Creatine kinase in cerebrospinal fluid

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The estimation of serum adenosine triphosphate-creatine phosphotransferase (C.P.K.) or creatine kinase has been proved valuable in the diagnosis of myopathy and in myocardial infarction (Dreyfus, Schapira, and Demos, 1960a; Dreyfus, Schapira, Scebat, Renais, and Lenègre, 1960b). As well as muscle tissue, the brain has been shown to contain a high enzyme concentration (Narayanaswami, 1952; Colombo, Richterich, and Rossi, 1962; Wood, 1963), but negligible activity is present in the kidney, spleen, liver, pancreas, lungs, fat, lymphatic glands, and erythrocytes. The role of the enzyme in the brain was considered by McIlwain (1963) to be the maintaining of the adenosine-triphosphate (A.T.P.) level. The importance of C.P.K. may lie in the relationship of this enzyme to carbohydrate metabolism and the movement of ions across the nerve cell membrane.

Knowledge of levels of C.P.K. in the cerebrospinal fluid from patients with neurological disorders would be of value if it could be shown that in destruction of cerebral tissues or alteration of cell permeability variations in enzyme levels take place. This paper records the results of experiments aimed at obtaining this information.

MATERIALS AND METHODS

Lumbar cerebrospinal fluid was obtained from 66 patients with various diseases and from 17 control subjects whose ages ranged from 10 days to 67 years. These last patients were not suffering from any organic disease. Serum was obtained from all 17 of these subjects and from some of the other patients. In addition, in 44 cases ventricular fluid was collected which was completely free of blood or from any evidence of contamination with fragments of brain tissue.

Tumour tissue obtained at operation or at necropsy and one normal brain from a patient dying suddenly were also examined. Tumour tissue and different areas of the normal brain were homogenized with 9 volumes of 0·1 M glycine buffer at pH 9 in a Potter-Elvehjem homogenizer at a constant speed for a constant time. The homogenate was centrifuged at 0°C. for 20 minutes at 20,000 g and the supernatant diluted up to a maximum dilution of 1 in 1,000 with glycine buffer.

All specimens were examined the same day they were obtained. The enzyme estimations for C.P.K. were performed in principle by the technique of Tanzer and Gilvarg (1959) but with a modification involving the use of automatic micropipettes and apparatus.

REAGENTS

Four solutions are made up as follows:

Solution 1 2 M glycine buffer, pH 9, 8 × 10⁻⁴M reduced nicotinamide-adenine dinucleotide, 6 × 10⁻³M adenosine 5'-triphosphate, and 2 × 10⁻⁴M phosphoenolpyruvate.

Solution 2 Lactate dehydrogenase, 2 mg./ml., 2 mg./ml. pyruvate kinase, and 0·5 M MgCl₂

Solution 3 Glycine buffer, 0·1 M pH 9, and 6·3 × 10⁻⁴M creatine.

Solution 4 Glycine buffer, 0·1 M pH 9.

ASSAY

Assay was performed using blank and test made up as below.

<table>
<thead>
<tr>
<th>Blank</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µl serum or cerebrospinal fluid</td>
<td>100 µl serum or cerebrospinal fluid</td>
</tr>
<tr>
<td>70 µl solution 1</td>
<td>70 µl solution 1</td>
</tr>
<tr>
<td>5 µl solution 2</td>
<td>5 µl solution 2</td>
</tr>
<tr>
<td>Mix and leave at room temperature for 10 minutes.</td>
<td>175 µl solution 3</td>
</tr>
<tr>
<td>175 µl solution 4</td>
<td>175 µl solution 3</td>
</tr>
</tbody>
</table>

Using the blank, the spectrophotometer (Unicam S.P. 500) is set to an absorbance of 0·300 at a wavelength of 340 mμ and the absorbance of the test solution is read (A₅). Exactly 10 minutes later, with the blank readjusted to 0·300, the test is again read (A₆). The difference in absorbance A₅ - A₆ = ΔA which multiplied by 56 is equivalent to the concentration of the enzyme in international units per litre.

If the absorbance is greater than 0·300/10 minutes the sample is diluted appropriately.

RESULTS

There was no recordable enzyme activity in 11 of the sera of the control subjects and in the other six subjects C.P.K. levels ranged from 0·1 to 0·6 international units (i.u.) per litre using this technique. There was no correlation between age or sex and enzyme content; thus a 22-day-old female gave a reading of 0·5 i.u. which was similar to that of a 42-year-old man. There was no detectable enzyme in 15 of 17 cerebrospinal fluids, while the other two

1 Reagents were obtained from Boehringer, Mannheim, Germany (English agents: Courtin & Warner, Lewes, Sussex)
fluids, one from a 38-year-old woman and the other from a 46-year-old man, gave readings of 0·06 i.u. The serum levels for this small series are similar to those obtained by other workers using comparable techniques (Colombo et al., 1962). Our normal figures for C.P.K. in cerebrospinal fluid will be considered as being up to 0·06 i.u. and in serum of up to 0·7 i.u. Further, no differences were found in ventricular fluid as compared with lumbar fluid.

The results of C.P.K. estimations in the cerebrospinal fluid in patients with a variety of neurological disorders are shown in Table I and Figure 1.

**TABLE I**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>No. of Cases</th>
<th>Creatine Phosphotransferase (i.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Hydrocephalus (non-progressive)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Hydrocephalus (progressive)</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Epilepsy (idiopathic)</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Epilepsy (symptomatic)</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Meningitis (bacterial and viral)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Cerebral atrophy</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Parkinson's disease</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Optic nerve disease</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Vascular insufficiency</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Benign, raised intracranial pressure</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Guillain-Barré syndrome</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Polynuertis</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Subdural haematoma (membrane)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Subdural haematoma (traumatic)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Subarachnoid haemorrhage</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

The patients with hydrocephalus could be divided into those in whom there was no evidence of increasing hydrocephalus, and in these no enzyme was detectable in the cerebrospinal fluid. Patients with an increasing hydrocephalus due to a variety of causes showed with only one exception raised C.P.K. activity. Only one patient with idiopathic epilepsy, irrespective of age, showed any enzyme activity, whereas in all except one with symptomatic epilepsy raised enzyme activity was found. Patients with meningitis or meningo-encephalitis, with cerebral atrophy or Parkinson's disease demonstrated no abnormal amount of enzyme in the cerebrospinal fluid, but variable results were obtained in other diseases although the numbers are too small in some of the conditions studied.

No correlation was found between levels of C.P.K. in serum and cerebrospinal fluid (Fig. 2), or between the cerebrospinal fluid levels of the protein content and C.P.K. (Fig. 3), or between the presence of small amounts of blood in the cerebrospinal fluid and C.P.K. levels.

The results obtained in 30 verified cases of
cerebral tumour are recorded in Figure 4. Twenty-one out of 30 showed an elevated level of C.P.K. in the cerebrospinal fluid, and apart from one patient with an astrocytoma grade III, the patients with normal levels were those with a craniopharyngioma or a meningioma. There were seven other patients in whom tumour was suspected by angiography but were not proven by operation and two of these gave no enzyme activity while the other five had raised levels of from 0·1 to 1·3 i.u. In addition one patient with a brain abscess had an enzyme figure of 0·3 i.u. in the cerebrospinal fluid while no activity was obtained in the cerebrospinal fluid from patients with a colloid cyst of the third ventricle and with a granuloma.

Estimations of C.P.K. were made in cerebrospinal fluid and tumour tissues in 10 patients, in three of whom brain tissue levels were also obtained. The results are shown in Table II together with figures for different areas of normal brain. It is seen that in none of the tumours was the C.P.K. level as high as in the brain, and that only small amounts of enzyme were present in meningioma, acoustic neuroma, and a colloid cyst. There was no evident correlation in the amounts of C.P.K. in cerebrospinal fluid and tumour.

**DISCUSSION**

The function of C.P.K. in the brain is not at present known, apart from a possible relationship with the level of A.T.P. (McIlwain, 1963). Details concerning the localization of the enzyme and whether within neuroglial or nerve cells are extremely meagre, but some preliminary experiments conducted by one of the authors (N.H.) have shown that between 59 and 60% of the total C.P.K. activity of brain tissue homogenate is to be found in the supernatant after 30 minutes' centrifugation at 0°C. at a speed of 100,000 g. Some of this, presumably cytoplasmic, readily diffusible, enzyme might pass into the cerebrospinal fluid in brain damage.

Previous studies (De Risio and Cumings, 1960; Szliwowski and Cumings, 1961) on enzymes in the cerebrospinal fluid as well as cyst fluids from cerebral...
tumours have indicated that increases in such enzymes as lactate dehydrogenase, phosphohexose isomerase, and alkaline phosphatase have correlated well with the degree of malignancy of the tumour. However, C.P.K. is present in brain tissue to a much higher level than in tumour tissue and this strongly suggests that breakdown of cerebral tissue may play a part in the presence of the enzyme in the cerebrospinal fluid, especially when consideration is given to the presence of C.P.K. in cases in which the tumour contained little or no activity. This suggestion is strengthened by the presence of small amounts of C.P.K. in the cerebrospinal fluid in such diseases as multiple sclerosis, cerebrovascular insufficiency, and in the Guillain Barré syndrome. The reason for an increase of enzyme in benign intracranial hypertension is not certain but could be related to the presence of cerebral oedema, for Sahs and Joynt (1956) have demonstrated oedema in such patients. Meningitis and meningo-encephalitis gave no evidence of enzyme activity despite the presence of cells in the fluid.

It is important to note that epilepsy when associated with a tumour was accompanied by a raised enzyme level, thus this estimation may be valuable in determining whether a brain tumour is present. Estimations of C.P.K. in the cerebrospinal fluid have been shown to be valuable in hydrocephalus for they indicate the nature of the condition whether progressive or not. This is especially true in older subjects in whom change in size of the head may be difficult to determine.

The content of creatine phosphotransferase in cerebrospinal fluid, in normal brain, and in tumour tissue has been investigated. Normal levels of C.P.K. in the cerebrospinal fluid range from 0 to 0·06 i.u. Raised levels of enzyme of up to 5·0 i.u. have been found in the cerebrospinal fluid from patients with astrocytoma, medulloblastoma, symptomatic epilepsy, progressive hydrocephalus, and in benign intracranial hypertension.

Brain tissue contains more enzyme than does tumour tissue and it is suggested that an increase of enzyme in the cerebrospinal fluid may reflect brain damage and necrosis.

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REFERENCES


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