Trans. Br. mycol. Soc. **88** (2), 229–236 (1987) *Printed in Great Britain*

A POSSIBLE RELATION BETWEEN CYCLIC-AMP LEVELS AND GLYCOGEN MOBILIZATION IN *COPRINUS CINEREUS*

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Addition of cyclic AMP to specific sites in plate cultures of a mature dikaryotic colony of *Coprinus cinereus* caused a twofold increase in the amount of glycogen over the whole culture within 19 h. There was no evidence for non-random distribution of glycogen around the site of administration of the cAMP. In the normally developing fruit body levels of endogenous cAMP were considerably elevated in fruit body initials and remained unusually high in the fruit body until very late stages of development, when they declined to about the concentrations observed in mycelium. These cAMP accumulations were positively correlated with localized accumulations of glycogen within the fruit body. Published data from in vitro analyses suggest that cAMP may inhibit glycogen synthesis and activate glycogen breakdown in *Coprinus*. It is suggested that observations reported here would be consistent with this view if cAMP were specifically involved in the bulk translocation of glycogen, i.e. promoted glycogen breakdown in order to mobilize carbohydrate for transport, rather than immediate metabolism.

Because of its involvement in the concerted migration and growth leading to fruit-body formation in *Dictyostelium discoideum* Raper (Newell, 1978) a great deal of interest has been focused on the likely role of adenosine 3′:5′-cyclic monophosphate (cAMP) in controlling differentiation in other organisms. A considerable amount of work has been carried out by I. Uno and T. Ishikawa and their colleagues on the basidiomycete *Coprinus cinereus* (mostly using the name *C. macrorhizus*). In a series of papers (recently reviewed in Uno & Ishikawa, 1982), these workers investigated cAMP metabolism during fruit-body formation in *Coprinus*. Most of this work has concentrated on certain mutant monokaryons able to produce monokaryotic fruit bodies, but the dikaryon (the normal mycelial origin of fruit bodies) was included as a control to relate the events to normal circumstances. Most recently, it has been shown that the *A* and *B* mating-type factors are involved in the co-ordinate regulation of adenylate cylase, phosphodiesterase and a cAMP-dependent protein kinase (Swamy, Uno & Ishikawa, 1985), such that fertile mycelia (the dikaryon normally, or fruiting-competent monokaryotic mutants) accumulate cAMP at the onset of fruiting. The accumulation proceeds to a late primordial stage, but then the amount of cAMP declines as the primordium matures (Uno, Yamaguchi & Ishikawa, 1974).

The nucleotide has been shown to activate glycogen phosphorylase and inhibit glycogen synthetase in vitro (Uno & Ishikawa, 1976, 1978), which is significant, since translocation of glycogen from the mycelium to the developing fruit bodies has been demonstrated (Madelin, 1960), the glycogen being first accumulated in the stipe base and then relocated to the cap of the primordium (Moore, Elhiti & Butler, 1978), where it appears to be utilized during spore formation. Although there is this implication of some sort of connexion between cAMP metabolism, glycogen metabolism and formation of the fruit body (perhaps mediated directly by modulation of glycogen synthetase/phosphorylase activities, or through the agency of the cAMP-dependent protein kinase), there is no serious suggestion as to the function of cAMP in this system.

Throughout their analyses, Uno and Ishikawa made extractions of mycelia together with any fruit bodies they might have produced. No attempt was made to separate the two structures. In the work reported here we have assayed both cAMP and glycogen in these structures separately, so as to partition these metabolites between the different parts of the organism. We have also tested the proposition (Moore, 1984) that cAMP serves to polarize the direction of glycogen translocation by examining the effect of applied cAMP on glycogen accumulation in the mycelium.

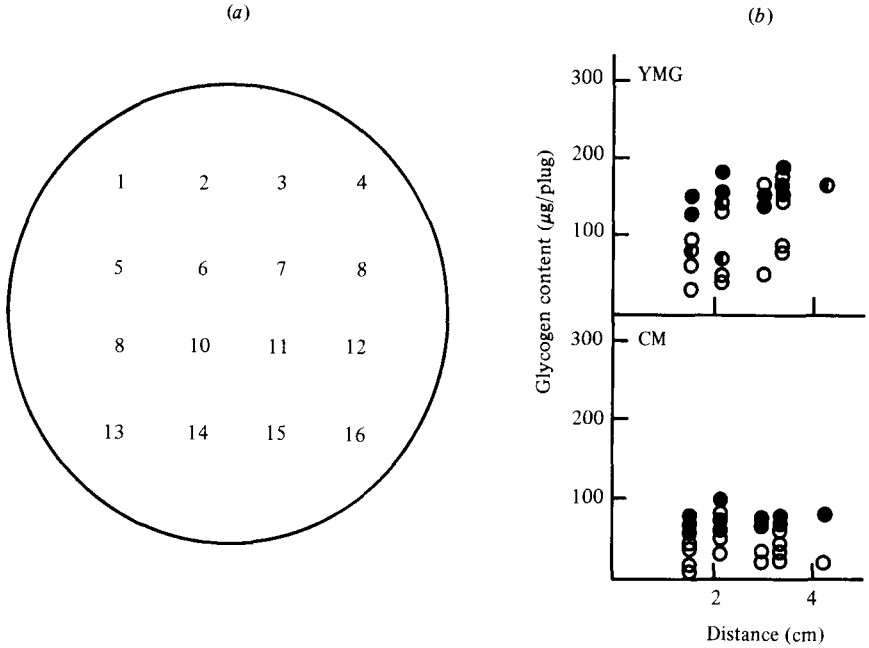

Fig. 1. (*a*) Sampling template used for removing plugs of mycelium for glycogen estimations in cAMP exposure experiments with Petri dish-grown dikaryotic cultures. Vertices of the matrix were at 1·5 cm centres. The cAMP solutions (etc.) were added to a well cut in the agar at position 10. (*b*) Example scatter diagrams comparing glycogen content of individual mycelial plugs with their Euclidean (straight-line) distance from position 10. The data shown depict results obtained on the two media used (YMG and CM) following 24 h exposure to 0·8 fmol of cAMP (closed circles) or 24 h after addition of water to the well at position 10 (open circles). Comparisons of this sort were made for all time intervals used, but no consistent distance dependency was observed.

MATERIALS AND METHODS

Organism and culture conditions

A dikaryon of *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray sensu Konr. was used throughout; it was originally isolated in Birmingham in 1973 by G. M. Butler and given the isolation number 177/1. It is deposited in ATCC under no. 42721. Moore *et al.* (1979) have described the six stages into which fruit-body development has been divided. Mycelial cultures were grown either on YMG medium (4 g yeast extract, 10 g malt extract, 4 g glucose l^{-1}) or *Coprinus* Complete Medium (CM: Moore, 1968), both being solidified when required with 1% agar. Fruit bodies were produced on cultures growing on horse dung using the incubation and illumination regime described by Moore & Ewaze (1976).

Effects of exogenous cAMP on mycelium glycogen content

The dikaryon was inoculated centrally on agar-solidified CM or YMG medium, contained in 90 mm Petri dishes, and incubated at 37 °C in the dark until the colonies had reached the edge of the dish (approx. 4 days). At this time a template, set out as shown in Fig. 1*a*, was used to remove a 2 mm diam plug with a sterile cork borer from position 10 in the matrix. The hole was completely filled with 20 μl of a solution normally containing 40 pM dibutyryl-cAMP. The dishes were then further incubated at 25° in a 16 h light : 8 h dark regime. After 0, 3, 19, 24 and 43 h incubation following addition of cAMP, the distribution of glycogen in the mycelium was determined by extracting the polysaccharide from 6 mm diam mycelial plugs cut from each position in the matrix (Fig. 1*a*). The experiments were done in triplicate and experimentals were compared with controls in which distilled water was added to the well cut at position 10. In other experiments the concentration of dibutyryl-cAMP used in the well was varied and the effects of cAMP (free acid) and butyric acid were examined.

Extraction and assay of glycogen, from both the mycelial plugs and from fruit body samples, were done using the methods described by Jirjis &

Moore (1978); authentic rabbit liver glycogen was used to prepare a standard curve.

Determination of cAMP in fruit bodies

Fruit bodies, representing different stages of growth, were excised from the cultures and dissected into separate cap and stipe samples. The stipes of stage 3, 4 and 5 fruit bodies were further subdivided into three segments; the top segment was delimited by the length of the cap and the remainder was bisected to give middle and bottom sections.

Assays of cAMP were done in triplicate, using an extraction method adapted from Brown *et al.* (1971). Tissue was pulverized under liquid nitrogen, then deproteinized with ice-cold 0·5 M perchloric acid in 25% (v/v) ethanol. After standing for one hour in an ice bath the slurry was filtered. The filtrate was brought to pH 7·5 with 1 M-KOH. The precipitate was removed by centrifugation and 2 ml portions of the supernatant were lyophilized in multi-dose vials and stored under vacuum at −20°. Shortly before assay, 2 ml of 0·05 M Tris/EDTA buffer, pH 7·5, was added to the sample vial. The cAMP content of 50 μl quantities of the resulting solution was measured with an Amersham cAMP assay kit, scintillation counting being done with a Packard 300CD spectrometer.

RESULTS

Glycogen contents at each of the 15 positions in the 4 × 4 sampling matrix (position 10 was not assayed) were measured to determine whether addition of cAMP had any effect on the distribution of glycogen through the maturing vegetative dikaryotic colony. Individual hyphal plugs cut from colonies at the start of the experiment varied in their content of glycogen within the range of zero to 44·5 μg on CM and 93·2 to 251·3 μg on YMG, depending on treatment conditions; in any one plate the individual samples differed from the plate mean by up to ±20% on CM and ±35 to ±52% on YMG. The glycogen contents of each point in the matrix in relation to position 10 (point of cAMP administration), were compared by plotting glycogen content of each plug as a function of its Euclidean distance from position 10 (examples

Table 1. *Effect of addition of 0·8 fmol of cAMP to a well in the Petri-dish culture, on accumulation of glycogen by a dikaryon of* Coprinus cinereus

Time after adding cAMP	Addition	Mean content of glycogen (μg)	Standard deviation	*t* value
A. Yeast extract/malt extract/glucose (YMG) medium				
0	None	186·5	49·5	
3	Water	120·0	36·6	1·7
3	cAMP	142·0	35·6	
19	Water	141·1	40·2	3·0**
19	cAMP	185·7	42·0	
24	Water	108·6	52·2	2·3*
24	cAMP	146·2	35·6	
43	Water	91·2	41·2	2·7**
43	cAMP	132·4	43·4	
B. Coprinus Complete Medium (CM)				
0	None	10·6	12·3	
3	Water	25·0	12·9	1·7*
3	cAMP	32·7	12·2	
19	Water	30·5	19·4	2·3*
19	cAMP	46·2	18·6	
24	Water	42·7	20·0	6·4**
24	cAMP	79·7	10·2	
43	Water	25·5	13·0	1·5
43	cAMP	49·6	17·9	

Glycogen contents shown refer to 6 mm diam plugs of mycelium and are the means of 15 replicates. *, *t* statistic shows a significant difference between the control (water added) and the experimental (cAMP added) at the 5% level; **, difference is significant at the 1% level.

Table 2. *Comparison of the effect of 24h exposure to dibutyryl-cAMP, butyric acid and cAMP on accumulation of glycogen by a dikaryotic mycelium grown on YMG medium*

Treatment: 20 μl of the solution shown added to a well in the Petri dish	Glycogen content μg/plug
Distilled water control	105·4 ± 46·0
80 pM isobutyric acid	101·1 ± 30·2
40 nM dibutyryl-cAMP	156·7 ± 49·3
40 μM dibutyryl-cAMP	148·9 ± 44·9
40 pM cAMP (free acid)	185·1 ± 50·5

Glycogen contents shown are the means ± standard deviation of 30 replicate mycelial plugs.

Table 3. *Cyclic AMP and glycogen contents of different parts of the fruit body of* Coprinus cinereus *at various stages of development*

		Glycogen		cAMP	
	Stage tissue	μg per fruit body	mg per g dry wt	pmol per fruit body	pmol per mg dry wt
0	Initials	69·5 ± 20·3 (7)	74·4 ± 10·7	53·1 ± 18·7	89·8 ± 49·5 (3)
1	Primordium	271·1 ± 120·0 (9)	79·7 ± 21·6	104·6 ± 80·0	13·6 ± 6·5 (4)
2	Cap	47·3 ± 12·3 (3)	21·1 ± 13·5	103·7 ± 33·4	36·5 ± 13·8 (3)
	Stipe	506·1 ± 241·3 (3)	67·6 ± 15·1	126·6 ± 30·8	18·5 ± 6·7 (3)
3	Cap	407·1 ± 244·7 (7)	39·7 ± 18·9	409·9 ± 152·4	39·3 ± 15·1 (3)
	Stipe top	28·3 ± 7·1 (7)	16·4 ± 8·3	95·8 ± 98·1	29·6 ± 23·8 (3)
	Stipe middle	64·1 ± 29·4 (7)	26·1 ± 13·5	169·1 ± 92·2	48·1 ± 29·9 (3)
	Stipe base	289·2 ± 156·4 (7)	56·0 ± 11·6	123·3 ± 64·9	34·9 ± 22·4 (3)
4	Cap	1419·6 ± 872·4 (8)	40·9 ± 11·7	274·4 ± 123·3	5·6 ± 1·4 (3)
	Stipe top	26·9 ± 24·9 (8)	5·4 ± 3·5	210·9 ± 117·3	31·4 ± 7·9 (3)
	Stipe middle	77·7 ± 79·5 (8)	10·3 ± 3·9	168·3 ± 59·4	20·0 ± 13·7 (3)
	Stipe base	209·2 ± 260·0 (8)	17·2 ± 8·2	174·7 ± 78·3	11·3 ± 4·8 (3)
5	Cap	28·3 ± 26·4 (6)	3·8 ± 2·0	47·8 ± 36·4	3·1 ± 2·0 (2)
	Stipe top	10·2 ± 5·3 (4)	4·0 ± 3·0	19·6 ± 3·5	5·0 ± 2·2 (2)
	Stipe middle	22·1 ± 7·5 (4)	6·4 ± 3·5	19·6 ± 0·0	3·1 ± 1·1 (2)
	Stipe base	37·5 ± 12·3 (4)	8·0 ± 4·7	18·4 ± 1·7	1·7 ± 0·9 (2)

Initials are spherical to subglobose aggregates up to 2 mm diam which appear about 88 h after inoculation, the larger initials have gill lamellae and dense polysaccharide deposits in the basal bulb of the stipe; stage 1 primordia are ovoid structures, 2–6 mm in height, appearing about 110 h after inoculation; they have well-developed gill tissues and dense polysaccharide deposits in the subhymenium as well as in the base of the stipe, and karyogamy occurs towards the end of this stage; stage 2 primordia are 6–9 mm tall fruit bodies found about 132 h after inoculation, the partial veil is intact initially but becomes ±free, histochemical staining of polysaccharide continues to intensify in the cap but that in the stipe base lessens, meiosis occurs during this stage; stage 3 fruit bodies occur about 144 h after inoculation, they are 10–20 mm tall, partial veil free, stipe begins to elongate slowly, meiosis is completed in this stage and basidia begin to form sterigmata; stage 4 fruit bodies occur 150–156 h after inoculation, they are 15–45 mm tall, basidiospore pigmentation begins to appear and polysaccharide deposits in subhymenium begin to disperse, stipe continues to elongate slowly; stage 5 fruit bodies are mature, they are found about 160 h after inoculation, the stipe elongates rapidly, the cap opens and spores are discharged. These developmental descriptions are taken from Moore *et al.* (1979), where illustrations can be found.

Entries are the means ± S.D. (± S.E. where appropriate) of the number of replicate assays shown in brackets.

shown in Fig. 1*b*). Despite the variability it is quite clear that no positional effect was evident. Further, the differences between positions along vertical and horizontal rows, their interaction with each other and their glycogen content in relation to position 10 (point of cAMP administration), were subjected to analysis of variance but no significant positional effect, i.e. no deviation from randomness, of any sort was recorded. In subsequent comparisons, therefore, the 15 sampling points on each plate were treated as replicate assays of the mycelium and bulked together.

Table 4. *Distributions of fresh weight and dry weight in the parts of the fruit body of* Coprinus cinereus *at various stages of development*

stage	Height (mm)	Fresh weight		Dry weight	
		Cap	Stipe	Cap	Stipe
0	Up to 2	1·7 ± 1·3		0·16 ± 0·07	
1	3–7	23·0 ± 13·4		2·3 ± 1·9	
2	7–10	52·3 ± 39·8	53·4 ± 35·1	4·7 ± 3·1	4·9 ± 3·2
3	10–15	121·1 ± 68·1	103·7 ± 50·8	11·8 ± 6·0	9·2 ± 5·5
4	16–53	351·8 ± 215·4	263·2 ± 212·3	25·6 ± 15·2	14·7 ± 12·4
5	54–129	471·7 ± 291·9	461·3 ± 325·7	34·4 ± 17·7	21·2 ± 17·8

All weights are shown in mg and are the means ± S.D. of from 5 to 67 replicates.

Mycelia grown on the two media differed in the amount of glycogen they contained after four days growth, i.e. at the start of the cAMP-exposure experiment, by a factor of about 20 (see zero time data in Table 1). Nevertheless, although the response to addition of cAMP to the colonies was small, it applied to both media: colonies grown on CM accumulated up to twice the amount of glycogen of the control within 24 h; those grown on YMG maintained their initially higher glycogen content, so that by 43 h the samples exposed to cAMP contained about 1·5 times the control glycogen content (Table 1). Tests showed that the effect was due to the cAMP moiety, rather than the butyric acid component of the dibutyryl–cAMP which was routinely used, and that it was independent of the applied concentration over a range of six orders of magnitude (Table 2).

Fruit bodies were sampled at different stages of development and assayed both for cAMP and glycogen contents (Table 3). As reported before (Moore *et al.*, 1979), glycogen is accumulated in the fruit body, reaching a maximum at stage 4 and appearing first in the stipe base, though later the greatest proportion is located in the cap. Although the total amounts of glycogen accumulated (approaching 2 mg per fruit body) were similar to those reported earlier, the fruit bodies obtained from the Birmingham dikaryon which were used here were rather smaller than those analysed by Moore *et al.* (1979) (Table 4). The greatest content of cAMP, about 90 pmol (mg dry wt)$^{-1}$ was observed in fruit-body initials. The amount of cAMP per fruit body increased during development to reach a maximum at stage 3, followed by a rapid decline. Tissue content (dry wt basis) varied with the nature of the tissue analysed and its stage of development, but in no case did the content in any part of the developing fruit body significantly exceed 50% of the level observed in fruit body initials (Table 3).

DISCUSSION

The medium on which the mycelium is grown clearly influences the amount of glycogen synthesized (Table 1). This may well be due to differences in the initial amount of glucose in the media (2·06%, w/v, in CM and 0·67% in YMG), though obviously these are complex media and other differences may also be important. Clearly, glycogen metabolism must be very different in mycelia grown on these two media, so it is highly significant that applied dibutyryl–cAMP led to an elevated accumulation of glycogen in both cases. The observations with YMG, that exogenous cAMP apparently prevented a decline in glycogen content which would otherwise have occurred as the colony aged, suggest that administration of cAMP to mature mycelial cultures enhances net accumulation of glycogen by delaying utilization of the polysaccharide. The cAMP was administered within the body of a preformed colony. The net accumulation of glycogen promoted by cAMP (which was detectable within 19 h on YMG and in 3 h on CM) occurred uniformly over the whole culture despite the non-uniform method of administration of the active chemical. Thus there was no evidence for cAMP polarizing glycogen transport; rather, the observations imply rapid transmission of some sort of signal to orchestrate a homogenous response. Lateral and radial transmission at a rate much faster than the rate of growth is indicated. Diffusion of a molecule about the size of dibutyryl–cAMP would achieve a radius of only about 12 mm from the point of administration within 24 h, yet by this time on CM even the most distant sampling positions (about 40 mm from the point of administration) were showing elevated glycogen contents (Fig. 1*b*). Significant, too, is the lack of concentration dependence (Table 2), which shows that administration of 20 μl of a 40 pM solution of dibutyryl–cAMP (= 0·8 fmol) was sufficient to saturate the mechanism leading to net accumu-

lation of glycogen. A high-affinity 'receptor' is implied.

With regard to the fruit body and the relationship of its glycogen metabolism to that of the parent mycelium, the following points are pertinent. Glycogen accumulated in vegetative cells of the dikaryotic mycelium is lost when fruit bodies are formed, and losses of mycelial mass are correlated with gain in mass of fruit bodies (Madelin, 1960). Glycogen accumulates in the stipe base of primordial fruit bodies but is then lost as development proceeds, and its disappearance from the stipe base is correlated with an increasing accumulation in the fruit body cap, which eventually contains over 95% of the fruit body glycogen (Moore *et al.*, 1979; Table 3); this changeover is accompanied by a change in the relationship between cap and stipe such that the cap becomes the heaviest part of the fruit body (Table 4). As much as 2 mg glycogen is eventually accumulated in each fruit body; it is utilized almost totally by the time mature spores are shed (Moore *et al.*, 1979; Table 3). The amount of glycogen utilized by an average fruit body would yield about 25 Joules of metabolic energy – if expressed instantaneously as heat energy, this would be sufficient to raise the temperature of an average fruit body cap by 17°.

For a dikaryon, the level of cAMP increased from about 4 to about 8 pmol (mg dry wt)$^{-1}$ in the whole culture (mycelium + any fruiting structures) during the first 10 days growth on a medium initially containing 0·4% glucose, then declined to about 3 pmol mg^{-1} over the next 4 days; whereas a culture grown on 5% glucose maintained an essentially constant level of about 2 pmol cAMP mg^{-1} (fig. 1 A in Uno & Ishikawa, 1974). Our own assays of dikaryotic mycelium grown on CM showed cAMP levels varying between 0·5 and 2·9 pmol mg^{-1} during 9 days growth at 37°. Thus fruit body initials showed a level of cAMP accumulation which was some 30 times greater than that of the parent mycelium (Table 3). A similar, but slightly less extreme accumulation in initials has been reported before (Milne, 1977) for the BC9/6,6 + H1 dikaryon of *C. cinereus*. This very high concentration of cAMP was not maintained, though it remained 5 to 10 times higher than the mycelial level for most of the life of the fruit body, and as the fruit body matured total content of cAMP increased in parallel with increasing content of glycogen. There was a broad parallel in terms of the proportional distribution of the two metabolites also, i.e. at stage 2 the largest proportion of both was in the stipe, at stage 3 the largest proportion of both was in the cap. However, cAMP content reached a maximum and started to decline much earlier than did the glycogen. At the stage when the majority of the glycogen was being utilized, the concentration (dry wt basis) of cAMP in fruit body tissues (particularly the cap) was in rapid decline, approaching the range found in mycelium. In terms of cAMP concentration, the most striking difference occurred in the top of the stipe at stage 4, where the concentration was about five times that of the adjacent cap and three times that in the stipe base (Table 3).

We neither wish nor intend to review the literature dealing with cAMP. Reference to any general text-book of biochemistry will reveal that accumulation of cAMP generally signifies a net deficit of glucose and that the involvement of cAMP in regulation of glycogen metabolism in animals consists in elevated cAMP levels enhancing glycogen utilization (by co-ordinated inhibition of synthesis and activation of breakdown) so as to relieve the glucose deficit and restore the energy charge. The negative correlation between glucose content of the medium and cAMP content of the *Coprinus* mycelium (Uno & Ishikawa, 1974); and the observed effects of cAMP on *Coprinus* glycogen synthetase (Uno & Ishikawa, 1978) and phosphorylase (Uno & Ishikawa, 1976), phosphodiesterase (Uno & Ishikawa, 1973), and location of cyclase (Uno & Ishikawa, 1975) imply close parallels between *Coprinus* and higher eukaryotes.

But this raises a paradox. The enzymic and other in vitro data seem to be consistent with the view that cAMP synthesis regulates the amount of glycogen in mycelia which are producing fruit bodies by inhibiting glycogen synthetase and activating glycogen phosphorylase (Uno & Ishikawa, 1982). Thus, in de-salted extracts of a monokaryotic culture, incubation with 5 μM cAMP + 3·3 mM ATP led to a threefold activation of glycogen phosphorylase activity, cAMP alone had no effect (Uno & Ishikawa, 1976) and 5 μM cAMP inhibited glycogen synthetase activity by 91%, 3·3 mM-ATP did not reverse this effect, but 2·5 mM glucose 6-phosphate restored activity to about 50% of the original (Uno & Ishikawa, 1978). The implication, then, is that accumulation of cAMP promotes the net utilization of glycogen. Yet, from the data presented here, it is clear that administration of cAMP to the mycelium rapidly results in an increase in the latter's content of glycogen. Furthermore, during development of the fruit body, elevated levels of glycogen were mirrored in elevated levels of cAMP, and the stage in fruit-body development during which the bulk of the glycogen was finally used was the stage which had the lowest levels of cAMP.

There are obvious difficulties in applying information obtained in vitro to events observed in vivo. However, diametrically opposed effects are

unlikely. The essential questions are whether the observed correlations between glycogen and cAMP in *Coprinus*, in vivo, always reflect utilization of glycogen to relieve a metabolic glucose and energy deficit, and what relationship they have to fruit-body development. For a vegetative mycelial hyphal cell suffering a glucose deficit the most immediate remedy lies in control of glucose uptake rather than polysaccharide degradation. It is thus likely that cAMP stimulation of glycogen accumulation observed in plate cultures reflects an additional level of control of the allosteric glucose transport system (Moore & Devadatham, 1979; Taj-Aldeen & Moore, 1982) which, by furnishing additional supplies of glucose 6-phosphate consequentially, overrides any inhibitory effect the cAMP may have on activity of glycogen synthetase. An effect of this sort on membrane-mediated processes may also have some relevance to transmission of the organizing signal referred to above.

In the fruit body, accumulation of cAMP occurs in locations which appear to be involved in mobilizing glycogen for translocation rather than for immediate metabolism. It is unlikely that glycogen is translocated as a polysaccharide. Such is the efficiency of storage of energy in glycogen that only about 6% of the energy obtainable by complete oxidation of each glucose residue, i.e. a little more than 2 out of 37 ATP molecules, would be used in the cycle (glucose → glycogen → glucose → glycogen). Advantages resulting from translocation of mono- or disaccharides rather than a polysaccharide could well compensate for this expenditure of energy. Thus, if translocation of glycogen requires its breakdown one could readily understand how, while the polysaccharide is in transit, high tissue contents of both cAMP and glycogen could be maintained (since the cell in which the glycogen is broken down is exporting the product, it could still experience a glucose deficit). Eventually, in the cap of the mature fruit body the glycogen is finally utilized and the consequent increase in energy charge results in a major reduction of cAMP level. Consequently, a specific role for cAMP in glycogen breakdown as part of a carbohydrate translocation process is likely.

We thank Drs R. S. Callow and G. S. Mani for assistance with the statistical analyses. R.C.K. thanks the Association of Commonwealth Universities for the award of a Fellowship, and the Ministry of Education and Culture of India and University of Bhopal for leave of absence.

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(*Received for publication 22 August 1986*)