Lactate Dehydrogenase in Cerebral Cyst Fluids
Total Activity and Isoenzyme Distributions as an Index of Malignancy

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The chemical grading of tumors is of interest in relation to tumor diagnosis and prognosis, as well as in the field of pure oncology. Our estimations of the lactate dehydrogenase (LDH) content of cerebral tumor tissue support those of others, demonstrating increased amounts of LDH3, the electrophoretically slowest moving fraction or M-type isoenzyme, in extracts of malignant tumors relative to more benign ones. Following the convention of numbering the five fractions of LDH separated by electrophoresis from 1, as the most anodic fraction, to 5 as the most cathodic fraction, this M-type of LDH, associated with anaerobic glycolysis, will be referred to as LDH5. It should be remembered when referring to the literature that there are a few authors who still use the reverse order of numbering, with the slow-moving fraction designated LDH1.

The fluid that accumulates in a cystic tumor has so far received less attention than the tumor tissue. High LDH activity in a specimen of cyst fluid from a cerebral astrocytoma was reported by Green, et al.,7 in an account of LDH and transaminase activities of the cerebrospinal fluid (CSF) of patients with various neurological diseases. Szilwowski and Cumings14 determined LDH activity in some of the 214 cerebral cyst fluids that they examined chemically and found a tendency for the higher levels to be associated with more malignant tumors. Buckell and Robertson2 estimated the total LDH activity in cyst fluids from 21 gliomas and 16 cerebral secondary carcinomas; there was an increase in enzyme activity parallel with the degree of histological malignancy. This change was not reflected in either the corresponding plasma or the ventricular CSF, where the slight increases in activity found were not related to the nature of the tumor. It was noted that, although estimation of the total LDH activity was an improvement over earlier methods of chemical grading, determination of isoenzymes would probably be a useful addition to the investigations. This has proved to be so.

Materials and Methods

In this study, 100 specimens of fluid from cystic lesions with histologically verified diagnoses were examined during the period from August, 1964, to November, 1967. The specimens represented 13 kinds of intracranial tumor and three non-tumorous cysts. Fluids from cases with no satisfactory histological material were also analyzed, and examples of these problem cases are included to illustrate some of the applications of LDH measurements.

Cyst fluid was obtained at craniotomy, ventriculography, or during burr-hole biopsy procedures from patients undergoing surgery either at Atkinson Morley’s Hospital or the National Hospital, London. All histological diagnoses came in the first instance through the routine neuropathological services of the two hospitals. To obtain more uniformity of histological opinion, the slides from the 79 Atkinson Morley cases were reviewed by one neuropathologist (MRC). Gliomas were classified according to the Kernohan grading systems,8 when the grade originally reported differed from that ascribed to the tumor under review the specimen was placed in the higher grade.

Samples were taken into sterile, screw-capped, glass containers, prepared with 50 units of heparin, and sent at once to the lab-
oratory where they were centrifuged and the supernatant fluid separated from the deposit. Early separation is important if there has been any contamination with fresh blood, as it is the red cells, not the plasma, that contribute significant amounts of extraneous LDH and so falsify the subsequent analysis. Fluid should not be allowed to stand on cells prior to enzyme estimation nor should the process of centrifugation be too forceful.

Details of our method of estimation of total LDH and of the separation and measurement of its fractions are to be found elsewhere. When possible, the total LDH activity was determined and the agar gel electropherogram was run and developed on the day of collection. Supernatant fluid from specimens not analyzed at once was kept in plastic vials at 4°C, and a reserve of fluid was stored at −15°C, in divided portions that were thawed once only and then discarded. Estimations on refrigerated samples were made during the 72 hours after collection, or an aliquot from the frozen reserve was used. There were 78 fluids analyzed within 3 days of collection, 11 between days 4 and 7, seven between days 8 and 21, and four stored frozen were analyzed between days 29 and 34. Storage experiments under similar conditions showed that cyst fluids could be kept at 4°C for at least 4 days, usually longer, and at −15°C for at least 21 days without any appreciable deterioration in the LDH content. Cyst fluids could not be kept for long periods because of an inconstant loss in the amount of LDH, that occurred in some specimens.

**Results**

The enormous range of LDH activity encountered is shown in Fig. 1; the mean values for the total LDH and relative isoenzyme distributions for different kinds of tumor are drawn to scale, with the inclusion of normal serum and CSF for comparison. The measurements for individual groups, however, overlapped, as can be seen from Table 1 where means and ranges for total LDH and its isoenzymes in the different tumors are given, with the standard deviations for the larger groups.

**Secondary Carcinomas.** Total LDH in the 20 cyst fluids from secondary carcinomas ranged from 29,000 international units (iu) per liter down to 380, with a mean of 10,090; 18 of the 20 cases contained more than 1,000 iu per liter and eight of these exceeded the highest value found for a malignant astrocytoma. The isoenzyme distribution showed an increase in LDH and LDH compared to the patterns given by the more

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Fig. 1. Relative amounts of LDH and LDH isoenzymes in cyst fluids. (Area of each division is equal to 200 iu of LDH per liter.)