medium. Since the *Candida* species, on the other hand, accumulated pyruvic acid in absence of nitrite and there was no other substance present known to be an inhibitor of carboxylase, it would seem probable that when *Candida* species are placed in a glucose-inorganic salts medium they are unable to synthesize thiamine at a rate sufficient for optimum development. That their ability to synthesize thiamine in such a medium varies in degree from one species to another was shown by the results of our tests carried out on cultures maintained in serial transfer (see Experimental and Results, paragraph on *Media and procedure*).

In those experiments in which arsenite was added to cultures of *C. krusei* (no. 5) there resulted a slowing down of the rate at which pyruvic acid was metabolized, but this did not serve to increase the quantity of accumulated pyruvic acid. It would seem probable that under normal conditions pyruvic acid is broken down by this organism partly by simple decarboxylation and partly by oxidative decarboxylation, but in the presence of arsenite the latter change is impeded, with the result that simple decarboxylation can occur to a greater extent, thus giving rise to a higher percentage yield of ethanol as compared with the arsenite-free control. Thus after 18 days (Table 6) the medium containing arsenite yielded 2.31% of ethanol and the control afforded only 1.73% of ethanol.

SUMMARY

1. Nineteen cultures representing not less than six species of *Candida* grew well in simple media of inorganic salts and glucose, with either ammonium phosphate or ammonium sulphate as source of nitrogen, and all the strains formed: (a) pyruvic acid, (b) another organic acid (or acids) and (c) ethanol, which in some cases was detected in traces only.

2. *Candida* species were unable to utilize nitrates as source of nitrogen, and were restricted in their growth and acid-forming capacity by addition of nitrite to the medium.

3. When thiamine hydrochloride (100 µg./100 ml.) was added to cultures of *C. krusei*, which normally produces an appreciably large yield of ethanol from glucose, the rate of utilization of glucose was increased, accumulation of pyruvic acid was prevented and the yield of ethanol was greater than that afforded by the control without added thiamine. Addition of thiamine hydrochloride (100 µg./100 ml.) to cultures of the very weakly fermentative *C. mycoderma* caused an increase in the rate of utilization of glucose and prevented accumulation of pyruvic acid, but did not promote formation of ethanol.

4. Addition of sodium arsenite (0.002M) to cultures of *C. krusei* caused the rate of utilization of glucose to fall and the yield of ethanol to be substantially increased.

REFERENCES

- Barron, E. S. G. (1943). *Advanc. Enzymol.* **3**, 166.
 Blom, J. (1926). *Ber. dtsch. chem. Ges.* **59**, 121.
 Caron, H. & Raquet, D. (1942). *J. Pharm. chim.* **2**, 333.
 Case, E. M. (1932). *Biochem. J.* **26**, 753.
 Janke, A. & Kropacsy, S. (1935). *Biochem. Z.* **278**, 30.
 Krebs, H. A. & Johnson, W. A. (1937). *Enzymologia*, **4**, 148.
 Lodder, J. H. & Kreger-van Rij, N. J. W. (1952). *The Yeasts. A Taxonomic Study*. Amsterdam: North Holland Publ. Co.
 Lu, G. D. (1939). *Biochem. J.* **33**, 249.
 Nord, F. F. & Mull, R. P. (1945). *Advanc. Enzymol.* **5**, 165.
 Nord, F. F. & Sciarini, L. J. (1944). *Arch. Biochem.* **5**, 435.
 Walker, T. K. & Wiles, A. E. (1952). *J. Inst. Brew.* **58**, 140.
 Willstätter, R. & Schüdel, G. (1918). *Ber. dtsch. chem. Ges.* **51**, 780.
 Wirth, J. C. & Nord, F. F. (1942). *Arch. Biochem.* **1**, 143.

mesoInositol in the Lens of Mammalian Eyes

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This work was undertaken for two reasons. First to confirm and extend the findings of Krause & Weekers (1938), who reported a high level of free inositol in the lens of three- to four-year-old cattle, considerably higher than that in other tissues of the eye. They used the method of Young (1934) and found the following values (in mg./100 g. of fresh tissue): lens, 126–177; optic nerve, 89–111; cornea, 6–8; iris, 22–35; retina, 13–17; choroid, 29–35;

sciera, 10–14; conjunctiva, 6; traces were found in the vitreous humour. The aqueous humour was not analysed. Secondly, the nature of a considerable fraction of the trichloroacetic acid-soluble phosphorus compounds in the lens is unknown (Pirie, van Heyningen & Flanders, 1955). It seemed possible that some of the phosphorus in this fraction might be in the form of inositol phosphates. The only acid-soluble phosphorylated inositol previously found in

normal mammalian tissues is phytic acid (inositol hexaphosphate), which occurs in nucleated erythrocytes (Rapoport, 1940). However, the search for such compounds does not appear to have been extensive.

Inositol has been found in the phosphatide fraction of the lipids of many animal tissues (Folch & Woolley, 1942; Folch, 1949; Hawthorne, 1955). The lens contains a small amount of phospholipid (about 200 mg./100 g. of cattle lens, aged one year; Krause, 1935), and consequently the protein plus lipid fraction of the lens has been examined for evidence of combined inositol.

METHODS

Preparation of lens extracts. Cattle, pig and sheep eyes were removed from the animals as soon as possible after death and brought to the laboratory on ice. They were used at once, or stored in the refrigerator at 0–4° for not more than an hour before use. The lenses from the eyes of laboratory animals were removed and extracted immediately after death. Lenses from human eyes were obtained *post mortem*. Two types of extract were made: (i) Trichloroacetic acid. The lens was ground in a known volume of ice-cold 5% (w/v) trichloroacetic acid, kept in the refrigerator at 0–4° for 30 min. and then centrifuged at 7000 g for 10 min. The extracts were stored at –15°. The trichloroacetic acid did not interfere with the inositol estimations. (ii) Acetate buffer at 100°. The lens was dropped into a graduated tube containing about ten times its weight of 0.1N acetate buffer, pH 5.2, standing in a boiling-water bath. The lens was thoroughly disintegrated by rubbing it with a glass rod against the side of the tube. After 4 min. the tube was removed and cooled. The contents were made up to a known volume and filtered or centrifuged. The clear extract was stored at –15°.

Hydrolysis of lens extracts and residues. A known volume of extract was boiled under reflux for 6 hr. with an equal volume of conc. HCl (Woolley, 1941). Most of the HCl was removed *in vacuo* and the hydrolysed extract was neutralized, made up to a known volume with water and its inositol content estimated. Any increase in the amount of free inositol in the extract after hydrolysis is assumed to give a measure of the amount of combined inositol originally present, since hydrolysis with acid under these conditions is believed to hydrolyse all forms of phosphorylated inositol (Woolley, 1941).

On some occasions the insoluble residues from the above-mentioned extraction procedures (i) and (ii) were examined for the presence of inositol. After three washings with large volumes of either trichloroacetic acid or acetate buffer they were hydrolysed as described above and the hydrolysates used for the estimations.

Estimation of inositol. This was estimated by microbiological assay with *Saccharomyces carlsbergensis* (American Type Culture Collection no. 4228). The method was based on that of Atkin, Schultz, Williams & Frey (1943) (see Snell, 1954). The basal medium described by these workers was modified by omitting inositol and adding pyridoxine (1 mg./l. of medium). Incubation was for 18–24 hr. at 37° in 18 mm. × 150 mm. tubes containing 2 ml. of medium; the tubes were incubated in a sloped position to increase

aeration. Growth was measured turbidimetrically with an EEL photoelectric colorimeter (Evans Electroselenium Ltd., Harlow, Essex). Inositol could be assayed by this method with an accuracy of $\pm 10\%$. The method is specific for free *meso*inositol (also called myo-inositol or inositol), the stereoisomer of hexahydroxycyclohexane, which is most widely distributed in nature (Woolley, 1947).

Estimation of glutathione. The method of Grunert & Phillips (1951) was used on trichloroacetic acid extracts of lens.

Irradiation of lens. The technique was as previously described (Pirie, van Heyningen & Boag, 1953; Pirie *et al.* 1955). The right eye of a seven-week-old rabbit was irradiated with X-rays (800–1000 r.) to produce the first signs of cataract (i.e. opacity) in the lens about 10 weeks later. Thereafter the cataract progressed rapidly. The left eye and lens were unaffected. The rabbits were killed and their lenses analysed at varying intervals after the first sign of cataract. On two occasions the lenses of rabbits with complete X-ray cataracts in the right eye were analysed. These rabbits were irradiated by the same technique, except that the right eye received 1400 r.; one (no. 9, Table 2) was seven weeks old at irradiation and the lens was fully opaque 10 weeks later. The other (no. 10, Table 2) was 26 weeks old at irradiation and the lens took about 30 weeks to become opaque.

RESULTS

Free inositol in the lens

Table 1 gives the values obtained for free inositol in the lens of various species of animal. The lens of each species examined contains inositol, though there appears to be considerable difference in concentration between species. The level of inositol in the lens of the very young animal (under two weeks old in cattle, and during the first week after the eyes are open in rabbits) was always lower than that in the adult animal of the same species (Table 1).

An adult cattle lens was divided into three concentric zones of approximately equal weight as follows. After removal of the capsule, the soft outer fibres (the lens cortex) were gently scraped off with the blunt side of a scalpel blade. The firmer layers beneath (middle zone) were then separated from the hard central core (lens nucleus) by more vigorous scraping. The three zones were analysed for inositol and it was found that the concentration decreased towards the centre of the lens; thus the three zones, lens cortex, middle layer and lens nucleus, contained 336, 307 and 213 mg. of inositol/100 g. of tissue respectively.

Free inositol in the cataractous lens

Table 2 gives the results of comparisons between the free inositol concentration in the normal rabbit lens (left) compared with its cataractous fellow lens (right). Glutathione (GSH) concentrations are included, as decrease in GSH is the earliest known chemical change in cataract development and may

Table 1. *Free inositol in the lens*

Species	Age	Lens wt. (approx.) (g.)	Inositol (each value is from a different animal) (mg./100 g. of lens)
Cattle			
Calf	Probably under 14 days	1.0	106, 128, 137
Adult	Probably 1-2 years	2.0-2.5	250, 298, 312, 343, 365
Rabbit	10 days	0.1	34, 58
	11 days	0.1	65
	13 days	0.1	63
	14 days	0.1	70
	17 days	0.1	32
	8-30 weeks	0.25-0.45	115, 117, 128, 136, 153, 156, 160, 175, 184, 190, 211
	2 years	0.55	106, 107
Pig	Unknown	0.4	315, 452
Rat	7 weeks	0.03	51
	35 weeks	0.05	83
Human	62 years	0.5	414
	69 years	0.3	509
Mouse	Unknown (adult)	0.01	142, 162
Guinea pig	Unknown (adult)	0.07	450, 490
Sheep	Unknown (adult)	1.0	490, 517, 553

Table 2. *Comparison of the concentrations of inositol and glutathione in normal and cataractous lens*

The right lens (R) of rabbits 1-10 was irradiated (see Methods). L, Left lens.

Rabbit	State of right lens*		Glutathione		Inositol	
			mg./100 g.	Difference (%) $\left(\frac{R-L}{L} \times 100\right)$	mg./100 g.	Difference (%) $\left(\frac{R-L}{L} \times 100\right)$
Control	(Not irradiated)	R	—	—	195	
		L	—	—	185	+5
Control	(Not irradiated)	R	—	—	103	
		L	—	—	110	-6
Litter mates	1	(+)	R	565	190	
			L	575	175	+9
	2	(+)	R	490	175	
			L	525	160	+9
	3	(+)	R	505	180	
			L	550	210	-14
Litter mates	4	(+)	R	415	146	
			L	440	156	-6
	5	+	R	450	107	
			L	511	128	-16
	6	+	R	449	146	
			L	489	117	+25
Litter mates	7	+	R	450	96	
			L	470	115	-16
	8	++	R	410	118	
			L	514	136	-13
	9	++++	R	99	13	
			L	485	153	-91
	10	++++	R	—	2	
			L	—	106	-98

* (+), Almost normal; +, small cortical opacities; ++, marked opacities; + + + +, completely opaque.

be used to assess other changes (Pirie *et al.* 1955). The table shows that nearly all the inositol is lost from a lens with a complete cataract. It is difficult, however, to decide at what stage in the development of cataract the reduction in inositol content begins. Whereas the GSH content of the lens can be measured with an accuracy of $\pm 3-4\%$ (Pirie *et al.* 1955), the limit of accuracy in the measurement of inositol is about $\pm 10\%$. The table shows that differences of 5 and 6% were found between the inositol content of two pairs of normal lenses; these differences are well within the limit of experimental error of the method of estimation. Of the right lenses showing early cataractous changes, some had a higher and some a lower inositol content than the normal fellow, and there is no evidence of a consistent decrease in inositol concentration. Decrease in GSH concentration was, as usual in X-ray cataract, a consistent finding. However, since the experimental error involved in inositol estimation is much greater than that for GSH, with the result that small differences in concentration cannot be detected, it is not possible to conclude that the GSH content decreases before inositol. Furthermore, for the same reason, it was not considered profitable to examine more X-ray cataracts at an early stage. The only conclusions that can be drawn from the figures are that at some stage after X-irradiation the inositol content of the lens starts to diminish, that by the time the first signs of cataract appear this diminution may or may not have begun, but that by the time the lens is fully opaque the inositol has almost completely vanished.

It can also be seen from Table 2 that variation between the inositol concentration of the normal (left) lenses of litter mates was considerable, though no greater than the variation in glutathione concentration. All three animals of a litter were killed on the same day.

Table 3. *Examination of lens for presence of combined inositol*

See Methods for preparation of extracts; hydrolysis was in 6N-HCl for 6 hr., boiling under reflux. TCA, Trichloroacetic acid.

Species	Material examined	Inositol (mg./100 g. of lens)	
		Before hydrolysis	After hydrolysis
Cattle			
	Calf		
	TCA extract	137	138
	TCA residue	—	1
Adult	TCA extract	343	323
	Acetate buffer extract	365	365
Pig	TCA extract	315	290
	TCA residue	—	3
Rabbit	Acetate buffer extract	184	184
	Acetate buffer residue	—	1

Combined inositol in lens

Table 3 shows that there is no evidence for the presence of combined inositol in acetate buffer or trichloroacetic acid extracts of calf, cattle, pig or rabbit lens. Furthermore, hydrolysis of the insoluble residue after trichloroacetic acid or acetate buffer extraction yielded negligible amounts of inositol, thus showing that inositol is probably not a constituent of the phospholipid of lens.

Inositol in lens after incubation in vitro

A calf lens was immersed in 10 ml. of Krebs-phosphate Ringer containing glucose (1 g./l.) in a wide-necked centrifuge tube (32 mm. diameter). The unstoppered tube was incubated in a water bath at 37° for 5 hr. with occasional gentle shaking. Under these conditions the lens continues to metabolize glucose to form principally lactic acid (Huysmans & Fischer, 1941; Merriam & Kinsey, 1950). Inositol was estimated in the lens and suspension fluid after incubation and was found to be the same (1.21 mg./lens) as in the fellow lens, analysed without incubation (1.27 mg./lens). Only traces (about 0.06 mg.) of inositol leaked out of the lens into the fluid during the 5 hr. incubation.

Inositol in the aqueous humour

Estimations of free inositol in the aqueous humour of the rabbit and the cattle eye gave values of 2 mg./100 ml. and 5 mg./100 ml. respectively. The level of inositol in rabbit blood is 0.8–2.7 mg./100 g. of blood (Campling & Nixon, 1954).

DISCUSSION

The high concentration of free *mesoinositol* in the lens has been confirmed (Table 1); in some species it is higher than that recorded for any other animal tissue (Woolley, 1947; Fleury & Balatre, 1947), although an animal secretion, that of the seminal vesicle of the boar, contains a very much greater concentration (2–3 g./100 ml.; Mann, 1954). In the cattle lens the concentration of *mesoinositol* is from 14 to 20 mm. This may be compared with the concentration in the cattle lens of three other low-molecular-weight, water-soluble organic compounds, which occur at rather high levels and which are known to be or are suspected to be concerned with its metabolism. They are all retained without loss within lens tissue during several hours of incubation *in vitro* under physiological conditions. These compounds are adenosine triphosphate (ATP), 1 mm (Müller, 1937; Nordmann & Mandel, 1952), glutathione, 5 mm (Dische & Zil, 1951; Kleifeld & Hockwin, 1956) and ascorbic acid, 2 mm (see Bellows, 1944). Thus *mesoinositol* is present in a considerably higher concentration than any of

these, but its function in the lens is entirely unknown and has scarcely been considered.

The lens of the rabbit has been examined more thoroughly than that of any other species for changes due to age or cataract formation. *meso*-Inositol, like glutathione (Pirie *et al.* 1955) and probably ascorbic acid (Kinsey, Jackson & Terry, 1945), but unlike ATP (Pirie *et al.* 1955), is at a lower concentration in the lens of the very young rabbit than in the adult rabbit (Table 1). Like glutathione (Pirie *et al.* 1953) and ATP (Pirie *et al.* 1955), it is mostly lost from a rabbit lens made completely cataractous by X-irradiation (Table 2), but limitations in the accuracy of estimation make it impossible to decide at what stage in the formation of cataract its concentration begins to decrease.

SUMMARY

1. Free *meso*inositol is found in the lens of several species of animal, ranging in concentration from about 50 mg./100 g. of lens in the rat to about 500 mg./100 g. of lens in the sheep and the human. This is higher than that recorded for any other animal tissue. It is present in the aqueous humour (rabbit, cattle) at a much lower concentration.

2. The concentration of *meso*inositol in the lens of the very young animal (cattle, rabbit) is lower than that in the adult animal of the same species.

3. There is no evidence of any combined form of *meso*inositol in the lens.

4. *meso*Inositol is virtually absent from the lens of a rabbit made completely opaque by means of X-rays, but limitations in the accuracy of its estimation do not permit conclusions as to the stage of cataract development at which the diminution begins.

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REFERENCES

- Atkin, L., Schultz, A. S., Williams, W. L. & Frey, C. N. (1943). *Industr. Engng Chem. (Anal.)*, **15**, 141.
 Bellows, J. G. (1944). *Cataract and Anomalies of the Lens*. London: Kimpton.
 Campling, J. D. & Nixon, D. A. (1954). *J. Physiol.* **126**, 71.
 Dische, Z. & Zil, H. (1951). *Amer. J. Ophthal.* **34**, part 2, 104.
 Fleury, P. & Balatre, P. (1947). *Les Inositols, Chimie et Biochimie*. Paris: Masson.
 Folch, J. (1949). *J. biol. Chem.* **177**, 497.
 Folch, J. & Woolley, D. W. (1942). *J. biol. Chem.* **142**, 963.
 Grunert, R. R. & Phillips, P. H. (1951). *Arch. Biochem.* **30**, 217.
 Hawthorne, J. N. (1955). *Biochim. biophys. Acta*, **18**, 389.
 Huysmans, J. H. B. M. & Fischer, F. P. (1941). *Ophthalmologica, Basel*, **102**, 275.
 Kinsey, V. E., Jackson, B. & Terry, T. L. (1945). *Arch. Ophthal., Chicago*, **34**, 415.
 Kleinfeld, O. & Hockwin, O. (1956). *Ber. dtsch. ophth. Ges.* **59**, 188.
 Krause, A. C. (1935). *Arch. Ophthal., Chicago*, **13**, 187.
 Krause, A. C. & Weekers, R. (1938). *Arch. Ophthal., Chicago*, **20**, 299.
 Mann, T. (1954). *Proc. Roy. Soc. B*, **142**, 21.
 Merriam, F. C. & Kinsey, V. E. (1950). *Arch. Ophthal., Chicago*, **43**, 979.
 Müller, H. K. (1937). *Arch. Augenheilk.* **110**, 128.
 Nordmann, J. & Mandel, P. (1952). *Ann. Oculist., Paris*, **185**, 929.
 Pirie, A., van Heyningen, R. & Boag, J. W. (1953). *Biochem. J.* **54**, 682.
 Pirie, A., van Heyningen, R. & Flanders, P. H. (1955). *Biochem. J.* **61**, 341.
 Rapoport, S. (1940). *J. biol. Chem.* **135**, 403.
 Snell, E. E. (1954). In *The Vitamins*, vol. 2, p. 361. New York: Academic Press.
 Woolley, D. W. (1941). *J. biol. Chem.* **140**, 453.
 Woolley, D. W. (1947). *Biol. Symp.* **12**, 279.
 Young, L. (1934). *Biochem. J.* **28**, 1435.

The Conversion of Glucose into Alanine and Glutamic Acid by Rat Liver, Liver Tumour and Kidney *in vivo*

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In studies on the metabolism of protein by tumour-bearing animals there often arises the problem whether a given protein has been synthesized by the tumour or by the normal tissues of the body. It seemed possible that this problem might be resolved by taking advantage of the differences in carbo-

hydrate metabolism between tumour and normal tissues first described by Warburg (1930). He showed that, in general, tumour tissue has a higher rate of aerobic glycolysis than normal tissue. It might, therefore, be expected that the rate of turnover of oxoglutarate in tumour tissue relative to that of pyruvate would be lower in tumour than in normal tissue. As a result the rate of conversion

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