The Metabolism of Glucose by the Rabbit Lens in the Presence and Absence of Oxygen

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1. The effect of the absence of oxygen on the metabolism of [U-¹⁴C]glucose by the rabbit lens has been examined. 2. Protein-free extracts of incubated lens were subjected to electrophoresis followed by chromatography on paper; radioautographs showed 10 compounds substantially labelled. In the absence of oxygen, less radioactivity appeared in glucose, sorbitol, fructose, alanine, glutamic acid, glutathione and nucleotides, and more in lactic acid, α -glycerophosphate and glycerol. 3. The radioactivity of lens protein was about two to four times greater after aerobic than after anaerobic incubation. 4. The radioactivity of carbon dioxide was 2- to 4-fold greater after aerobic incubation than after anaerobic incubation. 5. After aerobic incubation of the lens, the radioactivity of all the labelled compounds was higher in the outer part (cortex) than in the inner part (nucleus). 6. Free glycerol has been found to be present in the lens. Its concentration, and that of α -glycerophosphate, has been measured in the lens of several species of animal.

The lens is an avascular organ; the slowly circulating aqueous humour which bathes its front surface is the source of its raw materials and the route by which waste products are removed. At least three-quarters of the glucose it consumes *in vitro* is converted into lactic acid. Its consumption of oxygen (dissolved in the aqueous humour) is small, amounting to about 0.35 μ mole/g. of adult rabbit lens/hr. at 37° (Hans, Hockwin & Kleinfeld, 1956). Glucose consumption over the same period is about 1.0 μ mole/g. of lens (Wachtl & Kinsey, 1958).

Although the lens in organ culture consumes oxygen, it has not been proved that oxygen is necessary for maintenance of clarity *in vivo*. The absence of oxygen has not been shown to cause opacity (cataract) in cultured lens; severe anoxia can cause cataract in rats *in vivo* (Bellows & Nelson, 1944), but this is probably due to the increase in acidity in the aqueous humour.

The metabolic reactions in the lens in which oxygen participates have not been elucidated. The contribution of the citric acid cycle is small (Kinoshita & Wachtl, 1958). A number of comparisons between various aspects of the metabolism of the lens in the presence and absence of oxygen have been made. It has been shown that anoxia results in an increased production of lactate from the calf lens (Kern, 1962), an increased concentration of α -glycerophosphate within the bovine and rabbit lens (Pirie, 1963) and abolition of the oxidation by

calf lens of C-2 and C-3 of pyruvate (Kinoshita & Merola, 1961). Mamo & Leinfelder (1958*a,b*) have found that lens cells in culture accumulate globules of fat if incubated anaerobically. On the other hand, anoxia did not affect the concentration of ATP, ionic balance or incorporation of labelled arginine into protein in the calf lens incubated in a medium containing glucose (Kinoshita, Kern & Merola, 1961); only in the absence of glucose could the effect of oxygen be observed. Oxygen did not seem to be necessary for the concentration of hydroxyproline by the calf lens (Kern, 1962), though it somewhat enhanced the concentration of α -aminoisobutyric acid by the rabbit lens (Kinsey & Reddy, 1963).

The present experiments were undertaken to find out more about the oxidative side of lens metabolism. Rabbit lenses were used, instead of the more easily obtained bovine lenses, because the results could be more directly applied to any experiments *in vivo* which might be indicated. The metabolism of the bovine and rabbit lens differs in some respects (van Heyningen, 1958, 1959*a*). Old animals were used because there is some suggestion that the lens (bovine) from older animals depends more on oxidative metabolism for its energy (Kinoshita & Dillen, 1958), and also because interest in lens metabolism is geared to some extent to the study of cataract in man, of which senile cataract is much the most common form. Although little is known about the metabolism of

the human lens, it was thought that the use of the non-cataractous lens of the old animal might yield results more relevant to the study of human senile cataract than that of the younger animal more commonly used.

EXPERIMENTAL

Materials. Uniformly labelled D-[U- ^{14}C]glucose was obtained from The Radiochemical Centre, Amersham, Bucks. The contents of an ampoule (0.1mc) were dissolved in 0.5 ml. of water containing an amount of glucose calculated to bring the total amount to 30 mg.; the specific activity of the stock solution was therefore 3.3 $\mu\text{C}/\text{mg.}$ of glucose. It was examined by radioautography (see the Methods section) and contaminants were found to account for less than 1% of the radioactivity.

Animals. Rabbits, of various breeds, and usually 2–4 years old, were used. They were killed with intravenous Nembutal and the lenses were removed immediately. All experiments were done with rabbit lens, unless otherwise stated.

Incubation tubes. These were similar to those described by Merriam & Kinsey (1950a). They were modified by the addition of a bulb at one end into which a piece of yellow phosphorus could be introduced to remove the last traces of oxygen when anaerobic conditions were required. A bulb to contain NaOH could also be attached at the other end, for the collection of $^{14}\text{CO}_2$. Dr J. Kinoshita kindly supplied the prototype.

Incubation medium. This was freshly prepared as follows. TC199 (reconstituted from the dried form, Difco Laboratories Inc., Detroit, Mich., U.S.A.) (7.5 ml.) was diluted with NaHCO_3 (2.52%, w/v, gassed with CO_2 ; 1.5 ml.), antibiotics (sodium benzylpenicillin and streptomycin, 10 mg. each/ml.; 0.1 ml.), D-[U- ^{14}C]glucose (0.1 ml. containing 6 mg. of glucose; 20 μC) and water to 10.5 ml. As the TC199 solution contains 1 mg. of glucose/ml., the glucose content of this incubation medium is 13.5 mg./10.5 ml. A portion (5 ml.) of the medium was taken for each incubation, one aerobic and one anaerobic; the concentration of glucose was increased by addition of small volumes of glucose (10%, w/v), usually to bring the final concentration of the aerobic incubation to 7.6 mg. of glucose/5 ml. (8.45 mm) and the anaerobic incubation to 8.5 mg./5 ml. (9.45 mm).

TC199 is a synthetic medium, containing salts, glucose, amino acids, vitamins and other low-molecular-weight compounds; it was designed for use in place of serum ultrafiltrate in tissue culture (Morgan, Morton & Parker, 1950; Morgan, Campbell & Morton, 1955) and has often been used for studying the metabolism of the explanted lens.

Methods

Incubation of lens. Each lens of a pair was incubated in 5 ml. of medium, contained in incubation tubes, gently shaken in a water bath at 34°. One was gassed with air + CO_2 (95:5, v/v) and the other with N_2 + CO_2 (95:5, v/v), the gas mixtures being first passed through water at 34°. Gassing was for at least 30 min. before, and 30 min. after, introduction of the lens. Finally, a stick of yellow phosphorus was placed in the bulb of the anaerobic incubation tube, and both tubes were firmly stoppered with rubber bungs. Incubation was for 20 hr.

Treatment of lens and medium after incubation. The lens and medium were tipped into a shallow dish and examined for clarity. Normally both were clear. Bacterial infection, which occasionally occurred, was easily detected, both because the medium became cloudy and because there was a loss of total radioactivity. Metabolism of the lens itself produces so little CO_2 that there is no measurable decrease of total radioactivity in 20 hr. from the lens plus medium. The medium was transferred to a glass-stoppered tube, and the lens was briefly washed with a few millilitres of 0.9% (w/v) NaCl. The last traces of washing fluid were removed with scraps of filter paper without touching the lens, which was then dropped into a weighed centrifuge tube containing 5 ml. of 10% (w/v) trichloroacetic acid. The tube was reweighed, to give the weight of the lens. The lens was ground in the acid and, after chilling for 30 min., the tube was centrifuged at 4°, 7000g, for 15 min. The lens extract and precipitate were separated.

Preparation of lens extract for chromatography. The trichloroacetic acid extract of lens was extracted twice with 5 ml. of *N*-methyldiethylamine in water-saturated CHCl_3 (5%, w/v) (Hughes & Williamson, 1951). This extracted the trichloroacetic acid and brought the pH to about 6. After removal of a sample for determination of radioactivity, the volume of the remaining extract was measured, so that the weight of fresh tissue it contained could be calculated. It was then dried *in vacuo* and the residue dissolved in water (usually 3 mg. of fresh tissue/ $\mu\text{l.}$ of water) and stored at -20° .

Preparation of lens protein. The trichloroacetic acid precipitate was dissolved in *N*-NaOH (3.0 ml.), left for 30 min. at 37° and then reprecipitated with trichloroacetic acid (30%, w/v; 3.6 ml.). The purpose of this treatment was to remove amino acyl-RNA. The precipitate was then washed on the centrifuge at 4° three times with trichloroacetic acid (5%, w/v) followed by acetone and ether; its radioactivity was then determined.

Collection of $^{14}\text{CO}_2$. $^{14}\text{CO}_2$ was collected in NaOH (30%, w/v; 0.5 ml.) injected in the attached bulb through a rubber cap, at the end of incubation. Trichloroacetic acid (30%, w/v; 1 ml.) was gently blown into the incubation tube from the other end, the stopper being removed for as short a time as possible (about 5 sec.). The tubes were shaken for a further 4 hr. The NaOH containing $\text{Na}^{14}\text{CO}_3$ was removed from the bulb by means of a syringe, $\text{Ba}^{14}\text{CO}_3$ was precipitated, washed and the radioactivity measured by the method of Abraham & Hassid (1957).

The contents of the incubation tube were transferred to a centrifuge tube, the lens was ground, and the tube was then chilled and centrifuged. Radioactivity was measured in the supernatant (lens extract plus medium) and the protein precipitate was treated as described above.

Phospholipids. The trichloroacetic acid precipitates, from two lenses incubated aerobically, were pooled and those from the parallel anaerobic incubations also pooled. The precipitates were washed with water three times, stirred with chloroform-methanol (1:2, v/v; 20 ml.) for 1 hr. and then left overnight. This was followed by centrifugation and re-extraction with a further 20 ml. of chloroform-methanol. The extracts were combined, evaporated to 5 ml. at 30° in a rotary evaporator, left overnight and clarified by centrifuging. The resulting extracts were dried, subjected to mild alkaline hydrolysis and examined by electrophoresis and chromatography (Dawson, 1960).

The only modifications were the substitution of electrophoresis at pH 6 for that at pH 3.6, and the electrophoresis preceding instead of following chromatography. Radioautography was used to detect incorporation of ^{14}C into the hydrolysate of lens phospholipids.

Measurement of radioactivity. An end-window Geiger-Müller counter was used. Corrections were made for background, and a self-absorption correction curve of $\text{Ba}^{14}\text{CO}_3$ was made. Medium (0.02 ml.), lens extract (0.05 ml.) and protein (about 4 mg.) dissolved in formic acid (98–100%) were counted at 'infinite thinness' on 4 cm.² planchets. When extracts of lens before removal of trichloroacetic acid were counted, 0.02 ml. of a solution of N-NaOH (found sufficient to make 0.05 ml. of extract about pH 9) was added to the planchet. A comparison of the specific activities of the protein after aerobic and anaerobic incubations was also made by counting the protein at 'infinite thickness' (Waley, 1964).

Radioautography. This was on Kodak Royal Blue X-ray film, size 10 in. $\times$ 12 in. Films were usually left in contact with chromatograms for 2 weeks. Radioactive spots on the chromatogram detected in this way were cut out, counted on both sides and the average value was taken. This method was used to compare the relative radioactivity of a number of spots on the same paper. Afterwards the compounds were often eluted from the spots and subjected to further tests for identification.

Paper electrophoresis and chromatography. This was usually on Whatman no. 52 paper. Lens extracts and medium, after incubation, were examined by paper electrophoresis and chromatography followed by radioautography. Electrophoresis was performed in a ridge-pole apparatus at 10 v/cm., usually for 3 hr. The buffer was pyridine (2.5%, v/v)-acetic acid (0.25%, v/v), pH 6 (Runeckles & Krotkov, 1959). Chromatographic solvents used were: (A) butan-1-ol-acetic acid-water (40:9:20, by vol.); (B) phenol-water (25:7, w/v); (C) ethanol-aq. NH_3 soln. (sp.gr. 0.89)-water (16:1:3, by vol.); (D) ethyl acetate-pyridine-water (12:5:4, by vol.); (E) ethyl methyl ketone-acetic acid-water satd. with boric acid (9:1:1, by vol.).

For initial comparisons of lens extract after incubation, 10 μl . (25 or 30 mg. of lens) was subjected to electrophoresis for 3 hr., followed by chromatography in solvent (A) in a direction at right angles to the electrophoresis. These papers are referred to here as 'two-way papers'. Since the distance which the solvent could be allowed to run was limited to just under 30 cm. by the size of the X-ray film, the papers were developed in the solvent two or three times, and dried in air overnight after each run. This results in a better separation of the spots than one run of two or three times the distance. Markers were run in each direction. Medium (10 μl .) was subjected to the same procedure.

On some occasions the lens extract was applied to the paper in the form of a narrow streak; this was submitted to electrophoresis followed by radioautography. The various labelled bands which appeared were then eluted and examined separately, or cut out and sewn into a sheet of paper for chromatography. To establish the identity of labelled compounds in eluates, samples were subjected to electrophoresis or chromatography together with known compounds, which could be revealed by the sprays or dips given below. The method of 'finger-printing' (Moses, 1960) was used.

Incubation of the lens with labelled products of [^{14}C]-

glucose metabolism. To investigate the products of metabolism of some of the compounds, found to be labelled in the lens after incubation in [$\text{U-}^{14}\text{C}$]glucose, the following method was used. The labelled compound was eluted from several spots on 'two-way papers', or from labelled bands produced by electrophoresis or chromatography of strips of extract or eluate. The eluates were combined, divided equally between two centrifuge tubes, and evaporated to dryness in a desiccator. A rabbit lens was ground with water (0.5 ml. or less). Sometimes glucose (10%; 0.02 ml.) was added. The lens dispersion was divided into two parts: one part (control) was incubated without further addition; the other part was transferred to the tube containing half the eluted compound. Both parts were incubated for 3 hr. at 37°; trichloroacetic acid (6%; 1.0 ml.) was then added to each part, and the control mixture transferred to the tube containing the other half of the eluted compound. (The reason for this procedure was to determine whether any labelled substances contaminated the labelled compound under investigation.) Lens extracts were prepared as described above, and radioautographs were made, usually of 'two-way papers'. Sometimes the extracts were subjected only to electrophoresis at pH 6, because any breakdown products are more easily detected over an area of about 30 cm.² rather than the 500 cm.² (approx.) of the 'two-way papers'.

Detection of substances on paper. The AgNO_3 - NaOH method of Trevelyan, Procter & Harrison (1950) was used to detect carbohydrates, except after solvent (E), when the method of Metznerburg & Mitchell (1954) was used. The molybdate reagent of Burrows, Grylls & Harrison (1952) was used to detect phosphates, and the ninhydrin reagent of Wiggins & Williams (1952) to detect amino compounds. Nucleotides were located by means of a Hanovia Chromatolite ultraviolet lamp. Aniline hydrogen phthalate (Partidge, 1949) was used to detect aldoses, and orcinol-trichloroacetic acid (Klevestrand & Nordal, 1950) to detect fructose.

Estimations. Amino acids were assayed on paper by the method of Böde (1955), and sorbitol and fructose by comparing the size and intensity of spots revealed with AgNO_3 - NaOH with those given by spots of known concentration (Pirie & van Heyningen, 1964). The accuracy of these chromatographic methods was $\pm 20\%$. The method of Barker & Summerson (1941) as modified by Hullin & Noble (1953) was used to estimate lactic acid. For glucose, glycerol and α -glycerophosphate, enzymic methods were used (Wieland, 1963).

After elution from paper chromatograms, sorbitol was estimated by Faulkner's (1956) modification of the method of West & Rapoport (1949), and fructose by the reducing-sugar method of Nelson (1944). Equivalent areas of paper from the same chromatograms were eluted and assayed at the same time; in both assays the paper blanks were not more than 20% of the readings given by the carbohydrate to be assayed.

RESULTS

Glycolysis in the lens incubated aerobically and anaerobically. The Pasteur effect has been observed in the calf lens (Kern, 1962). For a comparison of the metabolism of glucose by the lens in air and in nitrogen therefore a higher initial amount of

glucose was put into the anaerobic incubation medium. The aim was to arrange that the average concentration of glucose throughout the incubation period was the same in the anaerobic and aerobic incubations. This is important since glucose, as well as being phosphorylated by hexokinase, is reduced to sorbitol, and the rate of reduction depends directly upon the concentration of glucose in the medium (Kinoshita, Futterman, Satoh & Merola, 1963). It may also be directly oxidized to gluconic acid (van Heyningen, 1964) and again the rate of reaction is faster at higher glucose concentrations. Table 1 shows that in the absence of

oxygen more glucose was used and more lactic acid formed, and that the average value for glucose in the medium (assuming a constant rate of usage) was slightly higher in the anaerobic incubation.

Glucose and lactic acid were measured only in the medium and not in the lens. However, the concentration of these two substances within the lens seems to be little changed during incubation. For glucose, this concentration is low (less than $0.1 \mu\text{mole/g.}$ of lens), as shown by the treatment of paper chromatograms of lens extracts with silver nitrate-sodium hydroxide (see the Methods section), and also by the fact that, after incubation with

Table 1. *Glucose used and lactic acid formed in the lens after incubation in a medium containing $[\text{U-}^{14}\text{C}]$ glucose*

The two lenses of a rabbit were incubated, one aerobically and the other anaerobically, for 20 hr. at 34° in 5 ml. of the medium described in the Methods section. The lenses were weighed after incubation. Glucose was estimated in the medium in series I and lactic acid in series II.

		Aerobic	Anaerobic
Series I	Lens wt. (mg.)	$611 \pm 32^*$	$627 \pm 28^*$
	Glucose in medium (μmoles)		
	Initial	$42.1 \pm 1.7^*$	$47.2 \pm 2.2^*$
	Final	$22.6 \pm 0.6^*$	$21.3 \pm 1.7^*$
	Amount used	19.5	25.9
	Average value†	32.4	34.2
	Glucose used ($\mu\text{moles/g.}$ of lens)	32.2	41.7
Series II	Lens wt. (mg.)	$710 \pm 28^*$	$736 \pm 44^*$
	Lactic acid in medium (μmoles)		
	Final	$35.6 \pm 2.2^*$	$48.9 \pm 1.7^*$
	Lactic acid formed ($\mu\text{moles/g.}$ of lens)	50.0	66.7
	Glucose converted into lactic acid (%)	78	80

* Mean value of five experiments $\pm$ standard error.

† Assuming a constant rate of usage.

Table 2. *Radioactivity of lens extract, protein and carbon dioxide after incubation of lens in medium containing $[\text{U-}^{14}\text{C}]$ glucose*

Details were as in Table 1, except that the lens was not weighed. The approx. weight was deduced from a table showing variations of lens weight with age, constructed from data accumulated in this Laboratory. The weight of lens protein was taken as one-third of the lens weight. Values are corrected to an initial total radioactivity of 15 000 counts/sec.

Age of rabbit (months)	Amount of radioactivity in CO_2 (counts/sec.)*			Sp. activity of protein (counts/sec./g. of protein)		
	Aerobic	Anaerobic	Aerobic Anaerobic	Aerobic	Anaerobic	Aerobic Anaerobic
2	—	—	—	400	220	1.8
4	610	162	3.8	610	330	1.8
13	250	63	4.0	150	100	1.5
26	182	114	1.6	360	200	1.8
35	152	87	1.75	110	60	1.8
36	486	130	3.4	160	50	3.2
42	317	187	1.7	120	30	4.0

* Normalized to the same weight of lens protein (1g.).

[U-¹⁴C]glucose, the radioactivity in the glucose spot was very faint (see below).

Lactic acid in the lens of the old rabbit is at a concentration of about 7 μ moles/g. of lens and, on the one occasion it was measured, the concentration was unchanged at the end of the incubation period. It is likely therefore that the amount of lactic acid in the medium is close to that formed by the lens during incubation.

The lens incubated anaerobically was about 3% heavier than the one incubated in air (Table 1). This difference is statistically significant (P 0.05).

Distribution of radioactivity after incubation of lens in medium containing [U-¹⁴C]glucose. Extracts of lens, made after 20 hr. incubation in a medium containing [U-¹⁴C]glucose, contained about 9% of the total counts; the gas phase (aerobic or anaerobic) did not have a consistent effect on this value.

As expected, the amount of glucose oxidized to carbon dioxide was low, but the total radioactivity in carbon dioxide formed under aerobic conditions was about two to four times as much as that formed anaerobically (Table 2). The radioactivity of the protein of the lens incubated aerobically was about two to four times that of the lens incubated anaerobically (Table 2). The specific activity of the protein tended to decrease with the age of the animal, possibly more precipitously under anaerobic conditions.

On three occasions the radioactivity of the lens extract was measured before and after treatment with methyl-di-octylamine in chloroform, to remove trichloroacetic acid. No decrease was detected in the radioactivity of 0.05 ml. of the extract, counted as described in the Methods section. The total chloroform extract contained about 5% of the total radioactivity of the water extract after the trichloroacetic acid had been removed. This was probably due to water dissolved and entrapped in the chloroform, but it is possible that one or more labelled compounds was extracted from the water by this treatment. All the compounds which were later found to be labelled in the lens, and of which a quantitative assay could be made, were shown not to be removed by the treatment.

Analysis of the medium. Samples of the media from aerobic and anaerobic incubation were applied to paper in the form of thin streaks and subjected to electrophoresis. Radioautography revealed only two bands, a neutral band and an acid band travelling in the position of lactic acid. Both were eluted with water and dried. Radioautography of two-way chromatograms of the eluted acid area in solvent (A) followed by solvent (C) failed to reveal any labelled spot other than lactic acid. The neutral band (from the aerobic incubation) was treated in the same way except that the two solvents were

Fig. 1. Tracing of a radioautograph (30 cm. $\times$ 25 cm.). A protein-free extract containing the equivalent of 25 or 30 mg. of lens was subjected to electrophoresis at pH 6 for 3 hr. in direction 1. The paper was then chromatographed two or three times in direction 2 in solvent (A) (see the Methods section). Broken lines indicate the removal of about 3 cm. Marker spots were run in each direction. Table 3 gives the identity of the labelled spots.

(B) followed by (A). Markers of glucose, fructose and sorbitol were used. There were one heavily labelled spot, glucose, and five faint spots, the most dense in the position of fructose. Some of the other spots may have been contaminants of the original D-[U-¹⁴C]glucose.

Thus lactic acid was the only product of lens metabolism found to be produced and appearing in the medium in a quantitatively significant amount.

Analysis of lens extracts. Radioautographs were made of papers on which lens extract (equivalent to 25 or 30 mg. of lens) had been subjected to electrophoresis followed by chromatography in

Table 3. *Radioactivity in the labelled products of metabolism of [U-¹⁴C]glucose in the lens*

The two lenses of a rabbit were incubated, one aerobically, the other anaerobically, for 20 hr. at 34° in 5 ml. of the medium described in the Methods section. Lens extracts were prepared and an amount equivalent to 25 mg. of lens was subjected to two-way electrophoresis and chromatography, followed by radioautography (Fig. 1). Radioactive spots were cut from the paper and counted on both sides. The two values for each spot were averaged and the background count (24 counts/100 sec.) was subtracted.

Spot (see Fig. 1)	Counts/100 sec.		Aerobic
			Anaerobic
a1 Glucose	trace	0	—
a2 Sorbitol	664	396	1.7
a3 Fructose	206	32	6.4
b Lactic acid	600*	536*	—
c α -Glycerophosphate	246	455	0.5
d Glutamic acid	81	32	2.5
e1 Nucleotides	122	55	2.2
e2 Glutathione	179	71	2.5
g Alanine	108	60	1.8
h Glycerol	112	130	0.9
j Glucose 6-phosphate (?)	trace	trace	—

* Lactic acid evaporates from the paper and therefore these values are too low.

solvent (A) ('two-way papers'; see the Methods section).

The results of three experiments were similar. Fig. 1 is a diagram of the radioactive areas detected by radioautography, and Table 3 shows the counts in each spot after incubation in air or nitrogen, and the nature of the compound identified as described in the paragraph below.

Identification of the labelled products of [U-¹⁴C]-glucose metabolism. Spots on radioautographs of 'two-way papers' (Fig. 1) were eluted for identification but more of the labelled substances were also collected by electrophoresis of bands of extract as described in the Methods section. This resulted in the separation of four clear-cut radioactive bands, as well as a few small weak bands which were neglected. The bands were: I, uncharged at pH 6; II, III and IV, negatively charged, which were separated from I in 4 hr. by distances of 14.6, 10.5 and 7.4 cm. respectively.

Band I. This band (neutral) was more heavily labelled in the extract of lens incubated aerobically. It was cut from the electrophoresis paper, sewn into another sheet of paper and subjected to chromatography three times in solvent (D). Five distinct labelled strips were revealed; they were named (i)–(v) in order of decreasing R_F ; several other weaker bands were not identified. Strip (i): this was identified as glycerol by co-chromatography in solvents (A), (B), (D) and (E); the eluate was also assayed enzymically. Strip (ii): co-chromatography of samples of an eluate of this strip in solvents (B) and (D) showed two compounds, one of which was fructose; the other was not identified. Strip (iii):

this was a weak band, scarcely discernible in the chromatogram prepared from extracts of the anaerobically incubated lens; it was identified as glucose by co-chromatography in solvents (B), (D) and (E). Strip (iv): this was the most heavily labelled, and was stronger in the extracts from aerobic incubations; by co-chromatography in solvents (B), (D) and (E) the radioactivity was found to be entirely in sorbitol. Strip (v): this was identified as alanine, by means of co-chromatography in solvents (A) and (B); the alanine was rather more heavily labelled in air than in nitrogen. This strip contained much inositol, which was scarcely labelled.

Band II. This was a diffuse, weakly labelled band, stronger in extracts from aerobic incubations. Evidence of the presence of adenosine mono-, di- and tri-phosphate, and oxidized glutathione, was obtained by co-chromatography in solvent (A) and electrophoresis at pH 4. All the glutathione became oxidized during the preparation of the lens extracts.

The spot j (Fig. 1) has the same mobilities as authentic glucose 6-phosphate; as this was not realized at the time that this band was being analysed, no attempt was made to confirm the presence of this compound.

Band III. This was shown to consist mainly of α -glycerophosphate and glutamic acid. Both were identified by co-chromatography in solvents (A) and (B) and electrophoresis at pH 6; α -glycerophosphate was also assayed enzymically. The labelling of glutamic acid was greater after aerobic incubation, whereas that of α -glycerophosphate was weaker.

Table 4. *Specific activities of some of the compounds labelled in the lens after incubation in a medium containing [U-¹⁴C]glucose*

In (A) and (C) radioactivity was measured in spots on paper (Fig. 1 and Table 3). In (B) it was measured in solutions eluted from paper. In (A) and (C) glucose, sorbitol, glutamic acid and glutathione were estimated on paper and α -glycerophosphate and glycerol enzymically in the lens extracts. In (B) all the compounds were assayed in the eluted solutions. The counts/sec./ μ mole of a solution of [U-¹⁴C]glucose counted in spots of 1cm. diam. on no. 52 paper were about half that given by the same solution counted on 4cm.² planchets at infinite thinness.

	Counts/sec./ μ mole			
	Aerobic		Anaerobic	
	Whole lens	Medium	Whole lens	Medium
(A)				
Glucose		340		310
Sorbitol	59		35	
α -Glycerophosphate	72		87	
Glutamic acid	10		4	
Glutathione	16		6	
Glycerol	86		77	
(B)				
Glucose		400		370
Lactic acid		185		170
Fructose	130		95	
Sorbitol	160		90	
Glycerol	80		80	
(C)	Cortex	Nucleus		
Glucose				240
Sorbitol	35	13		
α -Glycerophosphate	84	15		
Glutamic acid	14	3		

Band IV. This was identified as lactic acid, in the same way as in the medium.

Specific activity of compounds labelled in the lens. The concentrations of sorbitol, glutamic acid and glutathione were the same in the two lenses after aerobic and anaerobic incubations (4.5, 3.4 and 4.5 μ moles/g. of lens respectively for the lenses described in Table 3). Since the number of counts in these compounds were higher after aerobic incubation (Table 3) the specific activities were also higher (Table 4A).

α -Glycerophosphate and glycerol, on the other hand, were at higher concentrations after anaerobic incubation. The number of counts were also greater (Table 3) and the specific activity was probably unchanged (Table 4A).

The fact that less glutamic acid and glutathione are formed from glucose under anaerobic conditions is to be expected. The conversion of glucose into sorbitol by the lens has not previously been shown to be diminished in the absence of oxygen, however, and the conversion of glucose into glycerol has also not previously been shown in the lens. A further experiment was therefore done to confirm some of these findings, and also to measure the specific

activity of fructose in the lens and of lactic acid in the medium. Three pairs of lenses were used. Protein was removed with zinc sulphate-barium hydroxide. The extracts of the three aerobically incubated lenses, and those of the three anaerobically incubated lenses, were combined. Both were washed through a column of Amberlite IR-120 (H⁺ form). The extracts plus washings were then brought to pH 7 with a few drops of dilute aq. ammonia and taken to dryness in a rotary evaporator. The dried residues were dissolved in water, and bands of glucose, fructose, sorbitol and glycerol were separated on paper by means of electrophoresis followed by chromatography in solvent (D). Radioautographs showed that there was little glucose in the lens extracts, and it was not well separated from the strong bands of sorbitol; no attempt was made therefore to assay the glucose in the lens. Glucose and lactic acid in the combined media after incubation were separated by electrophoresis.

The carbohydrates were eluted from the paper with water for 3 hr. at room temperature, and the specific activity was determined. Table 4(B) gives the result of this experiment. It shows that the

Table 5. *Comparison of results from the cortex and nucleus of the lens after incubation in a medium containing [U-¹⁴C]glucose*

The two lenses of a rabbit (age 33 months) were incubated aerobically and extracts were made of the combined cortices and nuclei respectively.

	Cortex	Nucleus
Weight (mg.)	625	547
Protein (mg.)	192	281
Water (mg.)*	433	266
Dry wt. (%)	30.8	51.3
Counts/sec.		
Lens extract	1540	330
Protein	42	5
Per ml. of cell water	3560	1240
Incorporation into protein (%)†	2.7	1.5
Concn. of constituents (μmoles/ml. of cell water)		
Sorbitol	18	18
Inositol	18	18
Lactic acid	10.2	10.7
α-Glycerophosphate	3.15	2.75
Glutamic acid	2.6	2.6

* Calculated on the assumption that the dry weight of a lens is entirely protein.

† $\frac{\text{Counts in protein}}{\text{Total counts in tissue}} \times 100$

specific activity of the lactic acid in the medium was nearly at its maximum value of half that of the glucose. The specific activity of fructose is, within the limits of accuracy of the experiment, the same as that of sorbitol; both have higher specific activities after aerobic incubation. As in experiment (A) (Table 4) glycerol has the same specific activity after aerobic and anaerobic incubation.

Separation of the lens into cortex and nucleus after aerobic incubation. The finding that the labelled products which were examined in the lens had a much lower specific activity than that of the glucose in the medium (Table 4, A and B) prompted the separate analysis of the cortex (outer part) and nucleus (inner part) of the lens, after incubation. This was to determine whether all the substances in the cortex, into which the glucose first penetrates, have a higher specific activity. It had originally been thought that, although this state of affairs would be inevitable initially, after 20 hr. incubation there might be equal labelling throughout the lens.

The two lenses of a rabbit were incubated aerobically, each in 5 ml. of identical medium containing [¹⁴C]glucose. After incubation, each lens was divided into two parts, the soft outer cortex and the hard nucleus. Cortices and nuclei were combined and examined separately. The concentrations of protein, and of sorbitol, inositol, fructose and lactic acid, were measured, and also

Table 6. *Radioactivity of the labelled compounds in the cortex and nucleus of a rabbit lens after aerobic incubation in a medium containing [U-¹⁴C]glucose*

Details were as in Table 3, except that both lenses were incubated aerobically and extracts were made from the cortices (outer part) and nuclei (inner part) after incubation. Amounts equivalent to 25 mg. of cell water of cortex or of nucleus were used to prepare the radioautographs. Background: 25 counts/100 sec.

Spot	Counts/100 sec.		Cortex Nucleus
	Cortex	Nucleus	
Glucose	376	0	—
Sorbitol	1600	588	2.7
Fructose	461	304	1.5
Lactic acid	412*	245*	—
α-Glycerophosphate	662	100	6.6
Glutamic acid	90	16	5.6
Nucleotides	182	28	6.5
Glutathione	203	0	∞
Alanine	122	98	1.2
Glycerol	137	122	1.1

* Lactic acid evaporates from the paper and therefore these values are too low.

the radioactivity of the medium, lens extract and protein. The results are given in Table 5. Since the cortex of the lens is more hydrated than the nucleus, concentrations have been expressed in terms of cell water. The Table shows that the radioactivity in the cell water of the nucleus is about one-third of that of the cortex. The protein of the cortex is labelled eight times as strongly as that of the nucleus, but the percentage of label in the protein to label in the cell water is less than twice as much. Thus the proteins of the nucleus become labelled at about the same rate as those of the cortex.

The concentrations of some of the constituents were measured and expressed as μmoles/ml. of cell water. On this basis only α-glycerophosphate was at a lower concentration in the nucleus. Amino acids are generally evenly distributed throughout the lens water, but phosphate esters, nucleotides and glutathione are always more concentrated in the cortex (see van Heyningen, 1962).

Table 6 gives the counts in each radioactive spot of 'two-way papers'. Material equivalent to 25 mg. of cell water was applied to each paper. The Table shows that all substances were more strongly radioactive in the cortex. Glucose could be detected only in the cortex. Table 4(C) gives the specific activities of sorbitol, α-glycerophosphate and glutamic acid in the cortex and nucleus. It shows that, in all three cases, the specific activity in the nucleus is lower than that in the cortex, which, in turn, is lower than that of the glucose of the medium.

Table 7. α -Glycerophosphate and glycerol in the lens

Lenses were fresh unless otherwise stated.

Species	Description	α -Glycerophosphate (μ moles/g. of fresh tissue)	Glycerol
Cow		0.19	0.09
Guinea pig		0.94	0.32
Pig		0.33	0.10
Rat		0.30	0.08
Human	Senile cataractous	0.28	0.22
	Diabetic senile cataractous	0.29	0.13
Rabbit	4 years old	1.00	0.37
Rabbit	4 years old, cortex	2.14	0.90
	nucleus	1.42	0.44
Rabbit	Incubated in air*	1.03	0.52
	Incubated in nitrogen*	1.55	0.63
Rabbit	Incubated in air*	1.15	0.29
	Incubated in nitrogen*	1.77	0.64

* As described in the Methods section.

Metabolism of glycerol, α -glycerophosphate, fructose, sorbitol and lactic acid by the lens dispersion.

(a) Glycerol and α -glycerophosphate. Since the presence of glycerol in the lens has not been reported previously, its concentration in various species has been measured, together with that of α -glycerophosphate (Table 7).

The metabolism of [14 C]glycerol by lens dispersions was examined in the presence of added unlabelled glucose, as described in the Methods section. Radioautographs of 'two-way papers' were made. There was no evidence of any breakdown of glycerol under these conditions. The metabolism of [14 C] α -glycerophosphate was examined in the same way, and two breakdown products were formed, lactic acid and glycerol.

(b) Fructose. Sorbitol, α -glycerophosphate, glycerol, lactic acid and several unknown compounds became labelled after incubation of a lens dispersion with [14 C]fructose.

(c) Sorbitol. After incubation of a lens dispersion with [14 C]sorbitol, only faint spots, in the positions of lactic acid and fructose, were visible on radioautographs.

(d) Lactic acid. Since about 80% of the glucose used is converted into lactic acid by the lens, an experiment was done to see if any of the lactate formed is further metabolized. After incubation with [14 C]lactate in the presence and absence of glucose (see the Methods section), lens extracts were examined by radioautography after electrophoresis at pH 6. The radioautographs, which were developed for 6 weeks, showed that, after incubation another acid, with slightly greater mobility than the lactic acid at pH 6, becomes labelled. This is presumably pyruvic acid. The labelling was

Table 8. Radioactivity of labelled products of aerobic metabolism of [U - 14 C]glucose by the lens of the rabbit, rat and calf

Details were as in Table 3 with the following exceptions: one rabbit lens, one calf lens and ten rat lenses were incubated aerobically. Time of incubation was 20 hr. for the rabbit and calf lens and 5 hr. for the rat lenses. The cow lens was incubated for 20 hr. in 5 ml. of a modified Krebs-Ringer solution (medium A; Waley, 1964) containing [U - 14 C]glucose (10 μ C; 3 mg.).

	Rabbit	Rat	Calf	Cow
Age of animal (months)	42	2	—	—
Total lens wt. (mg.)	700	210	950	2000
No. of lenses	1	10	1	1
Spot (counts/100 sec.)				
Glucose	trace	91	311	508
Sorbitol	633	100	trace	359
Fructose	212	75	183	302
α -Glycerophosphate	305	271	171	134
Glutamic acid	84	44	68	77
Nucleotides	96	173	47	303
Glutathione	130	167	107	153
Alanine	78	81	78	72
Glycerol	61	31	110	78

stronger when the incubation was in the presence of glucose. No other labelled areas appeared.

Metabolism of [U - 14 C]glucose by the lens of the rat, calf and cow. Rat, calf and cow lenses were incubated aerobically under the same conditions as those described for rabbit, with the exceptions noted in Table 8. Lens extracts were prepared and examined in the same way. The labelled products were the same as with the rabbit, although the relative extent of labelling was different (Table 8).

Table 9. *Carbon-containing compounds of low molecular weight in the rabbit lens*

+ in the column 'radioautographic spot' means that the substance was found to be labelled when the lens was incubated aerobically in a medium containing [^{14}C]glucose and radioautographs of protein-free extracts were made, as shown in Fig. 1 and Table 3.

	Radioauto- graphic spot	Reference
Compounds at a concn. about 0.5 $\mu\text{mole/g.}$ of lens		
Glycerol	+	This paper
Tyrosine		Reddy & Kinsey (1962)
Proline		Reddy & Kinsey (1962)
Glycerophosphorylcholine		van Heyningen & Pirie (1958)
Phosphorylcholine		van Heyningen & Pirie (1958)
Compounds at a concn. about 1 $\mu\text{mole/g.}$ of lens		
α -Glycerophosphate	+	Pirie (1962) and this paper
Alanine	+	Reddy & Kinsey (1962)
Serine		Reddy & Kinsey (1962)
Glycine		Reddy & Kinsey (1962)
Asparagine and glutamine		Reddy & Kinsey (1962)
Ascorbic acid		Waters (1950)
Compounds at a concn. greater than 1 $\mu\text{mole/g.}$ of lens		
Lactic acid	+	Müller (1939) and this paper
Fructose	+	This paper
Sorbitol	+	This paper
ATP and other nucleotides	+	van Heyningen & Pirie (1958)
Glutamic acid	+	Reddy & Kinsey (1962)
Glutathione	+	Reddy & Kinsey (1962)
Urea		Reddy & Kinsey (1962)
Taurine		Reddy & Kinsey (1962)
Inositol		van Heyningen (1957)

Lactic acid was strongly labelled in all cases, but, owing to its volatility, its radioactivity was not measured. The most striking difference was in the labelling of glucose, sorbitol and fructose. Sorbitol was the most highly labelled substance in the (old) rabbit but was scarcely labelled in the calf. Sorbitol was also the most strongly labelled spot, apart from lactic acid, in 'two-way papers' prepared from extracts of the lens of a young rabbit after aerobic incubation in [^{14}C]glucose. Free glucose scarcely appears in the rabbit lens, but is the most highly labelled compound in the calf and cow lens.

Phospholipids. In the radioautographs of hydrolysed phospholipids, after exposure of the paper to the X-ray film for 6 weeks, seven spots were revealed which could have been due to the presence of the compounds found by Dawson (1960) after mild hydrolysis of phospholipids from various tissues. No further identification was carried out; treatment of the paper with ninhydrin followed by the phosphorus reagent (see the Methods section) showed one ninhydrin-positive spot (?phosphoserine) but phosphates were at too low a concentration to be shown. In this one experiment there appeared to be no difference in the labelling of

phospholipids between aerobic and anaerobic incubation.

DISCUSSION

Compounds in the lens labelled aerobically

After 20 hr. incubation the lens was still transparent and unblemished to the naked eye. But, even after such a long incubation, compounds were in all cases more heavily labelled in the cortex than in the nucleus (Table 6).

The nature of the compounds which appear as radioactive areas in radioautographs depends on the total radioactivity, which is determined by the amount of the compound present in the lens, and the extent to which it is labelled. With the exception of glucose, which was detected only in the cortex after aerobic incubation, no compound present in the lens at a concentration of less than 0.5 $\mu\text{mole/g.}$ of lens was found to be labelled. Table 9 gives the approximate concentrations of all the low-molecular-weight carbon-containing compounds known to occur in the lens at this concentration or a greater one.

The labelled compounds are discussed below. Of the compounds which occur at a relatively high concentration but are not labelled, tyrosine, proline, serine, glycine, ascorbic acid and urea may all be transported into the lens from the aqueous humour, though serine and glycine are interconverted in the rabbit lens (Merriam & Kinsey, 1950b). Asparagine and glutamine may have been broken down during the extraction procedure. Nothing is known of the metabolism of taurine, which occurs at a concentration of $5\mu\text{moles/g.}$ of rabbit lens, except that it can be formed from methionine in the rabbit lens (Mandel, Dardenne & Lessinger, 1957). Glycerophosphorylcholine is probably a degradation product of lecithin, and phosphorylcholine is probably formed by the phosphorylation of choline, so it is unlikely that ^{14}C from glucose would become incorporated into either of these during the time of incubation.

It is rather surprising that inositol, which occurs at a concentration of about $5\mu\text{moles/g.}$ in the lens of an old rabbit (van Heyningen, 1957), is not labelled, since Kinoshita *et al.* (1963) found some labelling in the inositol of the lens of a calf. It may be that inositol is synthesized by the lens but not broken down, or merely that the rate of turnover is lower in the lens from the older animal. Inositol is one of the few low-molecular-weight compounds to increase in concentration in the lens with age (van Heyningen, 1957).

Difference between aerobic and anaerobic incubation

Lens weight. The absence of air during incubation did not affect the clarity of the lens, which was, however, slightly heavier than the lens incubated in air (Table 1). This may be an indication that oxygen plays some part in the maintenance of the volume of the lens. The normal volume of cells is believed in general to depend upon active transport mechanisms for Na^+ (Whittam, 1960). Kinoshita *et al.* (1961), however, in experiments similar to those described in this paper, found no difference between the Na^+ and K^+ content of the calf lens after anaerobic or aerobic incubation; there was no decrease of '7 minute-hydrolysable' phosphate after anaerobic incubation; in the lens this gives a fair measure of the ATP content, which is believed to be the intermediate source of energy for Na^+ and K^+ transport in tissues.

Lactic acid. As in the calf lens (Kern, 1962), more lactic acid is formed by the rabbit lens in the absence of air. Under anaerobic conditions about 30% more glucose is used, and about 30% more lactic acid is formed; both in the presence and in the absence of oxygen, about 80% of the glucose used was converted into lactic acid. In both situations the lactic acid in the medium attained a specific

activity of about 90% of its maximum possible value, namely half that of the glucose (Table 4B).

α -Glycerophosphate. Accumulation of α -glycerophosphate within the lens under anaerobic conditions has already been shown by Pirie (1963), and in other tissues, for example, muscle (Peterson, Gaudin, Bocek & Beatty, 1964). Both lactate and α -glycerophosphate are formed by the reduction of glycolytic intermediates and these reactions are facilitated in anaerobiosis by the higher concentration of NADH. Peterson *et al.* (1964) suggest that the predominant formation of lactate over α -glycerophosphate in muscle is due to the fact that lactate readily diffuses out whereas α -glycerophosphate remains in the cells. A similar situation occurs in the lens; lactic acid diffuses into the aqueous humour whereas α -glycerophosphate does not.

Glycerol. Glycerol, like α -glycerophosphate, is found at a higher concentration after anaerobic incubation; it does not seem to be further metabolized by the lens. This finding, and the fact that labelled glycerol is formed when lens dispersions are incubated in labelled α -glycerophosphate, suggests that glycerol is probably produced by the action of a phosphatase on α -glycerophosphate. Mandel & Schmitt (1957) have shown that the bovine lens contains an α -glycerophosphate phosphatase. In most lenses of the species examined glycerol was at about one-third of the concentration of α -glycerophosphate (Table 7).

Gluconic acid. This is formed in the lens when the glucose concentration is high. In these experiments it was deliberately kept low and there was no appearance of labelled gluconic acid after incubation either in the presence or absence of oxygen.

Carbon dioxide. Carbon dioxide production is always very low in the lens and in the young rabbit lens, for example, that from C-6 oxidation is only, in air, a few per cent of that of C-1 oxidation (Kinoshita & Wachtl, 1958). The decrease in production of labelled carbon dioxide from $[\text{U-}^{14}\text{C}]\text{glucose}$ anaerobically (Table 2) cannot therefore be due to the loss of C-6 oxidation in the citric acid cycle. Presumably it is due to a decrease in the activity of the pentose phosphate pathway.

Nucleotides. The label in the nucleotides is probably derived from that in the pentoses formed by way of the hexose monophosphate pathway. Decrease in the activity of this pathway in anaerobiosis would account for decreased labelling of nucleotides.

Sorbitol, fructose and glucose. In the absence of oxygen there is a decrease in the incorporation of radioactivity into sorbitol and fructose. Sorbitol is formed in the lens by the reduction of glucose by aldose reductase (polyol-NADP oxidoreductase,

EC 1.1.1.21), and fructose by the re-oxidation of sorbitol with ketose reductase (L-iditol-NAD oxidoreductase, EC 1.1.1.14) (Hers, 1957; van Heyningen, 1959*a,b*). It seems likely therefore that the lowered production of carbon dioxide by the hexose monophosphate pathway is coupled via a lower rate of NADP reduction with the lower rate of reduction of glucose to sorbitol (Tables 3 and 4).

From the data of Table 2 it can be calculated that the decrease in total counts in carbon dioxide in anoxia, in the lenses from the four oldest rabbits, was 200–1300 counts/sec./g. of lens. The old rabbits have 5–10 μ moles of sorbitol/g., and in the experiment of Table 4(B) there was a decrease of 70 counts/sec./ μ mole of sorbitol in anoxia. This corresponds to a decrease in anoxia of 350–700 counts/sec./g. of lens, which is of the same order as the decrease in counts in carbon dioxide. These figures lend support to the idea that the lowered labelling of carbon dioxide in anoxia is coupled with the lowered labelling of sorbitol.

Britton (1962) suggests that the oxidation of sorbitol to fructose in seminal vesicles is impeded in anoxia. This may also be the case in the lens. In most tissues anoxia brings about an increase in NADH, which could lead to a suppression of sorbitol oxidation, and thence to a suppression of its formation.

Increased production of $^{14}\text{CO}_2$ by the calf lens incubated in [^{14}C]glucose is caused also by raising the concentration of glucose in the incubating fluid; in these circumstances the concentration of sorbitol in the lens increases (Kinoshita *et al.* 1963). However, in the experiments reported in this paper the glucose in the medium was kept as similar as possible in the two situations (Table 1), and no difference was detected in the sorbitol concentration in the two lenses after incubation. A lower concentration of glucose in the medium is not therefore the cause of the lower labelling of carbon dioxide in anoxia. However, the cause could have been the lower concentration of glucose within the lens after incubation in the absence of air. The presence of glucose was detectable only in lens incubated in air (Table 3). The reason for this is not known, but it may account for the lowered radioactivity in sorbitol, fructose and carbon dioxide in anoxia.

It should be noted that the specific activity of sorbitol and fructose, even after aerobic conditions, was at most about one-third of that of the glucose in the medium (Table 4), whereas that of lactic acid was 90% of its maximum possible value. In similar experiments Kinoshita *et al.* (1963) found that sorbitol and fructose reached a specific activity of about 80% of that of the glucose in the medium. However, in their experiments the lens of a younger animal of a different species (calf) was used and the

initial concentration of glucose in the medium was higher.

Glutamic acid, alanine and glutathione. Glutamic acid and alanine are concentrated by the lens, as in other tissues. These amino acids were present in the incubating medium at concentrations of about 107 and 30 mg./l. respectively; nevertheless, they both became labelled in the lens during incubation with [^{14}C]glucose. Labelled glutamic acid and alanine are generally formed in animal tissues from labelled glucose by way of the citric acid cycle; [^{14}C]glutamic acid is probably the source of the label in glutathione, which is synthesized in the lens from the component amino acids (Cliffe & Waley, 1958), as in the liver (Snoke & Bloch, 1954). Thus the suppression of the citric acid cycle in anoxia is probably the cause of the lowered incorporation of the radioactivity of glucose into glutamic acid, alanine and glutathione. It is not known if the labelling of these amino acids which occurs even under anaerobic conditions is due to their formation by pathways which do not use molecular oxygen (Krebs & Lowenstein, 1960), or simply to isotopic-exchange reactions (Haslam & Krebs, 1963).

Protein. The lowered radioactivity of the lens protein after anaerobic incubation is probably due to the lowered radioactivity in the free amino acids. It is known that amino acids are incorporated into lens proteins under conditions similar to those used in these experiments (Merriam & Kinsey, 1950*b*; Waley, 1964). Waley (1964) found that extent of incorporation of labelled glycine into the nucleus of the cow lens was only about 5% of that in the cortex; in the experiments described in this paper, the incorporation of the label in the nucleus was about 55% of that of the cortex (Table 5); this perhaps suggests that the labelling is due, not to incorporation of amino acid in protein, but to some other reaction. It is possible that the hexose moiety of glycoproteins, which are present in the lens (Dische, Smirnow & Zelmenis, 1962), become labelled under these conditions (Sarcione, 1964).

Comparisons with other tissues

Catanzaro, Chain, Pocchiari & Reading (1962) examined the effect of anaerobiosis on the metabolism of the [^{14}C]glucose by the explanted rat retina. They found that, of the seven low-molecular-weight compounds labelled in the presence of oxygen, only lactate was labelled in its absence. Beloff-Chain *et al.* (1959), in a similar comparison on rat diaphragm muscle, found that anaerobiosis caused diminished labelling in all metabolites.

Apart from these there appear to have been few comparisons on the lines of those described in this paper. The reason for this is probably that oxygen

plays a more obvious role in the metabolism of most tissues than in the lens, and deprivation of oxygen would rapidly cause swelling and structural abnormality.

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