CARBONIC ANHYDRASE IN THE VITREOUS BODY*

BY

J. GLOSTER

From the Ophthalmological Unit, Medical Research Council, Institute of Ophthalmology, University of London

Director of Research: Sir Stewart Duke-Elder

REVIEWS of some recent literature concerning the biochemistry of the vitreous body contain no references to the presence of enzymes (Bellows, 1952, 1953; von Sallmann, 1953, 1954; McLean, 1954). However, the possibility of the existence of enzymes in the vitreous body has not been overlooked. Lépine (1870) reported that the vitreous of dogs, rabbits, and frogs contained a substance which caused hydrolysis of starch, and Michel and Wagner (1886) mentioned that they had looked for "fibrinferment" in the vitreous but had found no evidence of its presence. Leber (1903) reported the presence in the vitreous of an amylase, and Lo Cascio (1922) found an amylase, catalase, and enzymes catalysing proteolysis, the reduction of methylene blue, and the hydrolysis of glyceryl monobutyrate; and, recently, anabolic enzymes have been demonstrated in the vitreous by Zeller (cited by Schwartz and Leinfelder, 1955).

Bakker (1941) reported briefly that the vitreous body of some mammals contains a minute amount of carbonic anhydrase. The occurrence of this enzyme in ocular tissues has become of great interest since Becker (1954) demonstrated that the systemic administration of a carbonic anhydrase inhibitor (acetazolamide, Diamox) often reduces the intra-ocular pressure in both normal and glaucomatous eyes. Carbonic anhydrase is known to occur in the lens (Bakker, 1939, 1941), in the ciliary body and iris (Wistrand, 1951; Gloster and Perkins, 1955), and in the retina (Leiner, 1940; Bakker, 1941; Kauth and Sommer, 1953). The experiments described in this paper were carried out to determine the carbonic anhydrase content of the vitreous of the rabbit and to investigate the relative amounts of the enzyme in the peripheral and central regions of the vitreous body.

Methods

(i) Preparation of Specimens of Vitreous.—The eyes were excised from dead rabbits, within half an hour of death, and were frozen rapidly in solid carbon dioxide and stored in a refrigerator at −5°C., usually for about 12 hrs. An equatorial cut was made through the sclera, choroid, and retina, and these tissues were peeled off, leaving the vitreous and lens frozen together. The outer layer of the vitreous was allowed to thaw and the lens was then removed intact, after which the vitreous was immediately frozen again in solid carbon dioxide. A few

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487
fragments of retina and choroid still adhered to the vitreous, and these were removed by shaving off a layer of frozen vitreous over its entire surface. The fresh surface was then washed rapidly with a small jet of glass-distilled water and wiped with a filter-paper. The vitreous was then allowed to thaw and was filtered through a 2-in. column of 1-mm. diameter glass beads in a vertical glass tube 4½ inch in diameter. The material obtained in this way could be pipetted easily if it was used undiluted in some experiments, while in others it was diluted with an equal volume of glass-distilled water.

In the experiments in which the carbonic anhydrase content of the central vitreous was compared with that of the peripheral vitreous, the above procedure was followed, but the eyes were excised and frozen immediately after the animal had been killed. After removal of the lens and other tissues, the vitreous was frozen again in solid carbon dioxide and then divided into central and peripheral portions with a sharp scalpel. The central and peripheral portions were filtered through separate columns of glass beads. Sufficient material was usually obtained from ten to twenty eyes. It should be added that, in preliminary experiments, it was found that placing the vitreous in contact with solid carbon dioxide for a short time did not alter its carbonic anhydrase activity.

In Experiment 5, the eyes of dead rabbits were punctured with a No. 0 needle and vitreous was withdrawn into a dry syringe. A few of these specimens contained fragmented tissue and were therefore rejected. The vitreous was filtered as described above.

It was important to ensure that the specimens of vitreous were not contaminated with blood, since erythrocytes are rich in carbonic anhydrase, and therefore the benzidine reaction was applied to the material before its carbonic anhydrase activity was determined. 0·5 g. benzidine was dissolved in 4·5 ml. glacial acetic acid at 50° C. and, after cooling, 20 ml. distilled water were added. 1·4 ml. of this solution, 4·7 ml. distilled water, and 0·4 ml. of a 3 per cent. solution of hydrogen peroxide were added to 0·5 ml. of the vitreous filtrate. None of the material used in these experiments yielded an appreciable blue colour. The sensitivity of this test was such that, if blood were added to vitreous, a recognizable blue colour developed when the concentration of blood in the vitreous was 1:5,000.

(ii) Determination of Carbonic Anhydrase.—A modification of the method of Roughton and Booth (1946) was used, which depends upon the rate of change of pH of a barbitone buffer after the addition of a saturated solution of carbonic acid in water at 0°C.

0·3 ml. vitreous was added to 3 ml. barbitone buffer (pH 8·4) and 2 ml. glass-distilled water in a stoppered glass weighing-bottle. Six drops of a 0·04 per cent. aqueous solution of bromthymol blue were added, and the weighing-bottle was then placed in ice-cold water for one hour. 5 ml. glass-distilled water, saturated at 0°C. with carbon dioxide, were added rapidly, and the time taken by the pH to fall to 6·3 was measured. This end-point was reached when the colour of the contents of the weighing-bottle matched a standard which consisted of six drops of bromthymol blue solution in 10 ml. of a Sörens test buffer pH 6·3. The rate of the uncatalysed reaction was determined by using 0·3 ml. glass-distilled water in place of the vitreous. Determinations were also made on vitreous which
CARBONIC ANHYDRASE IN VITREOUS BODY

had been heated previously to 80°C for one hour. In two experiments, 0.1 ml. of a 1 mg./ml. solution of sodium acetazolamide was added to the weighing-bottles containing vitreous, before determination of the carbonic anhydrase.

Results

(a) Carbonic Anhydrase Activity of the Whole Vitreous Body.—The results of five experiments are given in Table I, which shows that the time taken

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Uncatalysed</th>
<th>Undiluted Vitreous</th>
<th>Vitreous diluted 1:1</th>
<th>Heated Vitreous</th>
<th>Vitreous + Acetazolamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>167±1.4 (5)</td>
<td>63±1.3 (3)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>184±1.2 (10)</td>
<td>45±1.1 (10)</td>
<td>99±1.2 (10)</td>
<td>186±1.9 (10)</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>169±3.2 (10)</td>
<td>—</td>
<td>76±2.8 (6)</td>
<td>175±2.5 (7)</td>
<td>194±1.4 (6)</td>
</tr>
<tr>
<td>4</td>
<td>185±4.3 (6)</td>
<td>—</td>
<td>78±4.9 (6)</td>
<td>—</td>
<td>142±6.1 (3)</td>
</tr>
<tr>
<td>5</td>
<td>165±4.0 (4)</td>
<td>48±3.3 (4)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

for the pH of the contents of the weighing-bottles to change from 8.4 to 6.3 was reduced significantly by the addition of vitreous, either undiluted or diluted 1:1 with water. There was no acceleration of the reaction if the vitreous had been heated previously. Moreover, the addition of acetazolamide to the reaction mixture either abolished (Experiment 4) or reduced greatly (Experiment 5) the acceleration of the pH change which was caused by the vitreous. In Experiments 1 to 4 the vitreous was obtained by the freezing technique, while in Experiment 5 it was withdrawn by means of a syringe from eyes which had not been frozen.

(b) Comparison of Carbonic Anhydrase Activity of Central Vitreous with that of Peripheral Vitreous.—Table II shows that, when central or peripheral vitreous was added to the reaction mixture, the pH change occurred more

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Uncatalysed</th>
<th>Peripheral Vitreous (diluted 1:1)</th>
<th>Central Vitreous (diluted 1:1)</th>
<th>Heated Peripheral Vitreous</th>
<th>Heated Central Vitreous</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>185±2.3 (10)</td>
<td>116±1.9 (9)</td>
<td>153±2.6 (7)</td>
<td>191±3.1 (10)</td>
<td>187±1.5 (7)</td>
</tr>
<tr>
<td>7</td>
<td>180±3.7 (8)</td>
<td>112±1.5 (8)</td>
<td>140±2.0 (9)</td>
<td>187±1.5 (8)</td>
<td>187±1.3 (8)</td>
</tr>
<tr>
<td>8</td>
<td>199±1.8 (8)</td>
<td>147±1.7 (8)</td>
<td>173±2.5 (8)</td>
<td>200±1.8 (8)</td>
<td>190±2.2 (8)</td>
</tr>
</tbody>
</table>
rapidly than in the uncatalysed reaction. Furthermore, in all three experiments, the time taken for the pH change with peripheral vitreous was significantly less ($P<0.01$) than the time taken with central vitreous, but this difference was either eliminated (Experiments 6 and 7) or greatly reduced (Experiment 8) if the vitreous had been heated previously.

**Discussion**

The experiments described above demonstrate that carbonic anhydrase occurs in the vitreous body of the rabbit, and that higher concentrations of the enzyme are present in the peripheral area of the vitreous than in the central area. In order to assess the amount of enzyme present, a comparison was made with the acceleration of the reaction brought about by various dilutions of rabbit's blood, using the results of experiments reported previously, in which an identical technique of carbonic anhydrase estimation was employed (Gloster and Perkins, 1955). From these data, it was found that the concentration of carbonic anhydrase in the vitreous is approximately $\frac{1}{100}$ to $\frac{1}{600}$ of the concentration of the enzyme in the blood. Thus the concentration of carbonic anhydrase in the vitreous is considerably lower than that in the ciliary body and iris ($\frac{1}{50}$ to $\frac{1}{20}$ of the concentration in blood) and much less than that of the lens ($\frac{1}{4}$ to $\frac{1}{3}$ of the concentration in blood). These values for the ciliary body and iris and for the lens were obtained by the same technique as for the vitreous (Gloster and Perkins, 1955).

The tissues surrounding the vitreous body are relatively rich in carbonic anhydrase and it seems probable that the enzyme present in the vitreous originates from them, a view supported by the finding of more enzyme in the peripheral area than in the central area of the vitreous. The possibility that the carbonic anhydrase found in the vitreous had entered from the surrounding tissues as a *post-mortem* change is improbable, since it was found in the central region of the vitreous of eyes which had been excised and frozen rapidly within 2 to 3 minutes of the death of the animal.

According to Fischer-von Bünau and Fischer (1932), the vitreous body has no measurable metabolic activity. These workers injected bubbles of gas, of known composition, into the vitreous in living rabbits, and found that there was no change in the proportional concentrations of oxygen and carbon dioxide in the bubbles. On the basis of this evidence, it is almost inconceivable that the carbonic anhydrase in the vitreous body has any metabolic significance.

**Summary**

Carbonic anhydrase was demonstrated in the vitreous body of rabbits and a higher concentration was found in the peripheral area than in the central area. The concentration of carbonic anhydrase in the vitreous body is lower than that in the surrounding tissues.
REFERENCES

——— (1941). Ophthalmologica (Basel), 102, 351.