Differentiation of Early Subarachnoid Hemorrhage from Traumatic Lumbar Puncture

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SUMMARY The problem of early differentiation of "traumatic tap" from subarachnoid hemorrhage (SAH) was studied in the rabbit by determining the changes in percentage of hemolysis and in lactate concentrations in CSF within the first twenty-four hours following induced SAH. The 0.3 to 7% hemolysis which occurred was relatively independent both of the time following SAH and of the number of red blood cells (rbc) in the cerebrospinal fluid (CSF).

HOW TO DISTINGUISH true subarachnoid hemorrhage (SAH) from accidental blood contamination of the cerebrospinal fluid (CSF) at lumbar puncture, or so-called "traumatic tap," has been investigated for many years, and a number of clinically useful methods have been proposed. Unfortunately, many instances are encountered in which existing methods fail; these tend to be found in patients whose neurologic illness has been present only a few hours and in whom the need for a prompt diagnosis is greatest. Since the crucial difference between true SAH and "traumatic tap" is the length of time the blood has been present in the CSF, we considered several time-dependent processes for investigation, and finally selected two for study.

Tourtellotte et al.\(^1\) stated that 0.5 to 3% hemolysis occurs soon after red blood cells (rbc's) are introduced into the CSF and that essentially no further hemolysis occurs for at least six hours if the sample is stored at room temperature or at 4°C. Others found previously that incubation of rbc's at 37°C resulted in progressive hemolysis which was easily detectable at one-two hours.\(^2\) This suggests that progressive hemolysis also occurs with time after SAH, which might be considered to be similar to 37°C incubation, but a maximum of 3% hemolysis might be found following a "traumatic tap" providing the sample is kept between 4°C and room temperature for less than 6 hours prior to measurement. We elected to test this hypothesis as one possible approach to the problem of differentiating recent SAH from traumatic lumbar puncture.

A second hypothesis which seemed clinically feasible is based upon the fact that incubated red blood cells produce lactate as a result of the absence of an aerobic glycolytic pathway.\(^3\) If other sources of lactate can be excluded, lactate concentration in hemorrhagic CSF should increase as a function of time following the onset of a subarachnoid hemorrhage,\(^4\) whereas the lactate concentration in the spinal fluid obtained from a "traumatic tap" should be identical with that of uncontaminated fluid if determined immediately.

Preliminary work involving in vitro incubation of autologous rbc's in CSF of patients with non-hemorrhagic neurologic disease revealed that less than 3% hemolysis occurred between 0 and 24 hours.\(^*\) Obvious differences exist between in vitro and in vivo incubation (e.g. the conversion of oxyhemoglobin to bilirubin,\(^1\) significant sequestration of rbc's in the subarachnoid space,\(^6\) \(^7\) and the possibility of introduction of lactate from other sources besides blood cell metabolism). So we elected to study both percent hemolysis and CSF lactate concentration as a function of time following experimental SAH in rabbits.

METHODS

Twenty-two adult Kingswheel and New Zealand white rabbits were anesthetized with an intramuscular injection of 18 mg/kg of chlorpromazine and 55 mg/kg of ketamine, supplemented as needed with diethyl ether. In seven animals percutaneous cisternal puncture was performed using a ½ inch, 22 gauge needle attached to a 1 ml syringe, and ½ ml of autologous cardiac blood was injected slowly into the cisterna magna. The rabbit was then allowed to awaken. Four hours later, the animal was re-anesthetized with diethyl ether, another cisternal puncture was performed and ½ ml of CSF was removed for study. A simultaneous arterial blood sample was obtained. A group of four animals was similarly treated, except that the second cisternal puncture was performed one hour after the introduction of autologous cardiac blood; since these animals had not yet regained normal alertness by that time, it was believed that persisting alterations in blood gas values would invalidate lactate and pyruvate data. Therefore, the CSF samples from these animals were used only to calculate percentage of hemolysis. Control data were obtained from seven rabbits treated in the same way, except that no cisternal puncture was performed during the initial anesthetic procedure; the animal was allowed to awaken, and after four hours was re-anesthetized with diethyl ether and a ½ ml sample of CSF obtained via cisternal puncture. A simultaneous arterial blood sample was also obtained as before. Seven animals from this group then had ½ ml of autologous cardiac blood injected into the cisterna magna immediately following removal of the control CSF sample. The animal was then allowed to awaken and was re-anesthetized 24 hours later and the final cisternal CSF sample obtained. Arterial blood samples were also utilized for gas determinations and for lactate and pyruvate levels. CSF was analyzed for hemoglobin, bilirubin, lactate and pyruvate as described below.

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This investigation was supported by Central Ohio Heart Chapter, Grant RF 3777-AL.
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All CSF specimens were centrifuged at 3000 rpm (1,800 X g) for ten minutes within fifteen minutes of collection. It was found that centrifugation for less than eight to ten minutes or at a lower speed usually resulted in incomplete sedimentation of rbc’s in both human and rabbit CSF. Immediately after centrifugation the supernatant was removed carefully with a transfer pipette and placed in a stopped test tube. A volume of distilled water equal to the volume of supernatant removed was added to the sedimented cells, and the tube frozen to hemolyze the sample. A portion of the supernatant was pipetted at the proportion of 1 ml to 3 ml of cold 5% (w/v) metaphosphoric acid to precipitate proteins. After mixing and centrifugation, the resulting supernatant was separated and frozen for subsequent lactate and pyruvate assays. The remainder of the original supernatant was used for calculation of percentage of hemolysis.

**Percentage of Hemolysis**

Optical density of the spinal fluid supernatant was determined at 412 and 480 μM using a Beckman D.U. Spectrophotometer. Microcuvettes having a width of 3 mm and a 1 cm light path were employed. The total hemoglobin concentration in the supernatant was then calculated using the following expression:

\[ C_{Hb} = 8.7 \times (O.D._{412}) - 7.8 \times (O.D._{480}) - 0.1 \]

Where:

- \( C_{Hb} \) = Hemoglobin concentration in μM
- O.D._{412} and O.D._{480} refer to the optical densities at 412 and 480 μM respectively

The tube containing the hemolyzed sediment was then thawed and after vortex mixing, the optical density of the spinal fluid supernatant was determined using the expression:

\[ C_{Hb} = 29 \times (O.D._{412}) - 1.8 \times (O.D._{480}) - 0.2 \]

The bilirubin concentration may be calculated by use of the following expression:

\[ C_B = \text{bilirubin concentration in } \mu\text{M} \]

**CSF Lactate and Pyruvate**

CSF lactate was determined by the lactate dehydrogenase method. A glycine-hydrazine buffer was employed, and the change in O.D. was read at 340 μM.

CSF pyruvate was assayed by a somewhat similar procedure developed by Sigma Chemical Company. A Trizma® buffer was used, and the change in O.D. at 340 μM measured.

**Results**

(A) Eighteen rabbits were studied at intervals varying from one to 24 hours following subarachnoid hemorrhage, and percentage of hemolysis values between 0.3 and 7% were obtained. Though these values were independent both of the time after subarachnoid hemorrhage and of the magnitude of rbc count in the CSF, higher values tended to occur somewhat more frequently at the longer time intervals (table 1). Since generally lower rbc counts were also observed at these longer time intervals, despite the original introduction of equal volumes of autologous blood, the higher values probably reflect the sequestration of blood in the subarachnoid space rather than a greater quantity of lysed rbc’s. Rbc counts varied from 14,200 to 1,100,000 rbc/mm³ for all experiments. CSF bilirubin was calculated for each sample, and in no case was the bilirubin concentration greater than the hemoglobin concentration.

(B) CSF lactate and pyruvate concentrations were determined in 11 control rabbits which had been anesthetized, allowed to awaken, then re-anesthetized at four hours in order to eliminate the question of a residual effect of alterations of blood gases due to the first anesthetic procedure when compared with those values obtained at four hours after subarachnoid hemorrhage (table 2). Average CSF lactate concentration (control) was 1.99 mEq/L ± 0.12 S.E.M. Lactate and pyruvate concentrations from this control group were also utilized to calculate the lactate/pyruvate ratio, which provides a means of detecting significant excess lactate production as a result of hypocapnea or other factors leading to cerebral ischemia, hypoxia, or reduction in the phosphate potential. The average lactate/pyruvate (L/P) ratio for this group was 11.0 (table 2).

Seven rabbits were studied four hours after subarachnoid hemorrhage (table 3); the average value for CSF lactate in this group had increased to 3.16 mEq/L ± 0.24 S.E.M. and the L/P ratio had increased slightly to 12.7. The increase in lactate concentration is significant (p < .001) (Student’s t test), while the increase in L/P ratio is not (p < 0.2). Seven rabbits were studied 24 hours after subarachnoid hemorrhage, when the average CSF lactate had dropped to 2.46 mEq/L ± 0.13 S.E.M., with L/P ratio of 11.0 (table 4). The decrease in lactate concentration at 24 hours may reflect the clearance or isolation of rbc’s from the subarachnoid space, since average CSF rbc counts decreased from 846,000 in one hour samples to 467,000 at four hours and 170,000 by 24 hours despite the introduction of equal volumes of blood at zero time. As noted by others there was no correlation with CSF data providing the PC02 and PO2 were within reasonable limits (tables 2, 3).
Subarachnoid hemorrhage caused by the introduction of autologous blood into the cisterna magna of the rabbit, results in a limited degree of hemolysis which is essentially independent of the time (at least up to 24 hours) after initiation of the subarachnoid hemorrhage. This result is in general agreement with the in vitro findings of Tourtellotte et al. using lower incubation temperatures and mixed human blood and CSF. Kronholm and Lintrup studied seven patients with traumatic tap in which rbc contamination varied from 1,100 to 95,000 rbc/mm³. Their findings are in agreement both with Tourtellotte's and our own with similar patients (authors' unpublished data). On the other hand, Barrows et al. on the basis of a 37°C incubation experiment using mixed CSF and venous blood from a single patient, implied that significant hemolysis occurred by one hour, whereas CSF from seven patients with "traumatic tap" had revealed no detectable oxyhemoglobin. The authors did not, however, provide quantitative data. Matthews and Frommeyer also investigated the hemolysis of both homologous and heterologous rbc's in CSF, and reported a gradual decrease in number of rbc's during the first 12 hours of incubation at 37.5°C, with increasing xanthochromia visible to the naked eye" by four hours with rbc counts of 1,000 rbc/mm³ or more. Many other investigators refer to the appearance of significant xanthochromia in subarachnoid hemorrhage within four hours or less of the event. It is possible that, in addition to oxyhemoglobin, this may reflect serum contamination, increased CSF protein, or early conversion of hemoglobin to bilirubin. It is clear from the data presented that progressive hemolysis does not occur in CSF under conditions of simulated subarachnoid hemorrhage, at least within the time interval prior to the expected appearance of bilirubin. Early diagnosis of subarachnoid hemorrhage therefore cannot be made by the degree of hemolysis in the CSF. Our data demonstrate a significant increase in CSF lactate

**Table 1** Percentage of Hemolysis and Bilirubin Concentrations in CSF of Rabbits with Subarachnoid Hemorrhage

<table>
<thead>
<tr>
<th>Rabbit number</th>
<th>Time after onset of SAH (hrs.)</th>
<th>Calculated CSF rbc count (cell/mm³)</th>
<th>Percent hemolysis</th>
<th>CSF bilirubin concentration (µM)</th>
<th>CSF ratio bilirubin/hemoglobin</th>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1,100,000</td>
<td>4.6</td>
<td>0</td>
<td>0.4</td>
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<tr>
<td>2</td>
<td>1</td>
<td>859,000</td>
<td>0.27</td>
<td>1.2</td>
<td>0.42</td>
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<td>1</td>
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<td>1.8</td>
<td>0.4</td>
<td>0.03</td>
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<tr>
<td>Mean, (4 Animals)</td>
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<td>846,000</td>
<td>2.7</td>
<td>0.4</td>
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</tr>
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<td>5</td>
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<td>527,000</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>4</td>
<td>680,000</td>
<td>0.9</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>0.3</td>
<td>0.02</td>
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<td>0.05</td>
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<td>0</td>
<td>0</td>
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<td>Mean, (7 Animals)</td>
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<td>467,000</td>
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<td>0.3</td>
<td>0.01</td>
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<tr>
<td>15</td>
<td>24</td>
<td>90,600</td>
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<td>24</td>
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<tr>
<td>17</td>
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<td>18</td>
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<td>302,000</td>
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<td>6.0</td>
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<td>24</td>
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<td>21</td>
<td>24</td>
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<tr>
<td>Mean, (7 Animals)</td>
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<td>170,000</td>
<td>3.9</td>
<td>1.2</td>
<td>0.09</td>
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</table>

**Comment**

**Table 2** Results of CSF Lactate and Blood Gas Determinations on Control Rabbits

<table>
<thead>
<tr>
<th>Rabbit number</th>
<th>CSF Lactate (mEq/L)</th>
<th>Arterial blood p CO₂ (mm Hg)</th>
<th>p O₂ (mm Hg)</th>
<th>pH</th>
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<tr>
<td>12</td>
<td>1.40</td>
<td>26.3</td>
<td>43</td>
<td>76</td>
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<tr>
<td>13</td>
<td>1.68</td>
<td>20.7</td>
<td>44</td>
<td>80</td>
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<td>2.24</td>
<td>19.6</td>
<td>43</td>
<td>33</td>
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<tr>
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<td>51</td>
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<td>19</td>
<td>1.56</td>
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<td>1.93</td>
<td>24.2</td>
<td>64</td>
<td>48</td>
</tr>
<tr>
<td>22</td>
<td>2.68</td>
<td>30.5</td>
<td>55</td>
<td>42</td>
</tr>
<tr>
<td>Mean (11 Animals)</td>
<td>1.99</td>
<td>19.3</td>
<td>45</td>
<td>61</td>
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</table>
TABLE 3 Results of CSF Lactate and Blood Gas Determinations on Rabbits with Recent Subarachnoid Hemorrhage

<table>
<thead>
<tr>
<th>Rabbit number</th>
<th>Time after onset of SAH (hours)</th>
<th>CSF Lactate (mEq/L)</th>
<th>Lact./Pyr.</th>
<th>Arterial blood</th>
</tr>
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<tr>
<td></td>
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<td>Lact./Pyr. p CO₂ (mm Hg)</td>
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<td>4</td>
<td>3.13</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>6</td>
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<td>7</td>
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<td>3.18</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>Mean (7 Animals)</td>
<td>4</td>
<td>3.16</td>
<td>12.7</td>
<td></td>
</tr>
</tbody>
</table>

CSF, and of Shannon, et al. in hemorrhagic dog CSF. Hemoglobin. Our value of approximately 1.2 mEq/L lactate produced over the first four hours for the rabbit is comparable to these results, since our recovered total rbc counts averaged 467,000 rbc/mm³ to 1 Gm% hemoglobin in the rabbit which would represent approximately 477,000 rbc/mm³.

Our results suggest that the finding of a significant elevation of CSF lactate in hemorrhagic fluid with a normal or only slightly elevated CSF 1/ₚ ratio may be taken as presumptive evidence of early subarachnoid hemorrhage. These findings should be most applicable in the range of four–six hours after onset; i.e well before the expected predominance of bilirubin at approximately one–three days after subarachnoid hemorrhage.¹³ ¹⁹ ¹⁰ Note that a significant concentration of bilirubin did not occur in any of our samples. The applicability of these results to the clinical setting is being investigated in our laboratory. In the meantime, we share the opinion of others that within the period of time prior to the appearance of a significant concentration of bilirubin in the CSF, the most reliable and clinically feasible method of distinguishing traumatic lumbar puncture from subarachnoid hemorrhage is the determination of rbc counts or total hemoglobin concentrations¹³ ²³ in serially collected CSF samples.

References

13. Sigma Tech Bulletin 720 — U V
but one patient presented with cerebrovascular symptoms indicating significant clinical disease (table 1). Ten of the 25 involved only the carotid siphon. Hence, true intradural intracranial internal carotid and vertebral arteries. Intracranial from four to 71 years, with an average age of 45.6 years. All thought to involve only the renal arteries, FMD has been identified in a large number of small and medium-sized spectrum of angiographic findings in cephalocervical arteries and — most recently — in the renal and limited to the intrapetrosal internal carotid artery or in-...
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Stroke. 1977;8:613-617
doi: 10.1161/01.STR.8.5.613

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