2. All except three strains produced nodules on cowpea (*Vigna unguiculata*) var. C-152 which confirm earlier findings.\(^2\), \(^3\), \(^4\).

3. Interestingly enough, twenty-seven of these strains of *R. japonicum* also nodulated pigeon pea (*Cajanus cajan*) var. Prabhat which is a new finding worth recording.

4. Of the three strains (B-6, A-7, A-16) which did not nodulate cowpea, it was interesting to observe that A-7, A-16 could nodulate pigeon pea whereas strain B-6 isolated from Bragg variety did not nodulate the same.

Similarly, strains B-11, L-2, A-9, G-3, G-13, G-16, SB-16 which nodulated cowpea did not nodulate pigeon pea. These results point out that there are strains of *R. japonicum* which can differentially nodulate cowpea and pigeon pea.

May 11, 1981.


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**TISSUE LACTIC DEHYDROGENASE ISOENZYME ACTIVITY IN THE DIFFERENTIAL DIAGNOSIS OF TUMORS AND OTHER SPACE OCCUPYING LESIONS OF BRAIN**

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An increase in different metabolic enzyme activities in CSF of patients with a variety of disorders of central nervous system, presenting clinically as a space occupying lesion, led to the search for a characteristic biochemical pathology in the tissues which is responsible for this manifestation. Of the various enzyme systems, lactic dehydrogenase (LDH) and its isoenzyme pattern has been investigated in brain tumor tissue with a view to differentiate this neoplastic lesion biochemically, from the non-neoplastic space occupying lesions of the brain. Brain tumors have a greater tendency to employ anaerobic glycolysis, and there is a shift towards "heart pattern" of LDH isoenzymes to "liver pattern" along with an increase in total activity. These findings have been shown both in neuronal and glial tumors.\(^5\) The degree of shift has been shown to correspond with histopathological grading of the tumors. We have attempted to evaluate the utility of this methodology in predicting the biological behaviour of various histological types of tumors. The present paper reports observations made during studies on LDH and its isoenzyme pattern in histologically verified neoplastic and non-neoplastic lesions of the brain.

**Materials and Methods**

During the neurosurgical procedure, biopsy material taken from cases of space occupying lesions of the brain for histopathological diagnosis formed the source of the tumor material. Brain tissue removed at autopsy within 4 hours of death, from a case of road accident dying of non-neurological cause, a case of infarction of brain, two cases with tuberculous meningitis and a case of ischemia of brain formed the source of control and non-neoplastic pathological tissue of the nervous system.

The tumor material was obtained within 15 minutes of removal from the patient. The tissues were washed in cold buffered saline (pH = 7.4), one half of the material was used for biochemical analysis while the other half was submitted for histopathological study.

The histopathological and biochemical investigations were carried out in two different laboratories and the results were compared subsequently. The biochemical examination was carried out by making a 10% homogenate of all the tissues in glass-distilled water at 4°C.

**Methodology**

The total LDH was estimated by the usual method of measuring the change in absorbance of NADH at 340 nm in the presence of pyruvate at 25°C by using Gilford Statar—III System—4. The activity is expressed as units/L (of 10% homogenate). For isoenzyme separation the homogenate was centrifuged at 7000 rpm for 10 minutes and the supernatant was applied on a cellulose acetate membrane. Along with tissue homogenate, a standard preparation of LDH is spotted for reference (murine tissue extract of heart and brain in sucrose and human serum solution). The isoenzymes were electrophoretically separated by using Beckman micropore electrophoresis.
Table I

<table>
<thead>
<tr>
<th>Diagnostic Categories</th>
<th>Total LDH (U/L)</th>
<th>LDH₁</th>
<th>LDH₂</th>
<th>LDH₃</th>
<th>LDH₄</th>
<th>LDH₅</th>
<th>LDH₆/LDH₅</th>
<th>H/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Adult control brain</td>
<td>125</td>
<td>17·8</td>
<td>24·4</td>
<td>29·3</td>
<td>22·8</td>
<td>5·7</td>
<td>3·12</td>
<td>2·17</td>
</tr>
<tr>
<td>2. Infarct brain</td>
<td>50</td>
<td>15·1</td>
<td>14·7</td>
<td>35·6</td>
<td>37·4</td>
<td>8·2</td>
<td>2·00</td>
<td>1·59</td>
</tr>
<tr>
<td>3. Ischemia*</td>
<td>628</td>
<td>22·4</td>
<td>25·6</td>
<td>32·0</td>
<td>15·0</td>
<td>5·6</td>
<td>4·00</td>
<td>2·41</td>
</tr>
<tr>
<td>4. T.B. Meningitis*</td>
<td>524</td>
<td>21·0</td>
<td>26·0</td>
<td>25·5</td>
<td>22·5</td>
<td>5·0</td>
<td>4·00</td>
<td>2·28</td>
</tr>
<tr>
<td>5. T.B. Meningitis*</td>
<td>424</td>
<td>22·4</td>
<td>31·2</td>
<td>30·0</td>
<td>13·0</td>
<td>3·4</td>
<td>6·60</td>
<td>2·44</td>
</tr>
<tr>
<td>6. Medulloblastoma*</td>
<td>550</td>
<td>6·2</td>
<td>10·4</td>
<td>15·4</td>
<td>32·0</td>
<td>36·0</td>
<td>0·17</td>
<td>0·91</td>
</tr>
<tr>
<td>7. Secondary tumor in brain*</td>
<td>885</td>
<td>10·0</td>
<td>8·6</td>
<td>9·4</td>
<td>34·0</td>
<td>38·0</td>
<td>0·26</td>
<td>0·88</td>
</tr>
<tr>
<td>8. Meningioma*</td>
<td>490</td>
<td>10·0</td>
<td>20·0</td>
<td>22·5</td>
<td>22·0</td>
<td>25·5</td>
<td>0·40</td>
<td>1·28</td>
</tr>
<tr>
<td>9. Neuroblastoma</td>
<td>225</td>
<td>30·8</td>
<td>35·0</td>
<td>22·8</td>
<td>8·7</td>
<td>3·7</td>
<td>8·40</td>
<td>3·16</td>
</tr>
<tr>
<td>10. Astrocytoma</td>
<td>1055</td>
<td>50·8</td>
<td>27·8</td>
<td>13·6</td>
<td>5·2</td>
<td>2·6</td>
<td>20·00</td>
<td>4·83</td>
</tr>
<tr>
<td>11. LDH standard*</td>
<td>180</td>
<td>35·0</td>
<td>30·0</td>
<td>17·0</td>
<td>10·0</td>
<td>8·0</td>
<td>4·4</td>
<td>2·92</td>
</tr>
</tbody>
</table>

* Represented in Fig. 1.

apparatus in trisodium citrate and barbituric acid buffer (pH 7·4) and the bands are visualised by using tetra nitro blue tetrazolium (TNBT) and phenazine-methosulfate (PMS) reagent. The membrane is dried at 37°C and subjected to densitometric recording. The isoenzyme activity was expressed as percentage of total LDH activity. The ratio of LDH₄/LDH₅ was calculated and expressed as an index to differentiate the non-neoplastic and neoplastic lesions of CNS. Similarly the H and M ratio of the LDH isoenzyme was also computed and compared with the earlier index. An attempt has been made to evaluate the comparative usefulness of either of these indices in differentiating various types of the lesions.

Results

The histopathological study of the neoplastic tissues revealed the following tumors: astrocytoma, meningioma, neuroblastoma, medulloblastoma and a secondary tumor metastasizing to the brain. Total LDH activity is reduced in an infarct of the brain, while in the infarct, the activity is markedly elevated. The activity of LDH₄ and LDH₅ is raised in medulloblastoma, meningioma and the metastatic tumors (Table I). In tuberculous meningitis, ischemic brain, and frank infarct, the common denominator is atoxia of the brain though of different grades. While LDH₅ is low in these three, LDH₄ and LDH₆ are high in infarct when compared to the other two conditions. The LDH₄ isoenzyme level is also found to be low in tumors like astrocytoma and neuroblastoma. The ratio of LDH₄/LDH₅ was calculated in all cases and it is observed that in meningioma, medulloblastoma and in secondary tumors it is well below the normal ratio. In contrast, astrocytoma and neuroblastoma show very high ratio when compared to either infarct or tuberculous meningitis brain. On the other hand, the H/M ratio (Table I) does not appear to be efficient enough in resolving the differences either between non-neoplastic and neoplastic or between different types of neoplastic lesions. The pattern of isoenzyme in tumor and other cases is shown in Fig. 1.

Discussion

An increase in total LDH activity in patients with brain tumor and differential raise in various isoenzyme activities in contrast to cerebral infarct my have a potential for use in differentiating these two pathological states, which mimic each other clinically posing a diagnostic problem. As could be seen from Table I the ratio of LDH₄/LDH₅ for control group is around 2·4. The initial evaluation of study on a limited number of cases shows that low ratio of LDH₄/LDH₅ (< 1·0) is suggestive of tumors like meningioma, metastatic brain tumor and medulloblastoma. On the other hand, ratios > 5 are seen among tumors like neuroblastoma and a marked increase in the ratio (about 20·0) is observed in astrocytoma. Our reports agree with the reports of Shearin et al., who calculated the ratio of LDH₄/LDH₅ and used the ratio as an index for differential diagnosis.
INFLUENCE OF STORAGE TEMPERATURE ON SCLEROTIAL GERMINATION OF *CLAVICEPS FUSIFORMIS*

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*Claviceps fusiformis* Loveless, causing ergot of pearl millet (*Pennisetum typhoides* L.) Leake), is a serious pathogen in the arid and semi-arid tropics. A few workers have reported sclerotium germination under the field and laboratory conditions. However, no work has been done on the effect of different storage temperatures on the germination of sclerotia. Under the present investigation an attempt has been made to test the possibility of enhancing the germination of sclerotia by subjecting them to different storage temperatures.

Fresh sclerotia of *C. fusiformis* collected from rabi pearl millet crop of 1980 were used in the experiment. One set of sclerotia were bagged in polythene covers and kept at -10°C, 0°C, 15°C, 23 ± 1°C, 25°C and 37°C for 8 weeks. Another set of sclerotia was kept at 15°C, 23 ± 1°C, 25°C and 37°C and were chilled for 24 hours once in 4 weeks at 0°C. After 8 weeks 75 sclerotia were removed from each storage regime and plated 25 sclerotia/plate on water saturated coarse sand in perspex Petri plates. The plates were incubated at 23 ± 1°C with 12 h/12 h cycles of artificial day light (ADL) and darkness.

All storage temperatures, except -10°C, 0°C and 15°C (both in chilled and non-chilled), induced the sclerotia to germinate. Maximum germination (81-33%) was recorded in sclerotia stored at 37°C without chilling (Table I). The lower the storage temperature lesser was the germination percentage. In all cases, chilling reduced the sclerotial germination. This shows that *C. fusiformis* is well adapted to high temperature conditions prevailing in the semi-arid tropics.

The number of days required for sclerotium germination varied from 16-38 days (Table I). The germinating sclerotia showed white tuft of mycelium from which region, the stipes with the clavus started emerging. The average number of clavae per germinated sclerotium varied from 1-16-3-40. The number of clavae per germinated sclerotium ranged from 1-8.

In another experiment, newly collected sclerotia were incubated for germination as described earlier. It was observed that only 3-5% of the sclerotia germinated, that too after 60 days of incubation. This may be due to the dormancy of the sclerotia. However, it