Oxygen uptake by canine uveal tissue: In vitro and in vivo methods compared

J. O’Rourke and Charles Benson

This study was done to determine whether newer in vitro methods for measurement of uveal tissue respiration would corroborate QOt values previously obtained using such in vivo methods as isolated organ perfusion or sampling of ocular arteries and veins in the anesthetized dog. A good correlation exists among QOt values found with each of these three methods involving both iridociliary and choroidal tissue samples. Normal QOt values (one liter per dry milligrams per hour) ranged between 0.71 and 0.82 for both the in vitro Warburg-type measurement, using an oxygen electrode, and the in vivo sampling of the cannulated ocular vessels. Isolated organ perfusion with whole blood gives a somewhat higher value (1.90) when retinal uptake is obviously included. When retinal extraction of oxygen is suppressed, normal values representing uveal uptake are found. Serum uveitis reduces uveal extraction of oxygen in all three systems: hyperthermia increases it (Fig. 1).

Past studies of uveal tissue respiration in vivo have shown a small dimension of oxygen uptake in normal eyes and a further reduction below normal level during inflammation associated with uveitis. These findings have been based upon two kinds of experiments: first, measurements of uveal A-V oxygen differences utilizing blood samples recovered from the anterior ciliary vein and adjacent orbital arteries,1-3 and more recently by measurement of A-V oxygen differences across the intact isolated eye perfused with whole blood.4 Canine eyes were used because they provide ocular vessels large enough to permit repeated cannulation.

The present report concerns evaluation of a newer in vitro method for measurement of uveal tissue uptake of oxygen using a Clark-type electrode in a tissue bath chamber. Comparison is made with in vivo measurements just described and indicates generally a good correlation under conditions of normalcy, inflammation, and hyperthermia.

Methods

The in vitro measurements were made with fresh canine uveal tissue utilizing a biological oxygen monitor (Yellow Springs Inst. Co., Model No. 53), and attached strip chart recorder. The system contains two oxygen electrodes and three tissue bath chambers as the basis for measurements recorded. Calibrated against an air-saturated solution (modified Krebs-Ringer) of 3 c.c. volume (contained in lucite chamber bath assembly) the instrument records changes in oxygen content of the solution continuously (Hewlett-Packard Model No. 680). Each chamber of 3 c.c. volume contains 14.7 μl of oxygen at STP conditions. This value is computed on the basis of oxygen solubility of 0.0234 c.c. per cubic centimeter of Krebs-Ringer x 21 per cent air con-
UVEAL TISSUE OXYGEN UPTAKE:
IN-VIVO AND IN-VITRO MEASUREMENTS COMPARED

Fig. 1. Oxygen uptake by excised portions of canine choroid (top) and iris-ciliary body (second from top) correlates well with $Q_{O_2}$ values obtained in vivo by direct sampling of the ocular arteries and veins and by whole blood perfusion of the enucleated eye, after retinal suppression (see text). Each bar represents a value mean of six experiments, the range of which is shown by brackets.

tent of oxygen $\times 3$ c.c. chamber volume. This oxygen content of about 4.9 $\mu l$ per cubic centimeter of Krebs-Ringer solution lies closer to plasma content encountered in vivo than would a solution saturated with pure oxygen (which might show a content of approximately 23.4 $\mu l$ per cubic centimeter). The oxygen monitor system is calibrated to 100 per cent at the point of air saturation of the solution and subsequent readings are read directly as per cent of full scale. Thus a system containing uveal tissue and showing changes from 100 per cent to 80 per cent in 20 minutes would indicate that 20 per cent of available oxygen was consumed in that time (Figs. 2 and 3).

Modified Krebs-Ringer solution prepared daily from stock solution contained:

<table>
<thead>
<tr>
<th></th>
<th>Per cent</th>
<th>M</th>
<th>c.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.9</td>
<td>0.145</td>
<td>100</td>
</tr>
<tr>
<td>KCl</td>
<td>1.15</td>
<td>0.145</td>
<td>4</td>
</tr>
<tr>
<td>CaCl</td>
<td>1.22</td>
<td>0.11</td>
<td>3</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>2.11</td>
<td>0.145</td>
<td>1</td>
</tr>
<tr>
<td>MgSO$_4$·7H$_2$O</td>
<td>3.82</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Two milligrams of powdered, anhydrous dextrose, and 2 mg. of sodium bicarbonate were added for each cubic centimeter of Krebs-Ringer solution prepared. Finally, carbon dioxide was bubbled through the solution in order to adjust pH, after which 15 c.c. of serum recovered from the experimental animal were added.

Uveal tissue was dissected from eyes enucleated under anesthesia prior to death in mongrel adult dogs. Choroid and iris-ciliary body sections were dissected separately from chilled eyes and each sample was cut into three different sizes, giving a twofold weight difference as a check on accuracy of the $Q_{O_2}$ computations with this system. Three such portions were studied in each in vitro experiments. Twelve dogs were studied by the in vitro method in which one eye was studied for normal tissue values while the fellow eye was studied following induction of serum uveitis. For this purpose each animal was previously sensitized with horse serum adjuvant mixture by subcutaneous injection as previously reported. Uveitis was induced in this latter eye by the intravitreal injection of horse serum three weeks...
Fig. 2. Extraction of oxygen from modified Krebs-Ringer solution by 22 mg. samples of excised, canine choroid. Uveal inflammation was associated with reduced uptake and epinephrine with consistent acceleration.

Fig. 3. Oxygen extraction by excised 12 mg. samples of canine, iris-ciliary body tissues from Krebs-Ringer solution was considerably reduced in inflamed tissues and was, in normals, routinely accelerated following addition of epinephrine.
following onset of weekly sensitizing injections. Enucleations of the normal and inflamed eye were made simultaneously and the in vitro studies of each uveal tissue were made at the same session. Sensitization did not depress normal tissue respiration in the uninjected eye in preliminary experiments.

Following a 30 minute recording for each uveal sample, the tissue was removed, oven dried, and weighed to provide a dry milligram value required for computation of \( QO_2 \). Epinephrine HCl, 0.1 c.c., 1:1000 concentration was added to the tissue bath chamber during the final 10 minutes of each normal run in order to check responsiveness of that system. Hyperthermia in vitro was induced by increasing water bath temperatures surrounding the tissue bath chamber from a normal of 37° C. to a level of 42° C.

In vivo experiments, based upon direct sampling of the anterior ciliary vein as well as with isolated organ whole blood perfusions, were done in separate animals under conditions as previously reported, twelve paired eyes (6 animals) in each series. Hyperthermia was accomplished in the anterior ciliary vein studies by use of diathermy directed to the orbital region: in the isolated organ study it was induced by changes of temperature on the heat table, as previously reported.3,4 Tissue weight values employed in these in vivo studies were based on previous investigations as follows: canine iris-ciliary body 43 mg. dry: choroid 57 mg. dry. Preliminary dissection showed less than 10 per cent variation among animals in the dry weight of these uveal components in a body range of 15 to 18 kilograms.

Results

Concerning measurements on normal uveal tissues, the in vitro uptake values closely corroborated those obtained by sampling of the anterior ciliary vein (ACV), in vivo, while uptake registered by the isolated, whole eye perfused with canine blood was considerably higher. However, as previously reported, when this last value is corrected by suppression of retinal respiration with sodium iodate administration, the expected level of uveal values is obtained.4 Thus \( QO_2 \) values for in vitro choroid are 0.73 as a mean figure. For iris-ciliary body samples the corresponding value is 0.71. The \( QO_2 \) range of values is indicated in Fig. 1 for various experiments. These findings for each in vitro tissue study made with the oxygen electrode are close to the value 0.82 obtained for the total uveal \( QO_2 \) measured by ACV sampling.

The isolated eye preparation, which permits recovery of all venous effluent, shows a \( QO_2 \) value of 1.47 but as previously mentioned this is reduced to approximately 0.65 following iodate suppression. When uveal tissue samples were analyzed following iodate suppression there appeared to be no reduction in uveal \( QO_2 \) in vitro.

Changes associated with serum uveitis were of similar direction and degree in both the in vitro and in vivo systems. The \( QO_2 \) mean value in each situation was reduced and showed very slight overlap into the normal range. Thus in Fig. 1 values the normal vs. uveitis \( QO_2 \) ratio for choroid in vitro was 0.73/0.53; for iris-ciliary body in vitro, 0.71/0.63; for ACV sampling 0.82/0.25; for unsuppressed isolated organ perfusion 1.47/0.92 (Fig. 1).

Changes in uveal \( QO_2 \) induced by hyperthermia were consistently in the direction of increase. The normal vs. hyperthermic values as ratio for in vitro choroid were 0.73/1.24; for iris-ciliary body in vitro 0.71/0.93; for ACV sampling in vivo 0.82/1.03. Hyperthermia of the isolated organ system was associated with a marked increase in \( QO_2 \) representing in all likelihood a retinal response; normal vs. hyperthermic values in the isolated preparation were 1.47/3.10.

As shown in Figs. 2 and 3, as sample recordings, the uptake of oxygen from Krebs-Ringer solution proceeds linearly against time and presents similar differences between normal and inflamed uveal tissues as were observed with the in vivo methods. Thus, in the recording shown in Fig. 1, a 12 mg, portion of normal iris extracted oxygen at 1.34 per cent of 14.7 \( \mu l \) per minute giving a \( QO_2 \) value of 0.9; this value was reduced to 0.6 in iris tissue inflamed by serum uveitis: \( QO_2 \) was increased to 2.3 in normal tissue by the addition of epinephrine (0.1 c.c., 1:1000). Corresponding values for choroid shown as a sample record in Fig. 3 indicate a
normal uptake of 1.6 per cent per minute giving a QO₂ value of 0.73 for 22 mg. sample, which was reduced to 0.42 by serum uveitis. Epinephrine response of choroid in vitro under normal conditions was an increase to 2.3 per cent per minute uptake rate. Each of these sample records falls coincidentally on the upper end of ranges depicted in Fig. 1.

Discussion

Measurement of uveal oxygen uptake by a newer, in vitro method, appears to confirm two previous observations about uveal tissue respiration. The first is that uveal consumption of oxygen is very low in the normal canine eye, in spite of the rather vascular construction of this layer: The QO₂ values of iris and choroid are generally close to the level of 1.0. Prior to the employment of the in vitro methods herein described, one could not be certain that the consistent low values observed were unaffected by experimental conditions required, including general anesthesia, microcannulation, or some condition of the whole blood perfusate employed. It now seems apparent that the needs and state of the uveal tissue itself determine oxygen uptake observed: Such a determining role for various body tissues was recorded by Pfluger in 1872.

A second finding, confirmed by the in vitro, Warburg-type study, is that uveal inflammation following sensitization with serum is associated rather consistently with a reduction in uveal oxygen consumption. While this observation is not fully understood at present, it is likely associated with a phenomenon of reduced capillary blood flow, as evidenced in our other studies by retarded clearance of injected antipyrene.7 Implications of this observation for therapy of uveitis might include an emphasis on the use of agents which stimulate oxygen uptake including epinephrine and heat, which at least in these studies were shown to be effective.

Obvious advantages of the in vitro system, in view of the foregoing results, include naturally a saving in time and effort and the availability of continuous observation of rates of oxygen uptake. Such measurements under various conditions of disease and of therapy are believed to be a valuable adjunct in studies of uveal disease.

The assistance of Drs. Dean Burk and Mark Woods, National Cancer Institute, Bethesda, Md., in preparation of in vitro experiments is gratefully acknowledged.

REFERENCES

4. O'Rourke, J., and Berghoffer, B.: Uveal extraction and clearance of oxygen in normal, inflamed and hyperthermic eyes. part II. Whole blood perfusion of isolated organ and in-vivo canine studies compared, Ophthalmologica 155: 205, 1968.