molecules. EDTA-^{51}Cr is an extracellular anion with capillary permeability characteristics practically identical to those of sucrose (26). Albumin bound T-1824 served as intravascular tracer.

MATERIAL AND METHODS

Ten patients hospitalized for brain disorders indicating a carotid angiogram were studied. Focal brain diseases in the form of a small metastatic process or signs of a previous small ischemic infarct were present in the investigated hemisphere in two and in three patients, respectively. One of these patients suffered from a mild diffuse cerebral atrophy. In the remaining five patients the arteriogram and the brain radioisotope scan (chlormerodrin-^{197}Hg) were normal for the hemisphere. Essentially the same results were obtained in all 10 subjects. Therefore, mean values of the permeability characteristics of the entire group are reported below with the contention that much the same results would have been obtained had we been able to study normal human subjects. The informed consent of each patient was obtained.

The studies were performed in the morning after an overnight fasting period. Demerol (25 mg) and 0.5 mg atropine injected subcutaneously 0.5 hr before the investigation were used as premedication. With the subjects resting in the supine position, a polyethylene catheter (id 1.2 mm) was inserted percutaneously in the internal carotid artery. A Goodale-Lubin catheter no. 7 or 8 was advanced under fluoroscopic guidance from the cubital vein until the tip was located in the superior bulb of the ipsilateral internal jugular vein. After 30 min of rest, 1.5–2.0 ml of the injection mixture were injected into the arterial catheter at a rate of approximately 1 ml/sec. Starting at the time of injection, a continuous series of blood samples of 1.0 ml each was collected from the venous catheter, by means of a sampling machine, into dry heparinized tubes at the speed of one sample per second.

The injectate contained, at the same time, 10–20 μc ^{24}Na (AEK Risø, Denmark) or 10–20 μc EDTA-^{51}Cr (Farbwerke Hoechst AG, Frankfurt, Germany), and 30–40 mg inulin (Laevosan Gesellschaft, Linz, Austria), and in all cases 3–5 mg T-1824 (Evans blue, Warner-Chilcott) diluted in 5% human serum albumin in isotonic saline to a total volume of 1.5–2.0 ml. An aliquot of the injectate was added to heparinized venous blood collected immediately before the injection. Thus the composition of the injectate could be determined under circumstances similar to those of the blood samples.

The time until appearance of recirculation was measured in two patients by simultaneous collection of 1-sec-interval blood samples from the catheterized ipsilateral brachial artery. Significant recirculation did not appear in these blood samples until approximately 25 sec after the intracarotid injection (see Fig. 1).

The plasma concentrations of T-1824 and inulin were determined spectrophotometrically after adequate dilution

in isotonic saline (2, 19). The corresponding radioactivity of ^{24}Na and EDTA-^{51}Cr was counted in equally diluted amounts of plasma. At least 10,000 counts were registered using a well-type scintillation detector coupled to a conventional scaler. The concentrations in the blood samples were expressed in relative units by dividing by the corresponding injectate concentrations.

CALCULATIONS

As pointed out by Lassen and Crone (14), a certain degree of intravascular separation of tracer molecules with different diffusibility must be expected to occur in all segments of the vascular bed. This separation is caused by diffusion between laminarly flowing blood streams as first described by Taylor (25) for in vitro systems. The separation is most marked when one compares molecules that differ most with respect to diffusion constant, i.e., for ^{24}Na compared to T-1824-labeled albumin in this study.

The interlaminar diffusion effects imply that one cannot consider the albumin tracer as a reference tracer in the strictest sense: one cannot in each individual venous blood sample take the T-1824 concentration to indicate precisely the concentration of the smaller test molecules one would have found if no transcapillary exchange had occurred. But, as will be explained below, the T-1824 curve is, nevertheless, the appropriate reference curve for assessing the transcapillary exchange of the smaller molecules when two distinct points in time are being considered, t_∞ and $t_{40\%}$.

t_∞. This denotes time infinity. If one could follow the tracer curves until all tracer molecules had left the brain, and without recirculation occurring, then the area from $t = 0$ to $t \rightarrow \infty$ must be the same for all the tracers.

But, if some of the smaller tracer molecules penetrate the blood-brain barrier, then the area under the corresponding tracer curve may, nevertheless, appear smaller than that of the T-1824, provided the return of penetrated molecules is so much delayed that this return is not included. This situation would appear to exist in the brain where molecules having first entered the CSF and the brain's interstitial fluid spaces can be expected to be enormously delayed in their transit through the brain relative to the molecules (of the same species) remaining intravascularly.

The difficulty of correctly extrapolating to infinity is, however, considerable. We have followed the tracer curves down to very low concentrations in a number of cases and found a multiexponential downslope of the indicator curves (Fig. 1). Hence one cannot simply use the initial part of the downslope curve for extrapolation as is done customarily in indicator-dilution studies.

$t_{40\%}$. This denotes the time when the T-1824 curve has decreased to 40% of its peak value. As a consequence of interlaminar diffusion, the following general pattern of the venous dilution curves can be expected when comparing a highly diffusible tracer to a slowly diffusible one (Fig. 2): both during the upslope part and at the tail end, the curve for the highly diffusible molecule lies below that of the slowly diffusible molecule, while for the intermediary transit times the opposite must be the case. This pattern of two crossover points on the downslope, one near the peak and one at low relative concentrations, has been observed experimentally (Fig. 1). If, for the sake of the argument, we assume that ^{24}Na remain totally intravascularly, then the areas under the ^{24}Na and T-1824 curves are equal, i.e., the sum of the "positive" area between the curves during the upslope and at the positive area under the tail part must be precisely equal the "negative" area between the two crossover points. On this basis it can be predicted that, at one given point between the two crossover points, the areas under the two tracer curves will be precisely equal. Because of the skewness of the curves, one must expect that the upslope positive area is considerably larger than the corresponding positive area under the tail part. For this reason we have not used the time corresponding to a T-1824 concentration midway between the two observed crossover points (that would have been at ca. $t_{55\%}$), but have used $t_{40\%}$ (14). Perhaps one should approach the second crossover point even closer, e.g., go to $t_{30\%}$, to obtain an even more precise division of the negative area into its two components. This is, however, numerically without any importance whatsoever.

The $t_{40\%}$ integration procedure has the advantage that no extrapolation is involved, that recirculation does not occur, and hence need not be corrected for, and that backdiffusion (return of permeated molecules) is much less of a problem than with the t_∞ procedure. Hence, provided the general model of low backdiffusion prevails, it is likely that the $t_{40\%}$ integration gives a more correct value for the unidirectional fractional permeation (the extraction) than the t_∞ integration.

Recovery: R(40%). According to the above considerations, one may evaluate the fraction of diffusible test molecules that remains intravascular:

$$R(40\%) = \frac{\int_0^{t_{40\%}} C_{test}(t) \cdot dt}{\int_0^{t_{40\%}} C_{T\text{-}1821}(t) \cdot dt} \qquad (1)$$

The complement of R(40%), E(40%) = 1 − R(40%), is an estimate of the fractional unidirectional loss or extraction of indicator.

We have, for the sake of comparison with Crone's earlier studies (6), also calculated the relative recovery up to the time of peak concentration. Because of interlaminar diffusion effects, this calculation underestimates the intravascular fraction, respectively overestimates the extracted fraction, the errors being most marked for the smallest molecules.

In order to permit a comparison of the dilution curves for the brain directly with those of other organs, we have also plotted the apparent fractional loss of diffusible tracer in each sample, $E(t) = (1 - C_{test}(t))/C_{T-1824}(t)$, as a function of time (see Fig. 3).

Relative volume of distribution. V_{test}/V_{T-1824}. Despite the limitations inherent in extrapolation, this volume was calculated in the conventional manner, i.e., as the ratio of the corresponding mean transit times. Curves were only used when they had been followed for so long that the apparently biexponential downslope had been clearly defined without signs of recirculation intervening.

In order to evaluate the role of recirculation, we have in two cases measured the recirculating tracer concentration in a peripheral artery and assessed its impact on the cerebral

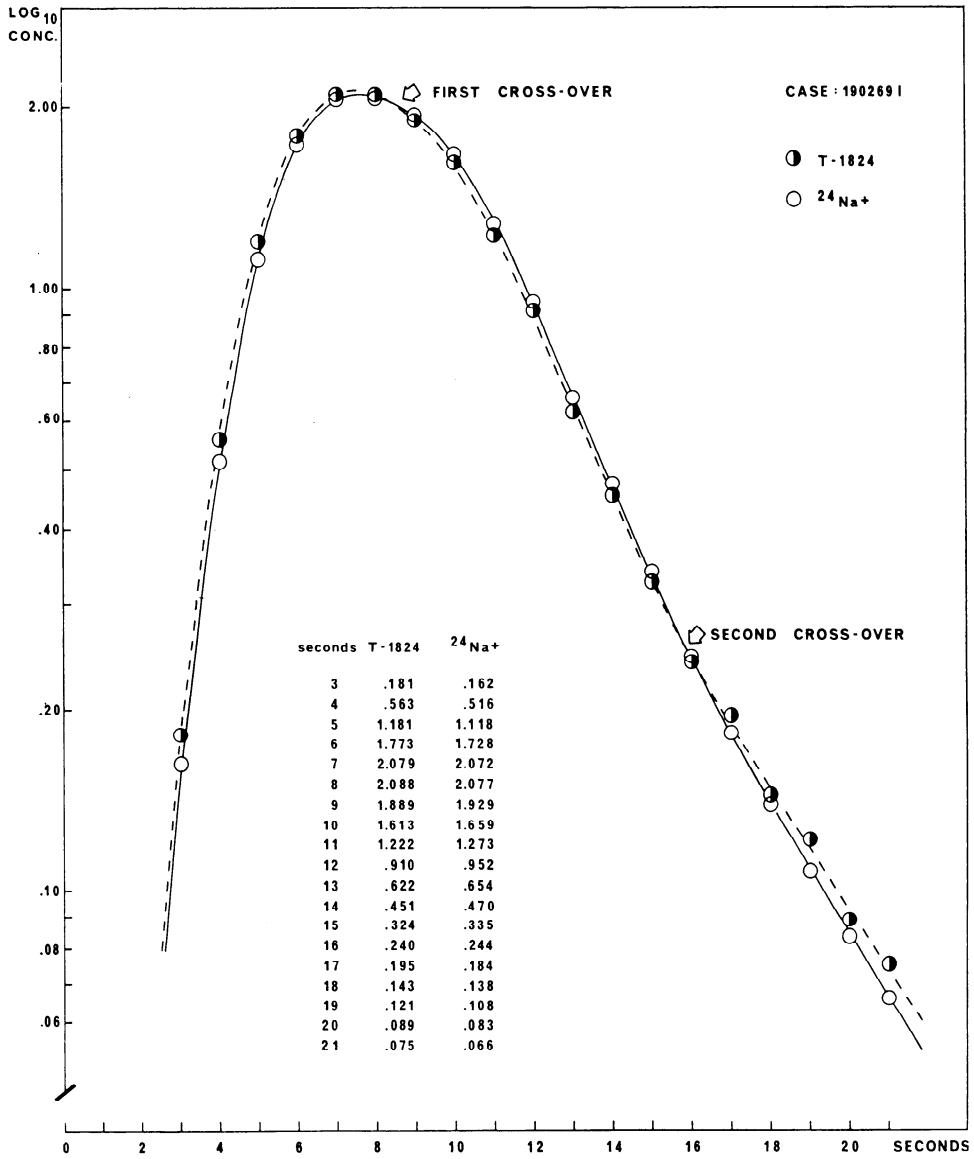

FIG. 1. ^{24}Na and T-1824 tracer-dilution curves from brain. In this case, recirculation was followed by sampling from brachial artery. Points with more than 2% recirculation effect (2% of relative concentration at that time) have not been included.

venous dilution curve by convoluting the observed arterial values by the early part of the cerebral venous curve. It was found that significant recirculation (amounting to 2% of the corresponding venous concentration) occurred first at the relative concentrations of 8 and 13% of the peak concentration in the two cases, respectively.

If no transcapillary exchange exists, or if the transcapillary exchange is essentially unidirectional, one will expect the various tracers to have relative volumes of distribution of 1.00.

RESULTS

The recovery values calculated to a time when the T-1824 curve had decreased to 40% of its peak value, R(40%), are listed in Table 1. The average values of these recoveries were 98.6% for ^{24}Na, 99.1% for EDTA-^{51}Cr, and 99.8% for inulin. The corresponding extraction values, E(40%) = 1 − R(40%), were 1.4, 0.9, and 0.2%, respectively. Only the extraction value for ^{24}Na was significantly different from zero ($P < 0.005$). Table 1 lists, in addition, the R(peak) values, i.e., the fractional recoveries calculated up to the peak of the time-concentration curves. These recoveries are all somewhat smaller than the R(40%) values. The corresponding values for E(peak) = 1 − R(peak) are also listed.

The recovery until time infinity, R(∞), was calculated in those cases where sufficiently long dilution curves were available. The mean values of R(∞) were: for ^{24}Na, 99.5% (n = 2); for EDTA-^{51}Cr, 98.5% (n = 5); and for inulin, 100.4% (n = 5). These results are essentially the same as those obtained as R(40%).

Figure 3 shows the E(t) curves, i.e., the apparent fractional loss of test molecules in each blood sample as a function of time. Corresponding to the first sample there is a considerable apparent loss of the test substance. The average values of this apparent loss amounted to 12.0% for ^{24}Na, 7.9% for EDTA-^{51}Cr, and 4.4% for inulin. As proposed by Martin and Yudilevich (18), we also used retrograde extrapolation to zero tracer concentration in order to calculate the apparent extraction at time zero. The E(0) values

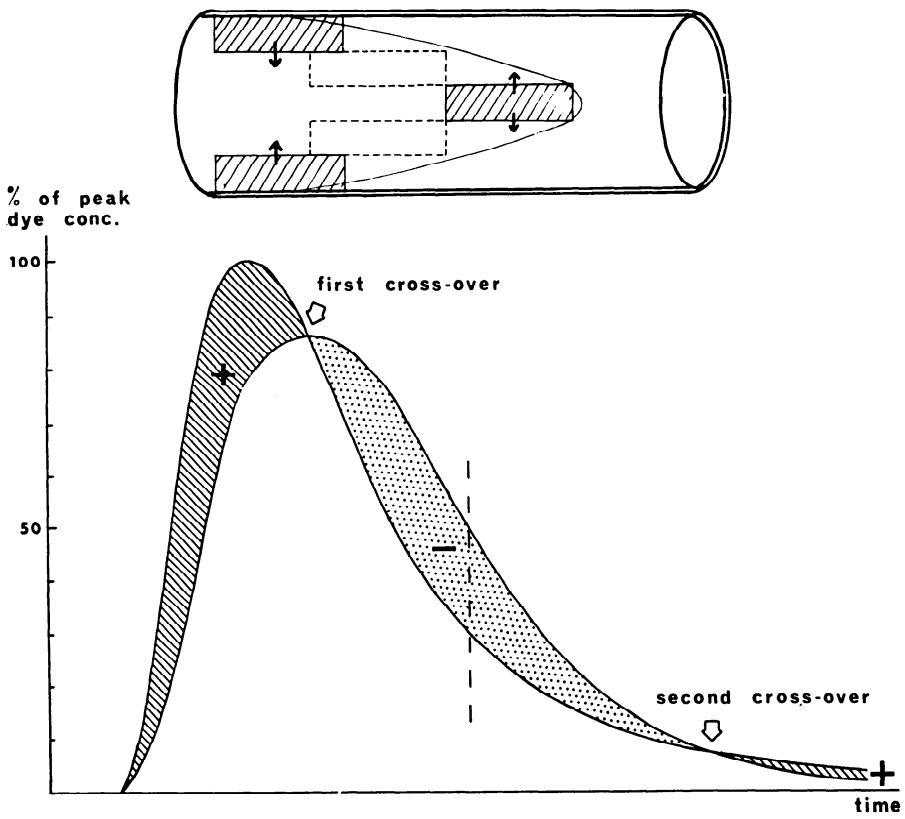

FIG. 2. Diagrammatic drawing of effect of interlaminar diffusion on intravascular dispersion of 2 tracers as described by Taylor (25). Upper diagram gives Poiseuille flow (represented by 3 laminae) and direction of diffusion causing predominantly movement of more-diffusible tracer molecules. Compared to less-diffusible tracer, it will be seen that interlaminar diffusion will slow down transit time of fastest transits and speed up transit time of the slowest transits. Lower diagram depicts these effects as they influence pair of tracer-dilution curves drawn in a linear scale. Broken line indicates approximately line dividing "negative" area into its 2 parts equaling, respectively, initial and terminal "positive" areas. For sake of clarity, curves have been drawn so as to magnify interlaminar diffusion effects manyfold compared to our experimental results in human brain.

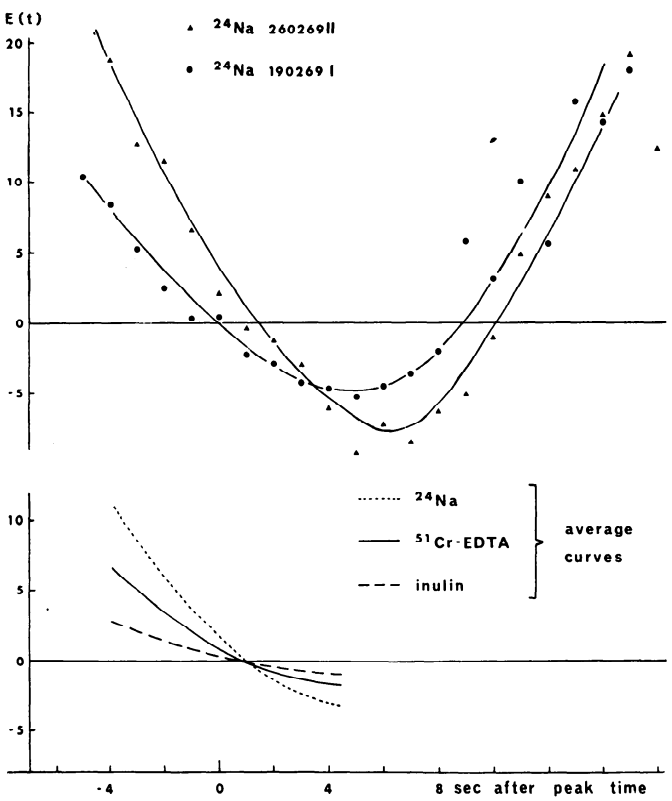

FIG. 3. Curves depicting $E(t) = (1 - C_{test}(t)/C_{T\text{-}1824}(t)) \cdot 100$ as a function of time. Average curves were obtained by taking mean value of samples with comparable concentrations relative to peak concentration.

thus calculated averaged 16.4% for sodium, 8.7% for EDTA-^{51}Cr, and 1.0% for inulin.

Table 2 lists the values for the mean transit times for the four substances. They do not differ significantly from each other. The relative volumes of distribution are not significantly different from 1.00.

DISCUSSION

Intravascular separation of tracers. It was the unexpected observation of a considerably lower concentration of ^{24}Na relative to T-1824, as well as of ^{42}K relative to T-1824 (8), in the earliest venous samples that led to a consideration of intravascular dispersive forces differing for different molecules according to their diffusion coefficients (14). Usually such effects are considered negligible and our data would have been interpreted as indicating a permeability of the blood-brain barrier almost as great as that of skeletal muscle (26, 27). In particular it was striking that the permeability to inulin appeared to be practically the same in the two organs. Inulin is well known to be virtually excluded from the interstitial spaces of the brain when injected intravenously. These paradoxical findings could be accounted for by intravascular separation due to interlaminar diffusion as described by Taylor (25).

Crone (6) calculated the transcapillary passage of the test molecules on the basis of the relative recovery up to the time of peak tracer concentration. If the true transcapillary loss up until this time equals that until $t_{40\%}$, then the erroneous "excess extraction" in Crone's calculations due to Taylor diffusion can be estimated (Table 1). This error is pre-

TABLE 1. *Individual values for recovery and extractions of ^{24}Na, EDTA-^{51}Cr, and inulin in human brain*

Case No.	Sex	Age, years	R(40%)·100			R(peak)·100		
			^{24}Na	EDTA-^{51}Cr	Inulin	^{24}Na	EDTA-^{51}Cr	Inulin
150169 II	M	31	97.8	102.1		95.1	99.7	
220169 I	F	74	99.6			95.0		
220169 II			97.4			92.8		
290169 I	M	56	99.1	100.5		94.5	96.9	
050269 I	F	52	96.9	98.0		94.6	96.9	
050269 III			98.7	98.8		95.4	97.1	
120269 I	M	69	98.9			93.7		
120269 II			100.5			96.4		
120269 III			99.2			94.7		
190269 I	M	64	100.1			97.8		
260269 II	F	69	96.8			94.2		
060270 I	M	78		98.3	103.8		96.8	103.7
060270 II				99.9	100.4		95.9	99.2
200270 I	F	58		95.7	102.1		95.2	101.1
200270 II				99.0	94.6		97.2	94.2
250270 I	F	56		99.5	98.2		98.3	97.1
Mean recovery ± 1 SEM			98.6 0.4	99.1 0.6	99.8 1.6	94.9 0.4	97.1 0.4	99.1 1.6
Mean extraction			1.4	0.9	0.2	5.1	2.9	0.9
Excess extraction, E(peak) − E(40%)*						3.7	2.0	0.7

* The part of the E(peak) value which is thought to be due to interlaminar diffusion effects (for details see text); similar calculation for ^{24}Na of E(30%/sec), i.e., the apparent extraction from the start of the dilution curve until the tracer concentration increase with only 30%/sec, yields an excess extraction E(30%/sec) − E(40%) = 6.6.

sumably also present in double-indicator studies of the capillary permeability in other organs. But, if the true transcapillary loss is considerable, as in hyperemic skeletal muscle, then the error in the extraction-to-peak-time calculation is of little importance (15).

Tracer separation due to interlaminar diffusion probably occurs in all vessels and catheters (13). It was not measurable in our venous catheter at the sampling rate used (1 ml/sec), while it was readily detectable in the larger veins (Fig. 4). Theoretically this separation should be most marked in the smallest vessels (21). The small venules of the brain and the eye have a very markedly laminar blood flow (10, 11) and hence afford an obvious site of this separation. Zweifach (personal communication) has recently confirmed, with accurate measurements, that the hematocrit in capillaries is considerably below that of large vessels. This means that true plug flow does not occur in capillaries, and it strongly suggests that the transcapillary transit times for albumin (and other tracers) in a single capillary are not all identical. Consequently Taylor diffusion probably also occurs in the capillaries.

It is interesting to note that the principle of Taylor diffusion is included in the theory of indicator dispersion in a tissue as presented by Perl and Chinard (22). In their convection-diffusion model the tissue functions like a thick and completely stagnant near-the-wall layer in a tube. If concurrent flow in straight capillaries is assumed, then two extravascularly penetrating indicators distributing in the same volume must be expected to separate (according to transcapillary permeability and extravascular diffusion coefficients) in practically the same way as shown in Fig. 1.

Transcapillary passage of tracers into brain. The very small capillary extraction of 1.4% for ^{24}Na does not, as it would appear, represent the extraction across the average capillary in the brain tissue, i.e., across the blood-brain barrier, because the system studied includes also venous blood from the orbital tissues, the choroid plexuses, and the minute brain areas without a blood-brain barrier (median eminence, area postrema). The extraction across the blood-brain barrier must thus be smaller than our values indicate. All that can be stated is that our studies have, when compared to double-indicator studies in other organs, confirmed the exceptional tightness of this barrier even to the very small hydrophilic tracers here studied.

TABLE 2. *Individual values for mean transit time and relative volumes of distribution for ^{24}Na, EDTA-^{51}Cr, and inulin in human brain*

Case No.	Mean Transit Time, $\bar{t}$, sec				Relative Volume of Distribution		
	$\bar{t}_{Na}$	$\bar{t}_{EDTA\text{-}^{51}Cr}$	$\bar{t}_{inulin}$	$\bar{t}_{T\text{-}1824}$	$V_{Na}/V_{T\text{-}1824}$	$V_{EDTA\text{-}^{51}Cr}/V_{T\text{-}1824}$	$V_{inulin}/V_{T\text{-}1824}$
190269 I	9.33			9.31*	1.002		
260269 II	9.61			9.48*	1.014		
060270 I		8.15	8.15	8.17		0.998	0.998
060270 II		8.91	9.16	8.96		1.994	1.022
200270 I		7.83	7.91	7.79		1.005	1.015
200270 II		7.33	7.22	7.21		1.017	1.001
250270 I		10.14	10.10	10.11		.003	0.999
Mean value	9.47	8.47	8.51	9.40* 8.45	1.008	1.003	1.007
±1 SEM		0.49	0.50	— 0.51	0.006	0.004	0.005

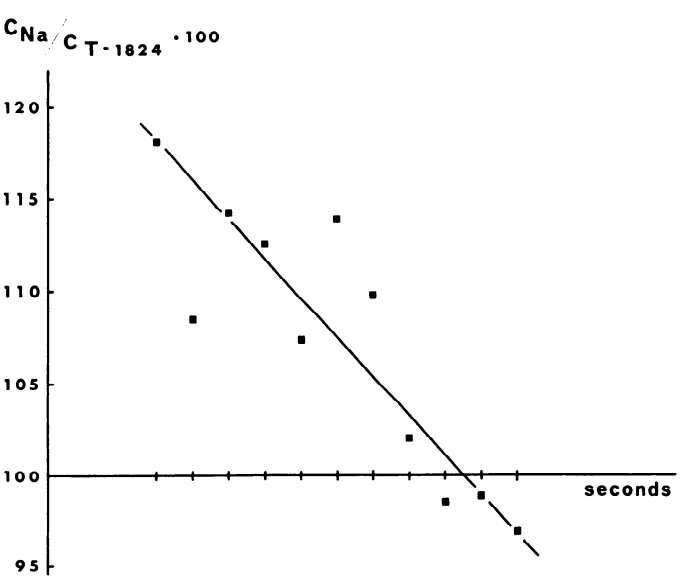

FIG. 4. Tracer separation in veins in man. A bolus of foot and T-1824 was injected in v. saphena magna at dorsum of ^{24}Na and samples were collected in right atrium.

Even if one considered the system as a whole, one could not calculate a correct value for the brain-choroid-orbital system. This is so because of the rather high permeability expected in some of the noncerebral tissues: a small degree of backdiffusion may then have been included in our extraction values. And, more significantly, a marked heterogeneity in extraction leads to an underestimation of the permeability (16, 27).

It may, nevertheless, be of interest to compare our results to those one might have predicted on the basis of animal studies. Davson (9) found, in the rabbit, that the rate of entry of ^{24}Na into the brain tissue and into the CSF followed approximately the same monoexponential function with a time constant of 0.004 min^{-1}[3]. Since the two curves are so similar, no major net ^{24}Na passage can be assumed to occur between the two phases, i.e., the brain tissue is primarily labeled by ^{24}Na crossing the blood-brain barrier. The Na pool of brain tissue corresponds to ca. 35 ml plasma per 100 g and hence the Na clearance of the blood-brain barrier is $0.004 \times 35 = 0.140$ ml/100 g·min. As the plasma flow in the normal brain is ca. 30 ml/100 g·min, the fractional extraction of Na in the average brain capillary is $0.140/30 = 0.005$. To this must be added about 0.001 in order to cover the Na in the CSF secretion of ca. 0.30 ml/100 g·min.

Thus, in the brain as a whole, a ^{24}Na extraction of 0.6% is to be expected. This value is in qualitative agreement with our data.

Is there a small rapidly exchanging extravascular space in brain for tracers studied? Bito and co-workers (2) studied the cerebral concentration of iodide-^{131}I after constant-level intravenous infusion of tracer amounts in rabbits. The apparent iodide space of the cerebrum was 0.94% after 15 min and 1.24% after 30 min of infusion. If this influx rate of 0.30%/15 min also applies for the first 15 min, then the results are compatible with the existence of a 0.64% very rapidly exchanging ^{131}I space. The authors speculated that this space might be located between the endothelial wall and the end feet of the astroglia surrounding the capillaries. This way of evaluating the ^{131}I data is open to some critique. There would, in our opinion, be little reason to expect that the uptake rate of the entire cerebrum (including the choroid plexuses) should be strictly constant in the first few minutes of the study.

Štulc (23, 24) found similar experimental evidence in mice, with ^{24}Na and ^{22}Na showing a fairly rapid initial tracer uptake. Assuming that the brain uptake curve had three monoexponential components (homogeneous compartments), a 2% rapidly exchanging Na space was postulated. This Na "space" is much larger than the one assessed for iodide and the existence of the Na space is even more hypothetical than that for iodide because it appears quite unlikely that the brain Na-uptake curve should be strictly triexponential. Also the permeability of the blood-brain barrier estimated by Štulc on the basis of his model is quite incompatible with our data. Using a value of 5,000 cm^2/100 g for the capillary surface area, Štulc's permeability value is 2.4×10^{-5} cm/sec, i.e., a value almost as high as that found for skeletal muscle in man (26).

In our studies, a small space freely exchanging the test molecules with plasma traversing the average brain capillary would manifest itself as a relative loss of diffusible tracer on the upslope part of the dilution curve with subsequent relative gain on the downslope due to tracer backdiffusion. Agnew and Crone (1) and Yudilevich (28) suggested this explanation to account for their observed tracer-dilution curves from the brain. However, as pointed out by Crone and Thompson (8), the relative loss of diffusible tracer at the tail part of the downslope curve disagrees with this concept. This finding, suggesting a loss-gain-loss of ^{24}Na, i.e., of two crossover points of the ^{24}Na and T-1824 curves (Fig. 1), speaks in our opinion quite decisively against the concept of a rapidly exchanging extravascular pool in the brain of a size influencing the tracer curves significantly.

It may also be commented that if the initial losses of the diffusible tracers were, in fact, due to transcapillary exchange into the brain tissue then all three test molecules, ^{24}Na, EDTA-^{51}Cr, and inulin, would appear to gain access to the hypothetical space; and inulin has a much higher molecular weight (mol wt = ca. 5,500) than the microperoxidase (mol wt = ca. 1,800) which neither crosses the brain capillary endothelial cell wall nor the tightly sealed slits between these cells (4).

Finally, and most significantly perhaps, the measured mean transit times were practically the same for all four tracers, i.e., the same for the test substances as for T-1824. The standard errors of the ratio of the mean transit times showed that if the test substances had had a 1% longer transit time than T-1824, then it would have been detected. Hence our data exclude a paravascular fluid volume (in rapid exchange with the plasma) of more than ca. $\frac{1}{100}$ of the plasma volume of the brain, i.e., any such hypothetical space in the human brain could not exceed ca. 0.04% of the brain weight.

Pappenheimer (20) has recently restated the small, rapidly exchanging, paravascular-space hypothesis. He proposes that the tight junctions between the endothelial cells constitute the barrier for hydrophilic molecules larger than glucose. Smaller hydrophilic molecules are supposed to pass relatively freely through these junctions and into the narrow space between the endothelial cells and the glial foot processes. For these molecules the barrier is the glial cells which are thought, in contrast to the endothelium, to exhibit a marked selectivity of permeability, e.g., to D-glucose (7) or to bicarbonate (12). The rapidly accessible space postulated by Pappenheimer's hypothesis is too small, ca. 0.02% of the brain volume, to be readily measurable with our technique. The histological evidence (4) argues, however, against the hypothesis, because the glial foot processes are not joined together by tight junctions but are separated by gaps ca. 100 A wide. The d-c potential and the concentration differences of ions between blood and cerebrospinal fluid could thus only be maintained, were ions free to penetrate the endothelium, by a very considerable ion pumping through the glial foot processes.

In general, spaces of different ionic composition are separated by cell membranes sealed together with tight junctions. There is, therefore, little doubt that the morphological substrate of the blood-CSF barrier is the tightly junctioned continuous layer of flattered arachnoid cells lining the inner surface of the dural membrane (T. S. Reese, personal communication). The demonstration of tight junctions between the endothelial cells of the brain capillaries, junctions impermeable to molecules of a molecular weight of 1,800,

affords persuasive evidence that the blood-brain barrier is simply the endothelial cell layer (4). According to this concept, the selective permeability of this barrier to D-glucose and certain amino acids, etc., is a function of the endothelial cell wall.

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