LACTIC DEHYDROGENASE OF CEREBROSPINAL FLUID
IN THE DIFFERENTIAL DIAGNOSIS OF CEREBRO-
VASCULAR DISEASE AND BRAIN TUMOR

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A simple, rapid, safe and effective means of distinguishing cerebrovascular disease from expanding intracranial lesions would be of use in those cases in which the history, examination and diagnostic study lead to uncertain conclusions. Apoplectiform symptoms as an early sign of an expanding lesion and the displacement of ventricles by thrombosis of major blood vessels simulating tumor have been confusing to many. The present work indicates that the level of the enzyme, lactic dehydrogenase, in cerebrospinal fluid is elevated in cases of cerebrovascular disease as compared with patients afflicted with intracranial tumors.

Although the utilization of the lactic dehydrogenase level as an index of cerebrovascular disease does not replace clinical judgment it may prove a useful technique in the armamentarium required for the differentiation of these two disease entities. The method is both simple and rapid and thus prompts the present preliminary report.

ASSAY METHOD

Lactic dehydrogenase catalyzes the following reaction:

\[ \text{Pyruvate} + \text{DPNH} + H^+ \rightleftharpoons \text{Lactate} + \text{DPN}^+ \]

Advantage is taken of the fact that one of the substrates, DPNH\(^*\), has a high light absorption \((\varepsilon = 6.32 \times 10^3)\) at a wavelength of 340 m\(\mu\) whereas the other components of the assay and the products of the reaction do not absorb significantly at this wavelength. The reaction may therefore be conveniently followed in a spectrophotometer by observation of the decrease in optical density at 340 m\(\mu\). Under the conditions to be outlined the initial decrease in optical density has been found to be linear with time and directly proportional to the quantity of lactic dehydrogenase.

In practice the following components were added per ml. of reaction mixture: water, 0.55 ml.; M phosphate buffer at pH 7.5, 0.05 ml.; 0.002 M DPNH\(\dagger\), 0.05 ml.; CSF, 0.3 ml.; 0.1 M pyruvate at pH 7.5, 0.05 ml. A blank cell contained only water. Pyruvate was added 3 minutes after the other components and was used to initiate

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* DPN and DPNH refer to the oxidized and reduced forms of diphosphopyridine nucleotide, respectively.

\dagger The compound is relatively unstable in solution. It has been found convenient to adjust DPNH solutions to pH 8.0 and, when not in use, to store them in a freezing compartment for no longer than 1 week.
the reaction. After the addition of pyruvate the optical density at 340 m\(\mu\) was determined at half-minute intervals for 3 minutes and the rate of change per minute was calculated. One unit of lactic dehydrogenase is designated as the amount of enzyme that results in a change in optical density at 340 m\(\mu\) of 0.001 per minute. Results are recorded in units of activity per 0.3 ml. of CSF and designated by the symbol, \(\mu\).

Curve A of Fig. 1 depicts the results of a typical assay. It will be noted that in this case the optical density decreased, i.e. DPNH was oxidized, without the addition of pyruvate. This reaction is caused by the presence of a substrate for an enzyme already present in cerebrospinal fluid. That this substrate is in fact pyruvate may be shown by the addition of a sample of spinal fluid which had been boiled so as to inactivate the lactic dehydrogenase of cerebrospinal fluid, to a cuvette containing DPNH and crystalline lactic dehydrogenase of muscle (Curve B). Furthermore, the 2,4-dinitrophenylhydrazine derivative of pyruvate isolated from a sample of cerebrospinal fluid had a melting point identical to that of an authentic sample. Assay of 50 samples of cerebrospinal fluid for pyruvic acid by an independent method\(^1\) revealed that the pyruvate content ranged from 0.04 to 1.5 micro-

![Fig. 1. Assay conditions. Curve A: CSF incubated in buffer. The arrows denote the time of addition. Curve B: Crystalline lactic dehydrogenase of muscle (0.1 mg.) incubated in the presence of buffer and DPNH. A boiled sample of CSF is added at the arrow. Curve C: Pyruvate and DPNH incubated in buffer. The same sample of CSF as is present in Curve A is added at the arrow. Note that the rates in Curves A and C are identical.](image-url)
moles per ml. of cerebrospinal fluid. Although pyruvate levels are elevated in pathological fluid, particularly in meningitis, an accurate correlation between content of pyruvate and pathology can not be made.

Dialysis of cerebrospinal fluid against a solution of 0.05 M potassium phosphate at pH 7.0 and 0.01 M thiocethanol for 12 hours removed all endogenous activity. When pyruvate was added the rate of the reaction was within 10 per cent of that of the original undialyzed sample. The rate of DPNH oxidation can be made independent of endogenous substrate by the addition of pyruvate to the reaction mixture (Curve C). Thus, the rate of decrease of optical density at 340 m\(\mu\) under the conditions outlined here is proportional only to the quantity of lactic dehydrogenase present in spinal fluid.

Samples of cerebrospinal fluid are stable for as long as 2 weeks at 4° C. Although obviously not desirable, samples may be kept at room temperature for several hours. In this study blood-tinged or cloudy samples of cerebrospinal fluid were rejected.

RESULTS

Patients in this series were divided equally with respect to sex and varied in age between 2 months and 81 years. Although no attempt was made to select cases it is inevitable that a certain natural selection is involved. Lumbar puncture is not a routine procedure so that even in those subjects labeled as “non-neurological,” suspicion of possible neurological involvement must have existed. With this in mind the data of Fig. 2 will be considered.

Non-neurological. The cases in this group include diagnoses of hysteria (Table 1), pneumonia (2\(\mu\), 4\(\mu\), 11\(\mu\)), cardiovascular disease (7\(\mu\), 10\(\mu\), 11\(\mu\)), cirrhosis (11\(\mu\), 18\(\mu\)), arthritis (16\(\mu\)), mastoiditis (2\(\mu\)), pyelonephritis (4\(\mu\)), gastric ulcer (3\(\mu\)) and diabetes (15\(\mu\)). An average of 10\(\mu\) was obtained for 20 cases.

Neurological. The results for many of the neurological cases are summarized in Table 1. Also included in this category were cerebral atrophy (13\(\mu\), 17\(\mu\)), pseudotumor cerebri (2\(\mu\)), aneurism of the internal carotid (10\(\mu\)) and Friedreich’s ataxia (14\(\mu\)). Among the head injuries is one case of subdural hematoma (8\(\mu\)) and a skull fracture (20\(\mu\); protein 95 mg. per cent).

It is of interest that in 2 cases of complete block resulting from a herniated intervertebral disc, lactic dehydrogenase levels of 7\(\mu\) and 10\(\mu\) were obtained although the cerebrospinal fluid protein was assayed as 96 and 114 mg. per cent, respectively.

The average level in 58 neurological cases was 10\(\mu\).

Although they have been excluded from statistical calculations, assays have been made in 4 cases of meningitis. Each of these patients yielded lactic dehydrogenase values (32\(\mu\), 44\(\mu\), 50\(\mu\), 65\(\mu\)) in the range obtained with cerebrovascular accidents.

Tumor. The diagnosis of brain tumor was confirmed at operation or necropsy in 13 of 17 cases. In 4 cases not so confirmed the diagnoses were metastatic lesions and a third ventricle tumor. Included in this series are
meningioma (8µ, 19µ), ependymoma (7µ, 23µ), third ventricle tumor (7µ), bilateral papilloma of the choroid plexus (2µ), pituitary tumor (17µ), astroblastoma (15µ), oligodendroglioma (15µ), astrocytoma (5µ, 19µ), glioblastoma (15µ, 18µ) and brain metastasis (Table 1). It is of interest that the cerebrospinal fluid protein was above the normal limits in most of these cases.

The average for all tumors was 14µ.

**TABLE 1**

*Lactic dehydrogenase in several disease states*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Cases</th>
<th>Lactic Dehydrogenase (µ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Hysteria</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Brain metastasis</td>
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<td>15</td>
</tr>
<tr>
<td>Convulsive disorders</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Head injuries</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>CNS syphilis</td>
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<td>12</td>
</tr>
<tr>
<td>Disc syndrome</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Meningitis</td>
<td>4</td>
<td>48</td>
</tr>
</tbody>
</table>

* Standard error of the mean.
Cerebrovascular Accidents. These cases were diagnosed by a typical history, physical findings and diagnostic tests. No distinction is made here between emboli, thrombosis and hemorrhage. In 12 of 18 cases the diagnosis was verified by postmortem examination. One case of subacute bacterial endocarditis (22μ) with brain emboli found at necropsy is included.

In cases in which more than one sample of cerebrospinal fluid was available, the highest value was recorded. It will be noted that assay values are generally higher when cerebrospinal fluid was obtained several days after the onset of symptoms (Fig. 3). The average value for this series was found to be 31μ.

![Graph](attachment:graph.png)

**Fig. 3.** Lactic dehydrogenase levels as a function of time after appearance of symptoms. Connected points refer to serial lumbar punctures on the same patient.

**DISCUSSION**

The discovery by Karmen et al.⁶ that the concentration of transaminases in human serum is elevated following myocardial infarction has led to the use of this valuable tool in the differential diagnosis of that disease.⁶,⁷ The cellular destruction occurring in the anoxic myocardium is thought to release the enzymes into the blood. Similar reasoning has been employed with respect to the formation of anoxic areas in the brain following cerebrovascular accidents. The results presented here clearly substantiate this argument. Comparison of the average value for lactic dehydrogenase in cerebrovascular accidents with that of all other samples of cerebrospinal fluid tested yields a ρ value of less than 0.001, i.e., the possibility of these differences being ascribable to chance is less than one in a thousand. While the present work
was in progress Green et al.\textsuperscript{2} have assayed the cerebrospinal fluid of 12 normal patients and 12 patients with cerebrovascular accidents for glutamic-oxalacetic transaminase and have demonstrated elevated transaminase levels in those with cerebrovascular accident.

Laetic dehydrogenase was used in this study because of the simplicity of the assay, i.e., only DPNH and pyruvate are required. Assays of aspartic-$\alpha$-ketoglutaric transaminase require the addition of malic dehydrogenase and, because of the presence of pyruvate in cerebrospinal fluid, are subject to errors because of the action of glutamic-pyruvic transaminase and, more particularly, of laetic dehydrogenase. The relatively large amounts of pyruvate present in cerebrospinal fluid, i.e., up to 0.0015 M, would not be removed during the preincubation period employed in the usual transaminase assay.\textsuperscript{4}

Clearly the usefulness of such assays depends on the ability to distinguish between disease states that may mimic cerebrovascular accident. Cases of cerebrovascular accident in this series fall between values of 20 to 57. Meningitis, a head injury and one tumor also yielded values in this range. Meningitis and injury can be ruled out on other grounds. Tumors, however, may give rise to anoxic brain with results similar to those caused by cerebrovascular accidents. From the data presented it would seem that such events are infrequent and that higher values of lactic dehydrogenase, i.e., above 30$\mu$, weighed with the clinical evidence should be diagnostic for cerebrovascular accident.

It should be noted here that the data presented for cerebrovascular accident are somewhat biased in that only those patients who required hospitalization were available for study and it is conceivable that in cases of "little-strokes" the assay values may be lower.

The pattern of enzyme increase is noteworthy. Lactic dehydrogenase levels may be low soon after symptoms appear and increase only after some days. In this connection it has been found\textsuperscript{9} that dogs subjected to cerebral anoxia simulating cerebrovascular accident yield very high levels of transaminase within hours after infarction and that the enzyme disappears from the cerebrospinal fluid within 10 days. Although experimental evidence is not available, it may be questioned whether patients with cerebrovascular accident who are seen clinically react in a manner and at a rate similar to those of the experimental animals. The possibility does exist that the increased levels of laetic dehydrogenase are not caused by leakage from anoxie brain but rather are a function of repair mechanisms.

**SUMMARY**

The concentration of the enzyme, laetic dehydrogenase, has been examined in spinal fluids from a series of patients with a variety of disorders. Significantly higher enzyme levels have been found in the cerebrospinal fluid of patients with cerebrovascular accidents as compared with normal individuals and patients with neurological disease including brain tumor.
The test is suggested as useful in the differentiation of cerebrovascular accident from other disease with similar clinical picture.

It is a pleasure to acknowledge the interest and cooperation of Dr. James W. Watts and Dr. George A. Kelser in this work.

REFERENCES