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

By A. K. HUGGINS and A. P. BOULTON. (*Department of Biochemistry, University College London, Gower Street, London W.C.1, U.K.*)

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

By J. S. D. BACON, A. H. GORDON and D. M. WEBLEY. (*Macaulay Institute for Soil Research, Aberdeen AB9 2QJ, U.K.*)

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 $\rightarrow$ 3)-glucan, and lutean, a β -(1 $\rightarrow$ 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