Levels of enolase and other enzymes in the cerebrospinal fluid as indices of pathological change

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SUMMARY   The activities of enolase, aldolase, pyruvate kinase, lactate dehydrogenase and creatine phosphokinase were measured in cerebrospinal fluid of 121 patients presenting with a range of disorders of the central nervous system. The results from 41 patients undergoing myelography were used as controls. An assessment was made of the relative merits of these five enzymes as markers of brain damage with special reference to brain tumours. Enolase was the most sensitive marker of pathological change and was the only enzyme raised in the CSF of patients with low grade astrocytomas.

The clinical significance of enzymes in the cerebrospinal fluid in the diagnosis of neurological abnormalities is still poorly defined and at present no specific biochemical marker of damage within the central nervous system is known. Enzyme activities in CSF have been measured from as early as 1939 when Kaplan[1] estimated a number of enzymes including diastase, lipase, and some proteolytic enzymes. Since then others[2-4] have examined a variety of other enzymes or isoenzymes in the cerebrospinal fluid, but the results of these studies have been relatively non-specific. In 1980 Willson *et al*[5] developed a radioimmunoassay technique for aldolase-C, an enzyme that is largely confined to the nervous system in man. Although the level of this enzyme was raised in several cases, there was no significant difference in the mean levels between groups of patients with different diseases.

It has been suggested that the cerebrospinal fluid is the lymph of the brain and that transit of proteins between the brain and the cerebrospinal fluid is relatively unrestricted,[6] whereas the blood-brain barrier prevents free communication with the blood. Damage within the central nervous system, therefore, should result in raised values of intracellular products within the cerebrospinal fluid considerably earlier than they can be detected in the bloodstream. The sensitivity of any assay technique will therefore depend in part on the freedom with which intracellular products are released into the cerebrospinal fluid. Un-bound products would be expected to appear earlier than those that are tightly bound to intracellular organelles or membranes.

We describe the results of an investigation of the cerebrospinal fluid of 121 patients presenting with a variety of neurological and neurosurgical disorders with a view to assessing their potential usefulness in diagnosis and prognosis. The enzymes assayed were enolase, aldolase, lactate dehydrogenase, pyruvate kinase, and creatine phosphokinase.

Methods

CSF samples were those taken for other routine clinical examinations, or in the case of ventricular CSF at the time of operation. CSF from 40 patients undergoing myelography was used as controls. Samples were frozen at $-16°C$ as soon as possible after extraction and kept at $-16°C$ until assayed. Any sample showing contamination with blood was discarded. All the enzymes assayed were performed on the LKB rate analyser at $37°C$. Enolase was assayed by the method of Rider and Taylor,[7] aldolase by the method of Underwood and Newshome,[8] pyruvate kinase by the method of Bucher and Pfleiderer,[9] and CPK (creatine phosphokinase) and LDH (lactate dehydrogenase) by the methods described by Bergmeyer.[10] Frozen aliquots of rat muscle supernatant suitably diluted to give activities in the same range as CSF were used as "internal" and "between-batch" standards. Wherever possible assays including

blanks were performed in duplicate and an average taken.

Results

The results for the enzyme activity of enolase, aldolase, pyruvate kinase, lactate dehydrogenase and creatine phosphokinase are given in figs 1 to 5

levels raised in the absence of demonstrable pathology.

Figure 1 shows that enolase activity was raised in all conditions examined except in the case of aneurysms that had not bled and in compensated hydrocephalus. The four cases where no clinical abnormalities were discovered all had normal enolase levels. Enolase was raised in the two cases of
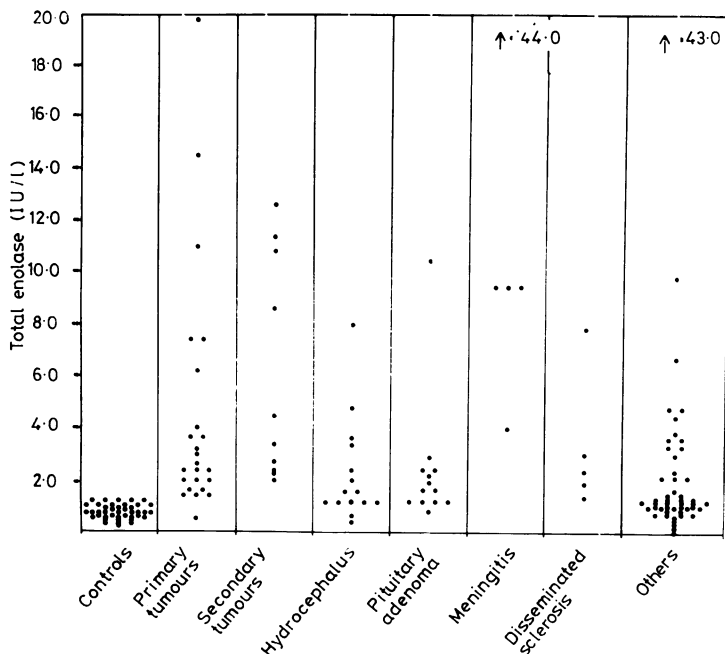

Fig 1 *Enolase activity in cerebrospinal fluid; results grouped according to diagnosis.*

respectively. The results were divided into groups according to final diagnoses. The last group designated "others" in figs 1 to 5 included patients with dementia, cervical spondylosis, benign intracranial hypertension, leukaemia, Hodgkin's disease, intervertebral disc disease, cerebrovascular disease including aneurysms, mental retardation, neuropathy and migraine. In some cases no clinical abnormality was detected. The range of enzyme activities in normal CSF was calculated as the mean ± twice the standard deviation found for the control group. Lumbar CSF was used for the controls whereas some of the samples tested, in particular those from patients with cerebral tumours, were ventricular. The correlation between normal values of the enzymes in the control group and in cases such as well-compensated hydrocephalus suggest that we were justified in making comparisons between ventricular and lumbar CSF. In no case were enzyme

leukaemia and the one case of Hodgkin's disease. These three patients were on cytotoxic drugs and it is not certain whether the rise in enolase activity was due to the disease, or the cytotoxic drugs or a combination of the two. All the cases of disseminated sclerosis studied when the samples were taken during an active phase of the disease had raised enolase levels. Only one patient with a brain tumour out of the 34 studied showed an enolase level within the normal range. This was a small benign meningioma. None of the patients with brain tumours was on cytotoxic drugs at the time the samples were taken. The two highest enolase values in CSF of patients with brain tumours were 14 units/l and 20 units/l (fig 1). These were from patients with a large craniopharyngioma (plus hydrocephalus) and a medulloblastoma respectively. It is notable that 40% of secondary tumours gave rise to CSF enolase activities ten times the normal
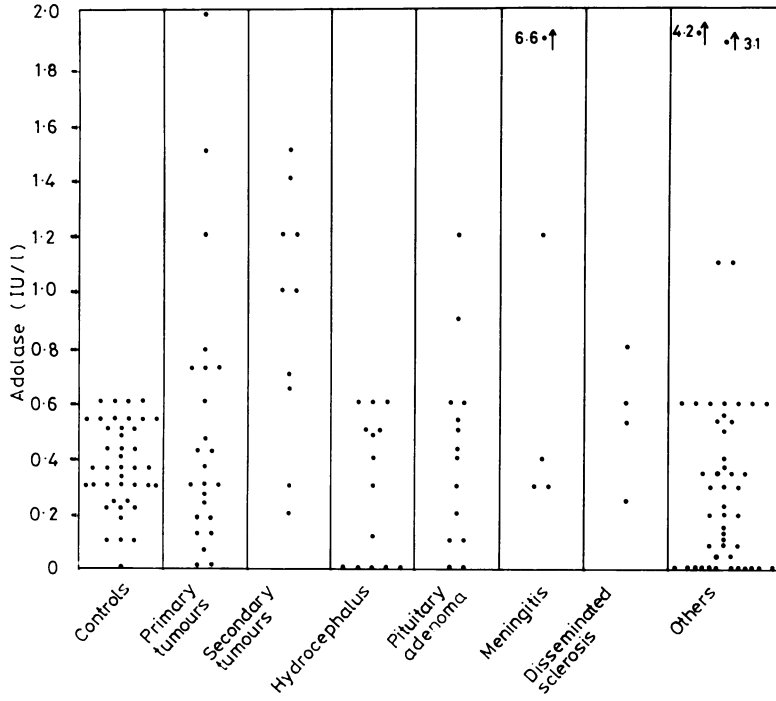
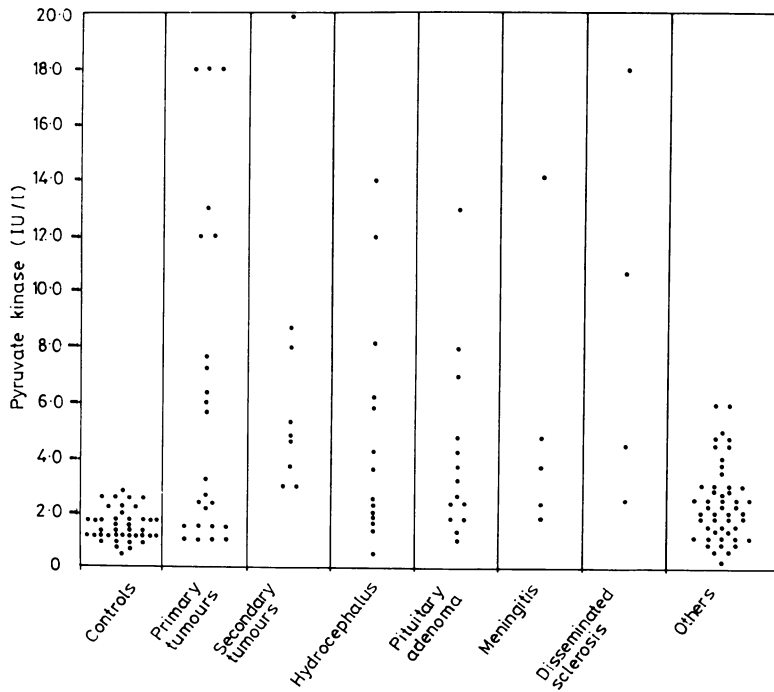Fig 2   *Aldolase activity in cerebrospinal fluid; results grouped according to diagnosis.*

Fig 3   *Pyruvate kinase activity in cerebrospinal fluid; results grouped according to diagnosis.*
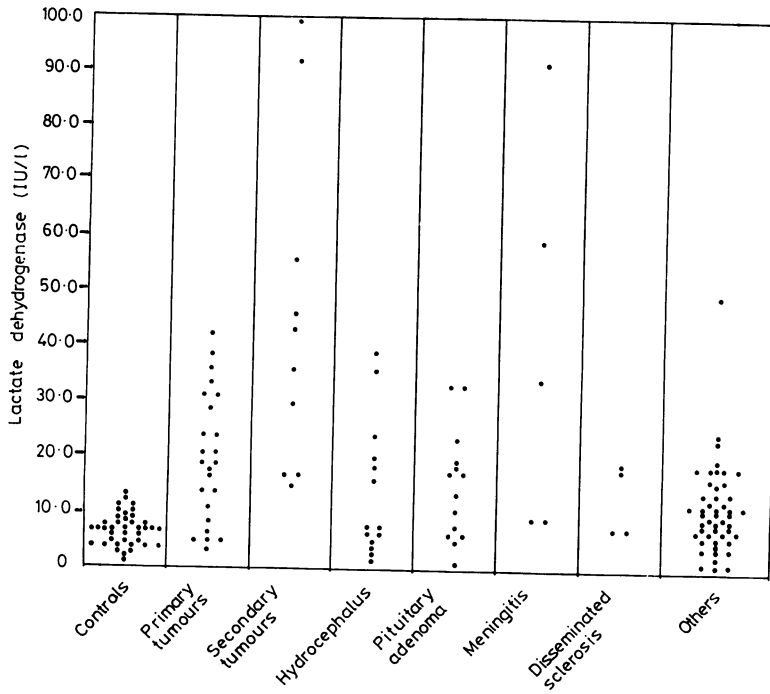
Fig 4 *Lactate dehydrogenase activity in cerebrospinal fluid; results grouped according to diagnosis.*
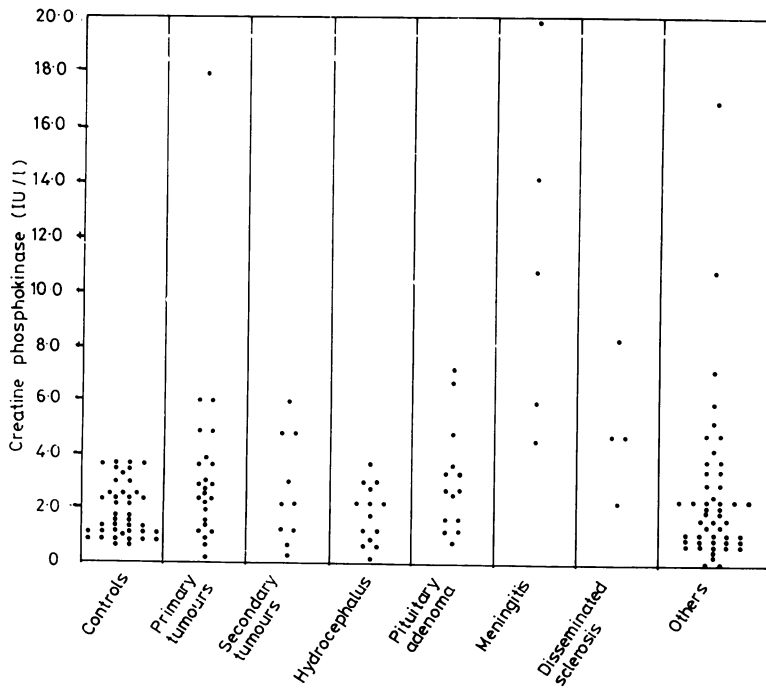

Fig 5 *Creatine phosphokinase activity in cerebrospinal fluid; results grouped according to diagnosis.*

mean (0·7 units/l) compared with 12·5% of primary tumours and 7% of pituitary adenomas.

Table 1 shows activities of the five enzymes

Table 1 *Enzyme levels in cerebrospinal fluid of patients with various grades of astrocytomas*

Astrocytoma grade	Enolase units/litre	Pyruvate kinase units/litre	Lactate dehydrogenase units/litre
I-II	1·56	1·8	8·2
I-II	1·32	2·4	8·0
I-II	3·6	1·56	4·8
I-II	3·6	2·3	1·44
I-II	3·0	1·56	3·0
I-II	3·6	2·30	11·0
III-IV	1·6	18·0	24·0
III-IV	3·12	18·0	19·2
III-IV	2·4	7·2	24·0
IV	1·98	6·4	16·8
IV	1·95	3·0	36·0
IV	6·2	18·0	21·6
Normal range	0·2-1·2	0·5-2·5	1·8-14·0

studied in CSF of patients with astrocytomas, the results being grouped according to histological grades. In the CSF of patients with low grade astrocytomas, enolase activity was raised whereas the other enzymes remained within normal limits. In CSF from patients with higher grade astrocytomas enolase activities were also raised, as were the activities of LDH and pyruvate kinase. In this group aldolase and CPK were elevated in only 30% of cases. In the study as a whole, nine patients had only enolase raised; out of these six had low grade astroxytomas, two long-standing hydrocephalus and one a small infarct. All nine had normal protein levels.

Galen and Gambino[11] published criteria for evaluating markers in diagnosis and screening: (1) Sensitivity is the fraction of patients with the disease giving positive results, (2) Specificity is the fraction of patients without the disease giving negative results, (3) Predictive value is the fraction of positive results that are true positives, (4) Efficiency is the fraction of all results that are correct. For any of these parameters, therefore, the higher the value the better the marker. A positive result is one higher than the mean plus two standard deviations as determined from the control group. The results are shown in tables 2 and 3. The predictive value depends on the prevalence of the disease in the population studied. In the present

Table 2 *Sensitivity, specificity, predictive value and efficiency of the five CSF enzymes for primary and secondary brain tumours*

	Enolase %	Aldolase %	Pyruvate kinase%	LDH %	CPK %
Sensitivity	97	44	64	75	38
Specificity	5	93	43	50	60
Predictive value	35	75	45	54	40
Efficiency	77	82	46	50	55

study brain tumours represented 24% of all the final diagnoses. The number of cases of the other diseases studied was not sufficient to allow the calculation of significantly accurate parameters but figures 1 to 5 enable us to study certain patterns.

Enolase was raised in approximately 80% of cases in the study, aldolase 20%, pyruvate kinase 37%, LDH 43%, and CPK 20%. CPK and aldolase levels were normal in all cases of hydrocephalus (figs 2 and 5). Both enolase and CPK were raised in all cases of meningitis. In the six cases where serial studies were possible, three involved meningitis and showed that CPK increased at the onset and continued to rise throughout the duration of the meningitis. At the onset of the meningitis enolase, PK (pyruvate kinase) and LDH also increased and in the one case where a brain tumour was present, aldolase was increased. During treatment of the meningitis CPK was the only enzyme not to stabilise. This could be purely a manifestation of bed-rest and release of enzyme from muscle.

The other serial studies involving patients with primary brain tumours showed that enolase exhibited the greatest change with growth of the tumour.

Discussion

These studies indicate that of the enzymes investigated enolase was the most readily released into the cerebrospinal fluid. We have previously demonstrated with anti-enolase serum using an immuno-peroxidase PAP technique on sections of brain tumours, that enolase is lost from ischaemic as well as necrotic tissue.[12] Enolase is present in relatively high concentrations in some cells, as much as 3% of the soluble protein, and it does not bind to actin as do PK, LDH and especially aldolase.[13] This is

Table 3 *Enzyme levels in cerebrospinal fluids from the control group*

	Enolase	Aldolase	Pyruvate kinase	LDH	CPK
Number of samples	41	41	41	41	41
Mean value iu/l	0·72	0·37	1·47	7·90	1·9
Standard deviation (SD)	0·26	0·15	0·49	3·05	0·95
Range = ±2 × SD iu/l	0·2-1·2	0·07-0·7	0·5-2·5	1·8-14·0	0-3·8

probably one of the reasons why enolase is such a sensitive marker of tissue damage.

No previous study has provided data that correlate tumour type with the pattern of enzyme elevation. The present study demonstrates that enolase is the only enzyme out of the five that were studied that had a raised activity in cerebrospinal fluid from patients with low grade astrocytomas; patients with high grade astrocytomas had raised levels for at least three of the enzymes studied. Moreover, two thirds of the patients that had only enolase raised, had low grade astrocytomas. Hence, raised cerebrospinal fluid enolase levels may be a useful means of detecting these tumours, particularly in their early stages.

The most rapidly growing neoplasms showed the highest levels of enolase activity in the CSF. Serial studies showed that tumour growth resulted in increasing enzyme levels. However, not all high grade astrocytomas showed enolase levels greater than those of low grade astrocytomas, indicating that factors other than tumour growth must also be involved. For instance, the time lapse between the period of maximal damage and the sample being taken, the presence of oedema which speeds up the movement of enzyme into the cerebrospinal fluid[14] and the localisation of the lesion may also be relevant factors. Moreover, Barrero *et al*[15] found that damage to the meninges produced far greater enzyme levels in cerebrospinal fluid than cerebral damage alone.

Enolase levels also appear to be a sensitive indicator of active demyelination. Levels were raised in all patients with disseminated sclerosis studied. Similarly, it is interesting that enolase was raised in the cases on cytotoxic therapy. With the exception of LDH, which was raised in one patient with Hodgkin's disease and one with acute lymphoblastic leukaemia, the other enzymes were within normal limits. Further work is clearly needed to determine how much the disease process itself contributes to the raised levels and how much relates to therapy. Quantitative studies on the different isoenzymes of enolase would be helpful to determine whether the damage is neuronal, glial or mixed in origin.

Raised levels of enalose were found in approximately half of the cases of hydrocephalus; the two patients with low levels both had long-standing hydrocephalus which was presumably well compensated, no longer resulting in damage to cerebral tissue with release of enzyme into the cerebrospinal fluid. Assays of this enzyme may well prove useful in assessing the rate of progress and activity of hydrocephalus.

In the cases of meningitis studied, levels of both enolase and CPK were raised and levels of CPK continued to rise during the duration of the disease.

This could be related to release of enzyme from muscle during prolonged bed-rest; further work is needed to assess this aspect.

In a multi-enzyme study on a mixed population of unselected patients, it is possible to calculate the specificity, sensitivity, predictive value and efficiency of each marker in the cerebrospinal fluid. Enolase activity appears to have a high sensitivity for brain tumours (fig 2), higher than any other enzymatic marker previously studied.[16] Enolase is the only marker to show 100% sensitivity in the case of low grade astrocytomas. Aldolase, on the other hand, appears to have a very high specificity, although it has a reduced sensitivity. In a similar study Man[17] found sensitivity of 53% and a specificity of 91%.

There is a change in the isoenzyme composition of aldolase in cerebrospinal fluid in many patients with cerebral neoplasms, resulting in an increase in aldolase-A,[17] whereas non-neoplastic diseases are associated with an increase in aldolase-C. Aldolase-C, which is the brain-specific form of this enzyme, shows an increase in binding to actin in high lactate conditions, whereas aldolase-A, the predominant type in neoplasms, shows a decrease in binding in the presence of high concentrations of lactate.[18] One would expect tumour tissue to be high in lactate concentration. The change in the isoenzyme type of aldolase to one which has reduced actin-binding in tumours could account for the high specificity of aldolase for tumours.

In conclusion, cerebrospinal fluid enolase levels appear to be a highly sensitive index of pathological abnormality within the central nervous system. Abnormalities are detectable even in the active phase of demyelination in disseminated sclerosis and in relatively small benign tumours such as some pituitary adenomas. Raised levels are demonstrable in these cases before abnormalities can be detected with any other known investigative technique, including CT scanning. Further work still needs to be done to determine precisely how sensitive a marker this enzyme really is and to clarify how levels vary in different phases of the same disease. It may be extremely useful in the differentiation of organic from psychological abnormalities. Assay techniques for the three different forms of enolase should further enable us to differentiate between damage to neurones and other cell types such as glial cells and muscle cells. Provided samples are taken during the acute phase of an illness, levels should correlate approximately with the severity of cellular damage. In conjunction with studies on a variety of different enzymes, it should be of some use in determining the rate of growth of tumours and in assessing the effects of therapy, particularly the toxic effects of CNS cytotoxic therapy in the treatment of lymphoproliferative

disorders and in particular in the prophylactic treatment of acute lymphoblastic leukaemia.

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