Biosynthesis of the Cyst-Coat Protein of *Colpoda steinii*

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About 90 min after the washing away of nutrient, ciliates of *Colpoda steinii* at 28°C stop swimming and start to secrete a protein cyst coat that is rich in glutamic acid residues (Tibbs, 1966). During encystment the total glutamic acid content of the cell increases by an amount that is the same as that present in the cyst coat (Tibbs & Marshall, 1970). This suggests that coat synthesis, rather than the secretion of a preformed protein, takes place after settling. L-[U-¹⁴C]Leucine has been used to confirm this and measure the degree to which the protein-synthetic machinery of the cell at this stage is directed towards coat synthesis.

L-[U-¹⁴C]Leucine was incorporated to the maximum extent into the coat subsequently formed when added to starving cells at all times up to 30 min after settling. Since incorporation of label into whole-cell protein occurred with no initial lag period, the results showed that no coat was synthesized between purification of the cells and 30 min after settling. The protein can hardly be present in the cells at the time of purification, for such cells are freshly excysted, a process that results in the loss of coat into solution. At low concentrations of added L-[U-¹⁴C]leucine rate of entry into the cell limited the amount of label incorporated, but this was not so above 5 mg/ml, where the specific radioactivity of the coat leucine was comparable with that of the added leucine when this was present over the whole of the synthesis period.

The total glutamic acid content of the cell reached a maximum at 2–2½ h after settling. Quantitative isolation of the coat also showed that its synthesis was now complete and that, for cells that have encysted for the second time, it contributed 25% to the total cell protein. At ½–2 h after settling 51% of the radioactivity incorporated was in the coat, which contains 4.47 mol of leucine/100 mol of amino acids compared with 8.54 mol/100 mol in the cytoplasmic protein. This suggests that 67% of the protein synthesized during this period is coat, but, because of the distribution of label among other amino acids, the real value is probably lower.

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Binding of D-Glucose to the Cyst Coat of *Colpoda steinii* and Metabolism of the Sugar during Excystment

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The protein cyst coat of the ciliate *Colpoda steinii* binds D-glucose in a reaction that is enzymically catalysed (Tibbs & Marshall, 1969; Marshall & Tibbs, 1969). As part of an attempt to assess the significance of this reaction *in vivo* it was found that, when the cell excysts, labelled glucose initially bound to the coat is metabolized and much of the radioactivity becomes incorporated into the ciliate.

Washed cysts from culture were shaken with [U-¹⁴C]glucose under conditions where about 50% of the radioactivity incorporated into the cell was concentrated in the coat. On 'chasing' of such cells with unlabelled glucose for up to 2 h, the radioactivity in the coat and cell protein did not decrease detectably. T.l.c. of hydrolysates of such coat showed that the radioactivity was mainly present as laevulic acid derived from the bound glucose. Amino acids were only slightly labelled. Cysts that had reacted with [U-¹⁴C]glucose and been 'chased' with unlabelled glucose were shaken at 28°C with a diffusate from hay infusion. At intervals during the subsequent excystment samples were filtered through glass-fibre discs and separated into cells and filtrate. Both fractions were assayed for radioactivity and the results showed that, when the cells had excysted, practically all the radioactivity present in the cysts originally was recovered in the motile ciliates.

Excystment occurred about 90 min after addition of the hay-infusion diffusate to labelled cysts. For about the first 75 min the coat appeared unchanged microscopically and could be isolated with a constant specific radioactivity. During the last 15 min of the period it was rapidly eroded away. When the filtrate from excysted cells was dialysed and freeze-dried, a protein with low specific radioactivity was obtained that represented 23% and 18% (two experiments) of the total protein present in the corresponding number of whole cysts. This protein is considered to have arisen from the coat that, for cells excysted for the first time, accounts for about 18% of the nitrogen content of the cyst (Tibbs & Marshall, 1970).

It is suggested that the reaction between D-glucose and the cyst coat may be part of the excystment trigger, since the bound material is apparently used by the excysting cell, and hay infusion itself contains glucose.

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Incorporation of [³⁵S]Sulphate into the Glycosaminoglycans of Rat Brain

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Many of the studies concerned with [³⁵S]sulphate metabolism in brain tissue have dealt mainly with the general radioautographical distribution (see, e.g., Boström & Odeblad, 1953) and with the incorporation into lipid and cerebrosides. Although [³⁵S]sulphate is known to be incorporated into 'chondroitin sulphate' (Guha, Northover & Bachhawat, 1960) and crude 'sulphomucopolysaccharide' (Robinson & Green, 1962) fractions from rat brain, there has been no attempt to analyse the distribution of radioactivity in the individual sulphated glycosaminoglycans that have been shown to be present in the tissue (Cunningham & Goldberg, 1968; Singh, Chandrasekaran, Cherian & Bachhawat, 1969).

Intraperitoneal injections of 2 μ Ci of sodium [³⁵S]sulphate/g body wt. were given to 40-day-old rats. The animals were killed 6h after the injection and the glycosaminoglycan-containing fraction was prepared from the excised brains by digesting the lipid-free material with protease followed by a precipitation with cetylpyridinium chloride and removal of nucleic acids. Resolution of the individual glycosaminoglycan components was accomplished by electrophoresis on cellulose acetate and cellulose micro-columns as described previously (Cunningham & Goldberg, 1968). Samples taken for determination of [³⁵S]sulphate were dried on planchets and their radioactivities measured by using a windowless scintillation counter. Labelled glycosaminoglycans were detected on the cellulose acetate strips by radioautography and by using a radiochromatogram scanner.

Protease treatment released 97% of the radioactivity into solution, 38% of this being precipitated from solution with cetylpyridinium chloride; the remainder was diffusible through heated dialysis tubing.

Hyaluronic acid, chondroitin sulphate, heparan sulphate and dermatan sulphate were present in the proportions 33:48:11:8 (measured as hexuronic acid). Cellulose micro-column results indicated that [³⁵S]sulphate was incorporated only into the sulphated glycosaminoglycans, namely chondroitin

sulphate (71%), heparan sulphate (14%) and dermatan sulphate (14%), one of the chondroitin sulphate fractions showing a higher rate of incorporation than the others. No radioactivity was found in regions or fractions corresponding to heparin or keratan sulphate, confirming that these glycosaminoglycans are not significant in brain tissue.

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Effect of Phenobarbital and other Compounds on Induction or Maintenance of Liver uridine Diphosphate Glucuronyltransferase during Organ Culture

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UDP-glucuronyltransferase activity towards certain substrates appears precociously, and above adult values, when early chick-embryo liver is cultured on chemically defined media; liver from older embryos develops UDP-glucuronyltransferase activity to adult values during culture (Ko, Dutton & Nemeth, 1967; Skea & Nemeth, 1969). Removal from an embryonic environment initiates this induction.

Phenobarbital, administered to chickens (I. H. Stevenson & G. J. Dutton, unpublished work) or mammals, subsequently increases liver UDP-glucuronyltransferase activity to many substrates. This communication reports that barbiturates, and possibly bilirubin, stimulate production of UDP-glucuronyltransferase activity in cultured liver when present in the culture medium (Dutton, 1970).

Phenobarbital (sodium salt) does not stimulate UDP-glucuronyltransferase activity towards o-aminophenol or p-nitrophenol *in vitro*, but added to the medium increases development, ultimate activity and persistence of the enzyme, most markedly with older-embryo liver; it prolongs survival of the enzyme in cultured hatched chick liver. High concentrations of phenobarbital are required: 1.0 mM is less effective than 10 mM, 0.1 mM being ineffective. Sodium diethylbarbiturate or sodium barbiturate, but not sodium acetate, could

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