Journal of Pharmacokinetics and Biopharmaceutics, Vol. 4, No. 6, 1976

The Application of Brain Capillary Permeability Coefficient Measurements to Pathological Conditions and the Selection of Agents Which Cross the Blood–Brain Barrier

Victor A. Levin,[1,2] Herbert D. Landahl,[1,3] and Mary A. Freeman-Dove[1]

Received Dec. 10, 1975—Final Apr. 29, 1976

Capillary permeability was analyzed in both normal rat brain and intracerebral 9L gliosarcoma tumor and in rhesus monkey brain using a newly derived formula for the capillary permeability coefficient (P). *The* P *value for isotopic urea and sodium was less in monkey brain than in rat brain. The* P *value for tumor was 1 log unit higher in tumor for all but the most rapidly transported drugs. Amphotericin B increased the normal brain* P *value for galactitol but not for urea. Glucocorticoids did not alter brain* P *values for sucrose or urea. Using* P, *drugs can be categorized in terms of blood–brain barrier (BBB) exclusion, slow BBB passage, moderate BBB passage, and rapid BBB passage. The technique described in this article is applicable to the study of regional differences in brain capillary permeability associated with disease states.*

KEY WORDS: brain capillary permeability; capillary permeability coefficient; brain tumor chemotherapy; blood–brain barrier.

INTRODUCTION

The number of available drugs that are effective against brain tumors is few. To date, those agents showing greatest success have been both lipophilic and able to cross normal brain capillaries with relative ease (1). As part of a continuing effort to characterize structural criteria for effective

This work was supported by NIH Grant CA-15435 and a gift from the Richard Jaenicke family.

[1]The Brain Tumor Research Center and the Howard Naffziger Laboratories for Neurosurgical Research, Department of Neurological Surgery, University of California, San Francisco, California 94143.

[2]Departments of Neurosurgery, Neurology, and Pharmaceutical Chemistry, University of California, San Francisco, California 94143.

[3]Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143.

chemotherapeutic agents and improve drug delivery to the brain and brain tumors, we have developed a rapid yet simple test of blood–brain capillary permeability. We believe that this technique will facilitate the process of drug selection as well as improve our understanding of the various pathological changes that can occur in brain tissue.

One of the major difficulties encountered in studying blood–brain capillary exchange of drugs in normal and disease states is the absence of a simple, precise method for quantitating changes in brain capillary permeability. Normally, the "blood–brain barrier" (BBB) excludes nonionized water-soluble molecules of greater than 5–7 Å (2). Although the "barrier" to these molecules has not been anatomically delineated, the "barrier" for protein exchange has been localized at the interendothelial "tight junctions" or zonulae occludens (3). Presumably the physiological BBB for smaller molecules resides at the capillary endothelia. Some molecules probably pass through the plasma membranes and cytosol of the endothelial cells, while others may be restricted by an inconstant basement membrane. In many pathological states (e.g., tumors, inflammation, trauma), the BBB becomes markedly altered both physiologically and anatomically, allowing previously excluded molecules ready access to intercellular spaces in the brain.

Brain capillary permeability has been measured by the arteriovenous method of Crone (4), the double-indicator method of Oldendorf (5), and the ventriculocisternal perfusion method of Patlak and Fenstermacher (6). All these techniques allow for the measurement of a permeability × capillary surface area constant ($P \cdot S$) and each offers certain advantages and disadvantages. Of the three methods, that of Oldendorf is the easiest and least expensive, requiring a single intracarotid artery injection of tracer in a rat with sacrifice and sampling of brain tissue for measurement of tracer radioactivity. The Crone technique uses a larger animal and relies on intracarotid artery and venous outflow cannulation; it is thus more expensive and technically more difficult. The technique of Patlak and Fenstermacher also requires large animals (cat, dog, monkey) and requires the greatest technical expertise of the three techniques. Ventriculocisternal perfusion must be initiated and tissue sampling at sacrifice must be very carefully executed; in addition, measurement of tissue radioactivity depends on computer assistance to make the necessary computations.

All three techniques have as their most serious limitation an inability to determine local brain capillary permeability changes in areas of pathologically altered function. Rather, these techniques all measure an *average* $P \cdot S$ value for an entire cerebral hemisphere or part of one. In a previous article, we described capillary permeability studies on the peritumoral brain surrounding 9L and Walker 256 tumors (7), using a simplified method which, in addition to measuring $P \cdot S$, approximates S and allows for computation of

the permeability coefficients (P). Because of the need for more information concerning capillary permeability in various focal and generalized disease processes of the brain, we expanded those studies and report our findings here.

The following studies were undertaken to analyze capillary permeability differences in cortex and subcortex of rat and rhesus monkey brain, using isotopic sodium and urea. In addition, we wanted to quantitate differences between rat brain and intracerebral 9L gliosarcoma tumor capillary permeability for a variety of cancer chemotherapeutic agents. We hoped that this information would expedite the selection of potentially active brain tumor chemotherapeutic agents.

MATERIALS AND METHODS

Intercapillary Distances and Capillary Length Measurements

Fischer 344 rats and rhesus monkeys were perfused at arterial pressures with a mixture of Pelikan Special Ink (Gunther Wagner, Pelikan-Werke, Hanover, Germany) and 10% formalin buffered to pH 7.3 (1 : 1) in order to fill, fix, and expose capillaries for photomicrographic measurements of intercapillary distances and capillary length in various brain regions.

Anesthetized animals were perfused through either the left ventricle or common carotid artery; the right atrium was opened at the time of perfusion to assure free flow of ink fixative through the brain. A peristaltic infusion pump was used to maintain pressures of 110–120 mm Hg in the rats and 120–140 mm Hg in the monkeys. Following 5–8 min of perfusion in the rats and 10–15 min in the monkeys, the brains were allowed to fix *in situ* for an additional 10–20 min. After removal of the brain, coronal sections were cut and measured in length and width to the nearest 0.5 mm. Fixation with 10% buffered formalin continued for an additional 1–2 days. Sections were cut approximately 3 mm thick, embedded in paraffin, cut by rotary microtome to 6–8 μm thickness, mounted, and stained with hematoxylin-eosin. The sections were remeasured in the two planes before and after fixation and the percent shrinkage was calculated (usually 10–20%).

The finished slides were photographed at magnification ×126 with Kodachrome-II, using an AO microscope and Kodak camera. A ruler with 10-μm divisions was also photographed under the same conditions as the slides. The slides were projected on a fixed screen, and the appropriate conversion factor was determined for each magnification. Only those vessels with diameters of 8 μm or less (before correction for fixation) were considered to be capillaries; agreement of two independent observers was required to make these designations. Only capillaries in proximity to each

other were used for ICD measurements. All capillaries on a slide were measured for capillary length; vessels on end were assumed to be 7 μm in length.

Radioisotopes

^{22}NaCl, [^{14}C]urea, [^{14}C]glycerol, [^{3}H]sucrose, ^{3}HOH, [^{3}H]antipyrine, [^{14}C]creatinine, and [^{3}H]galactitol were purchased from commercial sources. ^{14}C-Labeled dianhydrogalactitol (NSC-132313), dibromodulcitol (NSC-104800), procarbazine (NSC-77213), 5-fluorouracil (NSC-19893), BCNU (NSC-409962), and CCNU (NSC-79037) were supplied through the courtesy of Dr. Robert Engle, Drug Development Branch, NCI. Epipodophyllotoxin (NSC-122819) was kindly supplied by the Sandoz Company. Purity as determined by the supplier exceeded 98%. Reconfirmation of the purity of the chemotherapeutic drugs by either thin-layer chromatography or chemical ionization mass spectrometry was in agreement. In no case did we find any "contaminants" of less polarity than the parent compound.

Fresh samples of [^{99}Tc] human serum albumin (HSA) were prepared daily by Mr. John Huberty of the UCSF Nuclear Medicine Department. Ion-exchange chromatography and electrophoresis assured that there was greater than 99% bound isotope at the time of each experiment. [^{125}I]HSA was obtained commercially and dialyzed for 24 hr prior to use; precipitation with phosphotungstic acid confirmed greater than 99% bound isotope prior to each experiment. ^{51}Cr was used to label rat and monkey erythrocytes prior to use (8).

Rat Experiments

The experiments were conducted in normal male Fischer 344 rats weighing 180–200 g and in rats with 14- to 16-day-old intracerebral (i.c.) gliosarcoma 9L tumors (9). Animals were anesthetized with pentobarbital and the saphenous vein and femoral artery were cannulated with PE 10 tubing. Isotopes were injected over 0.5–2 min depending on the rate of plasma distribution and by i.v. infusion when necessary to maintain a constant plasma level of radioactivity. Sampling from the femoral artery was done halfway to the time of sacrifice. The sacrifice times varied from 5 sec to 30 min. This assured that levels of drug in the brain were measured during a constant blood level.

At sacrifice, a terminal blood sample was taken and the animal head was immersed in liquid nitrogen for 45 sec. The head was then cut with a Stryker cast saw, the brain was removed, and both cortical and subcortical sections were taken; nonnecrotic portions of tumor were removed from those

animals bearing an intracerebral 9L gliosarcoma. Samples were placed in tared scintillation vials, reweighed, and digested with a tissue solubilizer, and a toluene base fluor was added. Radioactivity was counted in a Beckman LS 250 scintillation spectrometer. Isotope decay and quench corrections were made using a Wang 600 programmable calculator.

Monkey Experiments

Animals were anesthetized with pentobarbital and phencyclidine prior to study. The femoral vein was cannulated by direct vision. For erythrocytes and albumin space measurements, [^{99m}Tc]HSA and [^{51}Cr]erthrocytes were injected 15 min prior to sacrifice with an overdose of concentrated barbiturate.

In the ^{22}Na experiments, the isotopes were injected at zero time as a bolus. Subsequently, 90% of the injected dose was delivered 5 min after the bolus as an exponential infusion (2 ml volume with a $t_{1/2}$ of 3.5 min). Blood samples taken from a cannula in the ascending aorta were taken at 5, 10, 20, 60, and 120 min after infusion as well as at termination of the studies.

In the [^{14}C]urea experiments, the isotope was injected as a bolus, followed by a slow infusion over 10 min.

After sacrifice, the calvarium was rapidly removed with a Stryker cast saw, the brain was carefully removed and dipped into liquid nitrogen for 45 sec, coronal sections of brain were taken, and slabs were cut in a plane from cortex to caudate nucleus. Tissue sections obtained from this profile were handled as described above for the rats.

Calculations

Calculation of the plasma volume, erythrocyte volume, blood volume, and tissue distribution space has been previously described (7,10). The distribution space (DS) is defined as

$$\mathrm{DS} = (R - \mathrm{PW})/(1 - \mathrm{PV}) \tag{1}$$

where

$$R = (\mathrm{cpm/mg\ tissue})/(\mathrm{cpm/mg\ plasma\ water}) \tag{2}$$

$$\mathrm{PV} = \text{plasma volume} = \frac{\mathrm{cpm}(^{125}\mathrm{I}\ \text{or}\ ^{99m}\mathrm{Tc})/\text{mg tissue}}{\mathrm{cpm}(^{125}\mathrm{I}\ \text{or}\ ^{99m}\mathrm{Tc})/\text{mg plasma}} \tag{3}$$

$$\mathrm{PW} = \text{plasma water} = \mathrm{PV} \times 0.93 \tag{4}$$

For those substances whose passage through brain capillaries is associated with little arteriovenous extraction, we have used the following

formula (7):

$$\text{permeability coefficient (cm/sec)} = P = \frac{0.28 \cdot \text{DS} \cdot \text{ICD}}{t\sqrt{\text{BV}}} \tag{5}$$

where ICD is intercapillary distance (cm), t is time (sec), and BV is blood volume, which is equal to PV plus erythrocyte volume (ml/g).

For molecules which rapidly cross brain capillaries and hence have a significant and constant arteriovenous drop passing through the brain, the following formulas were derived.

Assume a block of brain tissue as pictured in Fig. 1 with tissue and capillary length L, having a capillary of radius r, and a distance between capillary centers of ICD. C_B is the arterial blood concentration, C_V is the venous blood concentration, $\bar{C}$ is the average tissue concentration, and v is the velocity of blood flow through the capillary segment. For convenience, it was assumed that the capillary lattice within the tissue is in rectangular array.

In addition:

1. $(\text{ICD})^2 \gg r^2$.
2. $L \gg \text{ICD}$.
3. Short observation time so that $\bar{C} \ll C_B$ or $\text{DS} \ll 1$.
4. There is negligible longitudinal diffusion within the capillary (i.e., capillary is very long compared with ICD).[4]

Blood volume and blood flow are defined by

$$\text{BV} = \pi r^2 L/(\text{ICD})^2 L \qquad \text{BF} = \pi r^2 v/(\text{ICD})^2 L \tag{6}$$

[4]This is reasonable if (a) $\text{BV}/\text{BF} = t_r$, since $t_r = 1.5$–2.9 sec, and (b) the diffusion time constant for a 50% drop in concentration is proportional to $L^2/6D$, which is $(0.1)^2/6(2 \times 10^{-5}\ \text{cm}^2/\text{sec}) = 80$ sec.

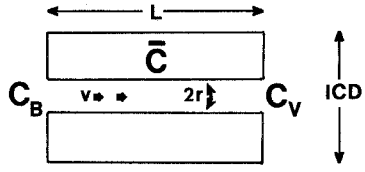

Fig. 1. Schematic model of a brain capillary of length L, diameter, $2r$, and distance between capillaries ICD. C_B, C_V, and $\bar{C}$ are the arterial, venous, and average tissue concentrations of tracer, respectively.

from which

$$r = \frac{(\mathrm{ICD})\sqrt{\mathrm{BV}}}{\sqrt{\pi}} = 0.564\,(\mathrm{ICD})\sqrt{\mathrm{BV}} \qquad L/v = \mathrm{BV}/\mathrm{BF} = t_r \tag{7}$$

where t_r is an average transit time for blood moving through the capillaries.

In a quasi-steady state with negligible backflow, the amount which enters a segment dx of capillary at x is $\pi r^2 v C(x)$. The amount which leaves at $x+dx$ due to blood flow is $\pi r^2 v C(x+dx)$. The difference is the amount leaving the segment due to permeability, $2\pi r\,dx P C(x)$; that is,

$$\pi r^2 v[C(x) - C(x+dx)]/dx = 2rPC(x) \tag{8}$$

or

$$\frac{dC}{dx} = -\frac{2P}{rv}C(x) \qquad \text{and} \qquad C(x) = C_B\,e^{-2Px/rv} \qquad C_V = C_B\,e^{-2PL/rv} \tag{9}$$

Balance Equations

The rate of increase of the concentration $\bar{C}$ in the tissue compartment equals the rate of the amount passing through the entire capillary wall from 0 to L; this is also equal to the difference between the rates entering and leaving the capillary, ignoring the rate of accumulation within the capillary:

$$(\mathrm{ICD})^2 L\frac{d\bar{C}}{dt} = 2\pi rP\int_0^L C(x)\,dx = \pi r^2 v(C_B - C_V) \tag{10}$$

For short times, $d\bar{C}/dt \simeq \bar{C}/t$. Also, $\int C(x)\,dx = (C_B - C_V)rv/2P$. Then from equation 11 we have

$$(\mathrm{ICD})^2 L\bar{C}/t = 2\pi rP(C_B - C_V)rv/2P = \pi r^2 v C_B(1 - e^{-2PL/rv}) \tag{11}$$

Using equation 7 to estimate v and r, and noting that $\mathrm{DS} = \bar{C}/C_B$, the above (equation 11) can be written as

$$\mathrm{DS}/t\mathrm{BF} = 1 - \exp\,(-\mathrm{DS}P/t\mathrm{BF}P_0) \tag{12}$$

where

$$P_0 = (\mathrm{ICD})\mathrm{DS}/2\sqrt{\pi\mathrm{BV}}t = 0.28(\mathrm{ICD})\mathrm{DS}/t\sqrt{\mathrm{BV}} \tag{13}$$

the value of P if blood flow is large enough.

Solving for P/P_0, we obtain

$$\frac{P}{P_0} = -\frac{t\mathrm{BF}}{\mathrm{DS}}\ln\left(1 - \frac{\mathrm{DS}}{t\mathrm{BF}}\right) = 1 + \tfrac{1}{2}\frac{\mathrm{DS}}{t\mathrm{BF}} + \tfrac{1}{3}\left(\frac{\mathrm{DS}}{t\mathrm{BF}}\right)^2 + \cdots \tag{14}$$

which becomes 1 if BF is large enough compared with DS/t. The quantity DS/t is the early rate of increase of the distribution space. But if the permeability is not high enough, the blood flow may not exceed DS/t by a very large ratio and the actual permeability will exceed P_0; equation 14 gives an estimation of the corrected value. Note, however, that since DS/t cannot physically exceed BF, the largest possible value of P_0 that can be obtained, P^*, will be found by replacing DS/t by BF in equation 13:

$$P^* = \frac{\mathrm{BF(ICD)}}{2\sqrt{\pi \mathrm{BV}}} = \tfrac{1}{2}\left(\frac{r}{L}\right)v = \tfrac{1}{2}\frac{r}{t_r} \tag{15}$$

The value of P^* is independent of drug properties. When $P_0 = P^*$, substitution of equation 15 in equation 14 would give an infinite value for P. Note that if $P_0 = 0.1P^*$, then $P = 1.05P_0$; that is, the actual permeability is 5% greater than the calculated value, P_0, from equation 13. The value of this corrected value of P becomes unreliable when P_0 is appreciably more than one-half of P^*. Thus, for example, if $P_0 = 0.5P^*$, $P = 1.38P_0$, and if $P = 0.9P^*$, then $P = 2.56P_0$.

In deriving equation 15, the tissue was represented by identical subunits. We can obtain an estimate of the effect of the actual variability in the geometric properties by assuming that the tissue is divided into a number of regions, each of which has a fractional volume F_i, and is characterized by values r_i, $(\mathrm{ICD})_i$, BF_i, etc. Then experimentally DS/t has the limiting value BF, the weighted average blood flow. Instead of P_0 being given by equation 13, the correct value of P_0, P_0^c, which would be the corrected value of P, for small enough permeability, will be given by

$$P_0^c = P_0 / \sum F_i \sqrt{\mathrm{BV}_i/\mathrm{BV}}/(\mathrm{ICD}_i/\mathrm{ICD}) \tag{16}$$

where P_0 is given by equation 13. This assumes that P is the same for every region i, or at least that it is not correlated with other variables.

There is no direct way to measure $(\mathrm{BV})_i$ and $(\mathrm{ICD})_i$ independently. However, since BV tends to decrease as (ICD) increases, it is likely that r_i is not appreciably correlated with $(\mathrm{ICD})_i$. Hence if we neglect variability in r, we may replace $\sqrt{\mathrm{BV}_i/\mathrm{BV}}/(\mathrm{ICD}_i/\mathrm{ICD})$ by its equivalent $(\mathrm{ICD}/\mathrm{ICD}_i)^2$ and estimate the denominator of equation 16, using the observed frequency distribution for $(\mathrm{ICD})_i$ from which ICD is obtained. For cortex and subcortex, the estimate for this correction becomes 1.23; for tumor, the value would be 1.29. Thus the effect of variability in geometric properties of tissues is not large in itself; even more important, there is only a negligible difference in the effect for normal brain as compared to tumor. Note that the effect of the correction is to reduce the estimate of permeability, but the

factor is independent of the drug and need not be considered in a comparison of different drugs.

In equation 7, variability in measurements has been ignored. If we take the BV measurement to be reliable, and if variability in r is ignored, then the sum of the individual BV_i values, which equals BV, is equal to $\pi r^2/\sum (ICD)_i^2$. From the measurements, the reciprocal of the mean reciprocal square, $1/\overline{(ICD)^2}$, is 0.78 for both cortex and subcortex. However, it should be noted that each region, BV_i, is associated with each of the neighboring capillaries and hence the variability is greatly reduced. If the variance is reduced by 4, then equation 7 should be multiplied by 0.94 or $r = 0.530(ICD)\sqrt{BV}$. If the hexagonal triangular model is used, then under the above assumptions $r = 0.506\sqrt{BV}(ICD)$, the uncorrected coefficient being 0.525. Unless the variability in r is greater than that of the variability reduced by $\sqrt{4}$, the factor is not altered by more than about 1%, for the case in which r_i and ICD_i are uncorrelated. Since 0.525 is between 0.506 and 0.530, 0.525 is used in the tables.

In deriving equation 10, we assumed (a) that $t \gg t_r = L/v$, the transit time, and (b) that the buildup of the compound in blood within the capillary was negligible. If this is not assumed, calculations show that as long as $t > 2t_r$, the results are very closely approximated by replacing t in equations 11–14 by $t - 0.6t_r$, the transit time t_r (in seconds) being given by equation 7. For large enough t, this correction is negligible.

RESULTS

Plasma and Blood Volume

Table I summarizes the plasma volumes in the rat cortex, subcortex, and 9L tumor from a previous study (7), and the values for rhesus monkey cortex and white matter as determined in the present study. In general, the plasma and blood volumes in the monkeys were lower than those found in rats.

Intercapillary Distances and Capillary Surface Area Computations

Table II summarizes the intercapillary distances (ICD) obtained previously in rat cortex and subcortex (7), and those determined in this study in i.c. 9L gliosarcoma tumors 14 days after implantation. Clearly, the ICD for the 9L tumor is considerably greater than for normal brain ($p < 0.001$). Based on a frequency distribution of tumor ICD values, 30% of all tumor capillaries were greater than 100 μm apart. This means that well over 30% of tumor cells are farther than 50 μm from a capillary. The calculation of tumor capillary radius indicates an enlargement of tumor capillaries. This is

Table I. Plasma and Blood Volumes (%)

	Plasma volume (±SD)[a]	Blood volume (±SD)[a]
Rat		
Cortex	1.5 (0.5)	2.4 (1.0)
	$N = 20/10$[b]	$N = 8/4$
Subcortex	1.0 (0.2)	1.6 (0.4)
	$N = 20/10$	$N = 8/4$
Tumor	2.9 (0.8)	5.7 (1.5)
	$N = 8/4$	$N = 8/4$
Monkey		
Cortex	0.9 (0.07)	1.4 (0.6)
	$N = 12/3$	$N = 8/2$
White matter	0.4 (0.06)	0.6 (0.1)
	$N = 33/3$	$N = 11/2$

[a] SD based on "N" animals.
[b] N = tissue samples/animal.

consistent with morphological studies of tumor vessels. Most of these vessels are abnormal in terms of structure and have a hyperplastic and "bizarre" appearance with a tendency toward sinuslike channels instead of the characteristic capillary structure.

The ICD values in the rhesus monkey brain differ considerably from the normal rat brain in that the ICD is greater in both cortex and subcortex. The cortex values are 50% larger in monkey cortex and 100% greater in monkey white matter.

Capillary length in cm/cm^3 was measured and found to be similar within specific regions, from different animal species; e.g., values were similar for rat and monkey cortex, and for rat and monkey subcortex (white matter), respectively. Since many vessels may have been angulated in the tissue, a capillary length was also computed assuming that vessels descended from the upper surface of the tissue to the lower surface at an angle $\geq 45°$. In addition, because of luminal collapse and variability in capillary cross-sectional diameters, all vessels on end were assumed to be 7 μm in diameter. From Table II it can be seen that the calculated capillary length is approximately 74% of the measured capillary length.

Using the median ICD value and BV for each tissue region, a functional *in vivo* value for the capillary radius was computed assuming that capillaries were in hexagonal array (method A) and using the actual capillary length in the volume of tissue actually measured (method B). From Table II it can be seen that the results of the two different methods of computing the capillary radius were not in agreement for the rat subcortex and monkey cortex. The reason for these differences is not immediately apparent. It would appear

Table II. Intercapillary Distance (ICD) and Length Measurements and Other Capillary Computations

Site	ICD (μm)		Capillary length (cm/cm^3)		Capillary radius (μm)		Surface area[d]
	Median	Mean (±SE)	Measured	Calculated[a]	Method A[b]	Method B[c]	(cm^2/cm^3)
Rat							
Cortex (n = 228/5)[e]	44	43.6 (1.01)	98,108	75,680	3.6	3.2	171
Subcortex (n = 137/5)	53	53.8 (1.53)	20,723	15,146	3.5	5.8	33
9L tumor (n = 247/3)	75.5	80.3 (2.47)	54,566	38,844	9.5	6.8	232
Monkey							
Cortex (n = 80/2)	67.1	62.0 (2.0)	97,741	72,644	4.2	2.5	192
White matter (n = 94/2)	106.6	104.5 (4.71)	19,402	14,028	4.3	3.7	38

[a] Calculated assuming tangential sections through capillaries at 45° angles to cut surface.
[b] From equation 6, r = median ICD $\sqrt{(0.276)(BV)}$.
[c] Since BV = (πr^2)(capillary length)/volume of tissue, $r = \sqrt{(BV/\pi) \cdot (\text{volume of tissue/calculated capillary length})}$.
[d] Area = $2\pi r$(calculated capillary length), where r is derived from method A above.
[e] Capillaries counted/animals; SE based on number of animals.

that the computation of capillary radius by method A is more consistent with literature values for the anatomical radius (4,11–13).

The capillary surface area (S) for the different regions also appears in Table II. It was calculated according to the following expression:

$$S = 2\pi rL$$

where r is the radius calculated by method A and L is the calculated capillary length. There was very good agreement of calculated S values between the rat and monkey for cortex and for subcortex (white matter), respectively. The subcortex S value was reduced to 19–20% of the cortex S for each species.

Permeability Coefficients for Rat and Monkey

Table III is a comparison of the permeability coefficients (P_0) for ^{22}Na (or ^{24}Na) and [^{14}C]urea in rat and monkey brain. The rat P_0 values were determined previously (7) and are summarized here for comparison. Two facts are apparent: (a) there is little intraspecies difference between cortex and subcortex (or cortex and white matter) values for the two isotopes, with white matter or subcortex being slightly larger than cortex values; and (b) interspecies urea permeability coefficients are more similar than sodium values; the P_0 value for sodium is four- to sevenfold greater in the rat than in the monkey, while the urea P_0 is only twofold larger.

Table III. Comparative Rhesus Monkey and Rat Brain Permeability Coefficients

Substance	Time (min)	Permeability coefficients (cm/sec)	
		Cortex	Subcortex or white matter
Rat brain			
Sodium	6	5.5×10^{-7} (12)[a]	10.0×10^{-7} (17)
Urea	3,6	1.9×10^{-6} (18)	2.5×10^{-6} (16)
Monkey brain			
Sodium	45–180	1.3×10^{-7} (4)	1.4×10^{-7} (4)
Urea	10	0.9×10^{-6} (2)	1.4×10^{-6} (2)

[a]Number of animals is given in parentheses.

Permeability Coefficients for Rat Brain and Intracerebral 9L Tumor

Since the cortex and subcortex permeability coefficients in the rat were reasonably similar, they were combined to give a single "brain" value. Table IV summarizes the P_0 for the various compounds and drugs studied in brain and i.c. 9L tumors. The "time" listed in the table was selected from the linear portion of the brain and tumor uptake curve.

The table designations of "BBB excluded," "slow BBB passage," etc., were independently determined by studies of blood–brain transport (7,10,15–19). In general, compounds with a "steady-state" brain distribution space of less than 4% (volume %) were considered to distribute in a pericapillary space and not in a true extravascular, extracellular brain space (10).

From Table IV one can appreciate that drugs which are excluded from the brain have an average $P \simeq 1 \times 10^{-7}$ cm/sec, while those that cross the BBB slowly have $P \simeq 2\text{–}7 \times 10^{-7}$ cm/sec. Drugs with moderate rates of BBB passage (i.e., $t_{1/2}$ for compartmental equilibrium of $\simeq$20 min) have an average $P \simeq 2 \times 10^{-6}$ cm/sec, or a full log difference from those that do not cross the BBB.

Blood Flow

If the correction factor suggested by Sokloff (20) for antipyrine blood flow determinations is applied to the regional blood flow measurements by DesRosiers *et al.* (21), one obtains a value for BF of 0.016 ml/g/sec for rat cortex pieces and 0.0055 ml/g/sec for subcortex. Using these values together with the values of ICD and BV in Tables I and II, P^*, the limiting value of P_0, was calculated for rat cortex to be 1.27×10^{-4} cm/sec and for rat subcortex to be 0.65×10^{-4} cm/sec.

Since the P_0 values in Table V for BCNU, CCNU, antipyrine, and tritiated water are close to the values of P^*, it is likely that they are at most only slightly determined by permeability values. The largest values are for tritiated water, BCNU, and antipyrine; none of these P_0 values is significantly different. It is unlikely that these three different molecules would have essentially the same P_0 values unless all were so permeative that their actual permeability values were much greater than P_0. Hence we shall use the average of the P_0 values for these compounds as an estimate of P^* to obtain estimates for blood flow; that is equivalent to estimating BF directly from the measured values of DS/t. For rat cortex we find BF = 0.016 ml/g/sec and for rat subcortex BF = 0.012 ml/g/sec. Note that for cortex the value is very nearly the same as that calculated from P^*, while for subcortex it is substantially higher than that given above.

Table IV. Permeability Coefficients in Rat Brain and Intracerebral 9L Gliosarcoma Tumor

Drug	Time (min)	Permeability coefficient (cm/sec)[a]		Molecular weight	log P[c]
		Brain (%SE/N)[b]	9L Tumor (%SE/N)[b]		
BBB excluded					
Galactitol	5, 10	0.9×10^{-7}(2.0/6)	3.9×10^{-6}(1.8/6)	182	−3.10
Sucrose	5, 10	1.2×10^{-7}(7.8/3)		342	−3.67
Epipodophyllotoxin	15	1.2×10^{-7}(1.0/2)	1.4×10^{-6}(3.8/2)	657	2.80
Slow BBB passage					
Creatinine	6, 12, 30	2.0×10^{-7}(1.0/14)		113	
Sodium (NaCl)	6	7.4×10^{-7}(1.1/17)	5.2×10^{-6}(2.0/4)	58	−2.95
Moderate BBB passage					
Dibromodulcitol	5, 10	1.9×10^{-6}(0.9/7)	1.0×10^{-5}(2.7/6)	308	−0.29
Dianhydrogalactitol	5, 10	2.5×10^{-6}(0.8/6)	1.0×10^{-5}(3.3/3)	150	−1.29
5-Fluorouracil	5, 10	1.7×10^{-6}(0.8/4)	1.7×10^{-5}(2.0/4)	130	−0.95
Urea	3, 6	2.2×10^{-6}(0.6/18)	1.8×10^{-5}(1.0/8)	60	−2.80
Rapid BBB passage[e]					
Glycerol	0.08, 5	2.4×10^{-5}(1.6/4)	2.6×10^{-5}(0.6/2)	92	−2.50
Procarbazine	2, 6	1.9×10^{-5}(2.7/4)	2.2×10^{-5}(2.9/3)	221	0.06[d]
BCNU	0.08	1.5×10^{-4}(3.2/2)		214	1.54
CCNU	0.08, 0.10	1.0×10^{-4}(2.3/3)		234	2.83
Antipyrine	0.08, 0.10	1.3×10^{-4}(3.3/3)		188	0.23
Tritiated water	0.08, 0.10	1.6×10^{-4}(2.1/12)	3.8×10^{-4}(1.2/5)	18	−1.15

[a] Calculated as P_0 without correction for blood flow.
[b] Percent log standard error/number of animals.
[c] Log of octanol/water partition coefficient (14).
[d] Procarbazine is rapidly oxidized to azo compound with log $P \simeq 1.1$ (personal communication from Corwin Hansch, 1975).
[e] Actual permeability values are $\geq P_0$ listed.

Table V. Permeability Coefficients (P_0)[a] for Molecules Which Rapidly Cross the Blood–Brain Barrier in Rats

	Cortex	Subcortex	*P* Value[b]
^{3}HOH (12)[c]	1.37×10^{-4}	1.88×10^{-4}	<0.001
[^{14}C]Antipyrine (3)	1.05×10^{-4}	1.63×10^{-4}	<0.005
[^{14}C]BCNU (3)	1.40×10^{-4}	1.60×10^{-4}	N.S.
[^{14}C]CCNU (3)	0.87×10^{-4}	1.19×10^{-4}	<0.005
Mean, all values	1.2×10^{-4}	1.6×10^{-4}	
Mean, less CCNU	1.3×10^{-4}	1.7×10^{-4}	

[a] Actual permeability values are $\geq P_0$.
[b] Paired *T* test on logs.
[c] Number of animals is given in parentheses.

It is perplexing at present why subcortex P_0 values are greater than cortical P_0 values in the rat (Tables III and V) but are more similar in the monkey. Clearly, one can be more precise in sampling white matter in the larger rhesus monkey brain; it is possible that irregular sampling error resulted in greater amounts of gray matter in some of the subcortical samples. This would result in an increase of exchange (i.e., DS/t) while P_0 would still be calculated using a lower blood volume and larger ICD than warranted. Alternatively, subcortical (and white matter exchange) may, in fact, be more rapid than cortex exchange. Investigations are under way to resolve these points.

It should be emphasized that for tritiated water, CCNU, and antipyrine the actual permeability values are very much larger than the P_0 values given in Table V. In the case of the cortex, for urea the correction factor from equation 14 is only about 1.01 and for glycerol 1.11. For CCNU, the factor is about 1.7, although this value is highly dependent on the limiting value of P_0 used.

Except for lipophilic drugs, P_0 values in all tumors studied so far are 1 log unit greater than in normal brain. The lipophilic drugs distribute equally in normal brain and tumor, which is consistent with previous studies (16–19).

Amphotericin B and Brain Permeability Coefficients

Table VI summarizes some preliminary studies of amphotericin B on BBB passage of urea and galactitol. Following a dose of 10 mg/kg amphotericin B, 24 and 12 hr prior to study, the P_0 for urea was found to be unchanged at $\simeq 2\times10^{-6}$ cm/sec. However, P_0 for galactitol was significantly greater by 1 log unit, indicating that amphotericin B reduced the brain capillary restriction for galactitol transport.

Table VI. Effect of Amphotericin B on Brain Permeability Coefficients in the Rat[a]

Site	Urea P values (cm/sec)		Galactitol P values (cm/sec)[b]	
	Untreated	Treated	Untreated	Treated
Cortex	1.9×10^{-6} (18)[c]	2.1×10^{-6} (4)	9.5×10^{-8} (6)	6.7×10^{-7} (4)
Subcortex	2.5×10^{-6} (16)	2.1×10^{-6} (4)	8.7×10^{-8} (6)	7.2×10^{-7} (4)

[a]Dose 10 mg/kg 24 and 12 hr before study.
[b]T test (on logs) P value <0.01 for comparison of treated vs. untreated groups.
[c]Number of animals is given in parentheses.

Effect of Glucocorticoids on Rat Brain and 9L Tumor Permeability Coefficients

Neither doses of methylprednisolone sodium succinate, 40 mg/kg for 2 days prior to study, nor doses of methylprednisolone acetate, 10 mg/kg for 3 consecutive days prior to study, altered the P for urea or sucrose in brain or i.c. 9L tumors. Table VII summarizes these results.

Table VII. Effect of Glucocorticoids on Rat Brain

Site	Urea P values (cm/sec)		Sucrose P values (cm/sec)	
	Untreated (%SE/N)	Treated (%SE/N)	Untreated (%SE/N)	Treated (%SE/N)
Depol-Medrol, 10 mg/kg, Q.D.×3				
Cortex	1.9×10^{-6} (0.5/18)[a]	1.4×10^{-6} (3.5/4)	2.4×10^{-7} (2.1/3)	2.1×10^{-7} (5.1/4)
Subcortex	2.5×10^{-6} (0.6/16)	1.3×10^{-6} (2.8/4)	0.7×10^{-7} (8.8/3)	1.0×10^{-7} (3.5/4)
Solu-Medrol, 40 mg/kg, Q.D.×2				
Cortex	1.9×10^{-6} (0.5/18)	1.5×10^{-6} (1.7/8)		
Subcortex[b]	2.5×10^{-6} (0.6/16)	0.8×10^{-6} (0.6/8)		

[a]Percent standard error/number of animals is given in parentheses.
[b]$P<0.001$ for treated urea subcortex compared to untreated; there is no statistical difference between the remaining untreated and treated urea P values, nor between treated and untreated sucrose P values.

DISCUSSION

Although there exists some controversy regarding the precise characteristics of effective chemotherapeutic agents for brain tumors, it appears that an ability to cross the normal BBB is a prerequisite for significant antitumor activity (1,19). To aid in the rapid evaluation of potentially active chemotherapeutic agents and to define the most efficacious chemical structure for drugs active against brain tumors, we have developed formulas for brain capillary permeability and tested their applicability in the rhesus monkey and Fischer 344 rat.

Our values for capillary surface area (S) for both the rat and monkey are in good agreement with those in the literature. Cobb (12) reported values in human cortex of 190 cm^2/cm^3 and white matter of 57 cm^2/cm^3. Crone (4) quotes a value for rat cortex of 240 cm^2/cm^3 based on measurements of capillary length in rat cortex of 110,000–140,000 cm^2/cm^3 made by Krogh (11) and Craigie (13). Krogh also measured subcortex capillary lengths and found them to be 30,000–40,000 cm^2/cm^3. Crone assumed a capillary radius of 3.8 μm, which is close to that actually determined in our studies (Table II).

Table VIII compares literature values for the "permeability coefficient" to those determined in this study for water, antipyrine, urea, and

Table VIII. Comparison of Permeability Coefficients to Calculated Literature Values

Substance	Study permeability coefficient (cm/sec)	Literature permeability coefficient[a,b,c] (cm/sec)
Water	1.6×10^{-4} (rat)	1.6×10^{-4} (rat; 5)
		1.5–2.0×10^{-4} (rhesus; 24)
		0.5–0.8×10^{-4} (dog; 6)
Antipyrine	1.3×10^{-4} (rat)	0.8×10^{-4} (rat; 23)
Urea	2.2×10^{-6} (rat)	1.9×10^{-6} (rat; 22)
	1.2×10^{-6} (rhesus)	1.0–1.5×10^{-6} (dog; 6)
Sucrose	1.2×10^{-7} (rat)	1.1×10^{-6} (rat; 22)

[a] Calculations for rats used equation from Bradbury *et al.* (23) for $P \cdot S = -\text{blood flow} \times \ln(1-0.8\times\text{BUI}/100)$, where blood flow = 0.01 ml/g/sec (20,21). BUI = brain uptake index, and $S = 100\ cm^2/cm^3$, which is the average from Table II.

[b] Calculations for rhesus monkeys used $P \cdot S$ value of 0.023 ml/g/sec (24) and $S = 100$ to 150 cm^2/cm^3, the latter to emphasize the predominance of cortical blood in the determinations of $P \cdot S$ in this study.

[c] Calculations for dog used $P \cdot S$ values of 7.7×10^{-3} $cm^3/g/sec$ for water and 1.5×10^{-4} $cm^3/g/sec$ for urea (6) and $S = 100$–$150\ cm^2/cm^3$ as an estimate of dog brain capillary surface area since no measurements are available.

sucrose. For the most part, the literature values have been computed by us with appropriate estimates of S and, when necessary, blood flow. It can be seen that there is good agreement with the intracarotid artery double-indicator studies of Oldendorf (5,22,23) for water, antipyrine, and urea; there is less agreement for sucrose, probably reflecting the reduced sensitivity of the intracarotid artery technique for slowly diffusing molecules. Results obtained in dogs for urea (6) and in monkey for water (24) also are close to the values obtained in this study.

Once values for intercapillary distance, blood volume, and plasma volume are established for a particular animal species and disease process, the routine determination of capillary permeability requires only a determination of drug levels in brain during the early linear portion of the brain uptake curve. For most drugs, sampling ranging from 5 sec to 15 minutes after drug delivery will suffice. The permeability value obtained is useful for separating (a) those drugs incapable of BBB passage from those which may cross the BBB slowly, (b) those which cross the BBB at a moderate rate, and (c) those which enter brain very rapidly by virtue of molecular size and lipophilicity.

Since we cannot determine the ability of all drugs to cross the human BBB, we must rely on other more indirect studies, and extrapolate to man as judiciously as possible. The monkey and the rat, although phylogenetically far separated from man, nevertheless share many characteristics of the BBB in common. In addition, we have found commonality among rabbit, cat, dog, and monkey in terms of brain extracellular space and the blood–brain barrier (25). Most drugs excluded from entering brain via the blood in primates and dogs are also excluded in rats and mice. The major discrepancy appears to be found in those molecules which cross the BBB at slow to moderate rates. For instance, we found previously that isotopic sodium crossed the rat BBB with a $t_{1/2}$ of <2 hr (7) and urea with a $t_{1/2}$ of approximately 1.2 hr (unpublished observations, V. Levin, 1974). In the monkey, Patlak and Fenstermacher (6), using a technique of ventriculocisternal perfusion, found that both urea and sodium crossed the BBB with a $t_{1/2}$ of greater than 6 hr (the limits of their technique). We have studied blood-to-brain exchange of sodium during the maintenance of a constant blood level of radioactive sodium and found that at 8.5 hr the brain level of sodium was approximately 30% of the equilibrium sodium "space" in the white matter, indicating that sodium transport across brain capillaries is extremely slow.

Thus the brain capillaries of rats appear to be somewhat more permeable to urea and sodium compared to those of the rhesus monkey. In addition, the discrepancy is more pronounced with sodium than urea (Table III). Further studies with a greater number of compounds will be necessary

to characterize and compare the primate blood–brain barrier to that of the rat before broad generalizations can be made.

For an inexpensive method to determine relative drug permeability in the brain, and alterations in such exchange in disease states, the technique described in this article is highly reliable. We have previously studied the pharmacokinetics of CCNU, 5-fluorouracil, and dianhydrogalactitol (15–18) and we demonstrated rapid-to-moderate blood–brain passage and intracellular entry. More recently, we evaluated procarbazine and found that it also crossed the BBB after first being oxidized to a lipophilic azo intermediate. All the other drugs in Table IV have also been investigated in terms of brain uptake. The findings in Table IV are in full agreement with the results of more complete pharmacokinetic studies of each of the compounds.

Preliminary studies with amphotericin B, an antifungal agent which has been demonstrated to alter lipid membranes to allow various compounds a greater permeation (26,27), suggest that some drugs may pass the BBB more rapidly in its presence. Further studies will be necessary to substantiate and extend these observations.

Glucocorticoids are used extensively in patients harboring brain tumors to reduce brain edema and improve clinical status, but the effect of glucocorticoids on tumor capillaries is poorly understood (28). Evidence implies that glucocorticoids reduce brain edema around, and possibly within, the tumor proper and under some circumstances slow the blood–tumor passage of some substances. In the studies involving methylprednisolone, summarized in Table VII, we noted a reduction in P_0 for urea and sucrose in rat brain in five of the six experimental situations, although we could not confirm this alteration in capillary permeability statistically. Other substances and drugs should be studied to determine if more rapidly transported drugs are affected by glucocorticoids. Interestingly, in diphtheritic-induced nerve edema, glucocorticoids improved blood–nerve transport of sodium by slowing transcapillary transport, reduced extracellular nerve edema, and reduced intracellular swelling (29). Paradoxically, there was little effect on the blood–nerve transport of inulin, a rather large molecule (molecular weight 5500).

In conclusion, the rat appears to be a satisfactory model system for evaluating and comparing the passage of chemotherapeutic drugs in screening procedures and some disease states. Drugs which cross the BBB at a moderate or faster rate of passage can be distinguished from those which are excluded or cross the BBB slowly. While amphotericin B may accelerate the rate of passage of some compounds through the BBB, preliminary studies with glucocorticoids indicate no effect on BBB passage. We have shown that the white and gray matter of rat and monkey brains demonstrate intraspecies similarities for 15 compounds in the rat and two in the monkey.

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