LACTIC DEHYDROGENASE OF CEREBROSPINAL FLUID IN THE DIFFERENTIAL DIAGNOSIS OF CEREBROVASCULAR 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:

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

Advantage is taken of the fact that one of the substrates, DPNH\(^*\) has a high light absorption \((\epsilon = 6.22 \times 10^3)\) at a wavelength of 340 mp 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 mp. 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

\(^*\) 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)