CNS, CSF, and extradural fluid uptake of various hydrophilic materials in the dogfish

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FENSTERMACHER, J. D., AND C. S. PATLAK. CNS, CSF, and extradural fluid uptake of various hydrophilic materials in the dogfish. Am. J. Physiol. 232(1): R45-R53, 1977 - The distribution of 10 radioactively labeled materials from blood to CSF, extradural fluid (EDF), telencephalon, medulla, and spinal cord was studied in the dogfish shark. Plasma volumes, blood flows, and blood-to-tissue or fluid transfer half-times ($t_{1/2}$) were calculated from these distribution data. Blood-EDF exchange occurred at slow but similar rates for all tracers. Urea uptake by the CSF was very rapid compared to that of the other compounds and may be facilitated by a special mechanism. The tissue plasma spaces of all three CNS regions were small (1.1–1.5%). The calculated rates of tissue blood flow (in ml/g-min) were: 0.11 for telencephalon and medulla, and 0.055 for spinal cord. Choroid plexus blood flow was estimated to be 2.3 ml/g-min, a surprisingly high rate. Transport between blood and tissue was most rapid for water ethylene glycol and slowest for inulin. The tissue $t_{1/2}$'s of urea indicated that a significant portion of this compound's net uptake by periventricular brain tissue occurred via the CSF.

Squalus acanthias; elasmobranch physiology; blood-brain exchange; blood-CSF exchange; CNS blood flow; CNS plasma space; urea; water; sodium; chloride

The morphology of the brain capillary complex (the capillary endothelium and surrounding perivascular space and glial cells) and the choroid plexus has been fairly extensively studied in both mammals and elasmobranchs (the cartilaginous fish), two quite divergent classes of vertebrates. The work of Brightman et al. (1) on three species of sharks indicates that the choroid plexuses in these fish are similar, with respect to both the fine structure and the distribution of intravascularly administered peroxidase, to those of mammals; however, this study plus those of Long et al. (16) and Hashimoto (11) demonstrate that the morphology of both the brain capillaries and the surrounding extracellular space and glial cells are quite different in these two vertebrate classes. In contrast to the mammalian situation, the endothelial cells in the brains of elasmobranchs contain numerous vesicles and are joined together by both open and tight intercellular junctions. Two unusual perivascular features—a relatively thick extracellular space which has numerous extensions projecting into the surrounding layer of glial cells and a pericapillary cellular cuff which is made up not only of glial end feet but also of glial cell bodies—are found in these fish.

Only a limited amount of information concerning the blood-CNS and blood-CSF transport of substances in the cartilaginous fish has been reported. Zubrod and Rall (29) included one shark, Squalus acanthias (the spiny dogfish), in their work on drug distribution in various vertebrate classes. They found that the brain:plasma distribution ratios of sulfanilamide at equilibrium were virtually identical in the dog and dogfish, but that the sulfanilic acid ratios were markedly higher in the dogfish. In agreement with the latter point, Cserr et al. (3) found that the brain:plasma inulin ratios at 20 h were 2–8 times larger in two shark species, Squalus and Gymnostomata cirratus (the nurse shark), than in the rat.

In work on a number of different fish species, Rasmussen (21, 22) observed that the rates of urea exchange between blood and brain and blood and CSF were very fast—indeed, much more rapid than in mammals—in all of the elasmobranchs studied. Just as the previously cited morphological reports showed structural dissimilarities in the brain capillary system in these two vertebrate classes, these few physiological studies suggest that differences in blood-CNS and blood-CSF transport exist between the cartilaginous fish and the mammals.

In the present work the movements of nine radioactively labeled, intravenously infused hydrophilic solutes plus tritiated water from blood to CSF, extradural fluid (EDF, a peculiar protein containing fluid which is found outside the brain), cerebrum (also called the olfactory lobes or telencephalon), medulla, and spinal cord were studied in spiny dogfish at times ranging from 10 min to 20 h after the initial injection. Apparent rates of tissue blood flow plus net blood-tissue and blood-fluid exchange were calculated from these data and related to reported values obtained with similar compounds in higher vertebrates.

Methods

Animals. The dogfish used in this study were caught in Frenchman Bay, Me., during July and August and
Materials were used: tritiated water (3H2O) [14C]urea, 35S, Corp.; [3H]ethylene glycol, [35S]thiourea, [3H]sucrose, also made up. The following radioactively labeled materials contained only 131I-labeled serum albumin (RISA) was also made up. The following radioactively labeled materials were used: tritiated water (3H2O) [14C]urea, 35S, and 22Na (all from International Chemical & Nuclear Corp.); [3H]ethylene glycol, [35S]thiourea, [3H]sucrose, and [3H]inulin (all from New England Nuclear Corp.); and RISA (from Mallinckrodt/Nuclear). The composition of the dogfish saline in grams per liter was: NaCl, 15.2; KCl, 0.3; MgCl2*6H2O, 0.51; NaHCO3, 0.38; trimethylamine oxide 2H2O, 8.04; urea, 21.6; NaH2PO4* H2O, 0.07; and CaCl2*2H2O, 0.37.

Injection solution. An injection solution was prepared which was composed of two radioactively tagged materials: a 3H-labeled compound plus either a 14C- or 35S-labeled substance or a 22Na or 36Cl salt in saline. For some experiments a second injection solution which was composed of two radioactively tagged materials: a 3H-labeled compound plus either a 14C- or 35S-labeled substance or a 22Na or 36Cl salt in saline. The RISA solution was intravenously injected 30 min before the end of the experimental period in 17 of the long-term experiments in which [3H]sucrose and [14C]inulin were used.

Sampling technique. Throughout the course of the experiment, the fish were allowed to swim freely in large plastic-lined swimming pools except for the times when blood samples were obtained and/or sustaining doses were given. At these times the animals were kept in water but gently restrained by holding. Blood samples were drawn from the caudal artery into heparinized syringes at three or four different times during the experimental period. Six experimental periods, 10 min, 30 min, 1 h, 4 h, 10 h (for sucrose and inulin only), and 20 h, were used. Blood samples were obtained at 2, 5, and 10 min for the 10-min experiments; 5, 15, and 30 min for the 30-min experiments; 15, 30, and 60 min for 1-h experiments; 1, 2, and 4 h for the 4-h experiments; 1, 4, and 10 h for the 10-h experiments; and 1, 4, 10, and 20 h for the 20-h experiments. All blood samples were centrifuged for 10 min to obtain plasma.

Immediately after taking the last blood sample, EDF was collected by inserting a needle through the chondrocranium into the extradural space in front of the olfactory lobes and withdrawing the fluid into a heparinized syringe. The animal was then removed from the water and decapitated. After exposing the cerebellum the CSF was drawn from the cerebellar ventricle, and the brain and spinal cord were removed from the carcass. The amount of the time that elapsed from the decapitation of the fish to the removal of the tissue samples was 1.0–1.5 min. Duplicate pieces of the telencephalon, medulla, and spinal cord were taken and placed in tared liquid scintillation vials to determine the weight of each tissue sample. For the medulla one sample was obtained from the ventricular edge and the other was cut from a site near the dural surface (only the averaged values for these two medullary sites are plotted in Fig. 3; however, the compartmental analysis was applied to both the averaged data and the two separate medullary sites). The EDF and CSF were visually examined and discarded if bloody. Duplicate 100-μ1 volumes of EDF, CSF, and the several plasma samples were pipetted into liquid scintillation vials in preparation for counting.

Sample analysis. To each vial was added 0.5 ml of a tissue solubilizer (NCS, Amersham/Searle); the vials were slowly shaken in a water bath set at 40°C until the tissues and fluids were fully digested (10–20 h). A toluene-based liquid scintillation fluid (Liquifluor, New England Nuclear) was added to each vial. All samples were analyzed for radioactivity by liquid scintillation spectroscopy using a three-channel counter with an external standard (Packard Instrument Co.) and the appropriate settings for separation of the two different isotopes present in the sample series. Samples from experiments in which RISA was used were initially counted for 131I at a setting which excluded all 3H- and 14C-derived disintegrations and subsequently recounted 6–8 wk later for the other two isotopes. Standard methods of correcting for background, quenching, and double-label overlap or splasherover were applied to the raw data, and the concentration of radioactive material in counts per minute (cpm) per milligram of tissue or per microliter of fluid were calculated.

Data processing. The first step in the analysis of the tracer data was the conversion of the corrected tissue and fluid concentrations of radioactivity to a simple distribution ratio (R) by dividing them by the final plasma sample’s concentration of radioactivity. Subsequently all these tracer ratios, except those of RISA, were changed into extravascular distribution ratios (R) by subtracting the respective RISA ratio from the simple ratio

\[
R = r - r_{\text{RISA}} \tag{1}
\]

The same vascular volume correction factor, the RISA ratio, was applied for all solutes regardless of their blood distribution volume since the plasma and blood spaces in the CNS of the dogfish are small (1–2%) and nearly equal (Hct = 18%, (12)).

The distribution space is defined as

\[
\text{space (\%)} = R \times 100 \tag{2}
\]

To obtain quantitative estimates of the net exchange rates from plasma to CSF or tissue, a simple closed two-compartment model (26) was employed. The working equation for a system in which the plasma concentration is assumed to be constant with respect to time is

\[
R = a (1 - e^{-kt}) \tag{3}
\]

The data (10 min–20 h) for each material was fit to this equation.
equation using a standard least-squares routine, and the values for \( a \), the infinite time or steady-state ratio, and \( k \), the exponential time constant, were determined. For the Tables 1–5 and the discussion, the time constant was converted to an exchange or transfer half-time, \( t_{1/2} \). The relationship between \( k \) and \( t_{1/2} \) is

\[
t_{1/2} = \frac{(\ln 2)}{k} \tag{4}
\]

The equation for calculating blood flow from tissue uptake data is derived from the Fick principle (for details of this derivation see, for example, reference 10). For the case in which the arterial concentration of the labeled marker is constant and diffusional equilibration between capillary blood and the tissue compartment occurs, the appropriate equation is

\[
F_p = \frac{a}{t} \frac{1}{\ln \frac{1}{1 - R/a}} \tag{5}
\]

In equation 5 \( F_p \) is the plasma flow. The plasma flow is converted to blood flow (\( F_b \)) by dividing \( F_p \) by the quantity, (1-Hct), which equals 0.82 in the dogfish.

Computer modeling of uptake. Equation 3 is appropriate for the situation in which the plasma concentration of the labeled material is constant over time, a condition that was not strictly met in these experiments. In view of this experimental difficulty, an evaluation of the error in our analysis of the exchange process which was caused by the assumption of a constant plasma tracer concentration was made. To do this “model” tissue uptake data (i.e., R vs. time) were generated by computer for a two-compartment system with specific values of \( a \) and \( k \) (two of the constants which were defined with equation 3 and hereafter symbolized in the development and discussion of the model data by \( a_i \) and \( k_i \)), but with either a continuously increasing or continuously decreasing plasma concentration (other plasma time courses were also used to produce model data, but these two were the most severe cases). Subsequently these model data were processed in the same manner as the actual experimental data, yielding new estimates of \( a \) and \( k \). These values for \( a \) and \( k \) were compared to the \( a_i \) and \( k_i \) which were used to produce the model data.

For the two cases modeled with increasing plasma levels, the concentration was considered to rise continuously from a value (in arbitrary units of concentration) of 0.5 at \( t = 0 \) to one of 1.0 at the conclusion of the several different experimental times: 10 min, 30 min, 1.0 h, 4 h, and 20 h. For one of these cases a rapid exchange velocity (\( t_{1/2} = 0.5 \) h) was used; for the other, a slow transfer rate (\( t_{1/2} = 5.0 \) h) was employed. For the remaining two cases which will be reported, the plasma concentration was assumed to decrease linearly from a value of 1.5 at \( t = 0 \) to one of 1.0 at the conclusion of the various experimental time periods. The same two exchange rates (\( t_{1/2} = 0.5 \) and 5.0 h) were used for the production of these data.

Criteria of plasma concentration constancy. Based on the analysis of the model data (see Table 5), the following criteria of “plasma concentration constancy” were used for determining the acceptability of a given experiment for inclusion in the reportable data: 1) the concentration of the next to the last plasma sample could differ from that of the terminal sample by 25%; 2) the concentration of the earliest sample could be as much as 30% above or below that of the final sample if there was a continuous increase or decrease in concentration with time for the sample series; 3) the first sample’s concentration could vary up to 50% from that of the terminal sample if the concentrations of the first and second samples straddled the concentration of the final sample. Application of these criteria eliminated more than 50% of the experiments that were tried during the course of this study. Of the experiments that were judged acceptable, the plasma concentrations of the earliest and the next to the last sample were, in general, within ±25% and ±10%, respectively, of the terminal plasma sample’s concentration.

Water and ion content. The water content of the tissues and fluids was measured on large pieces of tissue (0.5–5.0 g) and volumes of fluids (0.5–2.0 ml) which were placed in glass weighing bottles and dried to constant weight (2–3 days at 90°C). The fraction of water in the tissue and fluid was calculated from the wet: dry weight ratio.

The ion content was determined by placing samples of tissues of known weight into test tubes which contained 1.0 ml of distilled water. After being sealed, the tubes were strongly shaken with a vortex-type mixer, boiled for several minutes, and refrigerated. About 2 days later the tubes were centrifuged and the supernatant fluid was removed. The measurements of chemical sodium and chloride in the supernate were done with a flame photometer (Instrumentation Laboratory, Inc., model 343) and a Buchler-Cotlove Chloridometer (Buchler Instruments, Inc.), respectively. For the calculations of the tissue and fluid concentrations, the assumption was made that the ions were equally distributed throughout all the water within the tube; accordingly the flame photometer and Chloridometer readings were divided by the dilution factor (tissue water ÷ total water in tube).

RESULTS

Table 1 lists the values for the water, sodium, and chloride content of the fluids and tissues examined in

| Table 1. Water, sodium, and chloride contents plus RISA distribution ratios of various intracranial fluids and CNS regions in Squalus |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Water, g water/g sample | Sodium* | Chloride* | RISA Ratio* |
|                 |                    |         |          |             |
| EDF             | 0.858 ± 0.003      | 273 ± 4.0 | 260 ± 3.5 | 0.0          |
| CSF             | 0.970 ± 0.002      | 288 ± 5.5 | 283 ± 6.5 | 0.0          |
| Troleandomonalin | 0.643 ± 0.005     | 07 ± 0.0 | 82 ± 4.5 | 0.012 ± 0.002 |
| Medulla         | 0.748 ± 0.010      | 79 ± 2.0 | 72 ± 2.5 | 0.011 ± 0.001 |
| Spinal cord     | 0.703 ± 0.005      | 79 ± 2.0 | 69 ± 2.0 | 0.016 ± 0.004 |
| Plasma          | 0.837 ± 0.002      | 267 ± 1.5 | 257 ± 5  | 1.0          |

* Units for the fluids are meq/l, and for the tissues units are meq/kg wet tissue. ** RISA Ration is in (cpm/mg)/(cpm/μl plasma).
this study. The fluid:plasma and tissue:plasma ratios for water, Na, and Cl, respectively, are: 1.02, 1.03 and 1.01 (EDF); 1.04, 1.08, and 1.10 (CSF); .90, .33, and .32 (telencephalon); .80, .30, and .28 (medulla); and .75, .30, and .27 (spinal cord). The tissue:plasma ratios for RISA are also given in Table 1. This ratio is a measure of the size of the tissue’s plasma compartment; accordingly, the blood volume of these tissues would be about 20% larger than the plasma volume since the hematocrit in S. acanthias is 18% (12).

The extradural fluid results are presented in Table 2. The EDF ratios for all the labeled materials at 10 and 30 min were zero to virtually zero (<0.01) and are not included in this table. Statistical analysis (unpaired t test) of the 20-h data indicated that the inulin values were significantly lower (P < .01) than those obtained with any of the other compounds, whereas the chloride ratios were significantly higher (P < .02) than the inulin, sucrose, mannitol, urea, and thiourea ratios. For the other two materials, sodium and ethylene glycol, the differences between them and chloride at 20 h were possibly significant (P < .05). Similar analyses of the 4-h data supported the statistical differences between inulin and the other materials (P < .02) found with the 20-h results; however the discrepancies between chloride and the remaining substances at this point were slight and not statistically significant.

For six of the materials used in this study, experimental durations of 10 min, 30 min, 1.0 h, 4 h, and 20 h were chosen. With one compound, $^3$H$_2$O, only a single time point, 10 min, was used (the risk of losing sizable amounts of this highly exchangeable substance into the laboratory seawater system precluded longer duration experiments). The results with all seven of these materials are shown in Figs. 1 (CSF), 2 (telencephalon), 3 (medulla), and 4 (spinal cord). For inulin and sucrose a sixth time point, 10 h, was used in addition to the previously mentioned five. These data are presented in Table 3; the 10-min values are not included since in all cases the ratios were virtually zero (< .01).

### Table 2. Extraluminal fluid-to-plasma ratios of labeled materials at three times after initial injection

<table>
<thead>
<tr>
<th>Material</th>
<th>Ratios</th>
<th>1 h</th>
<th>4 h</th>
<th>20 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inulin</td>
<td>0.01 (1)</td>
<td>0.04 ± 0.003</td>
<td>0.27 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.01 ± 0.000 (3)</td>
<td>0.08 ± 0.02</td>
<td>0.57 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Mannitol</td>
<td>0.02 ± 0.006 (3)</td>
<td>0.11 ± 0.02</td>
<td>0.47 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Thiourea</td>
<td>0.03 ± 0.02 (3)</td>
<td>0.19 ± 0.02</td>
<td>0.62 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>0.01 ± 0.005 (5)</td>
<td>0.20 ± 0.02</td>
<td>0.65 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>0.01 ± 0.003 (3)</td>
<td>0.15 ± 0.02</td>
<td>0.64 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Chloride</td>
<td>0.02 ± 0.007 (3)</td>
<td>0.18 ± 0.03</td>
<td>0.87 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>0.02 ± 0.003 (4)</td>
<td>0.11 ± 0.01</td>
<td>0.71 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n indicated within parentheses.
entries for this fluid are given in these two tables. The agreement between the steady-state ratios of Na and Cl given in Table 4 and the previously mentioned water and ethylene glycol exchanged most rapidly (lowest $t_{1/2}$s), with urea and thiourea being, in most cases,

**TABLE 3. CSF or tissue-to-plasma ratios at various times plus exchange half-times and steady-state ratios calculated from these data**

<table>
<thead>
<tr>
<th>Fluid or Tissue</th>
<th>Molecule</th>
<th>0.5 h</th>
<th>1 h</th>
<th>4 h</th>
<th>10 h</th>
<th>20 h</th>
<th>Compartmental Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>Inulin</td>
<td>0.00</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.05 ± 0.01</td>
<td>0.07 ± 0.00</td>
<td>16.5 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>0.03 ± 0.01</td>
<td>0.04 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.18 ± 0.02</td>
<td>0.31 ± 0.01</td>
<td>21.7 ± 0.66</td>
</tr>
<tr>
<td>Telencephalon</td>
<td>Inulin</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.04 ± 0.00</td>
<td>0.04 ± 0.00</td>
<td>0.05 ± 0.00</td>
<td>5.0 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>0.02 ± 0.00</td>
<td>0.05 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.13 ± 0.01</td>
<td>2.0 ± 0.10</td>
</tr>
<tr>
<td>Medulla</td>
<td>Inulin</td>
<td>0.01 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.03 ± 0.01</td>
<td>0.04 ± 0.00</td>
<td>4.5 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>0.03 ± 0.00</td>
<td>0.04 ± 0.00</td>
<td>0.06 ± 0.01</td>
<td>0.08 ± 0.00</td>
<td>0.10 ± 0.01</td>
<td>3.6 ± 0.09</td>
</tr>
<tr>
<td>Spinal Cord</td>
<td>Inulin</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.04 ± 0.01</td>
<td>0.05 ± 0.00</td>
<td>0.07 ± 0.01</td>
<td>10.2 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>0.03 ± 0.00</td>
<td>0.03 ± 0.00</td>
<td>0.09 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.18 ± 0.01</td>
<td>8.4 ± 0.20</td>
</tr>
</tbody>
</table>

Values are means ± SE; n indicated within parentheses. * Exchange half-time.

**TABLE 4. Exchange half-times and steady-state ratios determined by compartmental analysis of data in Figs. 1-4 plus results from two separate medulla sampling sites**

<table>
<thead>
<tr>
<th>Tissue or Fluid</th>
<th>Parameter</th>
<th>Water*</th>
<th>Ethylene glycol</th>
<th>Thiosuccinate</th>
<th>Urea</th>
<th>Sodium</th>
<th>Chloride</th>
<th>Mannitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>$t_{1/2}$</td>
<td>0.14</td>
<td>0.45</td>
<td>1.50</td>
<td>0.16</td>
<td>5.10</td>
<td>6.50</td>
<td>9.90</td>
</tr>
<tr>
<td></td>
<td>Ratio</td>
<td>1.04</td>
<td>1.04</td>
<td>1.02</td>
<td>0.97</td>
<td>1.15</td>
<td>1.27</td>
<td>0.39</td>
</tr>
<tr>
<td>Telencephalon</td>
<td>$t_{1/2}$</td>
<td>0.12</td>
<td>0.60</td>
<td>1.80</td>
<td>1.10</td>
<td>3.00</td>
<td>4.00</td>
<td>4.70</td>
</tr>
<tr>
<td></td>
<td>Ratio</td>
<td>0.90</td>
<td>0.87</td>
<td>1.04</td>
<td>0.85</td>
<td>0.33</td>
<td>0.31</td>
<td>0.14</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>$t_{1/2}$</td>
<td>0.19</td>
<td>1.50</td>
<td>2.70</td>
<td>5.30</td>
<td>3.80</td>
<td>1.60</td>
<td>5.10</td>
</tr>
<tr>
<td></td>
<td>Ratio</td>
<td>0.79</td>
<td>0.73</td>
<td>0.91</td>
<td>0.64</td>
<td>0.31</td>
<td>0.27</td>
<td>0.19</td>
</tr>
<tr>
<td>Medulla (dural and ventricular)</td>
<td>$t_{1/2}$</td>
<td>0.12</td>
<td>0.55</td>
<td>1.90</td>
<td>1.70</td>
<td>3.90</td>
<td>4.00</td>
<td>3.30</td>
</tr>
<tr>
<td></td>
<td>Ratio</td>
<td>0.90</td>
<td>0.78</td>
<td>2.90</td>
<td>0.90</td>
<td>4.40</td>
<td>4.80</td>
<td>4.40</td>
</tr>
<tr>
<td>Medulla (ventricular)</td>
<td>$t_{1/2}$</td>
<td>0.11</td>
<td>0.50</td>
<td>1.80</td>
<td>2.30</td>
<td>3.50</td>
<td>3.20</td>
<td>3.10</td>
</tr>
<tr>
<td></td>
<td>Ratio</td>
<td>0.90</td>
<td>0.70</td>
<td>0.97</td>
<td>0.74</td>
<td>0.27</td>
<td>0.25</td>
<td>0.11</td>
</tr>
</tbody>
</table>

* Since there was only one time point for $^3$H-labeled water (10 min), the steady-state ratio ($a$ in equation 3) was calculated from the water content data in Table 1; the value of R was determined from data in Figs. 1-4. The estimation of $t_{1/2}$ was then made using Eq. 3 and 4. * Exchange half-time.
TABLE 5. Compartmental analysis of model uptake time courses and plasma-tissue exchange half-times

<table>
<thead>
<tr>
<th>Exchange time, h</th>
<th>Distribution Parameter</th>
<th>Analytical Estimate of Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rising plasma concentration</td>
<td>Falling plasma concentration</td>
</tr>
<tr>
<td>0.5</td>
<td>t_1/2_1</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>a/a_2</td>
<td>0.96</td>
</tr>
<tr>
<td>5.0</td>
<td>t_1/2_2</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>a/a_3</td>
<td>0.89</td>
</tr>
</tbody>
</table>

As presented in Methods, a is the steady-state distribution space determined by the least-squares fit of the model data to Eq. 3; a_i is the specific value of a which was chosen for the generation of the model data. * Exchange half-time.

clearly slower. Sodium, chloride, mannitol, and sucrose were intermediate in their uptake velocities, whereas inulin was consistently the slowest.

The compartmental analysis of the model tissue uptake data that were mentioned under Methods is presented in Table 5. These results indicate that the transfer rate or half-time would be 20–40% in error for the case of a rapidly exchanging material (t_1/2 = 0.5 h) with a continuously changing plasma concentration. For a more slowly exchanging substance (t_1/2 = 5.0 h), the error in the estimation of the exchange rate is only 10–20%. In all cases the calculated value of the steady-state distribution ratio, a, would be within 15% of the true value, a_i.

DISCUSSION

Extravascular fluid. In their comparative study of drug distribution between blood, brain, and CSF, Zubrod and Rall (29) noted in the dogfish that antipyrine, sulfanilamide, sulfanilic acid, and sulfadiazine eventually equilibrated between plasma and EDF, but that the rates of net drug transport were very slow (the half-times to equilibrium were 8 h or longer). These results cin analysis of plasma and EDF suggested to them that no barrier between these two fluids existed for these drugs and that EDF is formed slowly and directly from the blood. Rasmussen (21, 22) reported that the uptake of intravenously injected [14C]urea by EDF was slow in two shark species, Negaprion brevirostris (the lemon shark) and S. acanthias, but relatively rapid in two species of rays, Raja sabina and Rhinoptera bonasus.

The present study used nine different water-soluble substances; their net rates of entry into the EDF of the dogfish were low and comparable. The fluid:plasma ratios for 10, 30, and 60 min were equal to or less than 0.03 for all the substances. At 4 h the ratios were appreciably greater than zero; however EDF-plasma equilibrium (ratio =1.0) was not reached by any of these substances by 20 h. Of the tracers used, only inulin (slower) and [35Cl] (faster) appeared to distribute between plasma and EDF at speeds that were very different from those of the others. Attempts to analyze the EDF results with the compartmental model yielded senseless coefficients and time constants.

Cerebrospinal fluid. Among the CSF data, the most surprising finding was the rapidity with which the labeled urea exchanged between blood and CSF. The fluid:plasma ratios for urea and water were virtually identical at 10 min; the CSF time constant for urea is threefold higher than that of the next fastest substance, ethylene glycol (Table 4), a compound of similar molecular size and somewhat higher lipid solubility. Rasmussen (21, 22) reported that [14C]urea, when given by a single intravenous injection, very quickly reached a high concentration in the CSF of the three different elasmobranch species, N. brevirostris, S. acanthias, and R. sabina, that she studied. The present experimental results support Rasmussen's suggestion of rapid urea exchange between blood and CSF; furthermore, by their similarity to the [35Cl] data (Fig. 1 and Table 4), they imply that the uptake of this amide by the CSF is partly limited by the rate of blood flow to the tissues, choroid plexus, and brain, that surround the ventricular system. In contrast to the CSF findings, the transport of [14C]urea between blood and CNS was much slower than that of either water or ethylene glycol (Figs. 2-4 and Table 4).

Maren (17) has studied the transport of [22Na and [35Cl] from blood to CSF in S. acanthias and calculated equilibrium half-times of 3.7 and 5.0 h, respectively, from his data. Slightly larger values for these t_1/2's (5.0 and 6.5 h, respectively) were found in the present work (Table 4). The same equation was used to calculate the transfer times in both studies; however, since the plasma concentrations of the isotopes were declining during the course of Maren's experiments, an intermediate or mean plasma value was used. With such an approximation for the plasma concentration, the rates of exchange would be expected to be somewhat high and the t_1/2's low (see Table 5 and the related discussion). In mammalian species the plasma-to-CSF transfer half-times of Na and Cl are about 2–3 h (5, 15).

The slow transport of inulin and sucrose into the CSF has been noted in several mammalian species (23, 24). These reports and others like them indicate the presence of a permeability-limiting structure, the blood-CSF barrier, between these two fluids. A comparison of the inulin and sucrose steady-state ratios at the same times shows that higher values are found in S. acanthias than in most mammals. The significance of this difference is difficult to evaluate in terms of blood-CSF barrier permeability since the lower body temperature of the dogfish shark in Maine (12–15°C) would affect several other transport steps, such as diffusional movement between CSF and CNS tissue and CSF turnover times, in the overall exchange process.

CNS tissue uptake of tracers. For the simple kinetic analysis which was applied to these data, the tissue is assumed to be a single well-stirred compartment. This assumption was made since it seemed to be reasonably good for most of the substances used in the study (the likely exceptions are urea, thiourea, and ethylene glycol), and the limited number of experimental time points did not permit a more complicated analysis.

In all three areas the highest ratios at 10 min were obtained with [35Cl]; however, the apparent rates of exchange (t_1/2 =7–12 min; Table 4) were much lower.
than those reported for water in monkeys (6). After \(^{3}H_{2}O\) the most rapid transfer rates were observed with ethylene glycol, a compound that has a steady-state distribution ratio approaching that of water. The other two compounds that have large distribution ratios, urea and thiourea, entered these tissues at slower, but similar, rates. In their study of urea transport in the rabbit CNS, Kleeman et al. (14) determined exchange \(t_{1/2}'s\) of 2-6 h for cerebral gray and white matter and spinal cord; hence the rates of urea uptake by the brain of the dogfish are comparable to those observed in the rabbit. The peculiarly high distribution ratios for thiourea (the 20-h ratio is greater than the tissue water content) have been previously noted by Welch and Davson (28) in the sciatic nerve of rabbits. Several processes, e.g., tissue binding and/or metabolism, could account for these findings. The extracellular space in the CNS of the dogfish is around 14-24% (9); chloride and sodium appear to distribute in a space somewhat larger than the ECS as is indicated by the tissue:plasma ratios of both the tracer (Figs. 2, 3, and 4) and the chemical (Table 1) data. Therefore, although a two-compartment model was used with our \(^{22}Na\) and \(^{35}Cl\) data, such an analysis may not be entirely valid; nevertheless, such a model seemed to fit these data adequately and yield reasonable estimates of the spaces and \(t_{1/2}'s\). Several studies of tracer Na and Cl exchange between blood and brain have been performed with mammalian species; these authors have used either a two- (e.g., ref. 2) or three- (e.g., refs. 5 and 15) compartment model to analyze their data. Although any comparison must be qualified because of the differences in the models employed, the rates of blood-brain transfer of Na and Cl appear to be similar in magnitude in dogfish and mammals.

The remaining three compounds, mannitol, sucrose, and inulin, are mainly extracellular in their distribution, and their tissue uptake \(t_{1/2}'s\) are largely the product of the permeability and surface area of the capillary networks in the various regions of the CNS. Of these extracellular-type molecules, only mannitol in all three areas (Figs. 2-4) and sucrose in the spinal cord (Table 3) appear to have distributed in a space of a size approaching that of the ECS by 20 h, illustrating the relative tightness of the blood-brain barrier in these fish to such compounds. The 1- and 4-h mannitol and sucrose ratios of the telencephalon and medulla, when corrected for their respective tissue plasma volumes, are slightly higher but comparable to the whole-brain values of the rat reported by Siseon and Oldendorf (25) for the same two times. In addition our 1- to 20-h brain:blood ratios of sucrose and inulin for the dogfish are also somewhat larger than those reported by Reed and Woodbury (23) for the rat.

For seven of the labeled materials studied, the rates of uptake when compared among the four CNS regions were the lowest for the spinal cord; the compounds in this set ranged in size from water to inulin and in apparent distribution space for 7-8% (inulin) to 91% (thiourea). On the other hand, the two ions, \(^{22}Na\) and \(^{35}Cl\), seemed to exchange between blood and the various CNS regions, with the possible exception of Cl in the cord, at equivalent rates. No comparable data on blood-

spinal cord exchange exists for any other animal species.

**CSF-brain transport interaction.** In S. acanthias the arrangement of the CSF and surrounding brain tissue is quite unusual. There is a relative abundance of ventricular CSF (approximately 1.5 ml (8)) and no external or subarachnoid CSF. The brain in these animals weighs about 2 g and consists of a shell of nervous tissue which surrounds the ventricular fluid. The thickness of this tissue shell is greatest at the medulla (about 2.5 mm) and least at the telencephalon and cerebellum (approximately 1.0 mm). The net rates of tracer uptake in the thinner brain regions and the ventricular surface of the medulla would be influenced by the ventricular CSF's concentration of the labeled materials, whereas the net transfer rates for the tissue site most distant from the ventricular surface, the dural medulla, would be least affected by the ventricular fluid.

This brain-CSF transport interrelationship is illustrated in a simple manner by the differences between the net exchange rates of ethylene glycol and urea in the individual brain regions that were sampled (Table 4). For ethylene glycol all the brain regions, including the dural medulla and the CSF, have transfer \(t_{1/2}'s\) of approximately 30 min, and the effects of the ventricular fluid on the uptake of ethylene glycol by the surrounding tissue seems to be minimal. Urea, in contrast to ethylene glycol, enters the ventricular CSF much more rapidly than it enters the brain and spinal cord. Between the various brain sampling sites, however, the two that are adjacent to the CSF, the telencephalon and the ventricular edge of the medulla, have significantly shorter overall urea exchange rates \((t_{1/2}'s \sim 1 h)\) than the brain site that is most distant from the ventricles, the dural edge of the medulla \((t_{1/2} = 2.3 h)\). Since the individual exchange rates of all of the materials, except urea, were similar in both the ventricular and dural pieces of medulla (Table 4), the general transport parameters, such as blood flow and capillary surface area, do not appear to be markedly different for these two medullary sites; accordingly, these findings suggest that significant amount of \(^{14}C\)urea entered the peri-ventricular tissue via the CSF, thus augmenting the blood-to-brain transfer of urea.

**Tissue plasma spaces.** The plasma spaces, as indicated by the RISA ratios, were nearly identical for the three regions of the CNS that were sampled (Table 1). Tracer albumin spaces of equivalent size (1-2%) have been found in the rat (15, 23).

**Blood flow and water exchange.** Two recent studies (7, 19) have reported that the exchange of water between blood and brain is not completely flow limited at normal mammalian blood flow rates (0.5 ml/g-min) and that labeled water cannot be used to measure brain perfusion rates; however, the results of Raichle et al. (19) also indicated that the exchange of this molecule at low flow rates (<0.2 ml/g-min) in the rhesus monkey was virtually limited by the rate of brain perfusion. The flows calculated from the \(^{3}H_{2}O\) data in the present work indicated very low rates (<0.1 ml/g-min) of CNS perfusion; therefore if the water permeability of the CNS capillary system in the dogfish is similar to or higher...
than that in the monkey, the values presented herein are valid measurements of tissue blood flow.

In work with microspheres which were tagged with \(^{85}\)Sr, Kent et al. (13) reported that the minimum rate of cerebral blood flow in \(S.\) acanthias is 0.03 ml/g-min. In view of the authors’ qualification of their value as a minimal estimate, the agreement between that study and our own work (flow \(- 0.11\) ml/g-min) is fair. The telencephalon and medulla in the dogfish are regions of mixed gray and white matter; the flow rates that have been reported by Freygang and Sokoloff (10) for areas of the cat brain that have similar composition (reticular formation and hippocampus) and spinal cord are 0.6 and 0.4 ml/g-min, respectively. As would be expected for a cold-blooded animal like the dogfish, blood flow to the CNS is much lower than in mammals.

Using the CSF uptake data for \(\text{H}_2\text{O}\) and the assumption that all of this exchange took place across the choroid plexus, a blood flow of 2.0–2.6 ml/g-min was computed for the choroid plexus of the dogfish (a similar rate of choroid plexus perfusion by blood is obtained if the 10-min urea data are employed in the calculation). Welch (27) estimated that choroidal blood flow in the rabbit was 2.9 ml/g-min. The likeness of these rates in two such divergent vertebrate species is quite surprising.

In conclusion, among the observations reported in this work, two which apparently involve the choroid plexus are the most striking. First, the movement of tracer urea between blood and CSF was exceedingly rapid and probably limited by the speed of urea delivery by the blood to choroidal tissue. This transfer of urea seemed to be mediated by the choroid plexuses since the uptake rates of this compound by periventricular brain tissue, the other possible source, was relatively slow. Second, the flow of blood to the choroidal tissue was very high, being similar in magnitude to that reported for the choroid plexus of the rabbit (27).

In comparison with the reported mammalian values, the CSF-blood distribution ratios obtained with mannitol, sucrose, and inulin at the later times (4–20 h) were higher in \(S.\) acanthias, whereas the rates of blood CSF transfer of the other compounds plus the two ions were somewhat slower. With the exception of \(\text{H}_2\text{O}\), no marked differences in blood-brain exchange between mammals and dogfish were found in this study. The rate of water exchange was much slower in the dogfish, a likely result of the low rate of blood flow to the CNS (about 20% of those for comparable regions in mammals).

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