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Metabolism of Xylose by the Lens

2. RAT LENS *IN VIVO* AND *IN VITRO**

BY RUTH VAN HEYNINGEN

Nuffield Laboratory of Ophthalmology, University of Oxford

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When a diet containing 35 % of D-xylose is fed to a weanling rat, complete opacity of the lens (cataract) ensues. The relevant literature has been summarized earlier (van Heyningen, 1958).

Because the young rat lens is very small, the calf lens was used for a preliminary investigation of the metabolism of xylose by the lens *in vitro* (van Heyningen, 1958). In the present work, the metabolism of xylose *in vivo* and *in vitro* by the lens of the weanling rat was investigated. The cataract, which closely resembles that due to feeding with galactose or experimental diabetes, does not appear in its initial stages to be due to precipitation of the proteins of the lens, but to swelling of the outermost lens fibres (von Sallmann, Caravaggio, Grimes & Collins, 1958; Friedenwald & Rytel, 1955).

EXPERIMENTAL

Methods

Diet and examination of eyes. In feeding experiments weanling Wistar albino rats 22–24 days old were used,

except in one experiment where the rats were 14 weeks old. One or more litters of the same age were divided into two groups: one group was given a xylose-containing diet and the other group an identical diet except that glucose was substituted for xylose. The diet (Darby & Day, 1939) consisted of glucose or xylose 35 %, crude casein 18 %, cornstarch 26 %, dried baker's yeast 10 %, butter 6 %, salts 3 % and cod-liver oil 2 %. On a few occasions, indicated in the text, 25 % of xylose and 10 % of glucose were substituted for 35 % of xylose. Examination of the eye by ophthalmoscope or slit lamp showed early signs of cataract in the xylose-fed rats after 5–6 days on the diet. After 7–8 days cataract was usually well advanced and visible to the naked eye. The animals were killed with ether, usually after 1–5 days on the diet, and the tissues removed immediately for analysis.

Preparation of tissue extracts. The lens was removed from the eye, still in the animal, by cutting the cornea with a sharp scalpel and then applying gentle pressure to the eyeball. Each lens weighed 14–18 mg. The lenses from a group of xylose-fed or glucose-fed rats were pooled, weighed and ground in ten times their weight of 5 % (w/v) trichloroacetic acid; the protein precipitate was removed by centrifuging at 0° at 10 000 g for 20 min. The trichloroacetic acid extract was first treated with methyldiethylamine by the method of Hughes & Williamson (1951) to remove

* Part 1: van Heyningen (1958).

trichloroacetic acid. The volume of the extract was then measured so that the weight of fresh tissue it contained could be calculated. It was then taken to dryness in a desiccator. The dried extract was dissolved in a known volume of water (1–5 mg. fresh tissue/ μ l. of water) and stored at -10° . Extracts of other tissues, when required, were prepared in the same way. Aqueous humour, when required, was obtained from the eye before removal of the lens by pushing a fine capillary gently through the cornea. The aqueous humour ran into the pipette and was blown into a tared tube. This procedure was usually, but not always, successful. Between 5 and 10 mg. of aqueous humour per eye was obtained and samples from a group of animals were pooled and stored at -10° .

Blood, when required, was withdrawn directly into a tube containing 10% (w/v) trichloroacetic acid. When plasma was needed heparin was used as an anticoagulant. The heparinized blood was centrifuged, the plasma removed and mixed with an equal volume of 10% (w/v) trichloroacetic acid, and the protein precipitate removed by centrifuging at 0° at 10 000 g for 20 min. The trichloroacetic acid extract of blood or plasma was treated with methyldiethylamine (Hughes & Williamson, 1951) and then, if amino acids were to be measured, it was washed on to a column of Amberlite IR 120 (H^{+}); the amino acids were eluted from the column with aq. 2N- NH_3 soln. and the eluate was taken to dryness *in vacuo*. The dried extract was dissolved in a known volume of water and stored at -10° . Blood in which glucose and xylose were to be estimated was treated with $Ba(OH)_2$ and $ZnSO_4$ by the method of Somogyi (1945).

Preparation of lens extracts for measurement of enzyme activity in the absorption spectrophotometer. Rat lenses were ground in a mortar with five times their weight of deionized water, and the dispersion was dialysed overnight at 4° against deionized water. The dialysed dispersion was brought to pH 7.4 with dilute aq. NH_3 soln. with a glass electrode and the insoluble part was removed by centrifuging. The lens of the adult rat was used for the preparations of these extracts usually. Extracts of calf lens were made in the same way, except that only twice their weight of water was used to make the dispersion. Absorption measurements were subsequently made as described in Table 3 with a Unicam SP. 500 spectrophotometer.

Paper chromatography. Descending chromatography on Whatman no. 1 paper was used. The solvents used were: phenol–water (100:28, w/v; for the separation of amino acids, 0.3 ml. of aq. 3% NH_3 soln. was added to each 50 ml., and 6 ml. of aq. 3% NH_3 soln. in 200 ml. of water was put in the bottom of the tank); benzene–butan-1-ol–pyridine–water (1.5:3:3), upper phase (Hough, 1950); butanol–acetic acid–water (40:9:20; Waley, 1956); propan-1-ol–ethyl acetate–water (7:1:2; Albon & Gross, 1950).

For two-dimensional chromatograms a Multi-sheet Frame tank was used (Shandon Scientific Co.); the first solvent was butanol–acetic acid–water and the second phenol–aq. NH_3 soln.

Paper electrophoresis. Horizontal strips of Whatman no. 3MM paper between sheets of polythene and glass were used (Kunkel & Tiselius, 1951). The potential difference along the paper was about 10 v/cm. The method of Frahn & Mills (1956) was used for paper electrophoresis of sugar alcohols in 0.1M-sodium tetraborate, pH 9.2. A spot of phenol red (0.04%) was applied at the origin to follow the

course of the electrophoresis visually; it migrates just ahead of dulcitol, the fastest moving of the sugar alcohols examined. For electrophoresis at pH 4.0, pyridine–acetic acid buffer was used (50 ml. of acetic acid, 15 ml. of pyridine and 2.5 l. of water; Grassmann, Hannig & Plöckl, 1955).

Detection and estimation of substances after paper chromatography. The $AgNO_3$ –NaOH method of Trevelyan, Proctor & Harrison (1950) was used to detect carbohydrates which react under these conditions. The sensitivity of this reaction was found to be greatly reduced in the presence of sodium tetraborate, and after paper electrophoresis in a solution of this substance the method of Metzberg & Mitchell (1954) was used. Aniline phthalate was used to detect aldoses (Partridge, 1949). Carbohydrates were estimated on paper by comparing the size of the spot produced with $AgNO_3$ –NaOH with spots produced by a range of known amounts of the compound developed on the same sheet of paper.

Amino compounds were estimated quantitatively on paper by the method of Peart (1956), and compounds containing phosphorus were visualized and estimated as described previously (van Heyningen & Pirie, 1958).

Other estimations. Reduced glutathione (GSH) was estimated colorimetrically by the method of Grunert & Phillips (1951) and total glutathione chromatographically by the method of Waley (1957) or Peart (1956).

Oxidized glutathione (GSSG) in lens extracts was estimated enzymically with GSSG reductase prepared from yeast by the first three steps of the purification procedure of Racker (1955). Two rat lenses were ground with 2.5 ml. of 0.1M-veronal–acetate buffer, pH 7.2, and the dispersion was centrifuged. GSSG in 2.4 ml. of the extract was estimated by the addition of glutathione reductase (0.01 ml.) and reduced triphosphopyridine nucleotide (0.25 μ mole) in a final volume of 3 ml. Decrease of absorption was measured at 340 m μ . The reduction of 0.1 μ mole of GSSG took about 10 min. to go to completion.

Keto sugars were estimated by the method of Dische & Borenfreund (1951), pyruvic acid by the method of Friedemann & Haugen (1943), lactic acid by the method of Barker & Summerson (1941) and amino compounds by the method of Rosen (1956). Glucose was estimated by the method of Nelson (1944). To measure glucose in the presence of xylose, $Ba(OH)_2$ – $ZnSO_4$ extracts were analysed before and after removal of glucose by yeast fermentation (King & Wootton, 1956). Standards for glucose and xylose were included. The values for xylose were checked by the method of Barrenscheen & Peham (1941).

Materials

D-Glucose, D-xylose, D-fructose and D-galactose were obtained from British Drug Houses Ltd. and diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) from Sigma Chemical Co.

For enzyme experiments the D-xylose was recrystallized from ethanol. Arabitol was kindly given by Dr O. Touster, D-xylulose by Dr J. S. Fewster and glutathione reductase by Dr A. Pirie. Xylitol was made by the method of Hollmann & Touster (1957), except that freshly prepared Raney nickel catalyst was used. Reduced DPN (DPNH) was made by the method of Slater (1953) and reduced TPN (TPNH) by the method of Evans & Nason (1953).

Table 1. Glucose and xylose in whole blood and in the aqueous humour of weanling rats fed for 5 days on a diet containing 35 % of glucose or 35 % of xylose

Expt. no.	Fluid analysed	Sugar fed	No. of animals	Glucose (mg./ml.)	Xylose (mg./ml.)
1	Blood	Glucose	3	0.83	0
		Xylose	3	1.00	0.48
2	Blood	Glucose	3	0.70	0
		Xylose	3	0.70	0.39
3	Blood	Glucose	2	0.88	0
		Xylose	2	0.89	0.49
4	Blood	Glucose	2	0.83	0
		Xylose	2	0.89	0.54
5	Blood	Xylose	3	1.15	0.87
6	Aqueous humour	Xylose	2	0.77	0.88
7	Aqueous humour	Xylose	8	0.60	0.64

Table 2. R_f values and distances of various sugars and polyols in various chromatographic solvents

Solvents: (1) Phenol-water (100:28, w/v); (2) benzene-butan-1-ol-pyridine-water (1:5:3:3); (3) butanol-acetic acid-water (40:9:20); (4) propan-1-ol-ethyl acetate-water (7:1:2).

Solvent ...	R_f			
	(1)	(2)	(3)	(4)
Xylose	0.46	0.43	0.32	—
Glucose	0.40	0.32	0.24	—
Xylulose	0.61	0.54	0.39	—
Sorbitol	0.48	0.29	0.26	—
Xylitol	0.61	0.35	0.31	—
Distance travelled (cm.)				
Solvent ...	(2)			(4)
	—	—	—	—
Sorbitol	—	13.1	—	13.9
Xylitol	—	15.7	—	16.3
Arabitol	—	17.4	—	17.6
Ribitol	—	18.0	—	17.6

RESULTS

Glucose and xylose in the blood

Table 1 gives the levels of sugars in the blood of rats fed for 5 days on the diet with 35 % of xylose or 35 % of glucose and in the aqueous humour of xylose-fed rats. Xylose has been shown to penetrate readily from the blood into the aqueous humour (Harris & Gehrsitz, 1949). Chromatographic analysis also showed that the concentration of glucose was approximately the same in the aqueous humour of xylose-fed rats and glucose-fed rats.

Carbohydrate compounds formed in the rat lens from xylose

Extracts of the lens of rats fed on a diet of 35 % of xylose or 35 % of glucose (hereafter to be referred to as 'xylose-lens' and 'glucose-lens extracts') were compared by paper chromato-

graphy. The papers were treated with AgNO_3 - NaOH (Trevelyan *et al.* 1950). It was found that after 5 days on the diet both the xylose-lens and the glucose-lens extracts yielded faint spots of equal strength in the position of inositol and usually faint spots in the position of glucose. In addition the xylose-lens extracts showed spots corresponding to three other carbohydrate compounds, a very strong one which is almost certainly xylitol, a much weaker one corresponding in position to sorbitol and a very faint one in the position of xylose. There was no trace of xylonic acid, the oxidation product of xylose found in the calf lens after incubation in a medium containing xylose (van Heyningen, 1958). The R_f values, or distances moved, of xylose, glucose and a number of related compounds are given in Table 2. Sorbitol was identified only by its R_f in various solvents (Table 2), by the slow appearance of the AgNO_3 - NaOH spot, and by the fact that it did not migrate when subjected to electrophoresis on paper at pH 4.0, but migrated at the same rate as an authentic sample in sodium tetraborate at pH 9.2. These same criteria were also used for the identification of xylitol; further evidence for the identity of xylitol will be presented below. By running mixed spots in two solvents with the authentic compounds it was shown to be neither of the other two pentatols, arabitol and ribitol (Table 2).

Xylitol was often found in the xylose-lens extracts after the rats had been 1 or 2 days on the diet, at a concentration of about 2 mg. of xylitol/g. of lens. With one exception it always reached at least this concentration after 5 or 6 days and was usually at about 4 mg./g. After 2 days the sorbitol spot was very faint but after 5 days sorbitol was about one-tenth as concentrated as the xylitol, as judged by the strength of the chromatographic spots. In one experiment, where a weanling rat was fed on a diet with 25 % of xylose for 25 days, the lens extract contained xylitol and sorbitol in

approximately equal amounts. No sorbitol was ever seen in the lens of the glucose-fed rat, but chromatograms in phenol-water of extracts of calf lens incubated in a medium containing glucose (5 mg./ml.) show spots in the position of sorbitol.

To see if xylitol and sorbitol also accumulate in the lens of the adult rat, which is not susceptible to xylose-cataract (Booth, Wilson & DeEds, 1953), a litter of three 14-weeks-old rats was fed on a diet with 35 % of xylose and killed after 2, 4 and 13 days on the diet. There was no sign of cataract in any of the lenses. After 2 days the lenses contained a trace of both polyols; after 4 days xylitol had reached a concentration of 0.33 mg./g. and sorbitol 1.0 mg./g. After 13 days xylitol was at a concentration of 1.5 mg./g. and sorbitol 2.0 mg./g. There was no evidence of the presence of xylose or glucose in these lenses.

Incubation of rat lens in a medium containing xylose (van Heyningen, 1958) resulted in the formation of a high concentration of xylitol; no xylonic acid was found. In this experiment *in vitro* free xylose was also found in the lens at a high concentration, whereas *in vivo* it is found in the lens only in traces and is sometimes not detectable. However, the concentration of xylose (5 mg./ml.) in the incubation medium in the experiment *in vitro* was six to eight times as high as in the aqueous humour of the xylose-fed rat.

Extracts of calf lens, incubated in the medium containing xylose (van Heyningen, 1958), were re-examined and it was found that in addition to the high concentration of xylonic acid formed, a small amount of xylitol was also present. This was missed (van Heyningen, 1958) partly because it was mistaken for xylonic acid lactone, which has the same R_f as xylitol in phenol-water, and partly because it was in the 'barium-soluble ethanol-soluble' fraction which was analysed for xylulose and phosphates but not for AgNO_3 - NaOH -reacting substances. Thus the rat lens *in vivo* and *in vitro* makes only the reduction product xylitol, whereas the calf lens *in vitro* makes a little of this but mostly the oxidation product xylonic acid. Rabbit lens incubated *in vitro* under the same conditions was shown to make xylitol with a trace of xylonic acid.

Enzymic oxidation of xylose by extracts of rat lens

In experiments similar to those with calf-lens extracts (van Heyningen, 1958) it was shown that rat lens contains an enzyme system which reduces DPN in the presence of xylose at pH 9.0. This system was not examined any further since xylonic acid, the product of the reaction, is not formed by the intact rat lens.

Enzymic reduction of xylose, glucose, galactose and fructose by extracts of rat and calf lens

It was found that TPNH is oxidized by rat lens in the presence of xylose, galactose or glucose, but not fructose, whereas DPNH is oxidized in the presence of fructose, but not of xylose, galactose or glucose. Calf lens also contained similar enzymes but they were usually less active (Table 3).

Sugars are reduced to the corresponding sugar alcohols in the presence of polyol dehydrogenases and reduced coenzyme (McCorkindale & Edson, 1954; Hers, 1957). Thus xylitol and sorbitol are the expected products of the reaction in which TPNH is oxidized by lens extracts in the presence of xylose and glucose respectively. The formation of xylitol by an extract of rat lens in the presence of xylose and TPNH was shown as follows. Dialysed extract of rat lens (1 ml.) was incubated at 37° with TPNH (approx. 0.7 μ mole), 2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer, pH 7.4 (10 μ moles), and xylose (6.7 μ moles) in a final volume of 3 ml. After incubation for 1 and 2 hr. more TPNH (0.7 μ mole each time) was added, and after incubation for 3 hr. the reaction was stopped by the addition of 3 ml. of 10 % (w/v) trichloroacetic acid.

The extract was treated with methyldioctylamine (Hughes & Williamson, 1951), passed through a column of Amberlite IR 120 (H^+), neutralized with aq. NH_3 soln. and, after being taken to dryness *in vacuo*, redissolved in 0.1 ml. of water. Chromatography in phenol-water of 5 μ l. of this solution showed, in addition to a large xylose spot, a spot in the position of xylitol, which was not present in the mixture before incubation.

Table 3. *Oxidation of reduced tri- and reduced diphosphopyridine nucleotide by extracts of dialysed rat and calf lens in the presence of various sugars*

The reaction mixture contained dialysed lens extract at pH 7.4, sugar (670 μ moles of xylose; 560 μ moles of the others), tris buffer, pH 7.4 (100 μ moles) and DPNH or TPNH (approx. 0.22 μ mole) in a volume of 3 ml. Decrease in absorption at 340 m μ during 5 min. (ΔE) was measured.

Animal	Coenzyme	Sugar	ΔE /g. fresh wt. of lens
Rat	DPNH	Xylose	0
		Glucose	0
		Galactose	0
		Fructose	1.37
Rat	TPNH	Xylose	1.00
		Glucose	0.72
		Galactose	1.13
		Fructose	0
Calf	TPNH	Xylose	0.16
Cow	TPNH	Xylose	0.10
	DPNH	Fructose	0.49

Search for evidence of formation of D-xylulose

The results of experiments shown in Table 3 agree with the general finding that enzymes reducing aldoses use TPNH and those reducing ketoses use DPNH (see de Duve & Hers, 1957). There is one exception to this finding, however, namely an enzyme in liver mitochondria which reduces L-xylulose to xylitol, with TPNH as co-enzyme (Hollmann & Touster, 1957). In view of this, evidence for the formation by rat lens of xylulose from xylose was sought. An isomerase which converts D-xylose into D-xylulose has been found in bacteria (Kline & Baron, 1957; Litthauer, Volcani & Bergmann, 1955; Hochster & Watson, 1954) but not in animal tissues.

The method of Hochster & Watson (1954) was used, in which any xylulose which might be formed by the reaction of lens with xylose in the absence of TPNH is trapped by borate buffer, thus forcing the reaction in the direction of the formation of xylulose, and preventing it from being metabolized further. D-Xylose (1 μ mole), in 0.15 ml. of 0.2 M-borate buffer, pH 8.0, was incubated at 37° with 0.15 ml. of 1 in 4 dilution of dialysed rat-lens dispersion. The lens of the weanling rat was used in this experiment. Controls without xylose and without lens were incubated at the same time, and 0.1 ml. samples were pipetted into 0.5 ml. of 0.1 N-HCl at 0 and 4 hr. Analysis of the extracts for keto sugars showed no formation of xylulose from xylose by lens.

Enzymic oxidation of xylitol by extracts of rat lens

As expected, it was found that xylitol in the presence of dialysed rat lens and TPN was oxidized to the aldose sugar xylose whereas in the presence of DPN the keto sugar xylulose was formed.

Xylitol and diphosphopyridine nucleotide. The reaction with DPN was coupled with lactic acid dehydrogenase and pyruvate, in order to favour the formation of xylulose. Dialysed rat-lens extract (0.75 ml.) was incubated with xylitol (48 μ moles), sodium pyruvate (50 μ moles) and DPN (1 μ mole) in a final volume of 1.3 ml. at pH 7.5; rat lens contains an active lactic dehydrogenase. A control without DPN was prepared at the same time; after 6 hr. at 37° 2 ml. of 10% (w/v) trichloroacetic acid was added to each tube. The extracts were treated with methyldioctylamine, passed through a column of Amberlite IR 120 (H^+), neutralized with aq. NH_3 soln., taken to dryness *in vacuo* and redissolved in a small volume of water. Chromatography, followed by treatment of the paper with $AgNO_3$ -NaOH, showed a spot in the position of xylulose, which was not present when DPN was absent from the incubation mixture. This was confirmed by the finding that keto sugar was formed in the presence but not in the absence of DPN.

Xylitol and triphosphopyridine nucleotide. Dialysed rat-lens extract (1.5 ml.) and 0.5 ml. of 0.1 M-tris buffer, pH 8.0, were incubated at 37° with TPN (3 μ moles) and xylitol (20 μ moles) in a final volume of 3 ml. The rate of formation of TPNH was followed spectrometrically. After 6 hr., when about 0.5 μ mole of TPNH had been formed and the reaction had nearly stopped, trichloroacetic acid was added to the reaction mixture and to the control, which was identical except for the omission of TPN. The extracts were prepared for chromatography as described in the experiment above, and it was shown by means of the aniline phthalate spray of Partridge (1949) that the extracts incubated with TPN contained a reducing sugar with the same R_F as xylose and which was not present in the control.

Search for xylitol in tissue other than lens

The only animal tissues (apart from the lens) in which dehydrogenases reducing D-xylose to xylitol have been found are the seminal vesicles and placenta of the sheep (Hers, 1956, 1957).

In order to see if xylitol accumulates in tissues of the weanling rat other than the lens, trichloroacetic acid extracts of blood, kidney, lung, liver, heart, muscle, retina, testis and spleen were made from a rat fed with 35% of xylose for 5 days, and analysed chromatographically. No xylitol (or xylonic acid) was detected in an amount of extract equivalent to 15 mg. of wet tissue. Since 5 μ g. of xylitol is readily detected, the concentration in these tissues was less than 0.33 mg./g. An extract from 2 mg. of lens tissue gave a large spot corresponding to xylitol and an extract of 5 mg. of aqueous humour gave a faint spot of xylitol, presumably derived from the lens. No xylitol was detected in the seminal vesicles of a 16-weeks-old rat fed with a diet of 35% of xylose for 2 weeks.

Reversibility of xylose cataract

Booth *et al.* (1953) showed that regression of lens opacity occurred with time, even though the xylose diet was continued. This finding was confirmed. After 14 days on a diet with 25% of xylose a weanling rat had almost complete cataract in both lenses. After another 12 days regression had begun and when the rat had eaten the diet for 67 days the cataract had almost disappeared, and clear fibres had formed outside the remaining opacities. The rat was killed and the lenses were found to contain a high concentration of xylitol, about 1 mg./g. of lens, and a lower concentration of sorbitol.

Glutathione in the lens of the xylose-fed rat

In many types of experimental cataract, a decreased level of GSH within the lens precedes the appearance of the opacity; GSSG is not formed (see

Pirie & van Heyningen, 1956). Table 4 shows the difference in concentration of reduced and total glutathione in xylose-lens and glucose-lens extracts after the rats had been fed for 1-7 days on the experimental diet. Owing to the small amount of tissue used for the estimation of GSH (one or two lenses of 14-18 mg. weight) small differences between the xylose-lens and glucose-lens are not significant. Table 4 shows that, when xylose is fed to rats, the level of GSH in the lens probably begins to diminish at about the same time as the first appearance of opacities. No GSSG was found in the lens of the normal rat or of the rat fed on the xylose diet for 2, 4 or 7 days.

*Search for other changes in rat lens
as a result of feeding with xylose*

Groups of rats fed on a diet containing 35% of xylose, and their litter mates fed on a diet containing 35% of glucose, were killed at various intervals after the start of the experimental diets. The concentration of lactic acid, total P, inorganic P (P_i), P contained in compounds hydrolysed to P_i in 10 min. in N-HCl at 100°, and P contained in compounds not hydrolysed to P_i under these conditions, were compared in the two groups of lenses. Xylitol was usually present at a high concentration after 1 day on the xylose diet, although in one experiment it was at a low level after 5 days. On one occasion adenosine triphosphate (ATP) was kindly measured by Dr G. Born [by the specific firefly method (Strehler & Totter, 1954)] in the lenses of rats fed for 3 days on the diet and there was no difference between the two groups. There was also no significant difference in the concentration of the sum of the two compounds phosphorylcholine and glycerophosphorylcholine on the two occasions they were measured by their phosphorus content in chromatographic spots.

The concentrations of the compounds in the lens were: lactic acid 1.0-1.2 mg./g. of lens; total P, inorganic P, organic P hydrolysed to P_i in N-HCl at 100° for 10 min., and organic P not hydrolysed under these conditions, 0.81, 0.10, 0.24 and 0.47 mg./g. of lens respectively. ATP in the lens, measured by the firefly method, was at a concentration of 2.85 mm. This is equivalent to 0.18 mg. of 'easily hydrolysible' P/g. of lens and therefore accounts for most of the measured 'easily hydrolysible' P. Phosphorylcholine and glycerophosphorylcholine together accounted for 0.16 mg. of P/g. of lens.

Mandel, Schmit & Quaranta (1956) found a decreased level of pyruvic acid in the lens of the rat made diabetic with alloxan. Table 5 gives the value for pyruvic acid in the lens and blood of xylose- and glucose-fed rats. Very often there was a decrease of pyruvic acid in the xylose-lens but

this was not a consistent finding. On one of the two occasions on which the level in the blood was also measured it was raised in the xylose-fed rat and also in the lens. On the other occasion it was decreased in the blood and unaltered in the lens. Probably lens pyruvic acid at least partly reflects the level in blood and aqueous humour which in turn are dependent on many other variables.

Table 4. *Glutathione content of the lens of the xylose-fed rat relative to that of the glucose-fed rat*

Weanling rats were fed on a diet containing 35% of xylose or 35% of glucose for the stated number of days. For estimations of reduced and total glutathione, see Methods section. Concentration of GSH in the lens of the rat fed with glucose was 1.5-2.2 mg./g. of tissue.

Period on diet (days)	Concn. of glutathione in lens of xylose-fed rat expressed as percentage of that in lens of glucose-fed rat	
	Reduced glutathione	Total
1	110	—
1	—	89
2	111	—
2	—	89
2	—	100
2	—	111
3	117	—
3	—	78
3	—	100
4	81	—
4	—	89
5*	90	—
5*	—	53
6*	87	—
6†	34	—
7†	30	—

* Early stages of cataract.

† Lens severely cataractous.

Table 5. *Pyruvic acid content of the lens and blood of the xylose-fed rat, compared with that of the glucose-fed rat*

Weanling rats were fed a diet of 35% of xylose or 35% of glucose for the stated number of days. Pyruvic acid was estimated by the method of Friedemann & Haugen (1943).

Period on diet (days)	Concn. of pyruvic acid in lens or blood of xylose-fed rat expressed as percentage of that in glucose-fed rat	
	Lens	Blood
1	88	—
2	52	—
2	102	82
2	111	—
2	65	—
3	56	—
3	120	164
4	76	—
4	79	—

The concentration of pyruvic acid in the rat lens was 0.03–0.04 mg./g. of lens, a value similar to that found by Mandel *et al.* (1956).

Free amino acids in the lens

Comparison between the lens of xylose-fed and glucose-fed rats. By examination of extracts of xylose-lens and glucose-lens on two-way chromatograms it was possible to compare the concentrations of a number of the amino acids, by the method of Peart (1956). In lens extracts which had been taken to dryness and redissolved in water, all the glutathione had become oxidized and therefore ran as a single spot near the origin and well separated from all the amino acids. Table 6 shows

that after 3 days on the diet the concentrations of some of the amino acids, and of glutathione, were considerably reduced. In one experiment, in which the rats were killed after only 1 day on the experimental diet, there was 40 % less free alanine in the xylose-lens than in the glucose-lens, whereas there appeared to be no difference in the concentration of other amino acids or of total glutathione. Amino acids were identified only by their relative positions on the chromatograms, compared with the positions of known amino acids run at the same time on separate chromatograms.

Amino acids in blood plasma of xylose-fed and glucose-fed rats. To find whether the changes shown in Table 6 were a reflexion of similar changes in the

Table 6. *Free amino acids in the lens of the xylose-fed and glucose-fed rat*

Weanling rats were fed on a diet of 35 % of xylose or 35 % of glucose for 3 days. Free amino acids in 125 mg. of lens were compared on two-dimensional chromatograms (see Methods section) sprayed with the ninhydrin reagent of Peart (1956). The coloured spots were cut out, extracted with 0.7 or 3.5 ml. of 70 % (v/v) acetone (Peart, 1956) according to their intensity, and the extinctions of the coloured solutions at 580 m μ were measured in the Unicam spectrophotometer, in cells of 1 cm. length and capacity 0.5 ml. Extinctions of extracts made in a volume of 3.5 ml. are multiplied by five to make them comparable with those made in 0.7 ml.

Amino acid	<i>E</i>		Concn. of amino acid in lens of xylose-fed rat expressed as percentage of that in the glucose-fed rat
	Glucose-lens	Xylose-lens	
Glutathione	2.62	2.34	89
Alanine	2.35	0.83	35
Glutamic acid	2.21	1.40	63
Taurine and serine	1.97	1.57	80
Lysine and histidine	1.54	1.46	97
Threonine and glycine	0.70	0.39	55
Leucine and isoleucine	0.66	0.59	90
α -Aminobutyric acid	0.38	0.17	31
Glutamine	0.36	0.24	68
Methionine and valine	0.34	0.38	111
Aspartic acid	0.21	0.11	53

Table 7. *Difference between concentration of free amino acids in the blood plasma and lens of the xylose-fed and the glucose-fed rat*

Weanling rats were fed on a diet of 35 % of xylose or 35 % of glucose for 5 days. Free amino acids in 25 mg. of lens or 125 mg. of plasma were measured on two-dimensional chromatograms (see Methods section) and sprayed with the ninhydrin reagent of Peart (1956). The coloured spots were cut out, eluted with 0.7 ml. of 70 % (v/v) acetone and the extinctions measured at 580 m μ in the spectrophotometer, in cells of 1 cm. length and 0.5 ml. capacity. Values for the lens are all multiplied by five to make them comparable with those for plasma. Thus all the extinctions refer to 125 mg. of tissue.

Amino acid	<i>E</i>				Concn. of amino acid in tissue of xylose-fed rat expressed as percentage of that in glucose-fed rat	
	Glucose-fed rat		Xylose-fed rat			
	Lens	Plasma	Lens	Plasma	Lens	Plasma
Glutathione	5.07	—	2.67	—	53	—
Alanine	2.75	0.74	0.32	0.45	12	61
Glutamic acid	3.81	0.64	1.09	0.63	29	98
Taurine	4.60	—	2.29	—	50	—
Serine	—	0.35	—	0.38	—	109
Lysine and histidine	4.38	0.44	1.94	0.33	44	75
Threonine and glycine	2.37	0.62	0.88	0.49	37	78
α -Aminobutyric acid	0.81	—	0.92	—	113	—
Glutamine	—	0.45	—	0.32	—	72
Methionine and valine	—	0.10	—	0.09	—	88

blood, and therefore presumably in the aqueous humour, the concentrations of some of the amino acids of the blood plasma of xylose-fed and glucose-fed rats were compared by the method of Peart (1956) after 2–5 days on the diet. Table 7 shows that the decreases of amino acids in the lens are at least partly a reflexion of similar changes in the blood plasma, although the changes in the lens are greater than those in the plasma.

There were decreases in the amounts of other amino acids, which were at too low a concentration, or not sufficiently separated, for a quantitative estimation to be made. Comparisons of the amounts of free amino acids in the whole blood showed decreases in the xylose-fed rat, similar to those found in blood plasma.

There was a 31% reduction in the total amount of amino compounds in the aqueous humour of the xylose-fed rat compared with the amount in the glucose-fed control; but there was not enough aqueous humour for chromatography.

Apart from the fact that most of the xylose in the diet fed to the rats receiving xylose would not be utilized (McCormick & Touster, 1957), these rats usually ate a considerably smaller weight of food than did the glucose-fed controls.

Effect of xylose in the diet on free amino acids in blood plasma and lens. To determine whether the reduction of free amino acids in the plasma was due to the presence of xylose in the diet, or merely to the lowered food intake of the xylose-fed rats compared with the glucose-fed controls, the following experiment was carried out. A litter of nine rats, aged 23 days, was divided into three groups. The first group was fed for 5 days on the diet containing 35% of glucose *ad lib*. The second group was fed for 5 days on the diet containing 35% of xylose, also *ad lib*. The third group was given the weight of food eaten by the second group, less the xylose; thus it was given 65% of the weight of the food eaten the day before by this group, i.e. it had the same amount of food to eat as the xylose-fed rats, less the xylose. This 'sugar-free' diet was necessarily started 1 day later than the other two, but was continued for 5 days before the animals in this group were killed.

The same amino acids were measured. Whereas the glucose-fed rats gained weight steadily, both xylose-fed rats and those on the 'sugar-free' diet lost weight, the former more rapidly than the latter. (In experiments of longer duration, rats fed on the xylose diet gained weight steadily though more slowly than the glucose-fed rats.) It appeared that xylose was not itself responsible for the lowered level of amino acids in the plasma, since in the first of three experiments there was as great a decrease of amino acids in the plasma of the rats on the 'sugar-free' diet as in those fed with xylose. In

two other experiments only amino acids in the plasma of the xylose-fed rats were affected, those in the rats on the 'sugar-free' diet being the same as those of the glucose-fed controls. However, in one of these two experiments the lenses were also analysed, and it was found that although the amino acids were unchanged in the plasma of the rats on the 'sugar-free' diet those of the lens had decreased to 60–70% of their level in the lenses of the glucose-fed controls. The amino acids in the lenses of the xylose-fed rats had decreased even more, to 12–53% of the value in the glucose-fed controls.

DISCUSSION

Evidence of identity of xylitol in the lens of the xylose-fed rat

An attempt was made to isolate the compound formed by rat lens from xylose by chromatography of xylose-lens extracts in phenol–water and elution of the appropriate area. It was not found possible, however, with the small amounts which could be collected. That the substance is almost certainly xylitol is demonstrated by the following findings.

(1) It has the same R_F as xylitol in four solvents and is thus distinguished from arabitol or ribitol (Table 2). (2) It reacts slowly with the silver nitrate–sodium hydroxide spray. (3) It has no net charge at pH 4.0, shown by paper electrophoresis. (4) It migrates at the same rate as authentic xylitol in paper electrophoresis at pH 9.2 in sodium tetraborate. (5) A compound with the same R_F as xylitol is formed from xylose by dialysed lens extracts by the oxidation of TPNH but not of DPNH. (6) Authentic xylitol is oxidized by dialysed rat-lens extract with the reduction of TPN or DPN to form compounds with the same chromatographic R_F values as xylose and xylulose respectively.

Metabolism of xylose by the lens

It has been shown that xylose is reduced by the lens in one species of animal (the rat), whereas it is oxidized, and also to a lesser extent reduced, by the lens of another species of animal (the calf). These findings apply to the rat lens *in vivo* and *in vitro* and the calf lens *in vitro*. The rabbit lens *in vitro* behaves like the rat lens, reducing xylose to xylitol. In the calf lens it appears that the action of xylose dehydrogenase, in the presence of its substrate, interferes with the glycolytic cycle by competing with triose phosphate dehydrogenase for the available DPN (van Heyningen, 1958). The rat lens, on the other hand, reduces xylose through xylitol dehydrogenase and TPNH, and by analogy one might expect competition between this system and some other system which normally uses TPNH as coenzyme. Glutathione reductase answers this

description and is present in lens (van Heyningen & Pirie, 1953), but there is no evidence that it is affected by xylose since GSSG does not accumulate and total glutathione is not diminished at the time when xylitol has reached a high concentration in the lens.

In the lens the rate of the hexokinase reaction is believed to limit the rate of glycolysis (see Pirie & van Heyningen, 1956). Xylose has been shown to inhibit hexokinase *in vitro* (Sols & Crane, 1954) but does not appear to do so in the rat lens *in vivo* since glycolysis is unaffected. If glycolysis is not interfered with it seems likely that the initial disturbance occurs in the oxidative metabolism. Only about 2.3 % of the glucose used by the rabbit lens is oxidized (Kinoshita & Wachtl, 1958), but oxygen is essential for the lens and even a small decrease of the already small uptake might be disastrous to maintenance of transparency. The fact that nearly all the carbon dioxide formed by the rabbit lens is derived from oxidation of the C-1 atom of glucose implies that the pentose phosphate pathway is predominant over the citric acid cycle (Kinoshita & Wachtl, 1958). Possibly re-oxidation of TPNH produced in the two early stages of the pentose phosphate cycle is the process with which xylitol dehydrogenase competes. A cytochrome *c* reductase, with TPNH, is found in the lens of cattle and rabbit (Pirie, van Heyningen & Boag, 1953); unfortunately the amount of cytochrome *c* in the lens is so small that an investigation of the relative proportions of reduced to oxidized cytochrome *c* is not possible. On the other hand, if the rat lens contains a coenzyme transhydrogenase (Colowick, Kaplan, Neufeld & Ciotto, 1952), TPNH might be maintained in the reduced state in the presence of xylose while DPNH became oxidized. An analysis of the ratios TPNH/TPN and DPNH/DPN during the course of cataract formation might be illuminating. Furthermore, such an analysis applied to the normal lens of the calf and the rat might help to explain the finding that xylose is mostly oxidized in the former by an enzyme with DPN, and reduced in the latter by an enzyme with TPNH. There is little in the literature about the comparative concentrations of enzymes and coenzymes in the lenses of different species of animal; but these experiments suggest a great difference between some aspects of the metabolism of the calf lens and that of the rat and the rabbit.

Xylose metabolism by the intact animal

Xylose is metabolized only to a small extent by the rat (Miller & Lewis, 1932). About 1 % of injected xylose labelled with ^{14}C is probably converted into glycogen in the liver (Hiatt, 1957) and about 10 % is oxidized to carbon dioxide. In the

guinea pig 75 % of injected xylose is excreted in the urine (McCormick & Touster, 1957). In man, 13.5 % of administered [^{14}C]xylose is expired as carbon dioxide: all the ^{14}C excreted is in the form of unchanged sugar, no xylonic acid or xylitol being detectable (Wyngaarden, Segal & Foley, 1957). The present work shows that the level of xylose in the blood of the rat fed on a diet containing 35 % of xylose remains low and the level of glucose does not seem to be much affected.

Metabolism of xylose and xylitol by animal tissues

The way in which xylose enters the metabolism of the mammal is not known. No isomerase converting xylose into xylulose has been found and systems which phosphorylate xylose directly appear to be lacking. Apart from the lens, only in the seminal vesicles and the placenta has an enzyme catalysing the interconversion of D-xylose and xylitol, TPN being used as coenzyme, been found (Hers, 1956, 1957). The present work shows that, of all the organs examined, only the lens accumulates xylitol but this is perhaps not surprising in view of the fact that enzymes in the liver would convert any xylitol which might be formed from xylose into D- or L-xylulose and thence by recognized metabolic pathways (McCormick & Touster, 1957). Only 25 % of the ^{14}C of labelled xylitol administered to the guinea pig is excreted in the urine; 10 % is expired as carbon dioxide and the rest is presumably incorporated into metabolic intermediates, some of it possibly in the form of glycogen (McCormick & Touster, 1957). Evidence has been given that the lens contains an enzyme which converts xylitol into xylulose in the presence of DPN. The xylulose is presumably D-xylulose, since only this isomer has been shown to be formed from xylitol by other tissues in the presence of DPN (de Duve & Hers, 1957). The equilibrium of this reaction is in favour of the formation of xylitol, but removal of D-xylulose would force the reaction in the direction of breakdown of xylitol. The fact that no xylulose has been found in the lens of the xylose-fed rat, in spite of the high concentration of xylitol, suggests that it is removed as soon as it is formed, presumably by way of xylulokinase and the pentose phosphate cycle, and the limiting reaction is that converting xylitol into D-xylulose.

Formation of sorbitol

Extracts of rat lens reduce glucose to sorbitol with TPNH as coenzyme (Table 3); possibly the enzyme is the same as that which reduces xylose and galactose. There is a gradual accumulation of sorbitol in the lens of the xylose-fed rat, whereas it was not detected in the lens of the normal weanling rat. It is difficult to see why the metabolism of the

lens of the xylose-fed rat, which is presumably deflected by reduction of xylose in the direction of oxidation of TPNH, is further deflected in this direction by the reduction of glucose, which is apparently not reduced by the lens under normal circumstances. Possibly glucose is always reduced to sorbitol by the lens, and its utilization is defective in the lens of xylose-fed rats. Both xylitol and sorbitol accumulate in the lens of the adult rat which does not develop xylose cataract, and they were also found in a lens which had almost recovered from a severe xylose cataract, so neither of these compounds can in itself be responsible for the formation of cataract. The situation seems to be that formation of xylitol and sorbitol in the fast-growing fibres of the lens in the weanling rat deranges some part of the metabolism to the extent that transparency can no longer be maintained, whereas older fibres, which are not growing so quickly, can metabolize xylose and at the same time maintain transparency.

It appears from preliminary experiments that the lens of the weanling rat fed on a diet containing 35 % of galactose accumulates dulcitol, and the lens of the rat made diabetic with alloxan accumulates sorbitol (van Heyningen, 1959).

Lowered level of free amino acids in the lens of the xylose-fed rat

The lens of the xylose-fed rat contains a lower level of free amino acids than the lens of the glucose-fed control (Tables 6 and 7). This appears to be partly a reflexion of similar changes in the blood. The loss in amino acids in the lens seems to follow a fairly regular pattern, alanine being the first to diminish, followed usually by threonine plus glycine and aspartic acid; after 5 days on the xylose diet nearly all the amino acids and also glutathione were at a lower level in the lens of the xylose-fed rat than in the lens of the glucose-fed rat. It must be stressed that the assignment of the chromatographic spots produced by the individual amino acids is only tentative, but there is no reason to doubt that most of them are correctly assigned, since they are found in general in animal tissues (Tallan, Moore & Stein, 1954; du Ruisseau, Greenstein, Winitz & Birnbaum, 1957). The relatively high concentration of α -aminobutyric acid is an exception and this may well be wrongly named.

The present work shows that a lowered food intake alone can bring about a decrease of amino acids in the lens. Cataract has never been shown to be caused by starvation but it is caused in young rats by feeding with a diet lacking in certain amino acids, in particular tryptophan (Totter & Day, 1942). The clinical appearance of this type of

cataract is different from that of xylose cataract and it takes longer to develop (Buschke, 1943). The level of amino acids in the lens after feeding with a diet deficient in a single amino acid has not been examined, and the effect on the transparency of the lens of feeding with such a diet has been investigated for only a few of the individual amino acids. In the present work only the levels of those amino acids which normally occur at relatively high concentrations in the lens have been measured. A great deal more evidence is needed in order to decide whether the lowered level of amino acids in the lens as a result of xylose feeding is a primary cause of xylose cataract.

SUMMARY

1. The lens of the weanling rat fed on a diet containing 35 % of xylose for 1-7 days accumulates xylitol, which reaches a concentration of about 4 mg./g. of lens. The first signs of cataract usually appear after 5 days. No xylulose, xylonic acid or phosphorylated derivatives of xylose could be detected in the lens and very little free xylose is present.

2. Sorbitol also accumulates in the lens of the xylose-fed rat, but was not detected in the lens of glucose-fed controls.

3. Xylitol accumulates in the lens before clinical signs of cataract are present. Xylitol and sorbitol are found in the lens of the xylose-fed adult rat, which is not susceptible to xylose cataract.

4. Cataract caused by feeding with xylose was reversible and in the lens after recovery xylitol and sorbitol are found in high concentration.

5. Xylitol did not accumulate in other tissues of the xylose-fed rat, except in the aqueous humour where a small amount was found.

6. Extracts of rat lens reduce D-xylose to xylitol by means of an enzyme with reduced triphosphopyridine nucleotide as coenzyme. Glucose and galactose are also reduced by this system. Fructose is reduced by extracts of rat lens in the presence of reduced diphosphopyridine nucleotide. Xylitol is oxidized by rat lens to form xylose and xylulose in the presence of tri- and di-phosphopyridine nucleotide respectively.

7. No difference was observed in the concentration of lactic acid, inorganic phosphate, acid-labile phosphate or acid-stable phosphate between the lens of the xylose-fed rat and that of the glucose-fed control.

8. Glutathione in the lens does not decrease in concentration for the first few days of feeding with xylose, and no oxidized glutathione was found in the lens.

9. The lens and blood plasma of the xylose-fed rat contained lower amounts of free amino acids

than in the glucose-fed controls; the decreases in the lens were greater than those in the plasma.

10. The intact calf lens *in vitro*, as well as oxidizing xylose to xylonic acid (van Heyningen, 1958), also forms some of the reduction product, xylitol. The intact rat lens *in vitro* forms only xylitol from xylose.

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