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Conversion of δ -Amino Laevulinic Acid to Porphobilinogen by Different Tissues of the Polychaete Annelid *Neoamphitrite figulus*

THE heart-body, a small spongy mass of tissue within the dorsal vessel of the terebellid polychaete *Neoamphitrite figulus* (Dalyell), is almost certainly concerned with synthesis of the haemoglobin of the blood. The coelomocytes also contain haemoglobin, but this is different. Kennedy and Dales¹ have shown that the heart-body tissue and the coelomocytes in this species converted porphobilinogen (PBG) to uroporphyrin. Tissues were incubated with purified PBG suspended in sea water and at room temperature for varying periods and the products identified after extraction and purification by paper chromatography. Some similarly performed experiments using δ -amino laevulinic acid (ALA) did not give clear results. Subsequent work, using the method of McRae², whereby the conversion of ALA to PBG is identified against suitable controls by the positive reaction PBG gives with Ehrlich's *p*-aminobenzaldehyde reagent³, has, nevertheless, given quite clear results. McRae's method was followed in detail except that to quantify the results the tissues were merely dried with acetone and weighed, for it was desired only to compare the relative ability of different tissues to convert ALA to PBG. McRae used homogenates of whole animals (*Dugesia*). Tissues were deep frozen and homogenized on thawing in M/15 phosphate buffer, pH 7.0, the ALA being added to provide a concentration of 400 μ g/ml. of homogenate. Homogenates, suitably controlled, were incubated at 37° C for 2.5 h. The colour of the Ehrlich reaction was measured at 553 $m\mu$ after 15 min with a 'Unicam SP 500' spectrophotometer. While the activity of each tissue is expressed in arbitrary units, their relative activities in one typical experiment are shown in Table 1. The experiment was repeated five times with similar results. The tissues of five worms were pooled in each experiment. Table 1 shows that the heart-body is ten times more active than any other tissue determined. The body wall muscle is colourless and if it contains any myoglobin this is in a relatively low concentration and any ALAase present is insufficient to produce enough PBG to be detected by the method. On the other hand, Kennedy and Dales⁴ detected coproporphyrin III, which must have been formed during haem synthesis, in the body wall; whether this is formed in the skin or is deposited there, as this negative result suggests, is being investigated further. The hind stomach or gizzard has muscles of a deep red colour and some ability to convert ALA to PBG is not surprising. The ability of the coelomocytes to do so is

also expected, confirming the hypothesis that they synthesize their own haemoglobin from ALA and do not receive compounds later in the biosynthetic pathway to haem. The high activity of the gut (fore stomach plus intestine) from which the contents had carefully been removed is surprising, especially because Kennedy and Dales⁴ found no free porphyrins in these tissues. It may be that the coelomocytes, which contain haemoglobin, arise from the coelomic epithelium of the gut wall. This is being investigated further.

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Measurement and Significance of Weight Heterogeneity in Some Protein and Polysaccharide Preparations

MOST of the molecular weight values reported for macromolecules have been obtained by methods based on ultracentrifuge measurements. Many biologists and biochemists now have access to an analytical ultracentrifuge and their requirements can be satisfied by sedimentation velocity experiments.

Although macromolecules may sediment at similar rates and produce sharp boundaries, it is sometimes forgotten that this does not necessarily imply homogeneity of the system. Thus serum transferrins produce a single symmetrical boundary in ultracentrifuge experiments whereas under electrophoresis the proteins may be resolved into two or more discrete fractions, depending on the animal species. The transferrins may also exhibit some degree of association, for reported molecular weights of the transferrins^{1,2} of several species are between 66,000 and 90,000. The discrepancy in the reported molecular weights is of some clinical importance because it affects the reliability of iron binding measurements. Polysaccharides such as glycogen and amylopectin produce sharp boundaries as a result of the very large concentration dependence of the sedimentation coefficient³. These polysaccharides possess a very broad distribution with respect to sedimentation velocities. Several methods have been proposed in the past twenty years for the quantitative measurement of weight heterogeneity. In 1944, Gralén⁴ proposed that the width of the sedimenting boundary was an index of heterogeneity. More recently, Baldwin^{5,6} and other workers have made detailed analyses in which the complete distribution curve of the sedimentation coefficients is evaluated (compare Schachman⁷). Other more basic factors are often ignored, regardless of the method selected for quantitation of heterogeneity. Quite often the criteria of purity applied to the preparation are not sensitive enough to justify detailed mathematical analysis of the boundaries. The preparations may also undergo physicochemical changes during the experiment⁸ so that control experiments are essential.

We have been studying changes in the catabolic heterogeneity and weight heterogeneity of serum 7S gamma globulins in mouse and man after immunization with various antigens, including malignant tumours. Electrophoretically distinct fractions of 7S gamma globulin are broken down at different rates in the mouse^{9,10} and signi-

Table 1. CONVERSION OF ALA TO PBG BY DIFFERENT TISSUES OF *Neoamphitrite figulus*

Tissue	Weight of tissue (mg)	Activity (arbitrary units/mg of tissue)
Heart-body	1.8	89.00
Coelomocytes	64.9	8.50
Fore stomach + intestine	39.6	7.82
Hind stomach (gizzard)	58.0	4.08
Body wall	177.5	0.00
Blood	7.9	0.00