METABOLISM OF THE DEVELOPING RETINA*

I. AEROBIC AND ANAEROBIC GLYCOLYSIS IN THE DEVELOPING RAT RETINA

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IN recent years considerable interest has been focused on the role of oxygen in the genesis of retrolental fibroplasia, and careful control of ambient oxygen concentration has led to a considerably reduced incidence of this disease in premature infants. Much of the work which led to the ultimate elucidation of this problem and the demonstration that hyperoxia induced vaso-obliteration in the immature retina has been reviewed elsewhere (Ashton, 1957).

Although the effect of hyperoxia is now recognized as a practical phenomenon little is yet understood of the mechanism of the resulting vaso-obliteration, and why it should be confined to the immature retina. The importance of this distinction has been underlined by a recent report in which it has been demonstrated that intravitreal injection of the kitten with sodium fluoride or sodium iodoacetate produces total vaso-obliteration of the retinal vessels in a manner closely resembling that induced by oxygen exposure and that once again this effect is confined to the immature retina (Ashton, Graymore, and Pedler, 1957).

There is evidence to suggest that sodium fluoride inhibition promotes retinal oedema both *in vitro* (Graymore, 1958a, 1959), and *in vivo* (Pedler, 1958), and it has been tentatively suggested that external pressure resulting from this swelling may be a causative factor in chemically induced vaso-obliteration (Ashton and others, 1957). Whatever the actual underlying cause may be, the differing reaction of immature and mature retinae both to oxygen and metabolic inhibitors suggests some basic distinction between the two retinae, either structurally or metabolically or both. The present report is designed to indicate one aspect of retinal metabolism which exhibits a changing pattern during development.

Methods and Materials

The animals used in these investigations were female albino rats which had been maintained on a standard diet used in this laboratory (Bruce and Parkes, 1946). The ages were those quoted in the text.

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Removal of Retinae.—The animals were killed by a blow on the head followed by dislocation of the cervical vertebrae. Very young animals were killed by decapitation. The eyes were removed as rapidly as possible and the retinae dissected out according to the procedure reported previously (Graymore, 1958a).

Delay Periods.—The interval between killing the animal and commencing the incubation may be considered as two distinct periods. The first of these spans the interval between the death of the animal and the immersion of the isolated retina in chilled buffer. The second is the time which elapsed before the incubation was begun.

Preliminary investigations established that time intervals of 5 minutes in the former and 15 minutes in the latter did not result in any significant loss of activity, but nevertheless, these delay periods were made as brief as possible and kept well within the above limits. In the experiments reported here, the time interval between killing the animal and immersing the retina in chilled buffer was approximately 2 minutes, and in the aerobic determination each pair of retinae was taken through the entire procedure separately to avoid any delay in the second period.

Condition of the Retinae.—In the case of animals aged 7 days and above, only entire and intact retinae were used, but in the case of the very young animals (3 to 6 days) there was a tendency to slight fragmentation as a result of the extreme friability of the tissue combined with the difficulties inherent in the removal of the retinae from very small eyes (approximately $\frac{1}{8}$ " diameter).

It was found that the danger of fragmentation was greatest whilst transferring the retinae from one vessel to another and that this could be reduced considerably by employing a wide-nozzle pasteur pipette for this purpose. Experiments on whole retinae and on retinae which had been deliberately fragmented to an extent greater than that normally encountered showed that the average glycolytic rate of the latter was 97.1 per cent. of that of the former.

Incubating Medium.—After removal and during measurements the retinae were maintained in standard Krebs-Ringer-bicarbonate buffer (Krebs and Henseleit, 1932) fortified with 200 mg. per cent. glucose, and gassed with either 5 per cent. CO_2 -95 per cent. O_2 , or 5 per cent. CO_2 -95 per cent. N_2 according to the conditions required. Before incubation the buffer was gassed continuously and maintained in vessels packed with broken ice.

Period of Incubation.—All measurements were taken over an incubation period of 30 minutes, it having been established that under the conditions described the glycolytic rate was linear up to periods of at least one hour.

Measurement of Aerobic Glycolysis.—At zero time the retinae were dropped into 10 ml. incubating medium contained in 25-ml. capacity conical flasks immersed in a bath thermostatically controlled at 37°C. 5 per cent. CO_2 -95 per cent. O_2 was bubbled continuously through the medium at a rate sufficient to maintain the pH and provide optimal agitation for the flask contents. (Violent bubbling tended to produce tissue fragmentation.) After 30 minutes, 2 ml. 10 per cent. trichloroacetic

acid were added, and the flask was shaken and removed from the bath. The contents of the flask were centrifuged and 4 ml. of the clear supernate withdrawn for lactic acid assay (Barker and Summerson, 1941). The precipitated protein was retained for N_2 estimation. In the case of animals aged 20 days and over, one retina per flask was used. Two to three retinæ per flask were used from 12- to 14-day animals, and four to six retinæ per flask from the very young animals.

Measurement of Anaerobic Glycolysis.—Anaerobic conditions were obtained by gassing solutions with 5 per cent. CO_2 –95 per cent. N_2 . Lactic acid production was estimated by measuring the equivalent quantity of CO_2 liberated from the bicarbonate buffer by the acid, the volume of gas being determined by the classical Warburg manometric technique. The Warburg manometers and flasks were gassed for 15 to 20 minutes before readings were begun to ensure that true anaerobic conditions were established. Preliminary experiments were carried out in which both CO_2 evolution and actual lactic acid determinations were performed, and excellent correlation was obtained, thus demonstrating the adequacy of CO_2 evolution as a criterion of lactic acid production.

One retina per flask was employed for all experiments except those involving very young animals (3 to 6 days) when two to three retinæ were employed.

Estimation of Tissue.—The quantity of tissue was assessed as mg. protein calculated from the nitrogen content determined by the micro-Kjeldahl procedure.

Expression of Results.—All measurements of lactic acid production were expressed as the equivalent volume of CO_2 which would be liberated from bicarbonate solution by that quantity of acid. The final result is expressed as the volume of gas in micro-litres liberated in one hour by 1 mg. tissue protein at N.T.P. the symbol $Q_G^{O_2}$ (pr.) denoting aerobic glycolysis, and $Q_G^{N_2}$ (pr.) anaerobic glycolysis.

Results and Discussion

Figs 1 and 2 and Tables I and II show that the glycolytic rate of the rat retina is considerably increased during the second week of life, the activity of the retinæ from animals which had reached their weight plateau (3 to 4 months) being more than twice that of retinæ from animals in their first week of life.

FIG. 1.—Variation in the rate of anaerobic glycolysis in the rat retina during development.

TABLE I
ANAEROBIC GLYCOLYSIS IN DEVELOPING RAT RETINA

Age (days)	No. of Determinations	$Q_{\text{G}}^{\text{N}_2}$ (pr.)
3	3	28.5 ± 3.62
6	2	28.9 ± 4.30
8	5	29.0 ± 2.07
10	2	26.1 ± 2.25
13	2	40.4 ± 2.40
14	2	50.5 ± 6.45
16	1	48.3
29	3	47.4 ± 3.73
3-4 mths	7	59.1 ± 3.10

FIG. 2.—Variation in the rate of aerobic glycolysis in the rat retina during development.

TABLE II
AEROBIC GLYCOLYSIS IN DEVELOPING RAT RETINA

Age (days)	No. of Determinations	$Q_{\text{G}}^{\text{O}_2}$ (pr.)
5	2	9.6 ± 2.90
7	2	9.8 ± 0.10
12	3	12.7 ± 2.01
14	3	17.4 ± 1.87
20	3	21.5 ± 1.85
31	2	21.1 ± 0.40
3-4 mths	5	24.7 ± 1.46

The present results have been calculated on a mg./protein basis Q_{G} (pr.) instead of the more conventional mg./dry-weight basis, Q_{G} . Preliminary analyses indicated that protein accounted for approximately 85 per cent. of the total dry weight, so for purposes of comparison it should be remembered that $Q_{\text{G}} \equiv 1.176 \times Q_{\text{G}}$ (pr.).

Bearing this in mind, the anaerobic glycolytic rate of the mature retinae ($Q_{G^2}^N(\text{pr.}) = 59.1$, corresponding to $Q_{G^2}^N = 50.2$) is slightly lower than that of Dickens and Simer (1929) and Campos (1936), who recorded $Q_{G^2}^N$ values of 64.2 and 57.0 respectively, and considerably lower than that of Warburg (1927), who obtained a $Q_{G^2}^N$ of 88. The corresponding value for aerobic glycolysis ($Q_{G^2}^O(\text{pr.}) = 24.7$, corresponding to $Q_{G^2}^O = 21.0$) is much lower than that obtained by Warburg in the same work (45), but agrees well with that of Campos (21.8). Warburg and his associates have suggested that aerobic lactic acid production may be an artefact due to traumatic uncoupling of glycolysis and respiration, and if this is so it may well account for variations in levels according to isolation techniques and suitability of the incubating medium (Warburg, Posener, and Negelein, 1924; Warburg, Garwehn, and Geissler, 1957).

It is of particular interest that the sudden stepping-up of activity occurs around the time at which the eyes open (about 12 to 13 days), and one is tempted to suggest that it is a response to an increased energy demand coincident with the appearance of electrical activity in the retina. Zetterström (1956), investigating electrical activity in kittens, found that there was no electroretinogram during the first 5 days of life, but that a measurable electroretinogram appeared between 6 to 10 days. On the other hand, a litter of blind 12-day-old rats was kept in total darkness for 3 days and decapitated in the dark at 15 days of age. The retinae were removed in the light and found to have a mean $Q_{G^2}^N(\text{pr.})$ of 54.7; it is difficult to reconcile this with the above speculation, but more experiments are required to confirm this single observation (Graymore, 1958c). The alternative is to assume that the increased glycolytic rate is coincident with but not dependent on light stimulation. It is of interest in this respect that Zetterström (1956) found that kittens bred in darkness did develop a normal electroretinogram although its appearance was considerably delayed.

Histological evidence suggests that the rat retina is fully developed by about 17 days of age, and the results of the present investigation indicate that biochemical maturity may be reached about this time. Histological examination of retinae taken from rats of the same strain as those used in this investigation indicates that the outer limbs of the rods commence to differentiate at about 10 days. Intravenous injection of suitable doses of iodoacetate, a powerful inhibitor of glycolysis, produces a progressive degeneration of the rod cells, and Noell (1952) believes that glycolysis may have some special significance in these cells.

The general histological presentation, at the time at which the surge in glycolytic activity occurs, is one of preparation for vision. The occurrence of visual purple follows closely on the development of the outer limbs (Tansley, 1933) and Crozier and Pincus (1927) reported that young rats

were negatively phototropic 8 days after birth. It seems likely that the present results depict a similar immediately pre-visual change.

If the two curves showing the changing pattern of aerobic and anaerobic glycolysis were superimposed, they would be seen to follow each other very closely; assuming that aerobic lactic acid production reflects the relationship between glycolytic and respiratory processes, this fact suggests that respiration may show a similar increase at the same time. Aerobic glycolysis has, however, been measured *in vitro* under conditions favouring maximal respiration, whereas *in vivo* the picture is complicated by a changing pattern of vascularization, and hence of oxygen supply. Partial support for the suggestion that the present observations reflect the actual changes in activity in the living animal, as opposed to the potential activity dependent on adequate oxygenation, has been obtained by rapid freezing studies on the kitten retina (Graymore, 1958b), in which it was demonstrated that the lactic acid concentration increased during the first 3 weeks of life, after which time a plateau was reached.

Summary

(1) The aerobic and anaerobic glycolytic rates of rat retinæ have been determined at various stages of development ranging from 3 days to 3 to 4 months.

(2) In both cases there is a dramatic increase of activity during the second week of life. The possible significance of this is discussed.

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