PREFVIOUS STUDIES have established that blood-brain barrier (BBB) permeability to lactate is, in large part, carrier-mediated since it is saturable and stereospecific. The brain normally exhibits a net efflux of lactate, the jugular lactate concentration being greater than the arterial. Because of its very active glycolytic mechanism, the brain also produces considerable amounts of lactate when ischemic. Brain pH is reduced in ischemic foci and definition of any alteration in the rate of lactate efflux or influx in such pathological states would be of clinical relevance. In the present study, we have examined BBB transport of L and D lactate through a range of pH values. Nicotine was similarly studied. Since nicotine is a base and penetrates the BBB freely at physiological pH, it was thought to be of value to examine the effect of increased ionization at lower pH on its BBB penetration in the same experimental arrangement as that used for D and L-lactate. Additionally, D-glucose was similarly studied as a representative neutral substance. To establish whether the 3H-water reference changes with injected pH, 2 groups of animals were studied at high and low pH using 3H-water as the test substance and 14C-butanol as the reference. Radiolabeled butanol has been shown to be completely cleared through a wide range of brain blood flows.

Methods

The intracarotid single bolus injection technique has been employed in a variety of studies of the BBB. Adult Wistar rats (200–350 g) of either sex were used throughout this study. The right common carotid artery was surgically isolated after intraperitoneal sodium pentobarbital anesthesia. A small volume (0.2 ml) of the mixture of buffer and radiolabeled compounds (3H-water, 14C-test substance and 113m-Indium-EDTA, in the proportions listed below) were injected as an abrupt bolus into the carotid artery and 5 sec later the animal was decapitated. Because the bolus volume is much greater than the volume of the regional arterial lumen, the fluid injected displaces the regional arterial blood and the bolus passes through the brain microcirculation with approximately the same composition as when injected. In the present study the major variable is the pH of the injectate. The pH of the injectate was adjusted to a desired value and an attempt made to maintain it through microcirculatory passage by including a substantial concentration of an appropriate buffer having a pK at or near the injected pH.

The ipsilateral hemisphere was dissected from the cranium and extruded through a 20 gauge needle into two liquid scintillation vials each containing 1.5 ml of an organic base (Soluene, Packard Instrument Co., Downers Grove, IL). The tissue was dissolved and routinely prepared for liquid scintillation counting of the 3 isotopes and the brain uptake index (BUI) for each experimental state determined as described previously.

Appropriate time corrections were made to correct for the decay of 113m-Indium (physical T1/2 = 100 min) which occurred between the times the individual sample vials were counted.

The 113m-Indium generator used was obtained from New England Nuclear (NEN) (Radiopharmaceuticals Division, North Billerica, MA). To each ml of 113m-Indium eluted, 10 µl of sterile disodium edetate (150 mg/ml) solution (Endrate, Abbott Laboratories, North Chicago, IL) was added and the Indium-EDTA adjusted to a pH of approximately 7.4 with 7.5% sodium bicarbonate solution. Other radiochemicals used were purchased from NEN (Boston, MA 02118) and were of the highest specific activity available. In a total volume of 0.2 ml a mixture was prepared containing approximately 0.6 µCi of the 14C test substance (e.g. lactate, nicotine), 4-6 µCi of tritiated water and 50 µCi of Indium-EDTA, typically in a 10 mM buffer. The buffers and their respective pKs used in this study were piperazine-N,N-bis(2 ethane sulfonic acid) (PIPES), pK = 6.8; 2(N-morpholine) ethane sulfonic acid (MES), pK = 6.15; morpholinos propane sulfonic acid (MOPS), pK = 7.20; N-2-hydroxyethyl piperazine N-2-sulfonic acid (HEPES), pK = 7.55; N,N-bis(2 hydroxyethyl) glycine (BICINE), pK = 8.35; and cyclohexylamino propane sulfonic acid (CAPS), pK = 10.4. In each case the solution’s pH was adjusted to the pK of its buffer before injection except in the most acidic injection of nicotine where the pH was adjusted to 4.7, considerably below the pK (6.15) of the MES buffer used.

SUMMARY Brain uptake of radiolabeled D and L-lactate, D-glucose and nicotine, as measured by the intra-carotid bolus method, was examined over a range of pH of the injected solution. The uptake of L-lactate was highest at pH 6.1, and lowered significantly at pH 7.2, 7.5 and 8.4. In contrast, the uptake of the D-enantiomer was not as dramatically affected. Glucose uptake was not affected by alterations in pH. Nicotine uptake decreased with pH reduction through a range of 8.3–4.2. These data suggest that it is the uncharged molecule which penetrates the blood-brain barrier by both carrier and lipid mediation. A mechanism relating to these observations is postulated and possible relevance to lactate washout from ischemic brain discussed.
These buffers, in crystalline form, were obtained from Calbiochem (San Diego, CA).

To establish whether the $^3$H-water clearance changes with changing injected pH, 2 groups of 3 animals each were studied using $^3$H-water as the test substance and $^{14}$C-butanol as the diffusible reference. In one group the injected pH was 6.1 and in the other 8.4.

Unless otherwise indicated, all values for results are in the form of a mean (=x), standard deviation (=SD) and sample number (=n). The Student's $t$-test was used to test for statistical significance between differing experimental conditions. Counts per minute (cpm) recorded on the liquid scintillation counter were converted to disintegrations per minute by cubic regression analysis and compared with similarly quenched standard samples of known activity.

**Results**

As indicated in table 1, and figure 1, the brain uptakes of both D- and L-enantiomers of lactate increase with a decrease in pH of the injected bolus. These changes in L-lactate brain uptake index (BUI) were all statistically significant. The uptake of D-lactate was not altered as dramatically by pH changes as the L-enantiomer. Brain uptake of the neutral hexose D-glucose was not significantly altered by changes in injected hydrogen ion concentration (table 2).

The clearance of $^3$H-water was not different when injected at pH 6.1 or 8.4 (BUI at pH 6.1 = 84 ± 4, at pH 8.4 BUI = 85 ± 5; butanol used as the reference substance).

Since both lactate and glucose are transported across the BBB by specific (monocarboxyl and hexose, respectively) carrier systems, subsequent studies examined the effect of varying the injected hydrogen ion concentration on brain uptake of nicotine which penetrates the BBB by virtue of its lipid solubility. As indicated in table 3 and figure 2, this slightly charged base, which is not transported by a carrier system, exhibits pH-dependent changes in BBB penetration. Nicotine (pK1 = 6.16; pK2 = 10.96) uptake is reduced by lowering the pH of the injected solution.

**Discussion**

Several recent studies indicate that, in a variety of biological membranes other than BBB, transport is pH dependent. For example, the movement of phosphate, lactate, beta-hydroxybutyrate, propionate and octanoate, several anions, as well as threonine, and pentazocine, is subject to pH dependence. It has recently been suggested that within the clinical range of blood pH the blood-brain and blood-CSF distributions of morphine and its derivatives might be sensitive to alterations in pH.

The effects of pH on lactate uptake described here suggest that, within the range likely to be found regionally in pathological states, pH can be a substantial modulator of carrier-mediated BBB transport. These data indicate that BBB transport of lactate increases as hydrogen ion concentration is increased. This is in contrast to nicotine in which increased ionization (resulting from reduction of pH) reduces BBB permeability. The uptake of D-glucose was not measurably altered by changes in pH (of the injectate).

One possible interpretation of these data is that carrier-mediated lactate transport is of the un-ionized species rather than of the ionized. Such a conclusion contrasts with current understanding of ion transport.

**Table 1**

<table>
<thead>
<tr>
<th>pH</th>
<th>L-lactate BUI</th>
<th>D-lactate BUI</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.15</td>
<td>20.5 ± 2.2</td>
<td>6.8 ± 1.1</td>
</tr>
<tr>
<td>7.20</td>
<td>15.9 ± 1.5</td>
<td>5.4 ± 0.5</td>
</tr>
<tr>
<td>7.55</td>
<td>12.2 ± 0.8</td>
<td>4.8 ± 0.74</td>
</tr>
<tr>
<td>8.40</td>
<td>8.9 ± 0.4</td>
<td>4.0 ± 0.6</td>
</tr>
</tbody>
</table>

$n = 3$ for each $x$ ± SD. Alterations in L-lactate uptake were all statistically significant. Comparing pH 6.15 to 7.20, $p < 0.005$; pH 7.2 to 7.55, $p < 0.025$; and pH 7.55 to 8.40, $p < 0.005$. Alterations in D-lactate uptake were significant ($p < 0.05$) only when comparing changes of 1.2 pH units or more.

Injected L-lactate concentration was 0.009 mM and for D-lactate, 0.156 mM.

**Table 2**

<table>
<thead>
<tr>
<th>pH</th>
<th>Glucose BUI</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.15 (20 mM MES)*</td>
<td>34.4 ± 4.3</td>
</tr>
<tr>
<td>7.55 (10 mM HEPES)*</td>
<td>33.4 ± 3.3</td>
</tr>
<tr>
<td>8.40 (20 mM TAPS)*</td>
<td>35.6 ± 3.2</td>
</tr>
</tbody>
</table>

$n = 6$ for each $x$ ± SD. * = buffer abbreviations are defined in Methods. Injected glucose concentration was 0.005 mM.
TABLE 3  Effect of Intracarotid Injected pH on Brain Uptake of Nicotine

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pH</th>
<th>Nicotine Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>10mM MES</td>
<td>4.7</td>
<td>49 ± 10</td>
</tr>
<tr>
<td>10mM MES</td>
<td>6.15</td>
<td>77 ± 4</td>
</tr>
<tr>
<td>10mM MOPS</td>
<td>7.2</td>
<td>109 ± 3</td>
</tr>
<tr>
<td>10mM HEPES</td>
<td>7.55</td>
<td>120 ± 3</td>
</tr>
<tr>
<td>10mM TAPS</td>
<td>8.35</td>
<td>126 ± 12</td>
</tr>
<tr>
<td>10mM CAPS</td>
<td>10.4</td>
<td>127 ± 5</td>
</tr>
</tbody>
</table>

Nicotine pK1 = 6.16, pK2 = 10.96; n = 3. Injected nicotine concentration was 0.009 mM.

in mitochondrial membranes in which the charged species, rather than the un-ionized ones, are transported. The charged species, and is similarly transported in a protozoan, trypanosome, in its charged form. In rat erythrocytes and thymocytes, kinetic constants for beta hydroxybutyrate influx and efflux were found to be altered with changes in pH. Qualitative similarities in transport characteristics of the red cell membrane and the BBB have been noted previouly. It has also been demonstrated that in the BBB, neutral and basically charged amino acids are transported by separate carrier mechanisms, suggesting that the BBB may possess the ability to discriminate on the basis of ionic charge. [The possibility exists that this charge discrimination may be secondary to discrimination based on steric factors.]

Other data obtained in the present study suggest that, in the case of the substances reported here, it is the un-ionized species which penetrates the BBB. A finding related to this postulation is that in rat leukocytes there is an increase in uptake velocity of pentazocine with increases in hydrogen ion concentration. It was concluded that this analgesic drug was transported when it bore no net charge.

A possible explanation of a mechanism of BBB uptake, based on the assumption that the un-ionized molecular species are the transported, or membrane-penetrating form, appears in figure 3. When pH = pKa, any one molecule spends, on average, 50% of its time in the un-ionized form, and 50% of its time in the ionized state.

Lactate pKa is 3.83. At pH 7.4 only about one molecule in 2800 is un-ionized at any one time. When the pH is lowered one unit, the concentration of this un-ionized species is increased by a factor of 10 whereas the fractional change of the ionized species does not change appreciably; remaining in excess of 99% above a pH of 5.83 (pK 3.83 + 2 units). Thus, any major effect of pH change on BBB permeability to lactate suggests it is the un-ionized species which is being transported.

When ionized, the lactic acid molecule is much more polar and thus more lipophobic than when un-ionized. When the un-ionized state the residual polarity is due to hydrogen bonding (fig. 3). In this more hydrophobic state the relative affinities of the lactic acid molecule for the BBB carrier molecule, BBB lipid moiety and the adjacent blood plasma water favors its escape from plasma water and entry into the capillary endothelial cell membrane (the BBB).

![Figure 3](http://stroke.ahajournals.org/)

FIGURE 3. A lactate molecule is continually losing and gaining a proton. A single molecule alternates between these 2 states and the polarity of the molecule moves up and down through a considerable range. This graph diagrammatically follows a single lactate molecule through time as it alternates between its 2 states (shown here as it would appear at a pH of approximately 5). The fraction of the time it is protonated (and thus uncharged) is a function of the ambient proton (hydrogen ion) concentration. Thus, at lower pH values the molecule is un-ionized a greater fraction of the time. In this un-ionized state the small, remaining molecular polarity is due to hydrogen bonding capacity. At pH 7.4 any one lactate molecule is un-ionized only about 1/2800th of the time. At one pH unit lower, this changes to 1/280th of the time whereas the fractional change in the ionized species is virtually nil. If the un-ionized species is transported, lower pH should increase transport since the molecule is in the relatively non-polar state more of the time. In this latter state it is more hydrophobic and more readily detach itself from plasma water and attach itself to a BBB carrier site or enter the lipid moiety of the BBB.
The failure to see changes in BUI proportionate to the concentration of the un-ionized species when the pH is altered (see table 1) might be attributed to the possibility that pH changes in the present studies are limited to the region of the capillary lumenal mem-
brane and probably not throughout the entire brain capillary endothelial cell. Alternatively, Voorhees15 has indicated that pH-induced modifications of transport also involve changes in ionizable groups on the transport carrier. Such an explanation could not, however, be invoked in the case of membrane penetration of compounds such as nicotine which gain access to the brain by virtue of their lipid solubility.

In the early post-ischemic brain there often is a hyperperfusion commonly attributed to regional tissue acidosis due largely to accumulation of lactate and carbon dioxide. The local pH in ischemic brain has been calculated to be as low as 6.0 — 6.5.34, 35 The present data suggest that the increased permeability to lactate at this pH, particularly when amplified by increased regional blood perfusion, could substantially increase the efflux of lactate from the ischemic region. From common observation that clinical nuclear brain scans in the early post-ischemic period are normal, it can be concluded that the BBB is not non-specifically permeable in most such lesions and that trans-
capillary flux must largely be dependent upon carrier systems.

From previous work with L and D lactate it is assumed that transport of the D form probably occurs almost entirely by virtue of its lipid solubility and that the difference between the L and D forms represents carrier-mediated transport of the former.1 In the present study both L and D forms exhibit increased transport at lower pH. The effect on the D form is to be expected since an increased lipid/water partition coefficient is present at lower pH values.

If there were an immediate change in brain blood flow during the bolus passage through the brain microcirculation in response to the transiently abnormal pH, there could be an artifact introduced due to a change in the relative clearance by brain of the labeled lactate and the diffusible reference, 3H-water. The 3H-water is, under the conditions of barbiturate anesthesia used here, 85% cleared during one brain passage. This could be increased in regions of lowered flow and decreased in high-flow regions.

The independence of glucose uptake to pH changes suggests that the D-glucose carrier is not measurably altered by the transient pH shift produced during these experiments. This does not necessarily disprove that some of the changes of L-lactate uptake with pH changes are due to pH-induced alterations in the short-chain monocarboxylic acid carrier.

The present study indicates that in the BBB, as in other membrane systems, changes in pH result in permeability changes. Our data do not positively in-
dicate that either the charged, or the uncharged molecule is the form which penetrates the membrane; nor does it preclude the possibility that certain classes of molecules might be transported in the ionized state while other types of charged molecules penetrate membranes in the un-ionized state. The present study indicates that L-lactate is transported more effectively by the BBB at increased hydrogen ion concentrations. This could have clinical relevance by affecting the rate of washout of lactate from ischemic brain.

Acknowledgment

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The effect of ischemia on cerebral function, metabolism and structure is a highly controversial issue which, despite extensive research during the past years, has not been satisfactorily solved. Many investigators feel that duration and intensity of ischemia are the main determinants of tissue damage, whereas others, including ourselves, are of the opinion that post-ischemic events are of equal if not greater importance. Unexpected findings, such as the observation that severe incomplete ischemia is more harmful than complete ischemia, the site of occlusion seems to be of particular importance. For instance, following embolism of the capillary bed with microspheres, the net flow rate of the brain may remain constant because there is a redistribution of flow with

Arterial Air Embolism in the Cat Brain

H. Fritz, M.D. and K.-A. Hossmann, M.D.

SUMMARY In cats air embolism of the brain was produced by injecting 0.6 ml blood foam into the innominate artery proximal to the origin of both common carotid arteries. Air embolism caused transient ischemia of the brain, reaching a maximum within 1 min after injection. Resolution of the air embolism began a few minutes later and was completed within 15 min in the center and within 30 min in the border zone of the main supplying arteries. During this phase tissue perfusion was inhomogenous with reduced flow rates in some areas and reactive hyperemia up to 300% in others. This resulted in venous hyperoxia and a decrease of arteriovenous oxygen difference to as low as 2 ml/100 ml blood. Reactive hyperemia was accompanied by brain swelling and an increase in intracranial pressure from 3.6 ± 1.2 to 12.3 ± 2.0 mm Hg. The reason for hyperemia was a decrease of cortical pH which fell from 7.33 ± 0.03 to 7.03 ± 0.05, and which caused a dilatation of pial arteries up to 260%.

Immediately after embolism, the EEG flattened and oxygen consumption decreased. After normalization of flow, oxygen consumption returned to normal, but EEG only partially recovered. Air embolism had little effect on the water and electrolyte content of the brain, and produced very little damage to the blood-brain barrier.

THE EFFECT of ischemia on cerebral function, metabolism and structure is a highly controversial issue which, despite extensive research during the past years, has not been satisfactorily solved. Many investigators feel that duration and intensity of ischemia are the main determinants of tissue damage, whereas others, including ourselves, are of the opinion that post-ischemic events are of equal if not greater importance. Unexpected findings, such as the observation that severe incomplete ischemia is more harmful than total cerebrovascular arrest, have added to the confusion.

It is of little help when the different results are explained by differences in the experimental models as long as the pathophysiology of these models is not precisely known. We have, therefore, studied in previous investigations different forms of ischemia, and we found that, in fact, the pathomechanism of ischemia varies considerably. Besides the duration and completeness of ischemia, the site of occlusion seems to be of particular importance. For instance, following embolism of the capillary bed with microspheres, the net flow rate of the brain may remain constant because there is a redistribution of flow with hyperemia in the non-occluded vessels. The main injurious factor is the breakdown of the blood-brain barrier (BBB), leading within a few minutes to severe vasogenic brain edema and intracranial hypertension. On the other hand, ischemia produced by extracerebral interruption of blood supply (inflow occlusion), causes primarily a breakdown of the energy producing metabolism, and an inhibition of all endergetic processes. But there is no change in the permeability of the blood-brain barrier to serum proteins. Edema, if present, is of the cytotoxic type, and it is rapidly reversible upon restoration of blood flow. Finally, occlusion of the middle cerebral artery causes initially metabolic disturbances and a cytotoxic type of brain edema, but after a few hours vasogenic edema supervenes.

In the present series of experiments we have studied the pathophysiology of air embolism, which is a model of intracerebral small vessel occlusion. Air bubbles are trapped in small intracerebral arteries, leading to acute interruption of blood flow in the supplying territories of the affected vessels. The site of occlusion, consequently, is proximal to the capillary bed but distal from the large supplying arteries. We were, therefore, interested to know whether the pathophysiology of air embolism resembles the inflow occlusion or the microemboilism type of ischemia. Our results indicate that it combines elements of both types, and that, for this reason, it is a useful model for
pH dependence of blood-brain barrier permeability to lactate and nicotine.
W Oldendorf, L Braun and E Cornford

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