Lactic dehydrogenase activity of aqueous humour in retinoblastoma

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Lactic dehydrogenase (LDH) is an enzyme that catalyses the reversible reaction:

\[ \text{Pyruvate} + \text{NADH} \rightleftharpoons \text{NAD} + \text{Lactate} \]

NAD = nicotinamide adenine dinucleotide
NADH = reduced nicotinamide adenine dinucleotide
LDH can also react with NADP.

Although the levels of LDH have been estimated in the aqueous humour of animals (Kuhlman, and Kaufman, 1960), information regarding LDH levels in human aqueous humour appears to be scanty.

It is known that malignant tumours at different sites in the body are associated with an increased LDH activity in the corresponding body fluid (Wroblewski, 1957).

The purpose of the present study is to determine whether there is an increased LDH activity in the aqueous humour in cases of intraocular malignancy.

Materials and methods

Specimens of aqueous humour were collected from fifty patients coming for surgery to the Victoria Memorial Eye Hospital, Colombo. Of these, 46 had non-malignant intraocular disorders and four had retinoblastomata. The non-malignant intraocular disorders included the following:

- Cataract 33
- Adherent leukoma 4
- Aphakia 3
- Anterior staphyloma 3
- Lens-induced glaucoma 2
- Retinal detachment 1

The aqueous humour was collected using an Amsler's needle connected to a dry 2 ml. syringe, which was introduced at the limbus. These specimens of aqueous humour and blood were kept at 4°C and were analysed within 24 hours of collection.

The LDH activity in both aqueous humour and blood was estimated by the colorimetric method of King (1959). The principle of estimating the enzyme was first described by Wacker, Ulmer, and Vallee (1956), where lactate and NAD are used as substrates and the increase in the optical density at 340 m\(\mu\) is measured. The advantage of this technique is that the reagents are stable and cheap.

A unit of activity is the amount of LDH which will reduce 1 \(\mu\)mole lactate to 1 \(\mu\)mole pyruvate in 15 minutes at 37°C. Activity is expressed in units per 100 ml. of specimen.

The volume of aqueous required for the estimation is only 0.1 ml. and sufficient aqueous humour was obtained even from patients with a shallow anterior chamber. Care was taken to see that none of these specimens was contaminated with blood.

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Results

The LDH activity in the aqueous humour of the 46 non-malignant cases varied from 0 to 350 units/100 ml. (Table I).

Table I  LDH Activity in 46 patients with non-malignant intraocular disorders

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of patients</th>
<th>Range of LDH activity (units/100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum</td>
</tr>
<tr>
<td>Cataract</td>
<td>33</td>
<td>230 to 470</td>
</tr>
<tr>
<td>Adherent leucoma</td>
<td>4</td>
<td>150 to 240</td>
</tr>
<tr>
<td>Aphakia</td>
<td>3</td>
<td>160 to 480</td>
</tr>
<tr>
<td>Anterior staphyloma</td>
<td>3</td>
<td>400 to 420</td>
</tr>
<tr>
<td>Lens-induced glaucoma</td>
<td>2</td>
<td>250 and 400</td>
</tr>
<tr>
<td>Retinal detachment</td>
<td>1</td>
<td>160</td>
</tr>
</tbody>
</table>

The LDH activity in the serum collected and estimated simultaneously varied between 150 and 470 units/100 ml. The normal range is 160 to 400 units/100 ml. (King, 1959).

The four cases of retinoblastoma investigated gave the results shown in Table II. They all had a white mass behind the pupil which was clinically diagnosed as a retinoblastoma. Subsequent microscopic examination confirmed the diagnosis in all of them.

Table II  LDH Activity in 4 cases of retinoblastoma

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Sex</th>
<th>LDH Activity (units/100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum</td>
</tr>
<tr>
<td>1½</td>
<td>M</td>
<td>380</td>
</tr>
<tr>
<td>2½</td>
<td>M</td>
<td>380</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>280</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>480</td>
</tr>
</tbody>
</table>

Discussion

Since it was not possible to estimate the LDH levels in the aqueous humour of normal human eyes, these were estimated in the above 46 patients with non-malignant intraocular disorders.

The highest value obtained was 350 units/100 ml. in a patient with a cataract who had a pseudoxefoliation of the lens capsule. The lowest value obtained from a case of retinoblastoma was more than six times this value.

These results indicate that retinoblastomata are probably associated with a gross elevation of LDH activity in the aqueous humour.

Non-malignant growths are not associated with increased LDH activity (Erickson and Morales, 1961). The LDH activity in the body fluid bathing a non-neoplastic lesion in a
patient is lower than the LDH activity in the serum of that patient (Wroblewski, 1957). Disorders such as primary hyperplastic vitreous and intraocular tuberculosis, which may simulate a retinoblastoma, are therefore unlikely to be associated with gross elevations of LDH activity in the aqueous humour. It would seem, therefore, that the estimation of the LDH activity in the aqueous humour might be of value in the diagnosis of retinoblastoma.

Summary

LDH activity in the aqueous humour of 46 patients with non-malignant intraocular disorders ranged from 0 to 350 units/100 ml.

The four cases of retinoblastoma studied gave values of 1800, 2100, 1600, and 3250 units/100 ml.

Since LDH activity in the aqueous humour is greatly elevated in retinoblastoma, it seems likely that the estimation of LDH activity in the aqueous humour may be of value in the diagnosis of intraocular malignancy.

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