The Metabolism of Xylose by the Ocular Lens of the Rat

By S. LERMAN AND F. M. HEGGENESS

Department of Surgery, Division of Ophthalmology, and the Department of Biochemistry, and the Department of Physiology of the University of Rochester School of Medicine and Dentistry, New York, U.S.A.

(Received 17 June 1960)

Xylose and galactose differ markedly in their cataractogenic action. If young rats 28–32 days old are placed on a high galactose diet they develop dense lenticular opacity in 9–12 days, whereas rats 85–100 days old when placed on a similar diet require approximately 70 days for cataracts to develop (Mitchell & Dodge, 1935; Buschke, 1943; Babel, 1953; Lerman, 1960*b*). In contrast, when young rats are given a 30% xylose diet, although lens opacities do become manifest in 8–14 days, these lenticular changes are usually neither progressive nor permanent (with respect to lenticular transparency and function) in spite of the continued ingestion of xylose by the animal (Booth, Wilson & De Eds, 1953; van Heyningen, 1959). An apparent regression of the cataractous changes eventually occurs. Actually the lens (as well as the entire animal) is capable of recovering from, or adapting itself to, the initial deleterious effects of xylose. New fibres form around the original opacities and eventually the lens appears transparent. Furthermore, if older rats (14 weeks old) are placed on a prolonged 30% xylose diet, lenticular changes do not develop (Booth *et al.* 1953).

The sequence of events that occurs in the lenses of the young rats when these animals are fed on the 30% xylose diet was investigated in an attempt to elucidate some of the biochemical changes that occur in this organ during the period of the formation of lenticular opacities and the apparent regression of these changes.

MATERIALS AND METHODS

Holtzman-strain male white rats 28–32 days old, weighing approx. 45–55 g., were used in all the experiments. The experimental diet consisted of 30% of glucose, 30% of xylose, 21% of casein, 15% of fat, 4% of salt mixture W (Nutritional Biochemicals Corp., Cleveland, Ohio) and a complete vitamin supplement. Control animals received the same diet but with glucose replacing the xylose. Paired litter mates were used in these experiments; the animals were all started on their respective diets at the same time and were killed at specific time intervals as indicated in the Figures.

The relative activity of the hexose monophosphate shunt was determined on the basis of the recovery of $^{14}\text{CO}_2$ from the lenses incubated with glucose labelled in the C-1 or C-6 atom. The specific activity of this compound was

26000 counts/min./ μmole of $[1-^{14}\text{C}]$ glucose and 2700 counts/min./ μmole of $[6-^{14}\text{C}]$ glucose. The methods and materials employed in this experiment were reported by Lerman (1959*b*).

Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were assayed according to the method of Glock & McLean (1953).

Adenosine triphosphate (ATP) was determined by the firefly-luminescence method of Strehler & Totter (1952); disodium ATP (Sigma Chemical Co.) was employed as the standard.

Triphosphopyridine nucleotide (TPN)-xylitol dehydrogenase was assayed according to the method reported by van Heyningen (1959). Diphosphopyridine nucleotide (DPN)-xylitol dehydrogenase was assayed by a similar technique, in which DPN was substituted for TPN in the reaction mixture. D-Xylose (Mann Assayed Biochemicals, 136 Liberty St., New York) was used in these experiments. In the control reactions run concurrently with the TPN-xylitol-dehydrogenase assays, a significant amount of TPN was reduced although no xylitol had been added. It was thus necessary to determine the TPN-xylitol-dehydrogenase activity as the difference in the change in extinction at 340 m μ between the experimental and control reactions. This phenomenon, which occurred although the lens homogenates were dialysed for 16–18 hr. before assay, was never observed in the DPN-xylitol-dehydrogenase assays.

The oxidized and reduced di- and tri-phosphopyridine nucleotides DPN, TPN, DPNH and TPNH were determined by the method of Bassham, Birt, Hems & Loenig (1959), with slight modifications of the preliminary extraction procedure for the reduced pyridine nucleotides. Sodium carbonate (0.1 M) was used instead of NaOH (1 N) as greater stability and reproducibility were obtained. The standards employed (Sigma Chemical Co.) gave 80–85% recovery.

The soluble lens proteins were obtained by homogenizing single lenses in 1 ml. of water in micro-homogenizers kept in an ice-water bath. These tubes were then centrifuged at 3000 rev./min. for 30 min. and the supernatant fractions were retained for nitrogen determinations by the micro-Kjeldahl method.

RESULTS

The results of the experiment in which a single lens from each of eight control and 14 xylose-fed rats was incubated with $[1-^{14}\text{C}]$ glucose are shown in Fig. 1. The mean recovery of $^{14}\text{CO}_2$ from eight control lenses was 864 counts/min./g. wet wt. of lens (range 568–1060 counts/min.; figures uncorrected for self-absorption, which averaged 80%). This value is in good agreement with the data

Fig. 1

Fig. 2

Fig. 1. Recovery of ¹⁴CO₂ from the lenses of normal (O) and xylose-fed rats (●). Individual lenses were incubated for 21 hr. at 37° in 5 ml. of Krebs-Ringer bicarbonate buffer (pH 7.5) containing 50 μmoles of [1-¹⁴C]glucose.

Fig. 2. Recovery of ¹⁴CO₂ from the lenses of normal (O) and xylose-fed rats (●). Individual lenses were incubated for 21 hr. at 37° in 5 ml. of Krebs-Ringer bicarbonate buffer (pH 7.5) containing 50 μmoles of [6-¹⁴C]glucose.

reported previously for the normal rat lens (Lerman, 1959b). The recovery of ¹⁴CO₂ from the 14 lenses derived from the xylose-fed animals began to decline after the rats had been on the diet for 3 days. This decline became progressively more severe until the ninth day of the diet and then a slow progressive recovery took place and approached the control amounts by 15–16 days of the diet. These results correlate well with the time sequence of the development of lenticular opacities and the apparent recovery by this organ.

The amount of ¹⁴CO₂ recovered from the remaining lens of each of these rats (eight control and 14 xylose-fed) incubated with [6-¹⁴C]glucose is shown in Fig. 2. The mean recovery for the control lenses was 92 counts/min./g. wet wt. (figures uncorrected for self-absorption, which averaged 80%); the corresponding values for the lenses derived from the xylose-fed animals, though somewhat higher on occasion, showed no statistically significant difference (*P* > 0.05).

The results of the glucose 6-phosphate-dehydrogenase and 6-phosphogluconate-dehydrogenase assays are shown in Table 1. There was no significant variation in the activity of these two enzymes in the lenses from the xylose-fed rats as compared with the control lenses. These mean values (26.41 units of glucose 6-phosphate-dehydrogenase activity and 7.60 units of 6-phosphogluconate-dehydrogenase activity) for the control lenses are in close agreement with the data reported by Lerman (1960a).

Table 1. Glucose 6-phosphate- and 6-phosphogluconate-dehydrogenase activity in the lens of the normal and xylose-fed rat

For details see text. Enzyme activities are expressed as μg. of TPN reduced/mg. of soluble lens protein/hr.

Period on diet (days)	Glucose 6-phosphate-dehydrogenase activity		6-Phosphogluconate-dehydrogenase activity	
	Control lens	Lens from xylose-fed rat	Control lens	Lens from xylose-fed rat
3½	22.90	23.65	6.20	7.80
6½	31.90	32.90	8.30	8.10
9½	28.38	26.67	7.45	8.45
13½	26.05	27.67	8.85	6.85
16½	22.83	30.37	7.20	6.93

Fig. 3. Amount of soluble protein in lenses from normal (O) and xylose-fed rats (●) determined by the micro-Kjeldahl method.

The soluble-protein levels in the lenses derived from the xylose-fed rats remained relatively constant during the first 14 days of the diet, and then a small progressive increase in soluble-protein content per lens became evident, although by 21 days protein content in these lenses was still a good deal lower than the contents attained by the control lenses (Fig. 3).

Since the decreased level of soluble lens protein in the xylose-fed rats may be due to a lowered food intake (van Heyningen, 1959; Dische, 1959), the growth curves for these two groups of animals are shown in Fig. 4. After an initial failure to gain weight, the xylose-fed animals, after 6 days on the diet, showed an increase in body weight which paralleled that of the control animals.

The results of the ATP assays are shown in Table 2. In 14 control lenses the mean value was 38.41 μ g. of ATP/lens (range 34.80–42.0). After 4 days on the xylose diet the amount of ATP per

lens dropped by approx. 30 % and remained relatively constant at this amount until 16 days of the diet, when it returned to the control values.

The results of the determinations of the reduced and oxidized pyridine nucleotides are shown in Table 3 and Fig. 5. There was no change in the DPNH/DPN ratio in the lenses from the xylose-fed rats. However, the TPNH/TPN ratio showed a marked decline after 4 days on the diet and continued to fall until 6–8 days of the diet. A slow progressive recovery then ensued and the TPNH/TPN ratio returned to the control amount by the sixteenth day of the xylose diet. The marked fall in the TPNH/TPN ratio in these lenses was apparently due mainly to the decrease in total TPNH per lens. Once again this sequence of events is closely related to the clinically observed development and apparent regression of lenticular changes in these animals. Furthermore the high TPNH/TPN ratio in the control lenses provides added evidence about the high activity of the hexose monophosphate shunt in this organ.

TPN-xylitol (L-xylulose) dehydrogenase and DPN-xylitol (D-xylulose) dehydrogenase are both present in the lenses of the control and xylose-fed rats as previously reported by van Heyningen (1959). The activity of DPN-xylitol dehydrogenase is approximately ten times that of TPN-xylitol dehydrogenase (Tables 4 and 5). There is no change in the activity of the former enzyme in the lenses from the xylose-fed animals as compared with the control lenses. The activity of TPN-xylitol dehydrogenase in the xylose-fed rat lenses showed a marked fluctuation, but there was no

Fig. 4. Growth curves of paired litter-mate rats maintained on a control (O) and 30% xylose (●) diet.

Fig. 5. TPNH/TPN ratio in the xylose-fed (▲) rat lens; TPNH/TPN ratio in the control (△) rat lens; DPNH/DPN ratio in the xylose-fed (●) rat lens; DPNH/DPN ratio in the control (○) rat lens. Each plotted point represents a single determination performed on two lenses.

Table 2. Concentration of adenosine triphosphate in the lens of the normal and xylose-fed rat

ATP is expressed as μ g./lens. Each value represents the average of two lenses.

Period on diet (days)	Control lens		Lens from xylose-fed rat	
	ATP	Wt. of lens (mg.)	ATP	Wt. of lens (mg.)
4	42.0	16.8	26	15.0
			27.5	19.0
			25.5	21.3
6	41.8	15.0	27.5	22.4
			29.0	22.5
			32.0	19.0
9	38.5	18.7	22.0	22.7
			26.8	28.5
			21.8	20.0
13	35.0	30.5	23.3	21.8
			23.3	27.0
			26.0	26.1
16	39.8	26.2	32.0	29.3
			37.0	30.5
			35.5	27.8

significant difference between the activity of this enzyme when these values were compared with enzyme activity in the control lenses (Table 5).

DISCUSSION

The results of these experiments indicate that some of the biochemical difficulties that the lens of a xylose-fed animal encounters may be attributable to an impairment of the hexose monophosphate shunt. The decline in the C-1/C-6 and in the

TPNH/TPN ratios after the third day on the diet and their subsequent return towards normal amounts (after the animal has been on the xylose diet for approximately 16 days) are related to the clinically observed changes that become manifest in the lens during this period. The development and apparent regression of the lenticular opacities occurs between 5 days and 14–18 days on this diet.

Since the activities of both glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase remain unimpaired, the mechanism whereby

Table 3. *Reduced and oxidized di- and tri-phosphopyridine nucleotides in the lens of the normal and xylose-fed rat*

Concentration of the nucleotides is expressed as $\mu\text{g./g.}$ wet wt. of lens. Two lenses were employed for each determination.

Period on diet (days)	Control lens		Lens from xylose-fed rat		Control lens		Lens from xylose-fed rat	
	DPN	DPNH	DPN	DPNH	TPN	TPNH	TPN	TPNH
3½	141	180	124	169	51	247	29	221
5½	107	130	77	115	30	147	33	70
6½	191	206	103	170	36	131	35	79
9½	139	226	142	162	34	294	31	102
	160	273	—	—	31	201	—	—
12½	120	202	99	180	42	257	39	165
	118	273	—	—	38	211	—	—
16½	107	233	96	155	36	269	32	164

Table 4. *Diphosphopyridine nucleotide-xylose-dehydrogenase activity in the lens of the normal and xylose-fed rat*

DPN-xylose-dehydrogenase activity is expressed in units, 1 unit being defined as the activity which produces a change of E at 340 $m\mu$ of 0.001/min./g. wet wt. of lens at 24°.

Period on diet (days)	Wt. of control lens (mg.) (av. of two)	Enzyme activity in control lens	Wt. of xylose-fed lens (mg.) (av. of two)	Enzyme activity in xylose-fed lens
1	11.7	56.79	13.5	54.93
2½	18.6	59.14	12.0	98.61
3½	22.5	73.30	25.0	66.00
5½	19.4	73.79	21.8	72.63
6½	18.7	43.52	23.3	72.61
11½	23.2	37.35	24.8	45.03
		Mean 57.32		

Table 5. *Triphosphopyridine nucleotide-xylose-dehydrogenase activity in the lens of the normal and xylose-fed rat*

TPN-xylose-dehydrogenase activity is expressed in units, 1 unit being defined as the activity which produces a change of E at 340 $m\mu$ of 0.001/min./g. wet wt. of lens at 24°.

Period on diet (days)	Wt. of control lens (mg.) (av. of two)	Enzyme activity in control lens	Wt. of xylose-fed lens (mg.) (av. of two)	Enzyme activity in xylose-fed lens
1	11.7	5.12	13.5	3.70
2½	18.6	5.33	12.0	12.50
3½	22.5	2.96	25.0	2.67
5½	19.4	7.73	21.8	8.41
6½	18.7	7.84	23.3	1.43
11½	23.2	1.44	24.8	3.36
14½	19.2	5.65	26.8	7.46
		Mean 4.44		

the shunt is inhibited in these lenses differs from the previously reported effects of a high galactose diet on the rat lens (Lerman, 1960*a*; Lerman, 1959*a*). With the latter diet, a marked inhibition of the enzyme glucose 6-phosphate dehydrogenase rapidly develops. The mechanism responsible for an inhibition of the shunt in the lenses of xylose-fed rats is apparently a more complex one.

van Heyningen (1959) has reported the results of her studies on the metabolism of xylose by the rat lens. Her observations that the lactate concentration in the lenses of xylose-fed rats did not differ from the amount of lactic acid in the control lenses, together with our finding that the recovery of $^{14}\text{CO}_2$ from $[6\text{-}^{14}\text{C}]\text{glucose}$ from both xylose-fed and control lenses was essentially the same, would indicate that there is no gross impairment of anaerobic glycolysis in the lens of the xylose-fed rat. We were also able to corroborate her report of the presence of DPN- and TPN-xylitol dehydrogenase in this organ. Although she did not find any significant difference in the concentration of ATP between the lens of the control and xylose-fed rat, her assay was done only at the third day of the diet. Our results indicate that there is a significant decrease in ATP concentration in these lenses, which occurs after 3–4 days of xylose feeding and returns to normal after 16 days in the diet. The apparent inhibition of soluble protein synthesis follows a more prolonged course and shows only slight signs of recovery by 14 days.

Since the evidence thus far points to a possible inhibition of the shunt followed by a decrease in ATP concentration and an apparent failure to synthesize soluble protein, the shunt may in some way be an important source for the formation of high-energy phosphates. Pirie, van Heyningen & Boag (1953) have reported the presence of TPNH-cytochrome *c* reductase in the rabbit lens. A relatively active TPNH- as well as DPNH-cytochrome *c* reductase is also present in the rat lens (S. Lerman, unpublished work). The activity of the TPNH-cytochrome *c* reductase was found to be approximately one-third greater than the DPNH-cytochrome *c* reductase. Furthermore, there is no change in the relative activities of these two enzymes in the lenses derived from xylose-fed rats (S. Lerman, unpublished work). Thus this mechanism may be capable of providing a source of ATP for the rapidly growing rat lens by utilizing the relatively large amounts of TPNH formed by the shunt.

In the lens of the xylose-fed rats, there is a marked fall in the TPNH/TPN ratio after 3–4 days on the diet, and recovery occurs by 16 days. The basis for this decline is the decrease in TPNH, since the amount of TPN remains fairly constant throughout this period. This loss may be due to the

utilization of the TPNH by the rat lens in the reduction of D-xylose to xylitol (van Heyningen, 1959). This should become manifest quite early in the experimental period since the rat lens rapidly accumulates xylitol when the animal is placed on the xylose diet (van Heyningen, 1959). The equilibrium of this reaction apparently favours the formation of xylitol. van Heyningen has suggested that the limiting reaction might be the conversion of xylitol into D-xylulose. However, the activity of DPN-xylitol (D-xylulose) dehydrogenase remains unchanged in the lenses from the xylose-fed rats. It is thus possible that the rapid formation of and utilization of D-xylulose by the lens may also serve as a rate-limiting factor; that is, an inhibition of the hexose monophosphate shunt may develop.

SUMMARY

1. The lens of a 28- to 32-day-old rat fed on a 30% xylose diet shows an initial decline in the activity of the hexose monophosphate shunt as reflected by the C-1/C-6 ratio of $^{14}\text{CO}_2$ recovered from lenses incubated with $[1\text{-}^{14}\text{C}]\text{glucose}$ or $[6\text{-}^{14}\text{C}]\text{glucose}$.
2. This C-1/C-6 ratio returns to normal amounts after the animal has been on the xylose diet for 15–16 days.
3. The TPNH/TPN ratio in these lenses shows a parallel decline and recovery whereas the DPNH/DPN ratio remains unimpaired.
4. Both DPN- and TPN-xylitol dehydrogenases are present in the lenses of control and xylose-fed rats, as previously reported by van Heyningen (1959). There is no significant alteration in their activities in the lenses from xylose-fed rats.
5. The amount of soluble protein in the lenses of xylose-fed rats suggests an inhibition of soluble protein synthesis in these organs after 6–8 days on the diet, with a slow recovery beginning at 14 days.
6. There is a moderate decline of adenosine triphosphate in these lenses after 4 days on the diet with a recovery occurring by 16 days.
7. An hypothesis has been proposed to explain the biochemical changes that occur in these lenses during the period of development and apparent regression of lenticular opacities.

Acknowledgement is made to Dr E. Stotz and Dr M. Morrison for their helpful criticism and advice during the preparation of this paper and to Miss Suzzan Hawes for her technical help. This work was supported by research funds granted by the Rochester Eye Bank and Research Society Inc.

REFERENCES

- Babel, J. (1953). *v. Graefes Arch. Ophthalm.* **153**, 520.
Bassham, J. A., Birt, L. M., Hems, R. & Loening, U. E. (1959). *Biochem. J.* **73**, 491.

- Booth, A. N., Wilson, R. H. & De Eds, F. (1953). *J. Nutr.* **49**, 347.
- Buschke, W. (1943). *Arch. Ophthalm., Chicago*, **30**, 735.
- Dische, Z. (1959). *Amer. J. Ophthalm.* **48**, 500.
- Glock, G. E. & McLean, P. (1953). *Biochem. J.* **55**, 400.
- Lerman, S. (1959a). *Science*, **130**, 1473.
- Lerman, S. (1959b). *Nature, Lond.*, **184**, 1406.
- Lerman, S. (1960a). *A.M.A. Arch. Ophthalm.* **63**, 128.
- Lerman, S. (1960b). *A.M.A. Arch. Ophthalm.* (in the Press).
- Mitchell, H. S. & Dodge, W. M. (1935). *J. Nutr.* **9**, 37.
- Pirie, A., van Heyningen, R. & Boag, J. W. (1953). *Biochem. J.* **54**, 682.
- Strehler, B. L. & Totter, J. R. (1952). *Arch. Biochem. Biophys.* **40**, 28.
- van Heyningen, R. (1959). *Biochem. J.* **73**, 197.

Biochem. J. (1961) **79**, 229

The Effect of SKF 525A (2-Diethylaminoethyl 2:2-Diphenylvalerate Hydrochloride) on Organophosphate Metabolism in Insects and Mammals

By R. D. O'BRIEN*

Pesticide Research Institute, London, Ontario, Canada

(Received 21 July 1960)

The organophosphates are of two classes: those which are toxic directly, and those which require 'activation' in the animal body to produce the actual toxic compound. The directly acting compounds include the phosphates and phosphorothiolates, most of which contain the $-P(O)(OR)_2$ grouping, where R is an alkyl group. The other class includes the phosphorothionates, most of which contain the $-P(S)(OR)_2$ grouping, and the phosphorodiamidates that contain the $-P(O)(NR_2)_2$ grouping.

'Activation' is an oxidative reaction, involving the conversion in phosphorothionates of P(S) into P(O) (Gage, 1953) or, in phosphorodiamidates, of an alkyl into a hydroxyalkyl group (Heath, Lane & Park, 1955; Spencer, O'Brien & White, 1957). 'Activation' is accomplished in mammals almost exclusively by liver microsomes (Davison, 1955), and in insects principally by gut and fat body (Metcalf & March, 1953; O'Brien & Spencer, 1953).

It has been shown that, in mammalian-liver preparations, SKF 525A (2-diethylaminoethyl 2:2-diphenylvalerate hydrochloride) blocks the 'activation' of schradan (octamethylpyrophosphoramidate; tetramethylphosphorodiamidic anhydride), Guthion [*OO*-dimethyl *S*-(4-oxo-3H-1:2:3-benzotriazine-3-methyl) phosphorodithioate] and parathion (diethyl *p*-nitrophenyl phosphorothionate) (Davison, 1955; Murphy & DuBois, 1957). *In vivo*, SKF 525A is a potent antagonist of poisoning by schradan and Guthion, but not of poisoning by parathion (O'Brien & Davison, 1958). The present paper examines this paradox further.

* Present address: Department of Entomology, Cornell University, Ithaca, New York, U.S.A.

The effects of SKF 525A on the toxicity or metabolism of organophosphates in insects have not been reported previously. They are of interest because of the possibility that SKF 525A might be useful as an adjuvant in the treatment of livestock by systemic organophosphates for internal parasites. They may also help to elucidate the nature of the differences between the activating systems of mammals and insects.

METHODS

The mice were female albinos (20–35 g.) from Rolfmeyer Farms (Madison, Wis.). The houseflies (*Musca domestica*) were 1-day-old adult females of a normal CSMA strain. The cockroaches (*Periplaneta americana*) were adult females, about 28 days moulted.

The organophosphates used were: (1) Chlorthion (dimethyl *m*-chloro-*p*-nitrophenyl phosphorothionate); (2) Co-ral (diethyl 3-chloro-4-methylcoumarin-7-yl phosphorothionate); (3) Delnav [mixed isomers of 2:3-*p*-dioxandithiol *SS*-bis-(*OO*-diethyl phosphorodithioate)]; (4) Diazinon (diethyl 6-methyl-2-isopropylpyrimidin-4-yl phosphorothionate); (5) dimefox (tetramethylphosphorodiamidic fluoride); (6) dimethoate [*OO*-dimethyl *S*-(*N*-methyl-carbamoylmethyl) phosphorodithioate]; (7) Dowco 109 (*O-p-tert*-butyl-*o*-chlorophenyl *O*-methyl *N*-methylphosphoramidothionate); (8) EPN (ethyl *p*-nitrophenyl phenylphosphonothionate); (9) mipafox (*NN'*-diisopropylphosphorodiamidic fluoride); (10) paraoxon (diethyl *p*-nitrophenyl phosphate); (11) parathion; (12) ronnel (dimethyl 2:4:5-trichlorophenyl phosphorothionate); (13) schradan; (14) Systox (diethyl 2-ethylthioethyl phosphorothionate and its thiole isomer); (15) TEPP (tetraethyl pyrophosphate; diethyl phosphoric anhydride); (16) Tetram [*OO*-diethyl *S*-(2-diethylaminoethyl)phosphorothiolate]; (17) Thimet [*OO*-diethyl *S*-(2-ethylthioethyl)phosphorodithioate].