Discussion. According to WILLSTATTER[12], chlorophyllase could reversibly catalyze the reaction chlorophyll ⇌ chlorophyllide + phytol. It is, however, not known if/when the two roles of the enzyme occur in vivo. In the ripening bananas and apples, loss of chloroplast structure was said to hasten chlorophyll loss by enhancing enzyme-substrate proximity[13]. It appears possible that, for the same quantum of chlorophyllase, greater loss of chlorophyll could occur in a chloroplast losing its structure. Irrespective of the nature of the role the enzyme could have played in vivo, in the present study its activity was estimated in vitro in terms of its hydrolyzing activity on chlorophyll. Considering, the enzyme mainly a hydrolytic one, the initiation of the loss of chloroplast structure, even at the mature stage, and the increase in enzyme activity at the senescent stage in the diseased leaves, could explain the low levels of chlorophyll at these stages. The occurrence of high chlorophyllase activity at the time of chlorophyll accumulation, in both healthy and spiked leaves, apparently could not be explained on the basis of the hydrolytic activity of the enzyme. It is possible that the synthesizing activity of the enzyme might have occurred in vivo during this period.

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Capping of Concanavalin A- or Ricin-Binding Sites does not Influence Phagocytosis in Polymorphonuclear Leukocytes

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Summary. Rabbit polymorphonuclear leukocytes (PMNs) were capped with ferritin-conjugated concanavalin A or ricin, and then allowed to phagocytose yeast cells. Phagocytic activity and lectin distribution were determined by ultrastructural morphometry. Capped PMNs were found to phagocytose as efficiently as control PMNs, and always to ingest the particles with a lectin-free portion of their plasma membrane. This clearly indicates that concanavalin A- and ricin-binding sites of the PMN membrane are not involved in the recognition and phagocytosis of yeast particles.

Phagocytosis is thought to be initiated by the specific binding of a particle to the plasma membrane of the phagocyte[1]. No surface receptor has yet been characterized biochemically in phagocytes, although these cells are known to bear recognition sites for the F_c part of immunoglobulins and for C3b[1].

We report here first results of an investigation of the role of the lectin-binding sites of the polymorphonuclear leukocyte (PMN) plasma membrane in the recognition of phagocytosable particles.

Table I. Morphometric analysis of phagocytosis

Additions to PMNs	Cap surface area as percent of cell surface area (1)	Phagosome surface area as percent of phagosome plus cell surface area (2)	Lectin-bound phagosome surface area as percent of phagosome surface area (3)
RF, yeast	22.1±1.5 (15)	34.1±1.7 (15)	0.3±0.3 (15)
RF alone	18.2±1.1 (13)	–	–
CF, yeast	12.1±1.2 (12)	33.2±2.5 (12)	0 (12)
CF alone	9.9±0.7 (14)	–	–
No lectin, yeast	–	33.0±1.4 (25)	–

Numbers represent mean values ± SEM (n) from single cells which have endocytosed yeast. (1), IPlb/(IPlb+IPlf); (2), (IPgb+IPgf)/(IPlb+IPgb+IPlf+IPgf); (3), IPgb/(IPgb+IPgf). 'I' denotes number of intersections of test lattice with plasma membrane (Pl) or phagosome membrane (Pg) either lectin-bound (b) or lectin-free (f). For details of formulae derivations, see reference[7].

Methods. Rabbit PMNs were obtained from glycogen-induced peritoneal exudates[2] and were suspended (5×10^6 cells per ml) in a medium containing 122 mM NaCl, 4.9 mM KCl, 1.22 mM $MgCl_2$ and 16.7 mM sodium cacodylate buffer, pH 7.4. 1 ml of cell suspension was incubated at 0 °C for 10 min with an excess of either ferritin-conjugated ricin (RF, 18.4 µg) or ferritin-conjugated concanavalin A (CF, 18.1 µg). Unbound lectin was then eliminated by adding 5 volumes of ice-cold medium and centrifuging the PMNs at 1000 $g$min. The washed PMNs were resuspended in 1 ml of medium and incubated at 37 °C for 10 min in order to induce capping of the bound lectins[3]. Phagocytosis was then induced by adding a large excess of heat-killed bakers' yeast (5×10^8 cells in 0.1 ml of physiological saline). Phagocytosis was stopped 15 min later by adding 1 ml of ice-cold 3% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4. Control PMNs were handled exactly as the lectin-treated PMNs except that the lectin solution was replaced by the corresponding buffer. Fixed PMNs were pelleted in a microfuge (Beckman Instruments) and processed for electron microscopy[4,5].

Lectins. Ferritin conjugates of ricin (RF) or concanavalin A (CF), which can be visualized by electron microscopy, were prepared as described by BITTIGER and SCHNEBLI[6], and stored at 4 °C in 0.1 M sodium cacodylate buffer, pH 7.4.

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Morphometry. Relative surface densities of plasmalemma, granules and phagocytic vacuoles were calculated according to WEIBEL[7] on randomly selected single cells containing at least one yeast. This was the case in more than 80% of the PMNs examined. In order to avoid duplicity of sampling, only those cells appearing in a single grid space were photographed. The grid was then discarded in favor of the next one containing sections cut 10 μm deeper in the block. A curvilinear test lattice[8] was used for intersection counting in order to eliminate errors introduced by the anisotropy of the capped cell surfaces. Membrane was considered lectin-tagged if at the point of intersection with the test system a ferritin molecule was within 20 nm from the membrane[9].

Results and discussion. PMNs which were treated with either RF or CF in the cold and immediately processed for electron microscopy exhibited a uniform layer of

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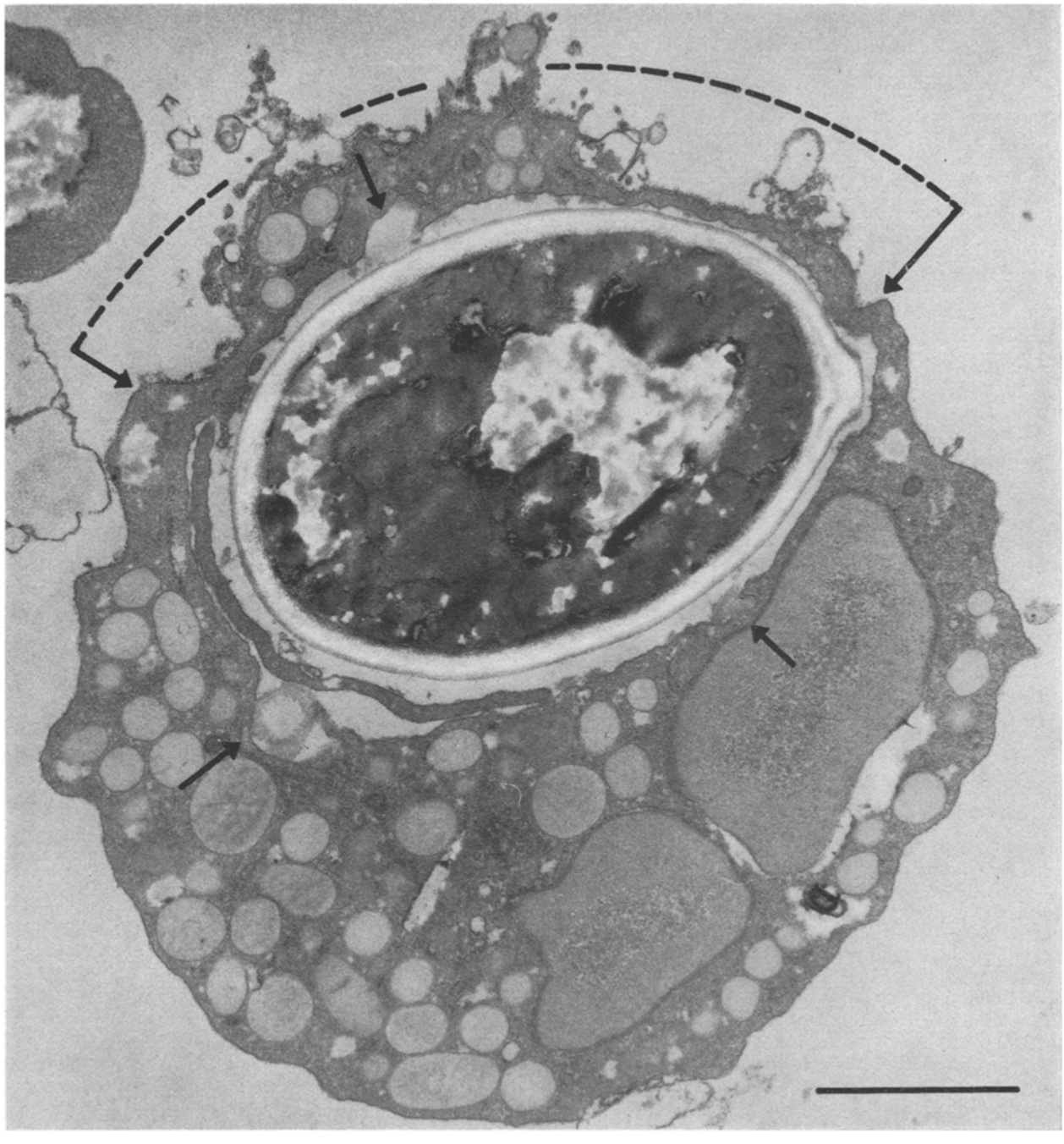

A ricin-ferritin capped PMN which has ingested a yeast cell. The capped portion of the cell is indicated by the dotted line. The phagosome membrane is devoid of ferritin marker indicating that ingestion was accomplished by lectin-free membrane. Note 3 fusion figures (arrows) which suggests that degranulation proceeds normally. Bar equals 1 μ*M*.

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-