Occurrence and Metabolism of *scyllo*Inositol in the Locust

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1. *scyllo*Inositol has been identified as a component of locust haemolymph, where it occurs in concentrations of 0.2–0.4 mg./ml. 2. A simple method for the identification of *scyllo*inositol is described. This has been used to demonstrate the presence of *scyllo*inositol in five other insect species. 3. Locust phospholipids contain *myo*inositol but no *scyllo*inositol. 4. Radioactivity from [^{14}C]glucose is incorporated into *myo*inositol and *scyllo*inositol by the locust *in vivo*. 5. Extracts of locust fat body catalyse the conversion of *myo*inositol into *scyllo*inositol. This seems to take place by a two-step process in which *myo*inositol is first oxidized with NAD^+ to *myo*inosose-2, and the *myo*inosose-2 is stereospecifically reduced with NADPH to *scyllo*inositol.

A number of different carbohydrates have been found in insect haemolymph. Trehalose is quantitatively the most important sugar in many insects (Wyatt & Kalf, 1957; Howden & Kilby, 1956) and in addition the presence of glucose, fructose, glycerol, amino sugars and glycogen has been reported (see Wyatt, 1961). The present paper describes the identification of *scyllo*inositol as a carbohydrate component of locust haemolymph. The synthesis of this compound by extracts of locust fat body has also been studied, and observations related to the possible metabolic importance of *scyllo*inositol are reported.

MATERIALS AND METHODS

Insects. Fifth-instar hoppers of the desert locust *Schistocerca gregaria* were obtained from the Anti-Locust Research Centre (London, W. 8). Except where otherwise stated the locusts were fed on the artificial diet described by Howden & Hunter-Jones (1958), supplemented with fresh barley seedlings. Other insect species were obtained from the Department of Zoology, University of Birmingham.

Special chemicals. *myo*Inosose-2 (m.p. 195–197°) was prepared by bacterial oxidation of *myo*inositol (Posternak, 1962) by using *Acetobacter suboxydans* N.C.I.B. 7069. A sample of *scyllo*inositol was kindly supplied by Professor A. B. Foster, and a further sample was prepared by the sodium borohydride reduction of *myo*inosose-2 (Reymond, 1957). NAD^+ , NADH, NADP^+ and NADPH were obtained from the Sigma Chemical Co. (St Louis, Mo., U.S.A.) and solutions of these were adjusted to pH 8 with sodium hydroxide soln. before use. D-[^{14}C]Glucose (3.9 mc/m-mole) and [^{14}C]myoinositol (150 mc/m-mole) were obtained from The Radiochemical Centre (Amersham, Bucks.).

Paper chromatography. The following solvent systems

were used: solvent 1, phenol–water (10:3, w/v); solvent 2, acetone–water (4:1, v/v). Whatman no. 1 paper was employed. After development with solvent 1, chromatograms were dried at room temperature for several hours and then well washed with acetone to remove any remaining phenol. Cyclitols were detected by the method of Trevelyan, Procter & Harrison (1950) or by the more specific method of Nagai & Kimura (1958).

Paper electrophoresis. Electrophoresis was carried out in 0.05 M-sodium tetraborate at 25 v/cm. for approx. 90 min. In some experiments samples (approx. 5 $\mu\text{g.}$) were treated by heating (100°, 10 min.) in 0.02 ml. of 0.05 M-sodium tetraborate before electrophoresis. As discussed below, *scyllo*inositol is characterized by a change in electrophoretic properties when treated in this way. After electrophoresis the papers were dried and treated with hydrogen fluoride in acetone (Britton, 1959). Detection was by the silver nitrate method of Trevelyan *et al.* (1950).

Estimation of haemolymph scylloinositol. For each estimation the haemolymph from at least five insects was used (total vol. approx. 0.5 ml.). Haemolymph was withdrawn from the thoracic haemocoel by means of a fine glass capillary, weighed, heated at 100° for 3 min., diluted to 2 ml. and centrifuged. The residue was washed twice with 2 ml. portions of water and the supernatants were passed through a small column containing 1 ml. of Amberlite IR-120 (H^+ form) resin and 1 ml. of Dowex 1 (OH^- form) resin. The column was then washed with 4 ml. of water and the combined effluents were evaporated to dryness under reduced pressure. The residue was taken up in a known volume of water (usually 0.2 ml.) and a series of dilutions of this were made. The *scyllo*inositol content of these solutions was then estimated by an adaptation of the chromatographic method of McFarren, Brand & Rutowski (1951): 0.01 ml. samples (2–10 $\mu\text{g.}$ of *scyllo*inositol) were applied to the base line of a chromatogram. At least three samples of the unknowns (at different concentrations) and four samples of a range of standard *scyllo*inositol solutions were applied per chromatogram. The chromatogram was then developed

in solvent 1 for 3 days (this separated *scylloinositol* from all other known deionized haemolymph components, including *myoinositol*). The chromatogram was dried and phenol removed as described above. It was then dipped quickly and evenly through a solution prepared by adding 98 ml. of acetone to a solution of 0.6 g. of silver nitrate in 2 ml. of water. After drying for 5 min. at room temperature, the chromatogram was again dipped in the silver nitrate reagent and dried. The chromatogram was then dipped in a solution of 2.5% (w/v) potassium hydroxide in ethanol and allowed to dry for 15 min. After dipping in aq. 10% (w/v) sodium thiosulphate, the chromatograms were well washed in distilled water and dried.

The density of the spot centres was measured directly (McFarren *et al.* 1951). A portion of the chromatogram was held flat against the light-source side of the cell housing of a Unicam SP.500 spectrophotometer, the light-source (at 600 m μ) being masked to a rectangle of 3 mm. $\times$ 5 mm. The instrument was first adjusted to zero on blank portions of the chromatogram, and then each spot was measured by repeated changes of the paper position to obtain a maximum density reading. The *scylloinositol* content of the unknowns was calculated by comparison of the densities of the unknown *scylloinositol* spots with those of the standard *scylloinositol* spots on the same chromatogram (McFarren *et al.* 1951). The standard deviation for a single spot was found to be 8% (17 estimations).

The overall recovery of inositol in this procedure was checked as follows. To haemolymph samples known to contain no *myoinositol* (checked by paper chromatography) were added known amounts of *myoinositol* to act as marker. These samples of haemolymph were subjected to the estimation procedure described above, except that additional standards of *myoinositol* were included on the chromatograms. The average recovery of *myoinositol* was 98.6% $\pm$ s.d. 7.2% (10 estimations).

Isolation of *scylloinositol* from haemolymph. Haemolymph was withdrawn from adult locusts (3–6 weeks after the final moult), heated at 100° for 3 min. and stored at –20°. Then 83 g. of haemolymph was extracted with 100 ml. of water and filtered. The filtrate was deionized by passage through a column of Amberlite IR-120 (H⁺ form) resin (100 ml.) and a column of Amberlite IR-4B (OH[–] form) resin (100 ml.). The effluent was evaporated under reduced pressure to 20 ml. and added to a column of Dowex 1 (borate form) resin (100 ml.). Elution was carried out with water, and the eluate was collected in fractions. Samples of the fractions were examined chromatographically for the presence of *scylloinositol*. The highest concentration of a compound chromatographically identical with *scylloinositol* was found after about 150 ml. of eluate had been collected. Appropriate fractions were combined and evaporated repeatedly with methanol to remove any boric acid. The residue was taken up in 0.5 ml. of hot water, 0.2 ml. of ethanol was added and the solution was cooled. Colourless crystals were obtained and were recrystallized from water–ethanol. The yield was 18 mg., m.p. 350–355°. This is referred to as the ‘haemolymph product’.

Isolation of *scylloinositol* from whole insects. The insect was first homogenized with 10 vol. of water, and the homogenate was heated to 100° for 3 min., cooled and filtered. The filtrate was deionized by passage through columns containing Amberlite IR-120 (H⁺ form) and Dowex 1 (OH[–] form) resins, and examined by paper chromatography

with solvent 1. The area on the chromatogram which corresponded to *scylloinositol* was eluted with water. One sample of the eluate was examined by borate electrophoresis, and another sample was first heated in 0.05 M-sodium tetraborate (10 min., 100°) and then examined by electrophoresis.

Locust phospholipids. Fifteen adult locusts (2 weeks after final moult, total weight 35 g.) were homogenized in chloroform–methanol (1:2, v/v), and the homogenate was treated by the procedure of Bligh & Dyer (1959) to give a chloroform-soluble fraction. This was evaporated to dryness under reduced pressure, taken up in 2 ml. of chloroform and the phospholipid was precipitated with 10 vol. of acetone. The precipitate was hydrolysed in a sealed tube with 2 ml. of 6 N-HCl at 120° for 16 hr., and the hydrolysate was deionized [Amberlite IR-120 (H⁺ form) and Dowex 1 (OH[–] form) resins] and examined by paper chromatography with solvent 1.

Isolation of inositols from locust injected with radioactive glucose. Saline (Hoyle, 1953), 0.02 ml. containing 10 μ C (2.5 μ moles) of D-[U-¹⁴C]glucose, was injected into the thoracic haemocoel of an adult male locust (7 days after final moult). After 24 hr. the animal was killed and ground in a mortar and pestle with 5 ml. of 6 N-sulphuric acid. The suspension was refluxed in 60 ml. of 6 N-sulphuric acid for 12 hr., diluted to 300 ml. with water, neutralized with barium carbonate and filtered. The filtrate was passed through columns containing 25 ml. each of Amberlite IR-120 (H⁺ form) and Dowex 1 (OH[–] form) resins. The effluent was concentrated under reduced pressure and diluted with water to a final volume of 5.0 ml. This solution is referred to as the ‘deionized hydrolysate’. Estimation of the radioactivity of *scylloinositol* and *myoinositol* in this solution was carried out as follows: 250 mg. of carrier *myoinositol* was dissolved in 2 ml. of warm ‘deionized hydrolysate’, and *myoinositol* was crystallized from this solution by adding a little ethanol and cooling. The product was acetylated by heating (10 min., 100°) with 12 ml. of acetic anhydride containing 0.4 ml. of conc. sulphuric acid and pouring the mixture into 2 l. of water at 0°. *myo*Inositol hexa-acetate was repeatedly recrystallized from ethanol to constant specific activity (m.p. 212–214°). This procedure was repeated for *scylloinositol*, except that 150 mg. of carrier was used, and that the *scylloinositol* hexa-acetate (m.p. 299–302°) was recrystallized from dioxan–ethanol. The inositol hexa-acetates were counted in solution in dioxan scintillator.

Radioactivity measurements. Scintillation counting was carried out in a Nuclear–Chicago liquid-scintillation system 725. The toluene scintillator consisted of 0.5% (w/v) 2,5-diphenyloxazole and 0.01% 1,4-bis-(5-phenyloxazol-2-yl)benzene in toluene, and the dioxan scintillator was a solution of 10% naphthalene, 0.7% 2,5-diphenyloxazole and 0.03% 1,4-bis-(5-phenyloxazol-2-yl)benzene in dioxan. Radioactivity on paper chromatograms was detected either with a strip scanner (Panax RCMS-2 with scintillation detector) or by radioautography with Kodirex X-ray film. Quantitative measurements of radioactive chromatograms were made by direct scintillation counting (Loftfield & Eigner, 1960).

Extracts of fat body. Adult locusts approx. 3 weeks after the final moult were used. The fat bodies were removed and transferred to ice-cold insect saline (Hoyle, 1953). Excess of saline was removed by blotting and the fat bodies were

Table 1. *Identification of 'haemolymph product'*

Figures in parentheses refer to values reported in the literature.

Experiment	'Haemolymph product'	<i>scyllo</i> Inositol	<i>myo</i> Inositol
(1) Colour test for cyclitols*	Pink colour	Pink colour	Pink colour
(2) m.p.	350–355°	351–355° (350–354°†)	(224–225°†)
mixed m.p.	350–355° (with <i>scyllo</i> inositol)		
(3) m.p. of acetate	298–300°	299–302° (300–301°†)	(213–214°†)
mixed m.p.	298–300° (with <i>scyllo</i> inositol acetate)		
(4) Chromatography in solvent 1 (40 hr.)	Moved 10.9 cm.	Moved 10.8 cm. (R_F 0.15†)	Moved 13.3 cm. (R_F 0.20†)
(5) Chromatography in solvent 2 (16 hr.)	Moved 15.2 cm.	Moved 15.0 cm. (R_F 0.17†)	Moved 16.0 cm. (R_F 0.18†)
(6) Electrophoresis	M_{Glc} 0.03	M_{Glc} 0.03 (0.02§)	M_{Glc} 0.48 (0.49§)
(7) Electrophoresis after heating with borate	M_{Glc} 1.13	M_{Glc} 1.13	M_{Glc} 0.48
(8) i.r. spectrum	Identical with <i>scyllo</i> inositol		
(9) i.r. spectrum of acetate	Identical with <i>scyllo</i> inositol acetate		

* Nagai & Kimura (1958). † Anderson & Wallis (1948). ‡ Angyal, McHugh & Gilham (1957). § Foster (1962).

homogenized with 2 vol. of water in a Potter–Elvehjem blender. The homogenate was centrifuged at 15000g for 30 min. and the supernatant fraction was dialysed for 24 hr. against 1 l. of distilled water.

Other methods. Infrared spectra were obtained by using a Unicam SP.200 spectrophotometer and the Nujol-mull technique. Melting points were corrected for the emergent stem of the thermometer.

RESULTS

Identification of scylloinositol. Table 1 lists experiments carried out to identify the 'haemolymph product' as *scyllo*inositol. Infrared absorption maxima were: for the 'haemolymph product', 1119, 1099, 1083, 1003 and 982 cm.⁻¹; for *scyllo*inositol, 1119, 1100, 1085, 1004 and 983 cm.⁻¹; for *myo*inositol, 1242, 1192, 1144, 1110, 1046, 1014, 1000, 930, 899, 894 and 730 cm.⁻¹. These values for the two standard compounds agree with those reported by Barker, Bourne, Stephens & Whiffen (1954).

*scyllo*Inositol concentrations in haemolymph. Table 2 shows the concentration of *scyllo*inositol in haemolymph from locusts of different ages and fed on different diets. The results show that the *scyllo*inositol content of haemolymph is greatly increased by feeding the insect on a diet with high *myo*inositol content.

*scyllo*Inositol in other insect species. Whole animals were used for these experiments since it was difficult to obtain sufficient quantities of haemolymph from insects of some species. *scyllo*Inositol was isolated from the insects as described in the Materials and Methods section. Evidence for the presence of *scyllo*inositol (i.e. a spot running as *scyllo*inositol on chromatography with solvent 1,

Table 2. *Concentration of scylloinositol in locust haemolymph*Figures are expressed as means $\pm$ s.d., with numbers of observations in parentheses.

Locust age (weeks after final moult)	Sex	Diet	Concn. of <i>scyllo</i> inositol (mg./g. of haemolymph)
2	M	Normal	0.19 $\pm$ 0.03 (5)
2	F	Normal	0.20 $\pm$ 0.05 (5)
6	M	Normal	0.43 $\pm$ 0.04 (3)
6	F	Normal	0.28 $\pm$ 0.02 (3)
1	M	<i>myo</i> Inositol*	1.45 (1)
1	F	<i>myo</i> Inositol*	1.02 (1)
3	M	<i>myo</i> Inositol*	2.8 (1)
3	F	<i>myo</i> Inositol*	2.2 (1)

* Fed (from final moult) on diet (Howden & Hunter-Jones, 1958) with addition of 5% (w/w) of *myo*inositol.

electrophoresis in borate buffer and electrophoresis after heating in borate) was obtained for the following insect species: *Locusta migratoria*, *Periplaneta americana*, *Periplaneta australasiae*, *Blaberus* species and *Calliphora* species. In the only other species examined (*Blaps mucronata*) no *scyllo*inositol was detected in one sample, although a trace spot was obtained from a second sample.

Inositols of locust phospholipids. The results described above show the presence of free *scyllo*inositol in insects. The possibility that *scyllo*inositol, like *myo*inositol, is present in locust phospholipids was examined. Phospholipids were extracted from locusts and hydrolysed as described in the Materials and Methods section. Paper chromatography

showed a large spot corresponding to *myoinositol* but no spot corresponding to *scylloinositol*.

Synthesis of inositols from glucose in vivo. *myo*-Inositol and *scylloinositol* were isolated from a locust injected 24 hr. previously with [^{14}C]glucose (see the Materials and Methods section). The results showed that 0.044% of the administered radioactivity was present in *myoinositol* and that 0.017% of the administered radioactivity was present in *scylloinositol*.

Confirmation of the presence of radioactive *scylloinositol* and *myoinositol* in the 'deionized hydrolysate' was obtained by chromatography with solvent 1. Radioactive areas corresponding to the two inositols were obtained. The radioactive materials were eluted from the chromatogram and

samples were examined by electrophoresis and by electrophoresis after heating in borate solution. The radioactive areas on the electrophoretograms corresponded to similarly treated standards of *myo*-inositol and *scylloinositol*.

Formation of scylloinositol from myoinositol in extracts of fat body. Table 3 shows that NAD^+ is required for *scylloinositol* synthesis from *myo*-inositol in extracts of locust fat body. No requirement for the other coenzymes tested was observed.

Reduction of myoinosose-2 by extracts of fat body. Fat-body extracts were also found to catalyse the reduction of *myoinosose-2* by reduced dinucleotide coenzymes (Table 4). The main product with NADH was *myoinositol*, and with NADPH was *scylloinositol*. Identification of the reaction products was confirmed by borate electrophoresis.

Enzymic reactions followed spectrophotometrically. The enzyme-catalysed reactions were followed by observing the oxidation and reduction of NAD and NADP in the spectrophotometer at 340 m μ . Table 5 shows the results. Reactions involving oxidation of coenzymes proceeded virtually to completion, but reactions involving coenzyme reduction stopped when only a small proportion of the coenzyme had been reduced. Presumably, at pH 7–9 the equilibrium position of the reactions was such that nearly all of the cyclitol substrate was in the reduced form.

Table 3. *Synthesis of scylloinositol from myoinositol by fat-body extract*

The reaction mixtures consisted of fat-body extract (0.3 mg.), tris buffer, pH 8.8 (5 μ moles), [$\text{U-}^{14}\text{C}$]*myoinositol* (0.05 μ mole, 0.05 μC) and coenzymes as listed (0.25 μ mole each) in a volume of 0.2 ml. After incubation for 2 hr. at 25°, the reaction mixtures were heated at 100° for 3 min., deionized by stirring with 0.05 ml. each of Amberlite IR-120 (H^+ form) and Dowex 1 (OH^- form) resins, made up to 0.4 ml., and 0.1 ml. was run on a chromatogram with solvent 1. Radioactive areas on the chromatogram were detected by radioautography and measured by scintillation counting.

Reaction mixture	<i>scyllo</i> Inositol formed (% of total radioactivity)
No coenzymes	0.2
NAD $^+$	13.8
NADH	0.35
NADP $^+$	1.7
NADPH	0.95
NAD $^+$ + NADPH	11.8
NAD $^+$ + NADPH, boiled enzyme	0.15

DISCUSSION

One point of particular interest in the identification of *scylloinositol* is its reaction with borate ion. Most neutral polyhydroxy compounds react reversibly and quickly with borate ions at pH 10 to form a complex, and consequently migrate towards the anode on electrophoresis in borate buffer. *scyllo*-Inositol is exceptional in reacting only very slowly with borate ion at room temperature, so that on borate electrophoresis the bulk of the material is non-migratory. However, *scylloinositol* will react

Table 4. *Reduction of myoinosose-2 by fat-body extracts*

The reaction mixtures consisted of fat-body extract (0.25 mg. of protein), *myoinosose-2* (0.25 μ mole), tris (3 μ moles, adjusted to pH 7.6 with HCl) and coenzymes as listed (0.5 μ mole) in a volume 1 ml. After incubation for 2 hr. at 37° the reaction mixtures were heated to 100° (3 min.), centrifuged, and samples of the supernatants were examined by paper chromatography with solvent 1. Estimates of the amounts of material present were obtained by the direct spectrophotometric method (McFarren *et al.* 1951) and the results are expressed as percentages of the total amount of inosose plus inositols.

Reaction mixture	<i>myo</i> Inosose-2	<i>myo</i> Inositol	<i>scyllo</i> Inositol
No additions	88	—	12
NADH	—	91	9
NADPH	—	2	98
NADH, boiled enzyme	100	—	—
NADPH, boiled enzyme	100	—	—

Table 5. *myoInositol dehydrogenase and scylloinositol dehydrogenase followed spectrophotometrically*

The reaction mixtures consisted of fat-body extract, substrate, (10 μ moles of *scylloinositol*, *myoinositol* or *myoinosose-2*), coenzyme (0.125 μ mole of NAD⁺, NADH, NADP⁺ or NADPH) and buffer (25 μ moles; phosphate below pH 8, tris above pH 8) in a total volume 1 ml. The reaction was followed by measuring changes in E_{340} in a 1 cm. light-path cuvette and the initial reaction rates were measured at 25° at the stated pH. A unit rate is defined as the oxidation or reduction of 1 μ mole of coenzyme/min./mg. of protein.

Substrate	Coenzyme	Approx. pH optimum of reaction	Reaction rate
<i>scylloInositol</i>	NADP ⁺	9.0	9 (pH 9)
<i>scylloInositol</i>	NAD ⁺		0 (pH 9)
<i>myoInositol</i>	NADP ⁺		3.4 (pH 9)
<i>myoInositol</i>	NAD ⁺	9.0	2.1 (pH 9)
<i>myoInosose-2</i>	NADPH	7-7.5	130 (pH 7.5)
<i>myoInosose-2</i>	NADH	7-7.5	57 (pH 7.5)

to form a complex if heated with borate, or if allowed to react at room temperature for 24 hr. (Weissbach, 1958). Weissbach proposed that the product of this reaction is *scylloinositol* diborate, but according to Vogl, Anderson & Simons (1966) the compound may be a polymer. This characteristic reaction with borate provides a convenient method for the identification of *scylloinositol*. First, unlike other inositols and related compounds (Foster, 1962), it does not migrate on borate electrophoresis; secondly, after heating in borate buffer at pH 10 the product migrates more rapidly ($M_{\text{glc}} 1.13$) than the other inositols.

scylloInositol has been found in fish tissues, in mammalian urine and in a number of plants (see Posternak, 1965). The experiments reported in this paper show that it is also present in locust haemolymph and in a number of other insects. It seems therefore that *scylloinositol* is a fairly common constituent of living organisms.

The functions of *scylloinositol* are still obscure. Hawthorne (1961) has examined the possibility that *scylloinositol*, like *myoinositol*, could be a constituent of phospholipids. He found that phospholipids from the acorn and from the liver of the common ray contained no *scylloinositol*, although both tissues are a source of free *scylloinositol*. The experiments reported here show that locust phospholipids contain considerable quantities of *myoinositol* but no *scylloinositol*.

In the locust, *scylloinositol* occurs in the haemolymph in fairly high concentrations (0.2-0.4 mg./ml.), but in mammals it is not a normal constituent of the blood (Malangeau, 1956). It is noteworthy that glycerol has been found in insect haemolymph (sometimes in high concentrations) and that another polyol, sorbitol, also occurs in insects (Wyatt, 1961). The main haemolymph carbohydrate in many insects is trehalose and this compound too has no reducing group. It may be of importance that all these compounds are chemically fairly unreactive.

For example, reactions between reducing sugars and amino acids are known to occur even at temperatures of about 30° (see Ellis, 1959), and amino acids are found in insect haemolymph in high concentrations. Gilmour (1965) has suggested that an advantage of trehalose to insects would be its lack of reactivity with amino acids. This advantage would also be possessed by *scylloinositol* and other haemolymph polyols.

In a study of nutritional requirements of the locust Dadd (1961) found that hoppers fed on a *myoinositol*-free diet showed poor growth, and that few hoppers survived the third instar. The evidence presented here suggests that adult locusts are capable of limited synthesis of inositols: radioactivity was incorporated from [¹⁴C]glucose into *myoinositol* and *scylloinositol*. The amounts incorporated (0.044% and 0.017% respectively) can be compared with values (0.08-1.4% and 0.0008-0.014% respectively) obtained in similar experiments with the rat (Posternak, Schopfer & Boetsch, 1959). The results do not exclude the possibility that symbiotic micro-organisms are responsible for inositol synthesis in the locust.

The insect fat body is the site of synthesis of trehalose (Candy & Kilby, 1961) and many other haemolymph components (Kilby, 1965). It is now shown that fat body can also synthesize *scylloinositol*.

The conversion of *myoinositol* into *scylloinositol* involves an epimerization at C-2 of the *myoinositol*. It is proposed that in fat-body extracts this conversion takes place as a two-step process with *myoinosose-2* as an intermediate (Scheme 1). The first step is an NAD-linked oxidation of *myoinositol* catalysed by *myoinositol dehydrogenase* (*myoinositol*-NAD oxidoreductase, EC 1.1.1.18). A similar enzyme is involved in the degradation of *myoinositol* by *Aerobacter aerogenes* (Lerner, Jackson, Graves & Stamer, 1956; Berman & Magasanik, 1966). The second step of *scylloinositol*

Scheme 1. Proposed pathway for conversion of *myo*inositol into *scyllo*inositol in locust fat body.

synthesis is an NADP-linked stereospecific reduction of *myo*inosose-2 to *scyllo*inositol; this is catalysed by *scyllo*inositol dehydrogenase (*scyllo*inositol-NADP oxidoreductase).

Radiotracer-incorporation experiments carried out on the rat (Posternak, Schopfer, Kaufmann-Boetsch & Edwards, 1963) and a plant (Scholda, Billek & Hoffman-Ostenhof, 1964) provide evidence that *myo*inosose-2 is also an intermediate in the interconversion of *myo*inositol and *scyllo*inositol in these tissues. However, the enzymic reactions were not investigated in either system.

The reduction of NADP⁺ by *myo*inositol in fat-body extracts (Table 5) is not readily explained since the results of Table 3 show that NAD⁺ is much more effective than NADP⁺ in stimulating the overall conversion of *myo*inositol into *scyllo*inositol. One possibility is that the product of *myo*inositol oxidation by NADP⁺ may be different from that by NAD⁺. It is likely that *myo*inosose-2 is the product of *myo*inositol oxidation by NAD⁺ since the reverse reaction is known to take place (Table 4).

Table 5 shows that the initial rates of *scyllo*inositol and *myo*inositol oxidation were lower than the rates of *myo*inosose-2 reduction. A similar effect is shown by reactions catalysed by the *myo*inositol dehydrogenase from *A. aerogenes* (Larner *et al.* 1956). The effect could be a reflection of the equilibrium position of the reactions (which is in favour of inositol synthesis). It may also be noted that for the *A. aerogenes* enzyme the K_m values for *myo*inosose-2 and NADH were lower than the K_m values for *myo*inositol and NAD⁺.

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