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,6,10-12 demonstrating increased amounts of LDH, 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 anodic fraction, to 5 as the most cathodic fraction, this M-type of LDH, associated with anaerobic glycolysis, will be referred to as LDH. 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 LDH.

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. Sziwowski 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 Robertson8 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 LDH₂ and LDH₃ compared to the patterns given by the more
benign lesions or to those obtained from extracts of various areas of normal brain or from normal serum or a hemolysate of red blood cells. This “malignant” type of isoenzyme spectrum was still seen in fluids from secondary carcinomas even though the total LDH activity was low. Two fluids with high total activities failed to show the malignant pattern and had less than 10% of the enzyme present as the fifth fraction.

An attempt was made to correlate the chemical results with the origin, site, and histological appearances of the secondary tumor. Insofar as conclusions can be drawn from these limited numbers, the total LDH appeared to be related to both mitotic activity and anaplasia of the tumor. Thus, two cases with total cyst fluid LDH activities of 29,300 and 27,000 iu were undifferentiated tumors showing numerous mitoses, while the tumors with the lower cyst fluid enzyme activity were the less mitotic well-differentiated specimens. The mean LDH₄ for the fluids from anaplastic secondary carcinomas was 29.8% while that from the differentiated secondaries was 23.1%; the difference between the means failed to achieve statistical significance but is suggestive. There were insufficient non-bronchial examples to determine whether the origin of the primary tumor had any bearing on the LDH of the secondary cyst fluid. The three secondaries from a source other than lung fell in the lower part of the LDH range, but all were well-differentiated tumors.

**Astrocytomas.** Grade IV astrocytomas yielded cyst fluids with total LDH activities from 8200 to 550 iu per liter with a mean of 3504. Ten of the 12 specimens were above 1000 iu and had 10% or more of LDH₄. Like the secondary carcinomas, the mean

### TABLE 1

Mean and range of lactate dehydrogenase activities found in the fluid from different types of cystic intracranial tumors

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>No. of Cases</th>
<th>Total LDH (iu per liter)</th>
<th>LDH isoenzymes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>secondary carcinoma</td>
<td>20</td>
<td>10,090 (380–29,300) SD 8523</td>
<td>8 (0–18) SD 5.6</td>
</tr>
<tr>
<td>astrocytoma grade IV</td>
<td>12</td>
<td>3,504 (550–8,200) SD 2638</td>
<td>14 (1–45) SD 12.1</td>
</tr>
<tr>
<td>astrocytoma grade III</td>
<td>32</td>
<td>3,170 (226–8,640) SD 2477</td>
<td>18 (1–68) SD 14.7</td>
</tr>
<tr>
<td>astrocytoma grade II</td>
<td>3</td>
<td>383 (240–600) SD 2477</td>
<td>28 (13–49) SD 14</td>
</tr>
<tr>
<td>astrocytoma grade I</td>
<td>5</td>
<td>168 (85–267)</td>
<td>37 (0–49) SD 2</td>
</tr>
<tr>
<td>hemangioblastoma</td>
<td>9</td>
<td>210 (110–387) SD 589</td>
<td>46 (26–77) SD 3</td>
</tr>
<tr>
<td>chromophobe adenoma</td>
<td>4</td>
<td>589 (140–1,055) SD 2,500 &amp; 3,000</td>
<td>34 (21–50) SD 10</td>
</tr>
<tr>
<td>craniopharyngioma</td>
<td>2</td>
<td>2,500 &amp; 3,000 SD 160</td>
<td>10 (2 &amp; 18) SD 1</td>
</tr>
<tr>
<td>neurilemma</td>
<td>5</td>
<td>160 (100–240) SD 48</td>
<td>48 (38–75) SD 3</td>
</tr>
</tbody>
</table>
distribution of isoenzymes for grade IV astrocytomas showed appreciable quantities of LDH 5 and 4, there being 19% of each compared to 27% and 26% respectively of these fractions in the fluids from the secondaries. In five of the 12 electropherograms fraction 5 or 4 was predominant so that the picture resembled that of the majority of carcinoma fluids.

Grade III astrocytomas, when cystic, produced fluids with a total LDH range of 8640 to 226 iu per liter with a mean of 3176. In 23 of the 32 (72%) grade III tumors, total cyst fluid LDH was above 1000 iu compared to 10 of 12 (83%) fluids from grade IV astrocytomas and 19 of 20 (95%) secondary carcinomas.

In all but five histologically grade III specimens, 10% or more of LDH5 was present. Four of these five, although graded III by virtue of their mitoses, form a rather different group from the rest of the grade III tumors; histologically all were gemistocytic tumors and chemically all had low total LDH activities and similar isoenzyme patterns in the cyst fluid resembling the lower grade fluids rather than the majority of the grade III samples.

There were only eight specimens of fluid from the lower grade astrocytomas, so further cases are required to lay down more precise limits. One of the three grade II tumors had a cyst fluid total LDH of 600 iu per litre with an isoenzyme distribution similar to that associated with the majority of the grade III astrocytomas. The other two grade II specimens had lower total LDH levels, 310 and 240 iu; in one of the latter no LDH5 could be demonstrated and only a trace was present in the other grade II specimen.

Five fluids from grade I astrocytomas ranged from 267 to 85 iu per liter. No LDH 5 or 4 was detected in four of these specimens.

Other Tumors of Glial Origin. These tumors are poorly represented. The only example from a cystic oligodendrogioma had a total LDH activity of 840 iu per liter and an isoenzyme pattern unlike that of any other tumor in this series, with 91% of LDH4. The one example of an ependymoma resembled the average grade II astrocytomas.

Extracerebral Intracranial Cystic Tumors. The nine specimens of hemangioblastoma had total LDH activities from 387 down to 110 iu per liter, with a mean of 210. In seven cases no LDH 4 or 5 could be demonstrated even in the undiluted fluid. No attempt was made in this study to concentrate any of the fluids, and traces of less than 1% of any fraction do not show up by the present method in fluids where the total activity is under about 150 iu per liter. The fifth isoenzyme fraction was just demonstrable in two of the hemangioblastomas, and these samples also showed 3% and 13% of LDH5.

The four specimens of chromophobe adenoma of the pituitary, with total LDH activities of 1055, 650, 514, and 140 iu per liter of cyst fluid, had a similar type of isoenzyme pattern with little activity in fractions 5 and 4.

Two fluids from craniopharyngiomas had total LDH activities of 3000 and 2000 iu per liter with 37% and 38% of LDH5. These levels are above those found for any of the other non-malignant tumors with the exception of the only specimen from an epidermoid cyst which had a total LDH activity of 2500, with 43% of LDH5.

The five specimens of neurilemoma ranged from 240 to 100 iu per liter with a mean of 160. Little or none of the fourth or fifth isoenzyme fractions were present.

Of the two truly cystic meningiomas, the fluid from one had a total LDH content of 290 iu per liter with the isoenzyme activities fairly evenly distributed between the five fractions. The other fluid had a very low activity, only 55 iu, so that the quantitation of isoenzymes is only approximate; it had, however, much less LDH5 than was found in the other meningioma specimen.

Fluids from Non-Neoplastic Cysts. Loculated fluid in contact with a meningioma had a protein content of 3.4 gm% and a total LDH of 70; only isoenzymes 1 and 2 could be demonstrated.

No LDH 5 or 4 was found in the fluid from an old organizing hematoma with a total LDH of 235 iu per liter, and a protein of 4.0 gm%.

The fluid from a porencephalic cyst resembled cerebrospinal fluid, with 40 mg% of protein and 8 iu LDH per liter.
Application to Problem Cases

Likelihood of Lesion Malignancy. The use of LDH analysis to determine whether a lesion is likely to be malignant is illustrated by the three problem cases reported below.

Case 1. A 62-year-old hypertensive man had had right hemiparesis and aphasia for 6 weeks and incontinence of urine for 4 days. On admission he had papilledema and right-sided pyramidal signs with no sensory loss. Blood urea and electrolytes were within normal limits. Chest x-ray showed an opacity 2 cm in diameter in the apical segment of the left lower lobe and a small lucent area in the right clavicle. A right carotid arteriogram showed a space-occupying lesion deep in the frontal region. Burr-hole biopsy of the frontal lesion yielded only cloudy, yellow fluid: this had a protein content of 4.7 gm% and total LDH activity of 15,200 iu per liter. The electropherogram was consistent with that for a malignant tumor, with 42% of LDH.

Case 2. This patient had received deep x-ray therapy for a bronchial carcinoma earlier the same year. The fluid submitted for analysis contained 4.0 gm% of protein, showed a total LDH activity of 12,000 iu per liter with 20% of LDH and indicated a malignant tumor.

Case 3. A 47-year-old normotensive man with a 9-month history of transient episodes of syncope followed by short periods of double vision experienced a sudden onset of expressive dysphasia and headache, without any loss of consciousness or localizing signs. He was confused, with a jargon dysphasia, but able to cooperate well. Physical examination was normal except for a blood pressure of 160/100 mm Hg. Lumbar puncture produced clear, colorless CSF under a pressure of 100 mm CSF. The Queckenstedt test was normal. The CSF protein was 40 mg% and less than one white blood cell per cu mm was seen. Chest and skull x-rays were normal. The blood urea was 31 mg%. Left carotid arteriography showed an infiltrating lesion in the left frontoparietal region, and technetium brain scan revealed a poorly defined area of increased uptake in the convexity of the left parietal lobe, which was interpreted as a tumor. At craniotomy the lesion was thought to be a metastasis; as it was in the dominant hemisphere only a biopsy was taken and about 5 ml of blood-stained fluid was sent to the laboratory. Histologically, no tumor was recognized in the biopsy material, which had the appearance of recent cortical infarction with some accompanying necrosis of white matter. The fluid had a protein content of 0.4 gm%, the total LDH was 1,380 iu per liter but the isoenzyme pattern did not suggest a malignant lesion. The patient made a good recovery and remains well 2 years later.

Histology Revision Because of Chemical Findings. The use of LDH analysis to confirm or revise histological grading of tumors is illustrated in the two cases reported below.

Case 4. A 30-year-old woman underwent a craniotomy for partial excision of a cystic cerebral glioma. The pathologist reported the specimen to be an astrocytoma grade I. The cyst fluid, not included in the analysis reported above, was examined; a total LDH of 3470 iu was found with 10% of LDH. When a background of histologically proven tumors had built up, this case stood out as an exception and the slide was given to the neuropathologist, without comment, who reported it as grade III. The patient died 3 months after operation.

Case 5. A 57-year-old woman had a cystic cerebral glioma partially excised. Her immediate recovery was good but before long she had to be readmitted to the referring hospital because of deterioration in her cerebral state, and soon thereafter died. The original report on the operation specimen had classed it as an astrocytoma grade II. The cyst fluid removed at operation had a total LDH activity of 1800 iu and 20% of LDH. The pattern did not look like that of a relatively benign fluid. Sections made from a specimen of the residual tumor obtained at postmortem examination showed an undoubted grade III astrocytoma. On careful review of the original slides, a few mitoses were in fact found to be present, indicating that it should probably have been classified as a grade III tumor.
**Discussion**

The purpose of this paper is to draw attention to practical applications of the estimation of the enzyme lactate dehydrogenase, and of its isoenzymes, in the fluid that occurs in cystic tumors of the nervous system. Started as a research project, these investigations are now used in this department to supplement, where appropriate, our routine neuropathology. They are found to be of particular value in the situation where fluid is withdrawn but no suitable material for histology is obtained. Knowledge as to whether the fluid in question is likely to have come from a benign or a malignant source may be of use in deciding the desirability of further procedures.

The chemical methods involved are not unduly complicated; estimation of the total enzyme activity presents few problems. It is now possible to obtain commercial kits for single determinations which ensure that reagents can always be at hand and fresh without wastage of expensive chemicals. Moreover, some sort of ultraviolet spectrophotometer is now to be found in most departments of chemical pathology. The work described here began without such an instrument, so the slower colorimetric method had to be used at the start and was later adhered to for the sake of uniformity. The colorimetric method utilizes the enzyme reaction in the direction of lactate to pyruvate, while the more commonly used version of the ultraviolet spectrophotometric method takes the reaction in the reverse direction. In a number of instances both methods have been employed on the same sample and have been in close agreement but, as mentioned earlier, there is variation between methods so that care is needed in comparing the results of one laboratory with those of another. Also, the different systems in use for the classification of cerebral tumors make it necessary to establish limits of LDH activity with regard to groups of specimens from tumors verified histologically by the particular neuropathology service concerned.

Demonstration and quantitative measurement of the isoenzymes are more laborious than the estimation of the total LDH activity, but they help to characterize some of the malignant cyst fluids where the total activity is low, such as in Case 20 with a total LDH of only 380iu per liter but with 15% of LDH5, or in Cases 56 through 60 with total enzyme activities of under 1000 but with more than 10% of LDH5. Demonstration of the isoenzyme pattern may also prove to be of use in helping to exclude malignancy when the differential diagnosis involves the probability of an old hematoma. If slides for electrophoresis are available, an isoenzyme preparation can be ready in about 2 hours. Agar-coated slides, prepared in batches and only cut from the surrounding gel just before use, give good banding of isoenzymes for at least a week after preparation, if not longer.

As LDH5 is more susceptible to heat denaturation than are the faster moving fractions, an attempt was made to distinguish these fluids with increased LDH1 by the proportion of activity destroyed by 30 minutes of incubation at 57°C, on the lines of the serum test proposed by Strandjord, et al.,16 for the detection of myocardial infarction. Increased LDH5 and 57°C-lability of the enzyme in the cyst fluids were found to go together, but the method requires very careful control of temperature and of pH and so far has not been as satisfactory as the electropherograms, although it has possible application where facilities for electrophoresis are not available. Neither urea inhibition4 of LDH nor the use of stilboestrol diphosphate5 has been tried in this context.

As demonstrated in the presentation of the results, the fluids can be divided broadly into two groups: 49 of the 64 (76.5%) from malignant tumors had a total LDH of over 1000iu per liter and 10% or more of LDH5; 30 of the 36 (83.6%) from benign lesions had a total LDH of under 1000iu and less than 10% of LDH5. Of the 12 fluids with either a total LDH of over 1000iu or 10% or more of LDH5 nine came from malignant tumors. One of the original aims of this study was to see if a distinction could be drawn, on chemical lines, between the malignant astrocytomas and the cerebral metastases. So far, this has only been partially successful; 14 of the 20 fluids from carcinomas had either a total LDH activity or an LDH5 content above the upper limit found for the astrocytomas.

Among the glial tumors and the secondary
carcinomas, the correlation of LDH, and especially of LDH₅, activity in cyst fluid with the histological assessment of malignancy was good. In fact, for tumor cyst fluids this was rather better than that found by similar measurements on extracts of tumor tissue. A possible advantage of working with this type of material is that it provides a fluid in equilibrium with the tumor tissue without involving the variable addition of enzyme derived from the inevitable residual amounts of blood introduced when a tissue extract is prepared. As in the case of tissue extracts, cyst fluids can only give an average of the types of enzyme in the tumor, and a small area of mitotic activity may be swamped by the bulk of the growth being of a more benign nature. On the other hand, the composition of the fluid might indicate a premalignant change as in Case 5. A similar shift toward the form of LDH associated with anaerobic glycolysis has been observed in the pre-invasive stage of carcinoma of the cervix.⁹

Summary

One hundred cerebral cyst fluids were analyzed for total lactate dehydrogenase (LDH) activity and distribution of isoenzymes. Results fell into two main groups; fluids from malignant tumors had a total activity over 1000 iu of LDH per liter and more than 10% of the activity as LDH₅; fluids from benign tumors had less than 1000 iu per liter total LDH and less than 10% of LDH₅.

Secondary carcinomas were most active; the highest LDH levels were associated with frequent mitoses and anaplasia.

Astrocytomas grades III and IV were very similar to each other and to the less active secondaries, and contrasted strongly with the astrocytomas grades I and II.

Fluids from hemangioblastomas, chromophobe pituitary adenomas, and neurilemmomas had low levels of total LDH activity, and little or no LDH₅. Activity was higher in the two craniopharyngiomas, and their iso-enzyme distribution was the reverse of that for the chromophobe adenomas.

The clinical application of these results has been briefly discussed. A detailed report of the analytical methods has been published elsewhere.

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