THE DISTRIBUTION OF CARBOHYDRATES IN CYSTS OF
HETERODERA ROSTOCHIENSIS

BY

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Alkaline digests of Heterodera rostochiensis cysts contained glycogen (1.4%), oligosaccharides (2.3%) and trehalose (5.3%). Glycogen was confined to the larvae and cyst wall, whereas trehalose was largely present in the perivitelline fluid.

The second stage larvae of cyst nematodes (genus Heterodera) may remain quiescent within the eggs contained in the cyst for many years until stimulated to hatch. Throughout the resting stage the larvae are sustained by materials stored within the egg. Hatching agents change the slow rate of metabolism of the quiescent Heterodera rostochiensis larvae, and an early sign of incipient hatching is an increase in the movement of the larva within the egg (Doncaster & Shepherd, 1967). We have examined the carbohydrate content of the egg and larva to obtain further information on the stored materials and their possible role in meeting the increased energy demands of the larvae immediately prior to hatching.

Experimental

The H. rostochiensis, British pathotype A, cysts containing eggs were raised on pot-grown potato plants and extracted by the usual procedures (Shepherd, 1970). Cysts (200 mg) were crushed with a glass rod on an aluminium slide (Shepherd, 1970) and the eggs and larvae separated from the debris as described by Clarke et al. (1967). The intact eggs containing larvae together with some free larvae were washed, freed from water by drying under reduced pressure over phosphorus pentoxide and then weighed.

Free larvae (630,000) were obtained by treating cysts with a 0.7 mM solution of picrolonic acid (Shepherd, 1970). The larvae were removed at 3 day intervals, washed with water by decantation and dried in vacuo over phosphorus pentoxide (29 mg).

Alkaline digestion. Batches of whole cysts containing eggs (40 mg, about 2,000 cysts) were hydrolysed with 1N- NaOH (1.0 ml) for 1 h at 100° C in a stoppered tube, following the procedure used for the determination of glycogen by Roe & Dailey (1966). Cyst walls (34 mg) obtained as described by Clarke (1968) were treated similarly as were preparations of whole eggs (50 mg) and larvae (29 mg). Undigested protein and glycoprotein were precipitated by the
addition of perchloric acid (160 μl) to the cooled digest. The acidified suspensions were centrifuged and the clear supernatant, together with washings, was used for total sugar determinations and column chromatography.

Ethanolic extracts. Dry whole cysts (8.1 g) were ground with a pestle and mortar. The powder was extracted with successive portions of 70% ethanol (8 × 100 ml). Evaporation of the combined extracts under reduced pressure left a resin (1.23 g), which was shaken with a mixture of methanol-water-chloroform (60 : 54 : 60, by vol., 100 ml) to remove lipophilic compounds. The mixture separated into two phases. The upper phase yielded a mixture of hydrophilic compounds (0.61 g) including much carbohydrate (0.36 g, 4.4% of wt. cysts).

Egg extracts were obtained by treatment of whole eggs with 70% ethanol in a small glass homogeniser. The homogenate was filtered and the filtrate, together with the washings, was taken to dryness.

Chromatography. A column (104 cm × 2.0 cm diameter) of Bio-Gel P-2 (polyacrylamide resin 200-400 mesh, Bio-Rad Laboratories; molecular exclusion limit approx. 1800, V, 102 ml) was used to separate carbohydrates according to their degree of polymerisation (D.P.).

The systems used for thin-layer chromatography were a) silica (Kieselgel G) impregnated with 0.2M-NaH₂PO₄, solvent: — acetone-methanol-chloroform-water (80 : 10 : 10 : 5, by vol.) (Lato et al., 1969), b) Kieselguhr-silica (Kieselgel G) (4 : 1, w/w) impregnated with 0.02M sodium acetate, solvent: — ethyl acetate-methanol-water (68 : 23 : 9, by vol.) (Prey et al., 1963), c) Kieselguhr, solvent: — butan-1-ol-ethanol-water (50 : 30 : 20, by vol.) and d) Kieselguhr, solvent: — butan-1-ol-pyridine-water (75 : 15 : 10, by vol.) (Weill & Hanke, 1962). The plates were sprayed with a solution of anisaldehyde (Stahl & Kaltenbach, 1961) or naphthoresorcinol (Lato et al., 1969).

Paper chromatography was on Whatman No. 4 paper developed with either solvent a) ethyl acetate-pyridine-water (60 : 25 : 20, by vol.) or solvent b) propan-2-ol-water (80 : 20, v/v). The chromogenic reagents were either alkaline silver nitrate, periodate-Schiff’s reagent or aniline oxalate.

Sugar determinations. The method of Dubois et al. (1956) was used to determine the total sugar content of the extracts and of fractions obtained by column chromatography. The results are expressed as glucose units. The total sugar content of whole cysts containing eggs was determined after hydrolysis with 1N-H₂SO₄ at 100°C. Fructose was determined by the method of Arni & Percival (1951) and glucose by the glucose oxidase method of Huggett & Nixon (1957). Reference samples of oligosaccharides for chromatography were obtained by hydrolysis of starch. Poly- and oligosaccharides were hydrolysed to monosaccharides with 2N-trifluoroacetic acid (Albersheim et al., 1967) at 100°C for 1 h. Glucose, fructose and trehalose were also determined by gas chromatography of their trimethylsilyl ethers following the procedure of Ford (1974). A Varian 1700 instrument with a flame ionisation detector was used and a 5’ × 1/8” column of 3% SE-30 on Varaport 30.