Lactate and Pyruvate Concentrations, and Acid-Base Balance of Cerebrospinal Fluid in Experimentally Induced Intracerebral and Subarachnoid Hemorrhage in Dogs

BY TOMEI SUGI, M.D., MASATOSHI FUJISHIMA, M.D., AND TERUO OMAE, M.D.

Abstract:
The effect of blood injected into either subarachnoid space or subcortical brain tissue upon lactate and pyruvate concentrations as well as acid-base balance of cerebrospinal fluid (CSF) was studied in the anesthetized dog.

CSF lactate and lactate/pyruvate ratio (L/P ratio) increased progressively following the intracranial injection of blood and reached the maximum level at six hours after injection. These changes were significantly greater in animals with intracerebral hematoma than in those with subarachnoid hemorrhage (SAH). An increase in CSF lactate and L/P ratio in hemorrhagic CSF seems to be caused by two different factors. Shed blood cells per se produce lactate and pyruvate, and blood in the subarachnoid space and intracerebral hematomas cause secondary changes in brain tissue metabolism by a probable reduction of cerebral blood flow. Therefore, an increase in CSF lactate with a concomitant rise in CSF L/P ratio is a useful indicator for brain tissue hypoxia, even when CSF is hemorrhagic.

The association of an increase in CSF lactate to a disproportionate decrease in CSF HCO₃ was also observed in these animals.

Additional Key Words:
cerebral hypoxia  lactate/pyruvate ratio  anaerobic glycolysis

Introduction:
In 1967, Froman and Smith reported that lactate concentration of cerebrospinal fluid (CSF) in patients with subarachnoid hemorrhage (SAH) markedly increased, and it was concluded that the increase was due to the glucose metabolism of shed blood cells per se in the CSF space. In our previous study in patients with cerebrovascular diseases, we also found that hemorrhagic CSF contained a higher lactate level and lactate/pyruvate ratio (L/P ratio) than did clear CSF.

An increase in lactate in CSF as well as in brain tissue has been reported in a variety of diseases or circumstances causing hypoxia in the brain. They include hypoxemia, hyperventilation, hypotensive shock, circulatory arrest, head injury, and cerebrovascular diseases. In our experience, an increase in CSF lactate and L/P ratio was significantly greater in stroke patients having disturbed consciousness or a poor prognosis than in those having normal consciousness or with a good recovery. L/P ratio of CSF reflects the redox state of the cytoplasmic NADH/NAD⁺ system when CSF is in diffusion equilibrium with brain tissue. These facts suggested that even in the presence of blood in CSF, anaerobic glycolysis of brain tissue, in addition to that of shed blood cells, could be reflected in CSF lactate level or CSF L/P ratio.

The present study was undertaken to clarify whether an increase in lactate and L/P ratio in hemorrhagic CSF represented the anaerobic state of the brain.

Methods:
Twenty-three mongrel dogs, weighing 8 to 15 kg, were anesthetized with 30 mg per kilogram of intravenous pentobarbital sodium and paralyzed with 2 mg per kilogram of gallamine triethiodide. Respiration was artificially controlled, and Paco, was maintained between 30 and 50 mm Hg as constantly as possible by adjusting respiration rate or tidal volume. Hypoxic animals with Po, below 60 mm Hg were excluded from the present study.

One femoral artery was cannulated for recording of blood pressure with a pressure transducer, and for sampling of blood. A 21-gauge needle was introduced into the cisterna magna, and through this needle CSF samples were obtained, and intracranial pressure was recorded by connecting the needle to a pressure transducer specific for low grade pressures. Physiological saline was continuously infused into the cannulated femoral vein throughout the experiment, and pentobarbital and gallamine were supplemented every hour.

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The animals were divided into three groups as follows: SAH, intracerebral hemorrhage (ICH), and controls. In ten dogs of Group 1 (SAH), 1 to 4 ml (2.6 ± 0.9 ml, mean ± SD) of autologous arterial blood were injected into the cisterna magna. In another ten dogs of Group 2 (ICH), a small burr hole was made in the parietal region of the skull and a 21-gauge needle was carefully inserted to reach the subcortical white matter over the lateral ventricle by using a stereotaxic apparatus. An intracerebral hematoma was made by introducing 2 to 3 ml (2.4 ± 0.3 ml) of arterial blood into the brain tissue. A small amount of injected blood leaked into the ventricles and subarachnoid space, which was confirmed at autopsy. In each group, 1 ml of CSF (1 ml for metabolite determination, 0.5 ml for gas analysis) and 0.5 ml of arterial blood were withdrawn twice during the control resting state, and every hour during the six-hour experimental period following the intracranial injection of blood. There were three dogs in Group 3: CSF and arterial blood were obtained in a similar manner as in Groups 1 and 2, without the intracranial injection of blood.

Lactate and pyruvate concentrations in CSF were determined enzymatically using Lactat-Test and Pyruvat-Test (Boehringer Mannheim). CSF L/P ratio was calculated from the measured values of lactate and pyruvate concentrations. In CSF and arterial blood, pH, PCO, and PO were determined at 37°C with IL-Meter Model 113. Bicarbonate (HCO3) was calculated by using Henderson-Hasselbalch nomogram and Mitchell's correction for CO2 solubility and pK' for CSF.15

The animals were killed at the end of the experiment, and the brains were examined macroscopically for the extent of blood in the subarachnoid space and for the location and size of the hematomas.

Results
In three control animals (Group 3) there were no remarkable changes in lactate, pyruvate, L/P ratio, pH and HCO3 of CSF during seven hours, as shown in table 1.

Mean values for each parameter during the control resting period of Groups 1 and 2 are tabulated in table 2. No significant differences in each parameter were noted between these two groups. In Groups 1 and 2, serial changes in CSF lactate and pyruvate concentrations, and L/P ratio following the intracranial injection of blood are illustrated in figure 1. CSF lactate in both groups increased progressively after injection of blood and reached the maximum level at six hours. Mean values for an increase in CSF lactate at six hours from the levels during the control period were

<table>
<thead>
<tr>
<th>Hour</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate (mM/L)</td>
<td>1.99</td>
<td>1.98</td>
<td>2.04</td>
<td>1.91</td>
<td>1.93</td>
<td>1.89</td>
<td>1.91</td>
</tr>
<tr>
<td>Pyruvate (mM/L)</td>
<td>0.237</td>
<td>0.297</td>
<td>0.289</td>
<td>0.269</td>
<td>0.288</td>
<td>0.270</td>
<td>0.270</td>
</tr>
<tr>
<td>L/P ratio</td>
<td>6.23</td>
<td>6.83</td>
<td>7.09</td>
<td>7.13</td>
<td>6.96</td>
<td>7.00</td>
<td>7.25</td>
</tr>
<tr>
<td>HCO3 (mEq/L)</td>
<td>24.7</td>
<td>26.0</td>
<td>26.0</td>
<td>27.6</td>
<td>26.9</td>
<td>26.0</td>
<td>27.4</td>
</tr>
</tbody>
</table>

2.06 mM per liter in Group 2 (ICH) and 1.03 mM per liter in Group 1 (SAH), respectively. The difference between the values of the two groups was of statistical significance at two, three and six hours following the injection of blood. At four and five hours, however,
EXPERIMENTALLY INDUCED INTRACEREBRAL AND SUBARACHNOID HEMORRHAGE IN DOGS

TABLE 1
Mean Values for Lactate, Pyruvate and Bicarbonate Concentrations, L/P Ratio and pH of CSF, and Pco₂ of Arterial Blood During Control Resting State Prior to the Intracranial Injection of Blood

<table>
<thead>
<tr>
<th></th>
<th>CSF (mM/L)</th>
<th>L/P ratio</th>
<th>pH</th>
<th>HCO₃⁻ (mEq/L)</th>
<th>Pco₂ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAH (n = 10)</td>
<td>1.95 ± 0.40</td>
<td>0.239 ± 0.068</td>
<td>8.47 ± 1.92</td>
<td>7.372 ± 0.034</td>
<td>25.9 ± 1.4</td>
</tr>
<tr>
<td>ICH (n = 10)</td>
<td>2.23 ± 0.63</td>
<td>0.280 ± 0.078</td>
<td>7.92 ± 1.08</td>
<td>7.393 ± 0.032</td>
<td>27.2 ± 1.5</td>
</tr>
</tbody>
</table>

Mean ± SD.

the difference was not significant because of the small number of dogs available for statistical analysis.

CSF L/P ratio remained unchanged for the first three hours after injection, and thereafter started to rise and reached the maximum level at six hours. The increase in CSF L/P ratio in ICH at three hours or later was significantly greater than in SAH. Mean values for an increase in L/P ratio at six hours were 4.04 in ICH, significantly higher than 1.71 in SAH. A maximum change in CSF lactate and L/P ratio of individual dog is shown in figure 2. According to the size of the hematoma produced, Group 2 was divided into three subgroups: small (S), moderate (M), and large (L) hematomas. There was a tendency for a greater increase in lactate or L/P ratio in dogs with large hematomas than small ones.

The changes in CSF pH and HCO₃⁻ are shown in figure 3. CSF pH in Groups 1 and 2 tended to fall at the late stage of the experiment, although it was not significantly different from the control resting state prior to injection. In spite of a progressive decrease in CSF HCO₃⁻ at four hours or later following the hematoma produced in Group 2, the variation of the individual values was too great to reach statistical significance. In Group 1, there were no consistent changes in CSF HCO₃⁻ during the period of observation.

Figure 4 shows the correlation of changes in CSF lactate with CSF HCO₃⁻ obtained at the various intervals during the experimental period. There was an inverse relation between the two values, i.e., an increase in lactate was accompanied by a decrease in CSF HCO₃⁻. The amount of HCO₃⁻ decrease was greater than the lactate increase, and the ratio of the former to the latter was roughly 1.5 to 1. There were, however, some dogs showing an opposite relation with an increase in HCO₃⁻ associated with an increase in lactate.

No significant relationship was obtained between the amount of blood injected and the maximal increase in either CSF lactate or L/P ratio as depicted in figure 5. Intracranial pressure rose slightly for a short period of time following the intracranial injection of blood, and then fell and remained below the control level because of repeated withdrawal of CSF samples. The maximum CSF pressure in most

![Graph showing changes in CSF pH and bicarbonate concentration following intracranial injection of blood.](http://stroke.ahajournals.org/)

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FIGURE 4

The correlation of changes between CSF lactate and CSF HCO₃ at various time intervals after intracranial injection of blood. An inverse relationship is obtained, although some showed an increase in HCO₃ in spite of a rise in lactate. The amount of decrease in HCO₃ is greater than the lactate increase.

animals was recorded two hours after beginning the experiments. The mean CSF pressures were 16.3 ± 8.4 mm Hg in SAH and 12.7 ± 4.3 mm Hg in ICH. There was no obvious relationship between a maximum rise in CSF pressure and a maximum increase in either CSF lactate or L/P ratio during the experimental period.

Discussion

The present study showed that when blood was introduced into either the subarachnoid space or the brain tissue, CSF lactate and the L/P ratio increased progressively in each experimental model. There have been observations suggesting that one of the causes of increased CSF lactate in SAH could be due to the glycolytic process in the shed blood cells.

In an in vitro study, Froman and Smith¹ observed a progressive increase in lactate with a concomitant fall in pH and HCO₃ in the mixture of blood and mock CSF tonometered for 12 hours. Similar observations showing a rise in lactate in the hemorrhagic CSF have been reported by Shannon et al.¹⁶ and Granholm.¹⁷ The latter noted the CSF L/P ratio to be unchanged despite an increase in both lactate and pyruvate.

The increase in CSF lactate and L/P ratio was significantly greater in dogs with ICH than in dogs with SAH in the present study. As the experimental conditions were the same in the two groups except for hematoma formation in ICH, the circulatory and the metabolic disturbances of the brain are assumed to be more severe in ICH than in SAH. These facts suggest that, in addition to a rise in lactate derived from shed blood cells, an increase in CSF L/P ratio as well as lactate in hemorrhagic CSF seems to reflect the anaerobic state of the brain. These parameters could be used as indicators for the severity of the cerebral hypoxia or the prognosis of patients with ICH.

In his in vivo study, Granholm¹⁷ also found an increase in CSF lactate and pyruvate, but no change in L/P ratio during the one to three hours following the intracisternal injection of blood in cats. In contrast, the present study showed that CSF lactate and L/P ratio increased progressively and reached their maximum level at six hours. This increase in CSF L/P ratio may indicate the anaerobic state of the brain tissue. This anaerobic glycolysis in the experimental SAH, as frequently seen in patients with SAH, was considered due to decreased cerebral blood flow resulting from cerebral vasospasm or from the increased intracranial pressure. Although we have no direct data to prove that a gradual decrease in cerebral circulation followed induced SAH, a delayed but consistent rise in CSF L/P ratio suggests that the intracranial blood injection might cause a reduction of blood supply to the brain or derangement of brain metabolism which results in brain hypoxia.

The relationship of changes in CSF lactate with CSF HCO₃ varies with the different experimental conditions. In hypoxia, MacMillan and Siesjö¹⁸ observed a one-to-one relationship between an increase in CSF lactate and a decrease in CSF HCO₃. In the present study, a larger decrease in CSF HCO₃ relative to the increase in CSF lactate was shown, and the ratio was 1.5 to 1, i.e., 1 mM per liter of lactate produced was coupled with 1.5 mEq per liter of HCO₃. Similarly, a 1:3 relationship was reported by Mines and Sørensen¹⁹ in hypoxic dogs. Shannon et al.¹⁶ studied the acid-base changes in hemorrhagic CSF following the intracisternal injection of red blood cells in dogs and found the rise in CSF lactate and pyruvate accounted for only
57% of the total fall in CSF HCO₃. The discrepancy between changes in CSF lactate and HCO₃ is not fully understood. It has been speculated that acid metabolites other than lactate exist or that an additional mechanism of acidifying CSF is operative in hypoxia or intracranial hemorrhages.

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References
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