bound lectin over their entire surface. Upon warming to 37 °C, however, the bound lectins accumulated rapidly (within 5 to 10 min) at one pole of the cell to form a cap (Figure). Lectin-induced capping in PMNs has been observed previously by RYAN et al.[3] using fluorescence microscopy. By electron microscopy, the cap appears as a multiple layer of ferritin dots on a frequently infolded segment of the plasmalemma. CF caps are similar but smaller than RF caps (Table I), indicating that PMNs bind concanavalin A to a lesser extent than ricin.

We have compared the phagocytic activity of capped PMNs with that of untreated cells. Of 5 such experiments, 1 has been evaluated by morphometry. Table I shows that the total relative surface area of the phagocytic vacuoles, which was our measure of phagocytosis, was the same in lectin-treated and in control PMNs. Treated and non-treated cells also showed identical amounts of intact granules remaining in the cytoplasm (Table II), suggesting that, under all conditions, a similar portion of phagosome membrane was contributed by granule fusion. These results clearly show that PMNs bearing either a CF- or an RF-induced cap phagocytose yeast particles as efficiently as untreated PMNs, and that lectin treatment does not influence the degree of granule fusion.

The use of ferritin as an ultrastructural marker has enabled us to observe the exact distribution of membrane-bound lectins. As shown in Table I, the membrane of the phagocytic vacuole was virtually free of lectin tag, which suggests that only membrane depleted of lectin-binding sites was engaged in particle recognition, and thus became internalized during phagocytosis.

We conclude that in rabbit PMNs the surface glycoproteins that bind concanavalin A and ricin are not required for the recognition and the uptake of yeast particles. Work in progress indicates that the same holds true for human PMNs which phagocytose opsonized yeast cells or staphylococci[10]. It has been shown previously that concanavalin A inhibits phagocytosis of polyvinyltoluene beads by PMNs adhering to glass[11]. Unfortunately, the differences between this and our experimental design do not allow a discussion of the contrasting conclusions.

Table II. Morphometric analysis of granule membrane

Additions to PMNs	Granule surface area as percent of total membrane surface area of cell
RF, yeast	34.7±2.0 (15)
CF, yeast	33.2±1.9 (15)
No lectin, yeast	33.0±1.4 (25)

Numbers represent mean values ± SEM (*n*) from single cells which have endocytosed yeast. No differentiation was made between azurophil and specific granules. Total membrane surface area includes membranes contributed by granules, phagosomes, plasmalemma, nucleus, mitochondria and Golgi apparatus. Derivations of formulae relating intersection counts to relative surface area are analogous to those given in Table I.

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The Role of Cyclic Adenosine Monophosphate in the Swarming Phenomenon of *Proteus mirabilis*

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Summary. The levels of cyclic AMP and adenyl cyclase in swarming and non-swarming cells of *Proteus mirabilis* and the effect of glucose on swarming have been investigated. The results indicate that cAMP is required for swarming, but that the flagellar derepression characteristic of swarming does not result from increased cAMP levels.

The onset of swarming in *Proteus mirabilis* is characterized by two physiological changes in the organisms at the edge of the growing colony. Inhibition of cell division occurs and at the same time there is flagellar derepression, resulting in the characteristic elongated, multiflagellate, multi-nucleate, highly motile swarming cells (swarmers)[2].

It has been claimed that swarming could be a consequence of nutrient depletion, caused by active growth (for a review of the factors influencing swarming see SMITH[3]). Under conditions of nutrient depletion, the internal levels of cyclic adenosine monophosphate (cAMP) would be expected to rise[4,5]. Cyclic AMP is needed for the synthesis of flagella in a number of organisms[6,7] and therefore the flagellar derepression associated with swarming might result from a high internal cAMP level, following nutrient depletion.

In this work we have examined the role of cAMP in swarming. The results indicate that cAMP is required for swarming, but that the flagellar derepression associated with swarming does not result from an increased level of cAMP in the swarming cells.

Materials and methods. The strain of *Proteus mirabilis* used was an isolate from University College Hospital. Stock cultures were maintained on slopes of Oxoid nutrient agar. Organisms were grown either on Oxoid nutrient broth No. 2, solidified with 1.75% (w/v) Difco Bacto Agar (swarming medium) or on the same medium to which 1% (w/v) activated charcoal was added (swarm-inhibiting medium[8]). Swarming cells were isolated from the swarming medium as described previously[9], after cutting out the inner part of the colony. Agar-grown, non-swarming cells were also isolated as before[9].

The intracellular cAMP level was measured using a cAMP assay kit from the Radiochemical Centre, Amersham, Bucks., England. The assay depends upon the competition, between added ^{3}H-cAMP and the cAMP in the sample, for added binding protein. The organismus under assay were harvested, resuspended in 5 ml 0.05 *M Tris*/EDTA buffer (pH 7.5) and sonicated for 3 min at 0 °C. Samples were centrifuged to remove debris and protein was precipitated with 5% perchloric acid. After removal of the precipitated protein by centrifugation, the super-

natants were neutralized and evaporated almost to dryness at 4 °C under nitrogen. The volume was made up to 0.5 ml with buffer and 50 µl samples added to 50 µl ^{3}H-cAMP (60 pmoles/ml, 1.6 µCi/ml) and 100 µl of binding protein. After 2 h at 4 °C, excess activated charcoal was added to remove all unbound cAMP, and the samples immediately centrifuged at 4 °C for 3 min. Samples (200 µl) of the supernatants were then carefully removed into 10 ml of toluene/TritonX100 (2:1) containing 4 g/l 2,5 diphenyloxazole (PPO) and 0.5 g/l 1,4 di[2-(5-phenyloxazolyl)] benzene (POPOP). Samples were counted in a Packard liquid scintillation spectrometer. Estimations of cAMP content were made by comparison with a standard curve.

Table II shows the activity of adenyl cyclase by a modification of the cAMP assay method and a method for measuring the adenyl cyclase activity[10] in *Escherichia coli*. After sonication of the organisms (see above) and centrifugation, the debris was retained and the supernatant evaporated down as above, but without deproteinization. The debris was taken up in 50 µl of 0.05 *M Tris*/HCl buffer at pH 9.0 containing 1 µmole $MgSO_4$ and 15 nmoles ATP; this represented the particulate fraction. The supernatant after evaporation to dryness was taken up in the same mixture (soluble fraction). After incubating at 34 °C for 30 min, the samples were placed in a boiling water bath for 5 min and the amount of cAMP present measured as described above. Results were corrected for the amount of cAMP already present in the samples to give values for that formed by adenyl cyclase action.

Results and discussion. Swarming apparently requires a basal level of cAMP, as 1% glucose added to the swarming medium delayed swarming for about 5 h; swarming began after about 10 h incubation at 30 °C as compared to 5 h if 1% glucose was not added. This delay was evidently caused by a fall in cAMP level in the cells during growth on glucose[11]; it was abolished by the addition of 1 m*M* exogenous cAMP. The dependance of swarming on a basal level of cAMP was not unexpected as in the bacteria so far studied cAMP is an absolute requirement for flagella synthesis, and growth with glucose reduces the synthesis of flagella and thus motility[6,7].

We measured cAMP levels in swarming cells to ascertain whether flagellar derepression was associated with increased cAMP levels. Measurements were made on 3 types of organisms; short non-swarming cells from solid media, early swarmers and swarmers. Early swarming cells were removed from plates just prior to swarming, whilst elongation and flagella synthesis was still actively occurring at the edge of each colony i.e. after $3^1/_2$ to 4 h incubation at 30 °C on swarming medium. This results in a mixture of actively growing swarmers, and short cells. Table I shows the cAMP levels in these three cell types. The level was clearly much lower in the late swarmer cells than in the other two cell types. There was apparently a loss of cAMP from the long cells during active swarming, as early swarming cells showed a similar cAMP level to the non-swarming preparation. Clearly derepression of flagellar synthesis is not associated with an increase in internal cAMP.

Table II shows the activities of adenyl cyclase in the three cell preparations. This enzyme was measured because the internal concentration of cAMP is dependent both on rate of leakage from the cells and the level of adenyl cyclase activity[12]. Table II shows that adenyl cyclase was, as expected, in the particulate fraction. It also shows that the active swarmers had less enzyme than non-swarming and early swarming cell types.

The above observations imply that although cAMP is required for swarming, the flagellar derepression observed is not caused by an increase in cAMP levels just prior to or during swarming. This indicates that nutrient depletion is not the cause of swarming, and therefore adds support to the hypothesis that swarming is the result of a negative chemotactic response to a toxic metabolic by-product[13].

Table I. Cyclic AMP content of swarming and non-swarming cells of *P. mirabilis*

Cell type	Extent of flagellation	Length of cells	Total cAMP (pmoles/g dry wt.)
Swarming cells	+++	All elongated (10–100 µm)	7.1
Early swarming cells	++	Mixed, normal and elongated (2–100 µm)	41.5
Short cells from solid swarm-inhibiting medium	+	All short (1–2 µm)	46.2

Organisms of the 3 types were examined microscopically for the extent of flagellation and for cell length. cAMP in samples from each preparation was assayed as described in 'materials and methods'.

Table II. The adenyl cyclase activities in swarming and non-swarming cells of *P. mirabilis*

Cell type	Cell fraction	Adenyl cyclase (pmoles cAMP/g dry wt./30 min)
Swarming cells	Soluble	0
	Particulate	2.2
Early swarming cells	Soluble	0
	Particulate	8.7
Short cells from solid swarm-inhibiting medium	Soluble	0
	Particulate	9.0

Adenyl cyclase was estimated in organisms from each type of preparation as described in 'materials and methods'.

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