Measurement and Diagnostic Value of Cerebrospinal Fluid Enzymes

JOHN SAVORY, Ph.D. and JUDITH P. BRODY, M.D.

Departments of Pathology and Biochemistry, University of Virginia School of Medicine, Charlottesville, VA 22908

ABSTRACT

The diagnostic value of cerebrospinal fluid (CSF) enzyme activities in neurological disorders has been evaluated most extensively with the enzymes aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH), creatine kinase (CK) and lysozyme. Methods used for performing the assays have been similar to those employed for serum analysis. Reference intervals for these enzymes in the CSF are given from several sources and demonstrate much lower activities than in serum. Studies of these CSF enzymes in cerebral infarction, brain tumors, central nervous system (CNS) infections and acute brain injury are reviewed.

Introduction

There is a great deal of uncertainty concerning the value of enzyme measurements in neurological disorders. Several enzymes have been measured both in serum and, more particularly, cerebrospinal fluid (CSF), and many studies have attempted to relate changes from normality with diseases of the central nervous system. Certainly profound changes are seen, but the value of many measurements as diagnostic and prognostic aids is questionable. As with all studies on CSF, estimation of adequate reference intervals is a major problem since large numbers of so-called normal samples are extremely difficult to acquire.

It is important first to discuss the nature and source of CSF in order that one might see how the appearance of intracellular macromolecules such as enzymes might relate to central nervous system diseases. Cerebrospinal fluid is found in the ventricular system and the subarachnoid spaces, serving as a cushion and support to the central nervous system. The fluid is clear and colorless, volume approximately 150 ml, with small amounts of glucose, protein and potassium, large amounts of sodium chloride and no cellular components. Most of the CSF is formed by the choroid plexuses in the lateral 3rd and 4th ventricles where it flows into the cerebello-medullary cistern then circulates in the subarachnoid space around the brain and spinal cord. The CSF is absorbed passively by the arachnoid villi into the cerebral venous sinuses.

The chemical milieu must be maintained with certain narrow limits for the functioning of all neurons. The “blood brain barrier” is directly responsible for this stable physical and chemical milieu.
There are three aspects to this barrier: blood-brain, blood-CSF and brain-CSF. The capillary endothelium, basement membrane and astrocytic processes are the structural components of the blood-brain barrier which act as a differential filter, permitting the passage of selective substances from the blood to the intercellular fluid. The “blood-CSF” barrier is in fact the epithelium of the choroid plexus which is an effective barrier as well as actively secreting certain substances into the CSF. The brain-CSF barrier, i.e., the lining of the ventricular system, is only a weak barrier.

Analysis of CSF has achieved considerable importance in the area of proteins, and measurements of immunoglobulins have been shown to be of considerable importance in the diagnosis of demyelinating disease and also subacute sclerosing panencephalitis. Total protein measurements in CSF of course are common laboratory tests in patients with central nervous system infections. Enzyme measurements in serum are extremely sensitive indicators of disease and in many instances can pinpoint the location of tissue injury.

For example, the measurement of serum lactate dehydrogenase, creatine kinase and their isoenzymes provide an absolutely specific test battery for the diagnosis of myocardial infarction. Therefore, it is reasonable to assume that the measurement of enzymes in the CSF might provide a sensitive means of studying disorders of the central nervous system where tissue injury occurs. Diseases and disorders of the central nervous system which fall into this category are: cerebrovascular accident, meningitis (particularly bacterial), central nervous system leukemia, hydrocephalus, epilepsy, brain tumors, increased intracranial pressure and subarachnoid hemorrhage. It is in these areas that most of the attention of CSF clinical enzymology has been focused. The enzymes studied most extensively in these and other central nervous system disorders are: lactate dehydrogenase (LDH) and its isoenzymes, creatine kinase (CK) and its isoenzymes, aspartate aminotransferase (ASAT) and lysozyme.

**Analytical Methods**

Enzyme activities in the CSF are much lower than in serum but in general the methods employed are the same as for the analysis of serum. Wroblewski and coworkers in 1958 used a conventional multipoint kinetic procedure for LDH employing the preferred pyruvate to lactate reaction. Hain and Nutter in 1960 also used multipoint kinetic procedures monitoring the absorbance at 340 nm for the measurement of ASAT, LDH and lactate dehydrogenase. Creatine kinase was determined by Wolintz et al using the forward reaction with creatine and ATP as substrates forming creatine phosphate and adenosine diphosphate (ADP), which is a procedure first described by Tanzer and Gilvarg. Owing to the unfavorable equilibrium, this procedure gives low activities and the reverse reaction gives better results for CK measurements in both CSF and serum. This method was adapted for measurement of CK in serum by Rosalki. Bayer et al and also Greenblatt used a modification of this Rosalki method to determine CK activity in the CSF of a patient with brain damage.

Many other approaches have been taken to measure CSF enzymes and have depended on the assays for serum enzyme determinations available to the investigator. There is every reason to recommend using state of the art serum methods for CSF enzyme determinations. Such methods are employed in the author’s laboratory for CSF analysis using a 35 cuvette centrifugal analyzer.* This type of

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* Rotochem IIa/36, American Instrument Co., Silver Spring, MD 20910.
instrument is under computer control with lag time and measurement time being controlled easily by the analyst. Reactions are monitored by nine successive absorbance readings at evenly spaced intervals and the computer determines the slope of the absorbance/time curve by regression analysis and calculates activity in IU/L from this slope.

The procedure for LDH is based on the pyruvate to lactate system described by Wroblewski and LaDue\(^70\) and an optimized version is recommended using a commercial kit.\(^1\) This method uses 20 \(\mu\)l of CSF, 80 \(\mu\)l saline flush and 500 \(\mu\)l substrate. The lag time is 60 seconds and the reaction time is monitored for a further 80 seconds.

Aspartate aminotransferase is based on the International Federation for Clinical Chemistry recommended procedure.\(^6\) Cerebrospinal fluid (50 \(\mu\)l) is preincubated with the cofactor, pyridoxal phosphate and other constituents of the substrate and \(\alpha\)-ketoglutarate. Following this 10 minute incubation, the reaction is triggered in the centrifugal analyzer by the \(\alpha\)-ketoglutarate and, following a lag time of 60 seconds, the reaction is monitored for 120 seconds and the ASAT activity calculated from the absorbance change.

The CK assay is based on that described by Szasz\(^61\) and a commercial kit is used.\(^2\) The method uses 10 \(\mu\)l of CSF, 100 \(\mu\)l saline flush and 400 \(\mu\)l substrate. The lag time is 65 seconds and the reading time is 120 seconds.

All enzyme measurements are carried out at 30\(^\circ\) which is the temperature recommended by the International Federation of Clinical Chemistry.

The methods described for the centrifugal analyzer provide precise results when used for CSF analysis. These methods using a centrifugal analyzer or some other recording spectrophotometric system are recommended by the authors for CSF ASAT, LDH and CK measurements.

Lysozyme activity in the CSF has been determined most commonly by the decrease in turbidity of a suspension of \textit{Micrococcus lysodeikticus} when destroyed by the enzyme. Problems associated with this method led Osserman and coworkers\(^48\) to develop an agar plate (Lyso-plate) technique where the \textit{Micrococcus lysodeikticus} organisms were spread uniformly in a buffered agar plate. Bacterial lysis produced translucent gel rings whose diameters could be related to CSF lysozyme activity.

Isoenzymes of LDH and CK generally have been determined by agarose gel electrophoresis. Beaty and Oppenheimer\(^5\) concentrated the CSF prior to electrophoresis in those instances where the LDH activity was too low to detect isoenzymes. Nelson and coworkers\(^45\) similarly used ultrafiltration through a collodion bag with a ten-fold concentration. Bayer et al\(^4\) determined the isoenzymes of CK by electrophoresis, DEAE-Sephadex chromatography, a modified dithiol activation and by an immunological method. Only the ion exchange chromatographic and, to a lesser extent, electrophoresis were able to detect the presence of the BB isoenzyme. Dithiol activation and the immunological method with anti-M antibodies was only shown to be useful in cases with increased MB isoenzyme without BB.

Reference Intervals

**TOTAL ENZYME ACTIVITY**

Reference intervals for CSF enzymes are not well defined because of the lack of
accessibility to CSF from normal healthy individuals and owing to the inconsistency of enzyme methods. The latter problem is more serious with enzymes than other analytes since primary enzyme standards do not exist and, therefore, different substrates and reaction conditions can produce widely variable results depending on the degree of optimization of the assay conditions.

Presumably the activity of enzymes in the CSF is due largely to the serum activity, molecular weight and the presence or absence of inhibitors. Molecular weights of the more common enzymes are: LDH-135,000; ASAT-100,000; CK-81,000; and lysozyme-15,000.

Summaries of some studies of ASAT, LDH and CK activities in control subjects are given in table I. In all cases, kinetic spectrophotometric assays at 340 nm were used; other procedures, which are inaccurate, have been ignored. These data are difficult to interpret as a means of predicting reference intervals for modern procedures with optimized substrates. However, the obvious observations are that CSF enzyme activities are lower than serum and that ASAT and LDH activities increase with age.

Lysozyme has been measured in control subjects and has very low activity in the CSF. Reitamo et al53 quote a range of 0 to 13 µg per ml, Hankiewicz et al27 0 to trace and Newman et al46 found no lysozyme in normal CSF.

**Isoenzymes**

Beaty and Oppenheimer5 have reported the percentage distribution of LDH isoenzymes in normal CSF as follows: LDH1 38 to 58 percent; LDH2 26 to 36 percent; LDH3 12 to 24 percent; LDH4 1 to 7 percent; LDH5 0 to 5 percent. This pattern is similar to serum except that in CSF LDH1 > LDH2.

**Clinical Studies of CSF Enzymes**

**I. ASPARTATE AMINOTRANSFERASE AND LACTATE DEHYDROGENASE**

Cerebrospinal fluid ASAT and LDH have been studied in a variety of neurological disorders, especially primary and metastatic brain tumors, cerebral infarctions, acute brain injury and infections.5, 8, 10, 15, 17, 19, 20, 23, 26, 30, 32, 34, 41, 44, 52, 59, 64, 68, 69

**A. Brain Tumors.** Studies on cerebrospinal fluid LDH in brain tumors have given contradictory results. Normal brain tissue has high concentrations of LDH with an isoenzyme pattern similar to serum,5, 19, 20, 52 the mean percentage values being LDH1 24 percent; LDH2 30 percent; LDH3 34 percent; LDH4 11 percent, and LDH5 0 percent. In patients with brain tumors, total LDH levels in the cerebrospinal fluid have been reported to be elevated in secondary malignancies (metastatic carcinomas and intracerebral or meningeal leukemia), while only highly malignant primary brain tumors had elevated levels.10, 69 Other workers have found elevated CSF total LDH levels in the majority of patients with primary brain tumors, with highest levels in those with malignancies.64 In another study, only mild elevations of CSF LDH were found in both metastatic and primary brain tumors.50 Rabow52 studied 28 patients with malignant and benign central nervous system tumors and found cerebrospinal fluid total LDH elevations in 50 percent of the tumor patients, not related to the degree of malignancy, although the two highest levels were in grade 3 and 4 astrocytoma patients.

Cerebrospinal fluid ASAT activity in tumors has been less well studied; however, elevations have been reported to correlate with LDH10 to have higher elevations than LDH,64 to be less frequently elevated23 and to be normal.41
### TABLE I
Cerebrospinal Fluid Enzyme Activities in Normal Cerebrospinal Fluid

<table>
<thead>
<tr>
<th>Aspartate Aminotransferase</th>
<th>Age</th>
<th>Sex (M/F)</th>
<th>No</th>
<th>Range (units)</th>
<th>Mean (units)</th>
<th>Temperature</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS NS 15</td>
<td>2-7</td>
<td>5</td>
<td>25</td>
<td>Green et al</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-86 NS 45</td>
<td>5-21</td>
<td>12</td>
<td>25</td>
<td>Katzman et al</td>
<td>33</td>
<td></td>
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<tr>
<td>NS NS 60</td>
<td>0.5-0.62</td>
<td>0.43</td>
<td>37</td>
<td>Green et al</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS 17/13 30</td>
<td>NS</td>
<td>0.9</td>
<td>38</td>
<td>Fleisher et al</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1 yr NS 23</td>
<td>2.3-13.3</td>
<td>5.6</td>
<td>25</td>
<td>Lending et al</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1 yr NS 18</td>
<td>2.0-15.0</td>
<td>6.2</td>
<td>25</td>
<td>Lending et al</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21-90 60/7 67</td>
<td>2.2-19.0</td>
<td>10.6</td>
<td>25</td>
<td>Hain and Nutter</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS NS 13</td>
<td>2.0-14.0</td>
<td>8.0</td>
<td>NS</td>
<td>Florez et al</td>
<td>17</td>
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<table>
<thead>
<tr>
<th>Lactate Dehydrogenase</th>
<th>Age</th>
<th>Sex (M/F)</th>
<th>No</th>
<th>Range (units)</th>
<th>Mean (units)</th>
<th>Temperature</th>
<th>Author</th>
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</thead>
<tbody>
<tr>
<td>21-90 60/7 67</td>
<td>2.5-38.5</td>
<td>20.5</td>
<td>25</td>
<td>Hain and Nutter</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 yr NS 23</td>
<td>1.1-36.8</td>
<td>13.7</td>
<td>25</td>
<td>Lending et al</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 yr NS 18</td>
<td>4.4-40.0</td>
<td>14.9</td>
<td>25</td>
<td>Lending et al</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS NS 13</td>
<td>10-50</td>
<td>30</td>
<td>NS</td>
<td>Florez et al</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>54-88 7/2 9</td>
<td>0-25</td>
<td>5</td>
<td>25</td>
<td>Wolintz et al</td>
<td>68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS NS 11</td>
<td>2.0-7.2</td>
<td>NS</td>
<td>25</td>
<td>Beaty and Oppenheimer</td>
<td>5</td>
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<table>
<thead>
<tr>
<th>Creatine Kinase</th>
<th>Age</th>
<th>Sex (M/F)</th>
<th>No</th>
<th>Range (units)</th>
<th>Mean (units)</th>
<th>Temperature</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>54-88 7/2 9</td>
<td>0-0</td>
<td>0</td>
<td>25</td>
<td>Wolintz et al</td>
<td>68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS NS 13</td>
<td>0-4.0</td>
<td>2.0</td>
<td>NS</td>
<td>Florez et al</td>
<td>17</td>
<td></td>
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</tr>
</tbody>
</table>

Lactate dehydrogenase isoenzymes of tumor extracts and homogenates have been studied, and there are consistent reports of elevated LDH₅ fractions in malignancy that correlate with the degree of malignancy seen in grade 3 and 4 astrocytomas and in metastatic carcinomas.¹⁸,¹⁹,²⁰,²⁰,²⁰,²⁰,²⁰,²⁰ The increased LDH₅ content has been considered secondary to the dependence of malignant cells on anaerobic metabolic processes.⁴⁸ In conjunction with the brain tumor LDH isoenzyme reports, the LDH isoenzyme composition of cerebral tumor cyst fluids have shown an increase in LDH₁. Meningiomas have been noted to have variable patterns.¹⁹,²⁰,²⁰,²⁰,²⁰ Meningiomas have been noted to have variable patterns.¹⁹,²⁰,²⁰,²⁰,²⁰
revealed similar patterns, i.e., increased LDH5 in malignant astrocytomas and in active metastatic carcinomas. Total LDH levels were also increased in the malignant tumor cysts.

The corresponding cerebrospinal fluid LDH isoenzymes have been less consistent, although Rabow did find an elevated LDH5 fraction in those patients with the highest total LDH elevations, which were two patients with grades 3 and 4 astrocytomas. Judging from these data, highly elevated CSF LDH levels with an increased LDH5 may be a useful indicator of malignancy, both in primary brain tumors and in metastatic carcinoma, while a normal activity is non-diagnostic. Isoenzyme patterns of tumors and cyst fluids may be of value when the tumor is difficult to classify histologically and may be of predictive or prognostic value in borderline tumors. Overall total LDH and LDH isoenzymes are of limited value in detection and classification of brain tumors. Elevations of CSF LDH occur in only 50 percent of tumor patients without significant differences between patients with benign and malignant tumors.

**B. Cerebral Infarction.** The concept of elevated CSF enzymes in cerebral infarctions was explored experimentally by Wakim and Fleisher. They produced acute cerebral infarcts in dogs by injecting vinyl acetate into one of their internal carotid arteries and found elevations of CSF ASAT that peaked at 100 hours and gradually fell to normal over 15 days with the proportion of elevation correlated with the histological damage. In light of these data, ASAT and LDH have been studied extensively in man in regard to cerebral infarction. Problems related to these reports are the vast discrepancy of methodologies and/or normal ranges of CSF ASAT (as shown in tables I and II).

Aspartate aminotransferase in CSF may become elevated in cerebral infarction unrelated to serum ASAT and it appears, therefore, that CSF and serum ASAT elevations occur independently, indicating that CSF ASAT is increased in cerebral infarction.

### TABLE II

<table>
<thead>
<tr>
<th>Age</th>
<th>No</th>
<th>Aspartate Aminotransferase Range(units)</th>
<th>Mean</th>
<th>No</th>
<th>Lactate Dehydrogenase Range(units)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-30</td>
<td>5</td>
<td>1.0-10.2</td>
<td>5.6</td>
<td>5</td>
<td>0-23.7</td>
<td>10.7</td>
</tr>
<tr>
<td>31-40</td>
<td>7</td>
<td>0.8-16.8</td>
<td>8.8</td>
<td>7</td>
<td>5.4-26.2</td>
<td>15.8</td>
</tr>
<tr>
<td>41-50</td>
<td>5</td>
<td>4.5-14.5</td>
<td>9.5</td>
<td>4</td>
<td>0-35.1</td>
<td>16.5</td>
</tr>
<tr>
<td>51-60</td>
<td>13</td>
<td>0-22.8</td>
<td>11.0</td>
<td>11</td>
<td>9.4-27.8</td>
<td>18.6</td>
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<tr>
<td>61-70</td>
<td>20</td>
<td>3.5-21.5</td>
<td>12.5</td>
<td>18</td>
<td>7.2-40.8</td>
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<td>71-80</td>
<td>12</td>
<td>5.7-16.9</td>
<td>11.3</td>
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<td>81-90</td>
<td>50</td>
<td>0-31.0</td>
<td>15.4</td>
<td>4</td>
<td>18.6-29.8</td>
<td>24.2</td>
</tr>
</tbody>
</table>

These data are summarized from the investigations of Hain and Nutter.
TABLE III
Enzyme Elevations in Acute Brain Injury

<table>
<thead>
<tr>
<th></th>
<th>Lactate Dehydrogenase</th>
<th>Aspartate Aminotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milliunits</td>
<td>Wroblewski units</td>
</tr>
<tr>
<td>Concussion</td>
<td>Contusion</td>
<td></td>
</tr>
<tr>
<td>1 - 12 hrs after trauma</td>
<td>4 ± 2</td>
<td>31 ± 3.4</td>
</tr>
<tr>
<td>72 hrs after trauma</td>
<td>5 ± 8.3</td>
<td>44 ± 5.6</td>
</tr>
<tr>
<td>Control</td>
<td>6 ± 1.6</td>
<td>19 ± 1.2</td>
</tr>
<tr>
<td>Contusion</td>
<td>34 ± 6.4</td>
<td>102 ± 15.7</td>
</tr>
</tbody>
</table>

These data are summarized from the investigations of Kaltiala et al. [32]

Eating an intact blood brain barrier. Elevations of CSF ASAT usually occur within seven days following the infarct and are unrelated to CSF protein. [33] The elevations may or may not be correlated with the severity of the infarct. [24, 26, 33, 68]

Lactate dehydrogenase in cerebral infarcts has also been studied and elevations have been felt to reflect tissue damage. [23, 30, 68] However, the sensitivity of this enzyme measurement in cerebral infarction seems poor, with elevations in only one of 15 patients, [69] and 13 of 30 patients with cerebrovascular accidents, [68] although more substantial lesions have shown correspondingly higher values. [68]

It appears that the measurement of CSF LDH or ASAT in cerebral infarcts is of questionable value.

C. Acute Brain Injury. The value of CSF LDH and ASAT has been examined in acute injuries of the brain both secondary to trauma or to chemotherapy or irradiation. [17, 32, 60]

In 30 patients studied by Kaltiala, [32] significant elevations of CSF ASAT compared to controls was seen at 1 to 12 hours and at 72 hours after cerebral concussion. The results are summarized in Table III. Significant elevations of CSF ASAT in contusion compared to concussion was seen at 72 hours. Lactate dehydrogenase was elevated in contusion patients only. Serum enzymes were measured but did not correlate with the clinical severity of the brain lesion or the CSF enzymes. Another study found neither CSF LDH or ASAT to be elevated in mild concussion, whereas moderate, severe, or fatal contusion showed significant elevations of CSF ASAT and LDH, as well as serum ASAT. [17]

In eight children receiving radiation therapy and intratheral chemotherapy for acute lymphoblastic leukemia, CSF LDH and ASAT were significantly elevated during therapy with 2 post irradiation syndromes later developing. [60] Florez et al. [17] felt that these enzymes were prognostic in severe head injury and in mild concussions, but the prognostic value was limited by the variation in enzyme content of different parts of the brain, the inability to localize the damage and the lack of differentiation of damage versus destruction of neurons.

It appears, therefore, that CSF LDH and ASAT may have some value in assessing the extent of head injury in conjunction with clinical assessment of the location of damage or destruction.

D. Central Nervous System Infections. The diagnostic significance of LDH and its isoenzymes in CNS infections has been studied by several investigators. Total LDH compared to controls were significantly elevated in acute
bacterial meningitis,\textsuperscript{5, 34, 44, 45} and was much higher than CSF LDH activities seen in aseptic meningitis where only mild elevations were observed. The activities of LDH fell with improving clinical condition.\textsuperscript{5, 44}

Cerebrospinal fluid LDH isoenzyme patterns revealed a predominance of LDH\textsubscript{4} and LDH\textsubscript{5} in bacterial meningitis,\textsuperscript{5, 44, 45} corresponding to elevated CSF granulocyte counts.\textsuperscript{5} Extracts of granulocytes have this isoenzyme pattern.\textsuperscript{5} Viral infections showed elevations of LDH\textsubscript{1} and LDH\textsubscript{2} which corresponded to elevations of CSF mononuclear cells.\textsuperscript{5, 45} However, this pattern is seen in serum and brain tissue also. In summary, CSF LDH may be of value in central nervous system infections in distinguishing viral, bacterial and conceivably partially treated bacterial meningitis, especially where the other CSF parameters are non-diagnostic. However, CSF protein and glucose measurements and cell counts continue to have greater value.

Cerebrospinal fluid ASAT activities have not been found to be altered significantly in acute central nervous system infections.

\textbf{E. Miscellaneous.} Several miscellaneous neurologic disorders are worth noting in regard to CSF LDH and ASAT activities. Cerebrospinal fluid ASAT has been found to be elevated in adult patients with seizure disorders if measured within 48 hours of a seizure.\textsuperscript{40} In children, CSF LDH and ASAT levels were discovered to be normal or minimally elevated in seizure disorder patients and normal in patients with febrile seizures.\textsuperscript{34}

Hydrocephalus and elevated intracranial pressure were also associated with increased CSF LDH.\textsuperscript{34, 45} However, after shunt procedures the levels returned to normal.\textsuperscript{34}

\textbf{II. CREATINE KINASE}

Creatine kinase is normally present in brain tissue, skeletal muscle and cardiac muscle and not in red blood cells, thus making measurement of this enzyme in cerebrospinal fluid possibly more specific as an indicator of brain damage in neurological disorders such as cerebral infarction, subarachnoid hemorrhage, acute brain injury and others. In cerebral metabolism, creatine kinase is thought to maintain an adequate supply of ATP at the expense of creatine phosphate reservoir.\textsuperscript{39, 68}

Acheson\textsuperscript{1} and Eisen\textsuperscript{14} have reported serum CK elevations in cerebral infarctions, and others have found CSF levels increased\textsuperscript{25, 42, 68} but not consistently.\textsuperscript{36} Greenblatt\textsuperscript{25} studied CSF CK in acute subarachnoid hemorrhage and found that elevations correlated significantly with evidence of a destructive process, i.e., hydrocephalus, infarction, parenchymal hematoma and intraventricular clot. The actual level of CK in this study and in the studies of cerebral infarction had no diagnostic specificity.\textsuperscript{25, 68} The study sited previously by Florez et al\textsuperscript{17} of acute brain injury showed (1) significant elevation of CSF CK in mild concussions compared to controls; (2) a significant elevation of CSF CK in moderate to severe concussions compared to mild concussions; and (3) fatal concussion compared to moderate to severe concussions.

Other clinical conditions reported to be associated with elevated cerebrospinal fluid CK levels include Duchenne muscular dystrophy,\textsuperscript{3} brain tumors\textsuperscript{29, 42} and hydrocephalus with increased intracranial pressure.\textsuperscript{13, 29, 42}

Contradictory reports of elevations in acute psychosis have been published.\textsuperscript{37, 63}

There are a few studies on the CSF CK isoenzymes, which indicate that their distribution is the same as in normal brain (97 to 98 percent BB, 0.9 percent MB, 1.7 percent MM).\textsuperscript{3, 4, 43, 58, 71}

Cerebrospinal fluid from patients with various neurologic disorders, including epilepsy, brain tumors, disk disease and multiple sclerosis have been studied for
elevations of CSF CK and determination of the isoenzyme pattern. Only 70 of 185 patients had elevations of CSF CK not specific for any disease process in particular, and only brain isoenzyme was detected. The isoenzyme separation involved polyacrylamide gel disc electrophoresis.

Bayer et al reported recently a case of severe brain damage in an 18 month old patient with an elevated CK BB fraction (and elevated CK MM in serum) demonstrating possible uses of CSF CK isoenzymes in distinguishing acute phase brain damage from clinically similar sequelae. The authors also showed permeability of the blood brain barrier with elevated activities of CK MM in the CSF.

Although CSF CPK may indeed be specific for brain tissue, an elevated value remains non-specific and the degree of elevation is not diagnostic of the clinical severity in a particular disease. The measure of this CSF enzyme is useful perhaps in cases of mild injury or very severe brain damage.

III. LYSOZYME

Lysozyme is a small (15,000 MW) basic protein that is enzymatically active in depolymerizing the mucopolysaccharides of bacterial cell walls. Lysozyme is found in high concentrations in the lysosomes of polymorphonuclear cells, phagocytes, monocytes, along the gastrointestinal tract and in the kidneys.

Lysozyme activity has been studied in inflammatory and neoplastic CNS diseases. Control patients have absent or just detectable levels in the CSF. High elevations were seen in bacterial meningitis but not in aseptic meningitis. This elevation was correlated with CSF protein concentration and with CSF protein and granulocytes. The CSF level of lysozyme was reported by Newman et al to be appreciable in patients with primary and secondary tumors and felt to reflect the tumor nature and degree of involvement. These studies found that other medical and neurologic diseases, such as Hodgkin's disease, and Guillain-Barre' syndrome, had no lysozyme in the CSF. This finding has been refuted with lysozyme being elevated to varying degrees in cerebrovascular disease, epilepsy, intracranial hemorrhage, cerebral atrophy and multiple sclerosis.

In addition, Di Lorenzo studied the correlation of CSF lysozyme elevations in tumors with CSF protein concentration and found that the presence of lysozyme was directly related to protein and not present when CSF cytology and chemistry were normal. Thus, CSF lysozyme appears to be of no practical help in the diagnosis of CNS tumors.

IV. MISCELLANEOUS ENZYMES IN CSF

There are a number of other enzymes that have been measured in the CSF as to their possible usefulness in CNS disease. Ribonuclease is a poly(c)-avid RNA-ase that has activity in the blood, urine, and CSF. The tissue source remains unknown; however, elevations in CSF have been shown in chronic cerebrovascular disease, tumors and cord compression. This enzyme is measured by double diffusion gel method with rabbit antibody to human urine ribonuclease.

Adenylate kinase is widely distributed in tissues and maintains adenylate. It has not been found normally in the CSF in adults but has been found in the CSF in patients with malignant tumors.

Cholinesterase and choline acetylase activity have been studied. Pseudo or true cholinesterase activities have been elevated in meningitis, Guillain-Barre' tumors and myasthenia gravis. The activities have been decreased in multiple sclerosis. Choline acetylase is present in
1/100 of the concentration \(^3\) of cholinesterase, is normally present in the CSF and is elevated nonspecifically in some neurologic diseases.

Arginine esterase activity in the CSF has been studied because of its possible etiologic role in the pathogenesis of migraine headaches.\(^7\)

Several enzyme activities are being determined in view of their possible role in activating neurotransmitters. Phenylethanolamine-N-methyltransferase which methylates norepinephrine to epinephrine has been detected in low levels in the CSF and in all areas of the brain.\(^6\) Dopamine-B-hydroxylase activity is proportional to norepinephrine release and its measurement in the CSF may be useful in determining the functional state of the noradrenergic system in the central nervous system.\(^21,47\)

Summary

In summary, several enzymes are detectable in CSF in normal individuals and, generally, methods used for serum are applicable to the analysis. Lactate dehydrogenase activities may be of value as indicators of malignancy. Cerebrospinal fluid ASAT levels in brain tumors do not have much value. Cerebrospinal fluid ASAT, LDH or CK may be elevated in cerebral infarction but are of little clinical value; however, in acute head injury these enzymes may be useful. In infections, these same enzymes may be clinically useful when used with conventional tests such as proteins, glucose and white cell counts. Creatine kinase in CSF may be an important assay in the acute phase of brain damage, especially the CPK BB isoenzyme. At one time CSF lysozyme was thought to be of value in CNS tumors, but it now appears that this test is not useful. Cerebrospinal fluid lysozyme is elevated in bacterial meningitis.

References

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