The relationship of rat lens respiration to oxygen concentration and pH

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As the oxygen content of the medium is raised, rat lens respiration increases abruptly to a plateau level at 20 to 30 per cent O_2, and then rises continually through at least 95 per cent O_2. Two oxidation reactions may be involved. Oxygen uptake is unexpectedly more active in the acid range than in the alkaline, in a pattern again suggestive of two oxidative mechanisms. The possible identity of the respiratory component active in high oxygen levels and at low pH is considered. It is probably not significant under conditions obtaining in vivo. On this assumption it is estimated that respiration accounts for less than 33 per cent of the energy derived from glucose catabolism in the lens.

A previous report[1] described the construction of an oxygen electrode flow respirometer and its use in measuring the respiratory rates of individual rat lenses. It was shown that substitution of Tris or phosphate buffering for bicarbonate or of Tyrode's solution for the complex medium TC199 was without effect on the rate of oxygen uptake. Characterization of aerobic metabolism in the rat lens continues in this paper with investigation of the effects of varied oxygen tension and pH. Bicarbonate-buffered TC199 remains in use because it more firmly guarantees a normal metabolic state of the lens. Nevertheless, unusually high oxygen uptakes are observed in unphysiological ranges of pH and oxygen concentration which suggests the presence of nonenzymatic oxidation reactions. Some properties of metal-catalyzed autoxidation of sulfhydryl and ascorbate, often proposed as possible factors in lens respiration,[2-5] are therefore briefly considered.

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Materials and methods

The respiratory rates of lenses of female Sprague-Dawley rats which weighed from 100 to 150 grams were measured in bicarbonate-buffered TC199 at 37° C. as has been described.[1] Different concentrations of oxygen were obtained by combining flows from the following tank mixtures: 95 per cent N_2 and 5 per cent CO_2, 95 per cent air and 5 per cent CO_2, and 95 per cent O_2 and 5 per cent CO_2. The exact fraction of oxygen in the dry gas phase was continually monitored with a Clark oxygen electrode* that registered on a 0.024 microamperes per millimeter Rubicon galvanometer. The electrode was calibrated against flowing air or tank O_2.

The bicarbonate concentration of the medium was varied from 0.001 to 0.1M to give final pH values betwen 5.2 and 8.0. The actual pH was checked to 0.05 unit on aliquots of medium gasses with 5 per cent CO_2 at 37° C., by means of Leeds and Northrop line-operated pH meter with microelectrodes. The double reservoir assembly of the respirometer[1] was used so that the respiratory rate of each lens could be measured first at pH 7.50 and then promptly at one other value without disturbing the setup.

*Yellow Springs Instrument Co., Yellow Springs, Ohio.

Table I. Respiratory rates of rat lenses at different oxygen concentrations

O_2 in dry gas phase (%)	Lenses (No.)	Mean lens weight (Mg.)	Oxygen consumption* (μl. O_2/lens-hours)
5	3	23.7	0.260 ± 0.032 (0.22 - 0.28)
10	5	23.0	0.481 ± 0.047 (0.43 - 0.54)
15	3	24.1	0.690 ± 0.082 (0.62 - 0.78)
20	32	23.9	0.747 ± 0.081 (0.63 - 0.88)
30	5	22.7	0.712 ± 0.066 (0.63 - 0.79)
50	6	24.2	0.900 ± 0.073 (0.79 - 0.98)
70	4	23.7	1.062 ± 0.156 (0.90 - 1.24)
95	9	23.8	1.236 ± 0.161 (1.01 - 1.50)

*Mean ± S.D. (range).

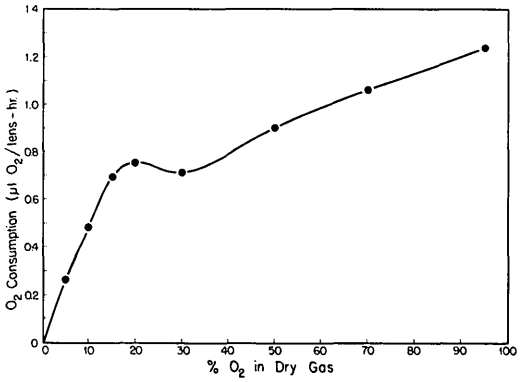

Fig. 1. Relationship between O_2 consumption of rat lenses and O_2 tension.

Studies on the autoxidation of cysteine and ascorbate were carried out in a closed system at room temperature. The electrode from the respirometer was placed in a chamber of about 0.5 ml. capacity with an inlet from a 10 ml. reservoir and an outlet closed by a stopcock. The chamber was initially filled with deionized water. One milliliter of neutral 0.05M cysteine hydrochloride or 0.01M ascorbic acid was combined in the reservoir with 4 ml. of 0.125M sodium phosphate buffer, pH 5 to 8. After aeration of the solution, 0.1 ml. of catalyst (0.01 mM. ferrous sulfate or cupric sulfate) was added and the solution run into the chamber. When necessary, concentrations were reduced proportionately so that the rate of oxygen utilization was always approximately linear in its initial portion. The rates were calculated in arbitrary units. In checking the final pH values on aliquots of solution, frequent restandardization of the meter was necessary because of the poisoning action of the cysteine.

Results

The oxygen uptake of rat lenses in different concentrations of oxygen is given in Table I. The relationship is shown graphically in Fig. 1. After an initially steep rise to a first maximum or plateau at 20 per cent O_2, there is a more gradual increase in the rate of oxygen consumption between 30 and 95 per cent O_2. It appears likely that the curve would continue to rise through oxygen concentrations above 1 atmosphere.

It was determined for every oxygen concentration used that the flow rate of medium in the respirometer was adequate for the measured rates of consumption to be maximal, i.e., flow independent. The critical volume flow rates[1] were all approximately 1 ml. per hour, and therefore the corresponding dissolved-oxygen flow rates were proportional to the oxygen content of the medium. Since the rate of uptake of oxygen by the lens is not proportional to concentration, the maximum allowable percentage utilizations of oxygen were smaller and the errors in measurement accordingly greater at the higher oxygen levels. For example, differences between inflow and outflow oxygen concentrations were 10 to 15 per cent with 20 per cent O_2 in the gas phase, but only 3 to 5 per cent with 95 per cent O_2.

Fig. 2 shows the manner in which the rate of oxygen consumption of the lens depends upon the pH of the medium when there is 20 per cent O_2 in the gas phase. The maximal rate of uptake is near pH 6, but the curve has a distinct shoulder in the range of pH 7 to 7.5. An attempt was made to determine the pH dependency of lens respiration in 95 per cent O_2 as well. However, largely because of the great error in measurements at this oxygen level, presentable data were not obtained.

The pH curves for the autoxidation of cysteine and ascorbic acid are shown in Fig. 3. The copper-catalyzed oxidation of ascorbic acid increases gradually with rising pH, as shown previously.[6] Oxidation of cysteine in the presence of copper has a

maximum near neutrality, whereas in the presence of ferrous iron the rate increases very sharply in the alkaline range. In the latter case, the rate of oxidation appeared to be proportional to the subjectively evaluated depth of color of the purple complex which forms upon addition of iron to cysteine.

Discussion

The biphasic curve relating oxygen uptake to the oxygen content of the medium (Fig. 1) probably represents the presence of two types of oxidatixe mechanism in the rat lens. Kleifeld and Hockwin[5] obtained very similar pictures for calf and cattle lenses in closed system analyses and proposed the same interpretation. It is based on the fact that cellular respiration ordinarily saturates at a particular oxygen tension (equivalent to only 0.2 per cent O_2 in the case of rat liver cells[7]) above which the rate of uptake is constant. The same pattern is found for tissue slices except that the critical oxygen tension is much higher, around 20 per cent O_2.[8] A presumably typical enzymatic component with just these characteristics can be picked out of the total curve for rat lens respiration shown in Fig. 1. The residual curve is a more or less linear relationship between uptake and oxygen concentration beginning at 20 to 30 per cent O_2. Its shape would not be especially different even if the supposed enzymatic component were depressed in the higher oxygen levels, as suggested by their apparent toxicity for human lens epithelium in culture.[9] The fact that the autoxidation rates of cysteine, glutathione, and ascorbic acid, all plentiful compounds in the lens, are directly proportional to oxygen concentration[10] suggests their identity with the linear component of lens respiration. Although Kleifeld and Hockwin[5] did not find differences in glutathione or ascorbate levels in cattle lenses cultured in 7 per cent and 95 per cent O_2, the difficulties in detecting the very small changes anticipated are such that the possibility remains.

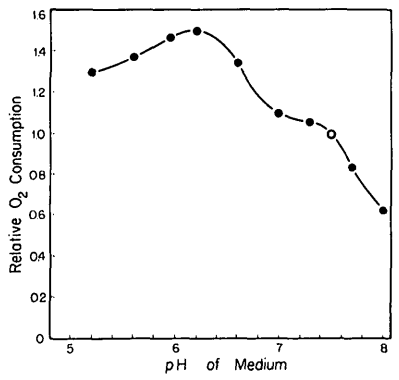

Fig. 2. Relationship between O_2 consumption of rat lenses and pH of the medium. Each solid symbol is the average O_2 uptake relative to that at pH 7.50 from determinations on at least 2 lenses.

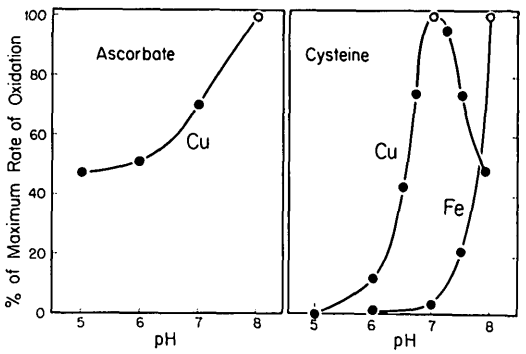

Fig. 3. Effect of pH on autoxidation of ascorbate and cysteine in solution relative to maximal rate observed in each case.

A similar analysis may be applied to the relationship of lens respiration to pH. The pH dependency curve typical of enzymatic respiration shows a maximum in the alkaline range[11] and is probably represented by the shoulder on the curve in Fig. 2. If a symmetrical curve with a maximum at pH 7.3 is subtracted from the relationship shown in Fig. 2, the residual curve indicates a reaction most active in the range below pH 7. It clearly does not correspond to any of the autoxidation reactions illustrated in Fig. 3. However, so many variables influence the effect of pH on thiol oxidation[12] that the cysteine curves at least are not necessarily representative of such reactions as they occur in the lens. It may well be that the phenomenon activated by acid medium is the release of autoxidizable

substrates or of heavy metal catalysts in a pattern overshadowing the pH dependencies of the autoxidations themselves.

Thus, lens respiration in vitro is probably compounded of a typical enzymatic mechanism (provisionally taken as the only component significant below 20 per cent O_2 and above pH 7) and potential autoxidation reactions brought into activity only under unusual conditions: in high oxygen tensions, in acid media, and upon injury to the lens by shaking[2] or boiling.[3] Only in the last instance is the oxygen uptake known to be catalyzed by heavy metals,[4] but this is probably true for all. The substrates are yet to be identified.

If one assumes that respiration is as efficient in energy production in the lens as in other tissues and that the conditions of respiratory measurement employed here are approximately normal, the importance in vivo of lenticular respiration relative to anaerobic glycolysis as an energy source may be estimated. At 20 per cent O_2, respiration accounts for less than 4 per cent of the glucose utilized by the lens.[1] Since the oxygen uptake is only 64 per cent as large in 10 per cent O_2 (the approximate equivalent of the oxygen tension in the rabbit aqueous[13]) it would consume less than 2.5 per cent of the total glucose utilization. The slight increase in glucose uptake due to a Pasteur effect[14] is neglected. According to current estimates, anaerobic glycolysis yields 2 moles and complete oxidation 38 moles of adenosine triphosphate from a mole of glucose. Hence, as a very conservative estimate, less than 33 per cent of the useful energy formation from glucose catabolism in the rat lens is attributable to respiration. The same figure is given by Kleifeld and Hockwin[15] for the rabbit lens.* Of course this fraction, although minor in the over-all economy of the lens, would be of great local significance if compartmentalized in the epithelium as other evidence strongly indicates.

*Based on yields of 4 and 36 moles of adenosine triphosphate by anaerobic glycolysis and complete oxidation, respectively. Recalculation from their data gives approximately 50 per cent of energy production as a result of respiration.

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Discussion

Dr. Jin H. Kinoshita (Boston, Mass.). Dr. Sippel is to be complimented for devising such an elaborate experimental setup to allow for measurement of the O_2 consumption of rat lens. The O_2 uptake of 0.75 μl per hour per rat lens can be transposed to 0.1 μl per hour per milligram dry weight. This value is very similar to that observed for rabbit lens of 0.09 μl per hour per milligram dry weight reported by Ely, who used the conventional Warburg technique. It appears that lens, regardless of species, has an extremely low O_2 uptake. Furthermore, as the author points out, the O_2 uptake of rat lens is even lower at the O_2 tension of the aqueous humor. From these observations Dr. Sippel has estimated that the biologic energy derived from respiration is less than 33 per cent of the total energy produced. In other words, according to his estimates, anaerobic glycolysis accounts for over 67 per cent of the energy available to the rat lens.

The determination of the contribution of respiration to energy production is an important aspect of lens metabolism. A definitive answer to the question of the degree of dependence of the lens upon oxygen is still not available. From a qualitative point of view, it appears that the lens depends primarily upon anaerobic glycolysis. We attempted to answer this question in experiments with calf lens. We found that although anaerobic glycolysis was able to supply all the energy necessary to maintain cation transport and amino acid incorporation—energy expending processes—there was also evidence indicating that limited amounts of energy were made available through the oxygen utilizing mechanisms. In bovine lens we estimated the energy produced from the aerobic and anaerobic phases of glucose metabolism from the amounts of CO_2 and lactic acid produced on incubation with C-14 glucose. Our estimates were similar to those made by Dr. Sippel. However, these estimates from the O_2 and CO_2 lactate data must be viewed with some caution since certain assumptions which may not be valid for the lens had to be employed.

I think it is very difficult to estimate the amount of energy produced through the aerobic mechanism from the oxygen uptake data. For one thing, as the author indicated, oxidizable components other than the cytochrome system may consume oxygen. Specific inhibitors may be used to aid in assessing the participation of the cytochrome system. Cyanide is most commonly used, but this inhibitor may not be helpful in lens since heavy metal catalysis of sulfhydryl oxidation is also inhibited by cyanide. Carbon monoxide is a specific inhibitor of cytochrome, and Dr. Sippel's experimental setup is ideally suited for testing this possibility. Information on cytochrome activity in lens would be extremely important.

I think the most direct way to assess the contribution of respiration to energy production is by the turnover rate of P-32 in ATP. Some experiments have been reported in which P-32 was used in lens studies, but not with this specific purpose in mind.

Dr. Sippel (closing). It may be misleading to compare the respiratory activity of the rat lens with the data obtained on rabbit lens by Ely with the manometric method. His oxygen uptakes are quite likely too high. The more reliable estimates recently obtained by the German workers, Hans, Hockwin and Kleifeld in 1955, are less than one third as great, thus placing the rat and rabbit lenses in the relationship expected for an avascular tissue: a lower respiratory activity correlating with the smaller relative surface area. I agree with Dr. Kinoshita that my estimate of the energy derived from aerobic metabolism is based upon several assumptions. I should reiterate, however, that it is a maximal estimate, and, as such, the figure of 33 per cent of the energy provided the lens attributable to respiration makes one wonder about the role of the hexose monophosphate shunt. If the shunt is important as an energy source, as Lerman proposes, it must be coupled to a critical reaction either through spatial cocompartmentalization or by some other means. Alternatively, it could provide not energy but a necessary intermediate. Dr. Kinoshita's suggestion concerning carbon monoxide is particularly interesting. I have recently submitted a proposal which includes study of the photo-reversal spectrum of CO-inhibited oxygen uptake of the rat lens, which should distinguish cytochrome-mediated respiration from autoxidations.