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The Metabolism of Trehalose during Morphogenesis of the Brine Shrimp *Artemia salina*

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High concentrations of trehalose are characteristic of many systems that are capable of undergoing cryptobiosis, e.g. plant seeds, fungal spores, diapausing insects and the brine shrimp *Artemia salina*.

Earlier work (Clegg, 1964, 1965) indicated that a rapid conversion of trehalose into glycogen and glycerol occurs on rehydration of dormant *Artemia* embryos. It was suggested that trehalose provides the necessary oxidizable substrate for the maintenance of the metabolic requirements during morphogenesis, and that the activation of trehalase might be important as a means of mobilizing this disaccharide.

In an investigation to study the metabolic changes underlying the initiation of morphogenesis in this species, we have attempted to correlate the changes in the concentrations of a number of carbohydrates with the enzymic profiles at different stages of development. Our findings show that trehalase activity (expressed as μmol of glucose formed/min per g dry wt.) does not alter significantly between dry cysts (0.16), cysts hydrated for 6h (0.18), 12h (0.17) and 24h (0.19), or adult shrimps (0.18); although the activities of maltase and sucrase were higher at the adult stage compared with dormant or hydrated cysts (maltase, 0.18 and 0.05; sucrase, 3.53 and 1.15 respectively). Thus an alteration in the total detectable trehalase activity does not appear to be a significant factor in initiating the metabolism of trehalose by developing *Artemia* embryos.

Further, although our results confirm those of Clegg in that there is a very much lower content of trehalose in hatched nauplii and adult shrimps compared with the encysted stage, we find that very considerable quantities (approx. 75%) of the trehalose initially present remains in the cyst remnants after hatching. Thus the low concentration of trehalose in hatched nauplii does not necessarily indicate that it has actually been degraded during morphogenesis. The functional significance of this residual carbohydrate is far from clear. It does not appear to provide a long-term energy store, since the concentration of trehalose in dormant cysts remains essentially unchanged for over 20 years (Clegg, 1962), and though it could conceivably provide an additional source of external nutrient after hatching this seems unlikely in view of the non-utilization by *Artemia* of various externally added substrates (Emerson, 1967; Huggins, 1969).

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Fractionation of the β-Glucanases in a *Cytophaga johnsonii* Culture Filtrate Lysing Yeast Cell Walls

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When grown in the presence of autoclaved yeast cell walls *Cytophaga johnsonii* produces extracellular enzymes that will lyse these cell walls *in vitro* under suitable conditions (Bacon, Milne, Taylor, & Webley, 1965). There is no evidence that mannan is degraded during the lytic process, and so attention has been concentrated on other cell-wall components, especially glucan.

We have fractionated the culture fluid, examining particularly its action on insoluble laminarin from *Laminaria cloustonii*, a β-(1→3)-glucan, and lutean, a β-(1→6)-glucan. An Auto-Diluter (Mk II; Hook and Tucker Ltd., London S.W.9, U.K.) was used to sample each chromatographic fraction, add buffered substrate and deliver the mixture into an Auto-Analyzer cup. Ribonuclease was added, a cap fitted and the cup incubated at 30°C, usually overnight, before being placed on the sampler module of the Auto-Analyzer for measurement of reducing sugar (Hoffman, 1937).

After concentration by freeze-drying and precipitation with ammonium sulphate the enzymes were applied to a Sephadex G-100 column and developed with 5mM-Na_2HPO_4. A variable amount of laminarinase emerged at the void volume, but the greater part was eluted later as a rather broad peak. A peak of luteanase followed closely on the laminarinase, and two further luteanase peaks were eluted with and after the ammonium sulphate; in this region there was also a small laminarinase peak.

When the fractions containing it were applied to

DEAE-cellulose and eluted with a gradient of pH and phosphate buffer the main laminarinase component was divided into three parts (A, B and C); the first (A) contained all the luteanase. The third peak (C) contained most of the laminarinase but did not lyse cell walls, either alone or in the presence of a purified luteanase. Fractions A and B both lysed cell walls and had little or no chitinase activity, which was found mainly in fraction C.

Fractions A, B and C are all endoglucanases, but, whereas fraction C produces mainly glucose, laminaribiose and laminaritriose from laminarin, fractions B and C do not seem to degrade this slightly branched polysaccharide (degree of polymerization about 25; Annan, Hirst & Manners, 1965) beyond the pentasaccharide stage.

It remains to be proved that the glucanase in fraction B is responsible for lysis of the cell walls, but evidently neither luteanase nor chitinase needs to be present. This fits the idea that the cell-wall β-(1→3)-glucan has practically no β-(1→6)-interchain linkages (Manners & Masson, 1969).

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Multiple Forms of Human Brain Monoamine Oxidase: Substrate Specificities

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Although pig brain monoamine oxidase (EC 1.4.3.4) is a single enzyme (Tipton & Spires, 1968), rat brain monoamine oxidase appears to exist in multiple forms (Johnson, 1968; Youdim, Collins & Sandler, 1969). Human brain mitochondrial monoamine oxidase has now been purified by the method described by Youdim *et al.* (1969); in all brain areas investigated (whole brain, cerebral cortex, cerebellum, basal ganglia, hypothalamus, pons and centrum ovale) multiple forms of the enzyme were detected. Four bands of activity separated by electrophoresis on 5% polyacrylamide gel (Youdim, Collins & Sandler, 1968); two bands migrated towards the anode (MAO_2 and MAO_3), one remained at the origin (MAO_1) and one migrated toward the cathode (MAO_4).

The specific activities (expressed as nmol of substrate deaminated/10min per mg of protein) of the intact mitochondrial enzyme and of the bands of enzyme activity, both prepared from the various brain areas, were measured with [^{14}C]tryptamine, [^{14}C]tyramine, [^{14}C]dopamine (3,4-dihydroxy[^{14}C]-phenethylamine) and [^{14}C]benzylamine as substrates (Robinson, Lovenberg, Keiser & Sjoerdsma, 1968). The activities of the mitochondrial preparations for the four substrates were 0.73, 1.74, 2.36 and 5.61 respectively. Each band of activity also differed in its substrate specificities; for example, the activity of MAO_1 prepared from whole brain homogenates towards dopamine and tyramine was 2.09 and 1.66 respectively whereas that of MAO_4 was 58.54 and 0.45. In addition, comparison of the specific activities of the enzyme forms isolated from the various brain areas investigated showed marked differences. The activity towards dopamine of MAO_4 isolated from basal ganglia, a brain area rich in this amine (Bertler & Rosengren, 1959), was 338.03 whereas that of the same band found in the cerebellum was 4.46. The corresponding activities of MAO_1 were 1.45 and 1.33 respectively. However, not all the substrates showed such variation. The specific activity of MAO_4 towards tryptamine was 0.71 in the basal ganglia and 0.78 in the cerebellum. These variations in specific activity were not accompanied by differences in the corresponding Michaelis constants.

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Insoluble Pronase

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Insoluble pronase (B grade; Calbiochem Ltd., London W.1, U.K.) was prepared by the method successfully used for papain (Cebra, Givol, Silman & Katchalski, 1961) and other proteases.

A synthetic polypeptide of *p*-aminophenylalanine and leucine (25mg) was diazotized and precipitated from solution at alkaline pH after partial self-coupling. The precipitate was washed with 50mM-tris–HCl buffer, pH 8.0, containing $CaCl_2$ (25mM), and a solution (1ml) of pronase (8mg) was added. The