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GLYCOLYSIS IN THE CORNEA OF THE RABBIT

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Numerous investigators have observed that the excised cornea respires and that glycolysis takes place in both aerobic and anaerobic conditions. In particular, Kohra (1935), Fischer (1940) and deRoetth (1951) observed that in the absence of oxygen the rate of glycolysis increased in all corneal layers—the epithelium, the stroma and the endothelium. Further, they demonstrated that the collagenous stroma, comprising nine-tenths of the corneal mass, possessed a very low metabolic activity compared to the outer cellular layers. Kohra studied rabbits, deRoetth cattle, while Fischer did not state the species employed. All these authors used a manometric technique to determine glycolytic rates, and deRoetth in addition made simultaneous analyses of the rate of accumulation of lactic acid in the cornea using a chemical technique. His findings differed from earlier workers in one important respect in that he observed no glycolysis or lactic acid accumulation in the epithelium in the presence of oxygen.

An absence of lactic acid production in the epithelium in aerobic conditions is not readily reconciled with the observation that lactic acid exists in this layer in a concentration exceeding that in the stroma both in bovine and rabbit cornea (Herrman & Hickman, 1948; Langham, 1952). In an attempt to elucidate this problem the rate of glycolysis in the whole excised cornea of the rabbit has been determined and related to the rate of accumulation of lactic acid in aerobic and anaerobic conditions, and to the rate of glycolysis in the component layers determined by a manometric technique.

The supply of oxygen to the cornea presents special problems, and it is still uncertain whether the tissue can obtain sufficient to respire at a maximal rate. In an earlier investigation (Langham, 1952) it was observed that the concentration of lactic acid in the cornea of the rabbit decreased when the atmospheric oxygen tension was raised. The possibility that this may reflect a change in the rate of accumulation of lactic acid has now been investigated by comparing the rates at which lactic acid accumulates in the cornea of the living animal and in the excised tissue.

METHODS

In all experiments corneae were removed from adult rabbits of each sex immediately following inducement of a general anaesthesia by a solution of pentobarbitone sodium (Nembutal) given intravenously.

Determination of lactic acid. The rabbit cornea weighing approximately 70 mg was placed in 2 ml. of a boiling 8% solution of trichloroacetic acid. With the aid of a glass rod the cornea was broken up and filtered into a 10 ml. graduated flask. To ensure complete extraction of the lactic acid the precipitate was washed at least 3 times with small volumes of hot distilled water. To partially neutralize the acid extract, 0·5 ml. of 1·7 N-NaOH was added before making up to the final volume of 10 ml. A sample of this extract, containing approximately 30 μg of lactic acid (normally 4 ml.) was then treated according to the technique described by Barker & Summerson (1941). In this concentration range the accuracy of the method was 1–2%. Estimations, which include two standards (lithium lactate), were made immediately after the experiment.

Manometric measurements. Confusion has arisen previously in the interpretation of results on the cornea over the use of the term 'rate of glycolysis' which has sometimes been used to denote rate of lactic acid production, measured chemically, and sometimes, rate of acid production, measured manometrically (deRoetth, 1951; Kinsey, 1952). To avoid any confusion the symbol $q_{L.A.}$ will be used to signify the rate at which lactic acid (μg) accumulates in 1 mg of dry tissue per hour whilst the more conventional Q_G will be used to denote the rate of evolution of carbon dioxide (μl.) by 1 mg of dry tissue per hour in a bicarbonate buffer. The Warburg method described by Dixon (1951) was used to measure the rate of acid production. A Krebs-Ringer bicarbonate solution (Umbreit, Burris & Stauffer, 1949) to which was added glucose to a concentration of 100 mg/100 ml. was gassed with a mixture of 5% CO_2 and 95% N_2 or O_2 before being run into the flasks. The flasks each containing one cornea were gassed with the same mixture at 37° C for a period of 30 min; this period was found necessary to get maximal anaerobic effects.

Following the period of gassing the flasks were allowed to equilibrate. Manometric readings were begun 1 hr after the excision of the cornea and were taken at intervals of 15 min. To determine the rate of acid production aerobically it has been necessary to assume an R.Q. of unity (Duane, 1949; deRoetth, 1950). Direct manometric determination of the rate of acid production in the stroma was made after removal of the epithelial and endothelial cells by scraping. Because of their small mass and the ease with which metabolic changes occur when these tissues are damaged it was not possible to measure the epithelial and endothelial activity by this means. The activity of the epithelium was calculated from a comparison of the activity of the whole cornea with that of a second cornea, in which the epithelium had been scraped off and taken from the same animal. An approximate value for the endothelium was deduced from figures obtained for the activities of the epithelium, stroma and whole cornea. More accurate data for the endothelium could not be obtained because of its very small mass compared with the stroma.

Lactic acid accumulation in the excised cornea. Two corneae removed from the same animal were used to determine the rate of lactic acid accumulation in the excised tissue. The corneae were equilibrated with the appropriate gas phase for 60 min in Krebs bicarbonate buffer containing glucose (100 mg/100 ml.). One cornea with its suspending medium was then added to a solution of trichloroacetic acid and the second cornea treated similarly after a further time interval. The concentration of lactic acid in both corneae was then determined.

The estimation of the rate of accumulation of lactic acid in the excised cornea in the time immediately following excision of the cornea was made by placing the control cornea immediately into trichloroacetic acid solution. The second cornea was placed epithelial face upwards in a dry glass well in a flask containing a small volume of physiological saline. Substrate was unnecessary in this series of experiments and owing to the shorter experimental periods no suspending fluid was used. In this series of experiments the average weight of the corneae was 77·0 mg (mean of 68) and the ratio of the control to experimental corneae 1·007 (34).

RESULTS

The rate of accumulation of lactic acid in the component layers of the excised cornea. The rate of accumulation of lactic acid in aerobic and anaerobic conditions remained constant over an experimental period of at least 3 hr. The results recorded in Table 1 show that a maximal utilization of oxygen by the excised cornea leads to a decrease of approximately 70% in the rate of accumulation of lactic acid. Damage to the epithelial and endothelial cells leads to an increase in the rate of lactic acid production, but errors from this cause should be small for the epithelial and endothelial layers were examined and if found to be damaged after the experimental period the results were rejected.

TABLE 1. Glycolysis in whole excised cornea in N_2 or O_2. $q_{L.A.}$ represents μg lactic acid/mg dry wt./hr. Q_G represents μl. CO_2 evolved/mg dry wt./hr in Krebs bicarbonate medium. Duration of experiments 2 hr. $Q_G^{O_2}$ has been calculated on the basis of an R.Q. of unity. Results are expressed as arithmetic mean $\pm$ standard error of the mean.

	Chemical $q_{L.A.}$	Manometric Q_G
Anaerobic (95% N_2 + 5% CO_2)	10·5 ±0·97 (6)	2·62±0·12 (18)
Aerobic (95% O_2 + 5% CO_2)	2·76±0·20 (12)	1·43±0·15 (18)

TABLE 2. Acid production and respiration in the three main component layers of the cornea of the rabbit. Q_G represents μl. CO_2 evolved in Krebs bicarbonate buffer/mg dry wt./hr on the basis of an R.Q. of unity. Q_{O_2} represents μl. O_2 absorbed/mg dry tissue/hr (Q_{O_2} values taken from Langham, 1952). Values for the whole cornea and the stroma were obtained by direct measurement. Epithelial values were calculated from the difference in the activity of the whole cornea and a second cornea, whose epithelium had been scraped off. Endothelial values were computed from a comparison of grouped data on the whole cornea, epithelium and stroma. Results are expressed as arithmetic mean $\pm$ standard error of mean.

	Q_G *Aerobic*	Q_G *Anaerobic*	Q_{O_2}
Whole cornea	1·43±0·15 (18)	2·62±0·12 (12)	0·864±0·032 (27)
Epithelium	9·93±1·0 (6)	18·9 ±0·87 (6)	6·25 ±0·54 (6)
Stroma	0·38±0·04 (6)	0·51±0·05 (6)	0·23 ±0·04 (6)
Endothelium	7·04	17·7	5·7

Similar experiments on the component layers of the excised cornea were made using the manometric technique to determine the total rate of acid production; the results are recorded in Table 2. Values for the whole cornea and stroma were obtained by direct measurement, but values for the epithelium and endothelium had to be deduced indirectly. Thus the epithelial activity was calculated from the difference in the values found for the whole cornea and one from the same animal which had its epithelium scraped off; endothelial activity was computed from grouped data of results on the whole cornea, epithelium and stroma. It is apparent from Table 2 that the rate of acid accumulation in the epithelial and endothelial layers decreases greatly in the presence of oxygen. In the stroma, which has a low (Langham, 1952) or negligible respiratory activity (Duane, 1949) the change between the anaerobic and aerobic values is barely significant ($0{\cdot}1 > P > 0{\cdot}05$).

In using data from manometric observations it is to be noted that total acid accumulation may not be equivalent to the rate of lactic acid accumulation, and in consequence experiments were made to investigate this question. At 37° C and 1 atm. pressure 1 μl. of carbon dioxide is equivalent to 3·9 μg lactic acid. The values found for the whole cornea are included in Table 1 and showed that in anaerobic conditions the total acid accumulation was equivalent to the accumulation of lactic acid. In contrast, when respiration was maximal the amount of lactic acid accumulated accounted for only half the total acid, assuming that the R.Q. of this tissue is unity (Duane, 1949; deRoetth, 1950).

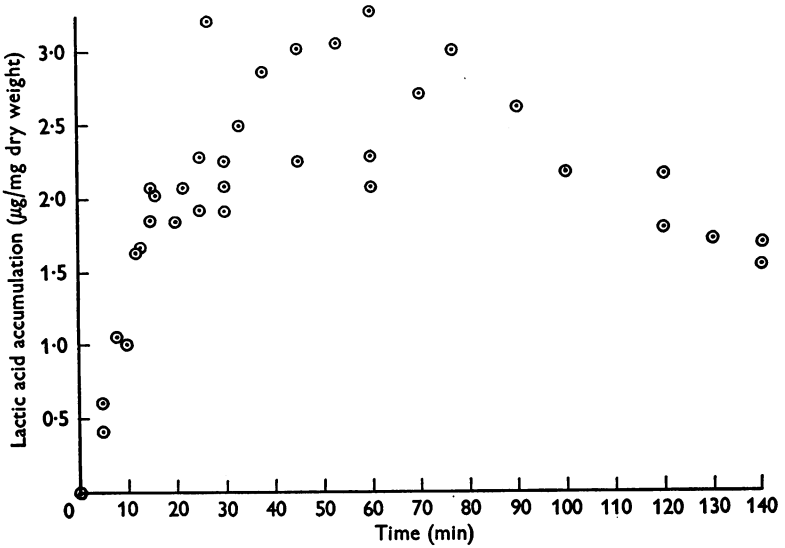

Fig. 1. The accumulation of lactic acid in the freshly excised cornea. Each point has been calculated from the difference in the concentration of lactic acid in the control cornea analysed at time of excision and the experimental cornea analysed T min after excision from the animal.

The rate of accumulation of lactic acid in the freshly excised cornea. Corneae removed from the two eyes of the living animal were found to contain equal concentrations of lactic acid, a ratio of $1{\cdot}00 \pm 0{\cdot}019$ being observed in a series of eight rabbits. The relation between the concentrations of lactic acid in the excised cornea and time will be determined by the nature of the atmosphere, the availability of substrates, and possible metabolic changes within the cornea; at the beginning of the experiment these factors would be minimized. The increase in concentration of lactic acid in the freshly excised cornea in humid air at 37° C is recorded in Fig. 1. Each point represents the difference in concentration of lactic acid between the two corneae of the same animal, and as this is zero at the moment of excision the relation must pass through the origin. For small time intervals the points are best represented by a

straight line passing through the origin. The slope of this line is a measure of the increase in lactic acid concentration in the cornea and a value of $0{\cdot}121 \pm 0{\cdot}0087$ (A.M. $\pm$ S.E.) μg lactic acid per mg dry weight per min was calculated for the six values from $T = 0$ min to $T = 12$ min. The slope begins to decrease beyond this point, and it was therefore considered inadvisable to use values greater than 12 min to obtain a more accurate estimate of the slope in the initial period.

The decrease in the rate of accumulation of lactic acid to zero in approximately 60 min is in agreement with the observation of numerous workers using the manometric technique that no glycolysis occurs in the excised cornea in the absence of substrate. Utilization of lactic acid by the excised cornea was investigated by prolonging the experimental period beyond the time at which accumulation of lactic acid ceased. The results for experimental periods up to 140 min are included in Fig. 1 and are suggestive that lactic acid may be utilized in the excised cornea. However, in similar experiments in which the cornea was maintained sterile for 24 hr the concentration of lactic acid still exceeded that in the control cornea.

DISCUSSION

The rates of glycolysis in the whole excised cornea of the rabbit in aerobic and anaerobic conditions agree with those reported by Kohra (1935). Similarly, the observation that the most active metabolism occurs in the epithelial and endothelial layers agrees with the same author but differs as to the absolute values recorded. This discrepancy could be readily explained if the epithelial and endothelial layers were assumed to occupy a greater corneal volume than actually is the case for it is necessary to know these to calculate their activities. Unfortunately, Kohra did not state how these were determined.

It is of interest that there are certain species differences in respect of corneal metabolism. deRoetth (1951) determined the rate of accumulation of lactic and total acids in excised bovine corneae, and concluded from his observations that the epithelium had no aerobic glycolysis. This contrasts with the present findings and those of earlier workers that aerobic glycolysis takes place in the corneal epithelium of the rabbit. Again working on bovine cornea Herrman & Hickman (1948) concluded that the oxygen uptake of the cornea was sufficient for the complete oxidation of the carbohydrate utilized, and that oxidation of lactic acid took place only in the presence of the epithelium. In rabbit corneae there can be little oxidation of lactic acid since the production of $7{\cdot}26$ μg per mg dry tissue per hr would necessitate an uptake of $6{\cdot}7$ μl. of oxygen for complete oxidation, whereas the maximal uptake of oxygen is $0{\cdot}86$ μl. per mg dry tissue per hr.

The conclusion of deRoetth (1951) that no aerobic glycolysis occurs in bovine corneal epithelium is not readily reconciled with the observation of

Herrman & Hickman (1948) that the concentration of lactic acid in the epithelium of bovine corneae exceeds that in the stroma, unless an active transfer of lactate from the stroma to the epithelium occurs, as suggested by Friedenwald (1948). In rabbits the concentration of lactic acid in the epithelium is greater than in the stroma (Langham, 1952), but this is readily explicable from the observation that lactate production occurs within the layer.

In the absence of an appreciable loss of lactic acid by oxidative or non-oxidative metabolism a substantial fraction of the lactate ions produced in the cornea must pass into the surrounding tissues. Diffusion across the corneal scleral junction into the capillaries will be limited by the small area of contact, and the adverse concentration gradient existing between the scleral tissue surrounding the corneal scleral junction and the stroma (Langham, 1952). Diffusion through the epithelium is likely to be small in view of its keratinized multicellular structure and its high content of lactic acid. In contrast, the concentration of lactic acid in the aqueous humour is well below that in the stroma and exchange here will be limited only by the properties of the thin unicellular layer of flattened endothelium.

The exchange across the endothelial barrier may be represented by the equation

$$\frac{dC_s}{dt} = \frac{AK_{ac}}{M_s}\left[\frac{C_s - C_a}{r_{sa}}\right],$$

where dC_s/dt is the rate of production of lactic acid in the stroma in unit time, M_s is the weight of the stroma, A is the superficial area of the stroma and C_s and C_a are the concentrations of lactic acid in the stroma and aqueous humour respectively. This equation is analogous to that used by Maurice (1951) in his study of the permeability to sodium ions of the living rabbit cornea. All the terms are known except r_{sa}, the steady state ratio of the activities of the lactate ion between the aqueous humour and the stroma; however this may be expected to be approximately the same as chloride, namely 0·7 (Davson, 1949). Applying this data K_{ac} for the lactate ion has been evaluated and found to be within the limits of 0·069 and 0·075 cm.hr^{-1} which agrees with the value of 0·072 cm.hr^{-1} found for the sodium ion by Maurice (1951). This result was to be expected if all the lactate diffuses across the endothelial barrier as Maurice (1953) has shown that its permeability to all the ionized substances of small molecular weight that have been measured is of the same order of magnitude.

It is difficult to say why the rate of accumulation of lactic acid in the freshly excised cornea ($q_{\text{L.A.}}$ 7·26) is higher and lower than in the excised tissue bathed in Krebs phosphate in presence of oxygen ($q_{\text{L.A.}}$ 2·76) and nitrogen ($q_{\text{L.A.}}$ 10·7) respectively. The possibility that the cornea of the rabbit produces lactic acid

at a high rate because of an insufficient supply of oxygen to maintain a maximal respiration is in agreement with the observations of Langham (1952), that an increased oxygen tension in the tear fluid leads to a decreased concentration of lactic acid in the cornea. On the other hand, it is quite possible that the rate of production of lactic acid in the excised cornea immersed in a Krebs phosphate medium does not reflect the true limits of glycolytic activity in the cornea of the living animal for it is well known that the limiting layers of the cornea are very readily injured. Similarly, the decrease in concentration of lactic acid in the cornea of the living animal in presence of excess oxygen may result from a changed diffusion gradient across the endothelial-aqueous humour barrier.

A definite answer to this problem will, it is hoped, be obtained by the more direct approach of measuring the rate of passage of oxygen across the cornea (Heald & Langham, 1953).

SUMMARY

1. The rates of aerobic and anaerobic glycolysis in the component layers of the excised cornea have been measured, both aerobic and anaerobic activity having been observed in all three layers. In the stroma the rate of acid production is unaffected by oxygen tension in contrast to the marked changes found in the epithelium.

2. In the whole excised cornea the rate of acid production in anaerobic conditions was found to be equivalent to the rate of accumulation of lactic acid. In contrast in aerobic conditions accumulation of lactic acid accounted for only half the total acid formed.

3. The rate of accumulation of lactic acid in the whole excised cornea has been found to decrease 74% in the presence of an atmosphere of oxygen.

4. The rate of accumulation of lactic acid in the cornea of the living rabbit has been estimated from the rate at which this acid accumulates in the excised tissue immediately following excision and found to exceed that in the excised cornea in aerobic conditions. The reasons for this difference are discussed.

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