cycloheximide–chloramphenicol-treated cells, suggested that this process did not require new protein synthesis and that transport protein(s) may be present on cell membranes of both phases of *H. capsulatum*, but only active at 37 °C.

If the action of temperature is to change membrane redox potential, and this can be facilitated by activating a permease for cysteine uptake, fungal dimorphism may be controlled by some critical sulphydryl groups, or by a general change in reducing state. To test this, *p*-chloromercurphenisulphonic acid (PCMS), a sulphydryl-blocking agent, and dithiothreitol (DTT), a sulphydryl protector, were tested for their effects on the phase transition. Mycelia were exposed to 100 μM PCMS for different lengths of time (5 min to 2 weeks) and were then washed twice, resuspended in fresh medium without PCMS and plated on GYE agar plates at 37 °C. The cells grew continuously after this treatment but never reverted to the yeast form (Fig. 2). PCMS (100 μM) also accelerated the transition of yeast to mycelia at 25 °C. After exposure to PCMS and incubation at 25 °C, yeast transformed to mycelia in 5–6 d instead of the usual 12–14 d. Even yeast incubated at 37 °C began to transform to mycelia in the presence of PCMS, but reverted to normal yeast when PCMS was washed out.

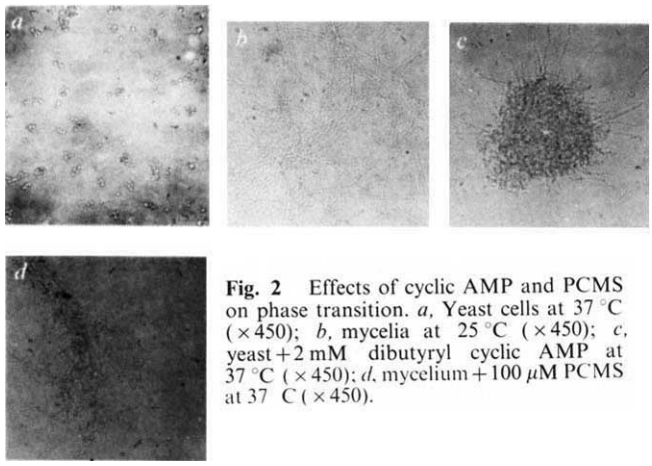

Fig. 2 Effects of cyclic AMP and PCMS on phase transition. *a*, Yeast cells at 37 °C (×450); *b*, mycelia at 25 °C (×450); *c*, yeast + 2 mM dibutyryl cyclic AMP at 37 °C (×450); *d*, mycelium + 100 μM PCMS at 37 °C (×450).

Opposite effects were found when cells were treated with DTT. For example, when yeast were incubated at 25 °C in the presence of 5 mM DTT, transformation to mycelia did not occur (although in this case the transition to mycelia still occurred when DTT was removed from the medium). Neither PCMS nor DTT were toxic to either phase of *H. capsulatum* at the concentrations used.

The effects of PCMS and DTT argue that sulphydryl groups, most likely at the surface of the cell, are critical for determining the phase of *H. capsulatum*. The irreversibility of the PCMS effect was unexpected, but also suggested that some surface feature was critical. The next question was how critical sulphydryl groups, especially at the membrane of the organism, could regulate a complex transition of yeast to mycelium. One possible explanation was that this phenomenon was similar to the effects of many hormones on eukaryotic cells, which often begin with a membrane change that modifies the intracellular level of a cyclic nucleotide.

In *H. capsulatum*, we found that the level of cyclic AMP was about five times higher in the mycelial phase than in the yeast phase. Cyclic AMP was assayed in yeast and mycelia by the radioimmunoassay of Steiner *et al.*[13] or with the Amersham–Searle cyclic AMP assay kit. The cells were collected by filtration, washed three times in ice-cold water, suspended in either 5% TCA, 0.1 N HCl or 0.05 M Tris-HCl buffer, *p*H 7.5, containing 1 mM 3-isobutyl-1-methylxanthine (Aldrich Chemicals). Glass beads (diameter 0.45–0.50 mm) were added to the suspension and the cells were disrupted at 4,000 r.p.m. for 3 min in a B. Braun Cell Homogeniser MSK, cooled with liquid CO_2. The resulting homogenates were centrifuged and an aliquot of the supernatant was lyophilised directly, or, in the case of cells homogenised in TCA, after extraction in ether. The dried material was resuspended in the appropriate buffer, and the cyclic AMP content determined. The recovery of added cyclic AMP in controls

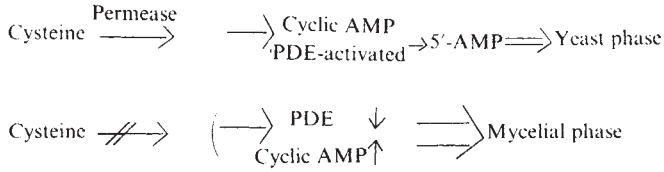

Fig. 3 Working hypothesis for role of cysteine and cystine in morphogenesis of *H. capsulatum*.

was 80–90%. The supernatant of cells homogenised in Tris buffer containing methylxanthine and precipitates of HCl and TCA-extracted cells redissolved in 1 N NaOH were assayed for protein according to Lowry *et al.*[14]. The average of three determinations showed that mycelia contained 46.9 and yeast 9.8 pmol cyclic AMP per mg protein. In addition, 2 mM dibutyryl cyclic AMP or 5 mM theophylline added to yeast cultures resulted in the conversion of yeast to mycelia, even at 37 °C, the temperature characteristic for the yeast phase (Fig. 2).

Thus, although the role of cysteine and cystine in morphogenesis of *H. capsulatum* is not understood, it seems likely that they satisfy a requirement for critical sulphydryl groups that then determine the level of intracellular cyclic AMP. We do not know how this might occur, but possibly, as in *Escherichia coli*, the activity of cyclic AMP-phosphodiesterase (cyclic AMP-PDE) requires the presence of a reducing substance[15]. The redox potential, governed by sulphydryl groups, could then have a regulatory role in the level of cyclic AMP, which in turn would control morphogenesis. Therefore, we suggest as a working hypothesis the schema of Fig. 3. At 37 °C the cells are able to bring cysteine to critical membrane sites. There it would activate cyclic AMP–PDE, thereby lowering intracellular cyclic AMP and maintaining the yeast phase. When the temperature is switched to 25 °C, transport of cysteine decreases and cyclic AMP–PDE activity falls. This results in an increase in cyclic AMP levels and a shift to the mycelial phase.

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The role of chemotaxis in the ecology of bacterial pathogens of mucosal surfaces

ALTHOUGH the ecological role of bacterial chemotaxis has been the object of considerable speculation, little is known of its role in nature[1]. Moreover, chemotaxis as a factor in the interaction between the mammalian host and its indigenous or pathogenic microflora remains unexplored. Studies with *Vibrio cholerae* and other intestinal pathogens have shown that bacterial association with the mucosa is influenced by bacterial motility, adhesion to brush border membranes, the presence of an indigenous flora and by the inhibitory effects of local antibodies

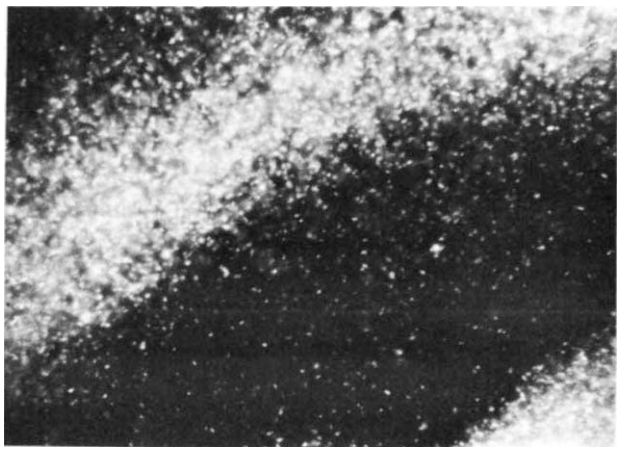

Fig. 1 A chemotactic strain of *S. typhimurium* forming a receding band at some distance from the mucosal surface. The bright triangular area in the lower right corner is the surface of the mucus gel overlaying the villi. The band had originally formed within the mucus gel and some bacteria have been retained there. The diffuse light within the band emanates from bacteria outside the focal plane of the microscope. The diffuse light in the mucus is due to light scattering by the gel itself. Darkfield (×110).

(summarised in refs 2, 3). We have shown previously that a pepsin digest of rabbit intestinal mucosa (PMS) inhibits the association of cholera vibrios and *Salmonella enteritidis* with rabbit intestine, and speculated that this extract was inhibitory because it neutralised the adhesion of the bacteria to a hypothetical receptor in the mucus gel[3]. We report here that PMS neutralises a positive chemotactic response of several bacterial species to the mucosa and in that way reduces bacterial association with intestinal tissue. Chemotaxis thus seems to be one of several mechanisms controlling interactions of bacteria with mucosal surfaces.

Observation under a phase-contrast or darkfield microscope of pieces of mouse or rabbit small intestine immersed in a suspension of *V. cholerae* revealed that very high concentrations of vibrios aggregated at the villous surface of this tissue in approximately 1 min. Three non-chemotactic vibrio mutants were selected using a modification of the method of Aswad and Koshland[4] and differed from the parent strain in that they did not accumulate at the surface of intestinal tissues, but remained randomly distributed throughout the suspending buffer. Similarly rapid attraction to mucosal tissue was shown by *Escherichia coli* (AW 405) and *Salmonella typhimurium* (SL 1634), but not by their non-chemotactic mutants (cheB 590 and SL 2538, respectively). None of the mutants used in this study differed from their respective parents in the rate of motility as determined by direct microscopic observation.

After chemotactic strains of the three bacterial species had accumulated on the surface of intestinal tissues for a period of approximately 2–5 min, the bacterial accumulation began to move away to form a clearly visible band of motile bacteria some distance from the tissue surface (Fig. 1). The distance of separation increased with time until the band eventually reached the edge of the coverslip. No such bands were formed by any of the non-chemotactic mutants. The formation of these bands may be caused by the local consumption of oxygen at the tissue surface or by the generation of metabolites with negative chemotactic activity by the tissue and/or by the bacteria. Such negative chemotactic gradients would compete with the positive chemotactic gradient generated by the tissue. Accordingly, the bacteria would be expected to accumulate in an area defined by the interaction of opposing gradients. Similar banding phenomena in broth or agar cultures of various bacteria have been described by others[5–7]. Whatever the actual mechanisms may be, our direct microscopic observations indicate that strikingly effective chemotactic attraction and repulsion of a variety of bacteria can occur at the mucosal surface.

Agar blocks infused with an ultrafiltrate of PMS could be substituted for the mucosal tissue slices. Such blocks attracted *E. coli*, vibrios and salmonellae to their surfaces, indicating that PMS contained one or several attractants. Agar blocks soaked in buffer had no effect. Moreover, the addition of PMS to the bacterial suspension reduced or prevented the chemotactic attraction of vibrios to rabbit mucosal tissue in the microscopic assay. By analogy with other chemotactic systems[7] it seems likely that the attractants in PMS saturated those chemotactic receptors of bacteria which normally reacted with the attractants emanating from the mucosa, thereby blocking the reactivity of the bacteria. These interactions were also demonstrated with Adler's capillary test for chemotactic activity[8]. Cholera vibrios were attracted into capillaries containing dilutions either of PMS or of a soluble fraction obtained by centrifuging freshly prepared scrapings from the mucosa of rabbit small intestine (Muc. Sn.) (Table 1). The latter preparation presumably contained the chemotactic mediators of mucosal tissue in relatively unaltered form. When PMS was added to the bacterial suspension as well as to the capillaries, no chemotactic gradient could form and no migration into the capillaries occurred. Similarly, the presence of PMS in the vibrio suspension considerably reduced the entry of vibrios into capillaries containing Muc. Sn. (Table 1), suggesting that at least some of the attractants in PMS and Muc. Sn. were similar. No response in the capillary test was observed with the non-chemotactic mutant vibrios.

The effect of chemotaxis on the association of vibrios with intact slices of rabbit ileal tissue was determined as described before[3], with the modification that the tissues were incubated in buffer containing 5×10^5 cells per ml of each of the chemotactic parent strain plus one non-chemotactic mutant. The proportions of mutant to parent strains that remained on the washed tissues were determined by preparing agar plate cultures and subsequently transferring a number of individual colonies to semisolid tryptone agar[6]. The proportion of non-chemotactic mutants associated with the tissue slices was 3.0 to 3.8 times lower than their proportion in the suspending fluid, indicating that the mutants had a significantly lower chance of associating with the mucosal tissue than the parent strain (Table 2). PMS in the suspending medium had no effect on the association of the non-chemotactic mutants with tissue slices. In contrast, PMS in the suspending medium reduced the association of the parent strain with mucosal slices by a

Table 1 Chemotactic attraction of *Vibrio cholerae* into capillary tubes and its inhibition by a preparation derived from intestinal mucosa

Content of capillary	Buffer	50% PMS*	5% PMS*	50% PMS*	Muc. Sn.† 2%	Muc. Sn.† 2%	Muc. Sn.† 2%
Content of bacterial suspension	Buffer	Buffer	Buffer	50% PMS*	Buffer	50% PMS*	5% PMS*
No. of bacteria in capillary‡	3.5§	142	51	4.0	40	8.5	9.5
($\times 10^{-4}$)	7.0§	97	51	7.0	34	14	12

* Ultrafiltrate of pepsin digested rabbit mucosal scrapings.
† Supernate of centrifuged, freshly prepared rabbit mucosal scrapings.
‡ Capillaries of 0.27 mm diameter, filled with 5 μl Tris buffer (*p*H 7.4) containing the materials indicated, were dipped for 60 min into vibrio suspensions. The number of vibrios which had invaded the previously sterile capillaries were then counted in a Petroff–Hausser chamber.
§ Duplicate determinations.

Table 2 Reduced invasion of mucosal tissue by non-chemotactic mutants of *Vibrio cholerae*

Parent strain plus mutant no.*	Fraction of non-chemotactic vibrios in suspending fluid*	Fraction of non-chemotactic vibrios on washed mucosa†	Factor: fluid / mucosa
8	41/93 (44.1%)	23/155 (14.8%)	3.0
6	39/73 (53.4%)	22/155 (14.2%)	3.8
31	61/137 (44.5%)	23/155 (14.8%)	3.0

* Slices of rabbit mucosal tissue were incubated without shaking for 8 min in a suspension of vibrios containing approximately equal concentrations of the chemotactic parent strain plus one non-chemotactic mutant as indicated.
† Based on counts of 31 colonies each cultured from 5 mucosal tissue slices.

factor of two to four[3]. Consequently, when the chemotactic attraction of the parent strain towards the mucosa was blocked by PMS, its ability to associate with the mucosa was reduced to a level similar to that of the non-chemotactic mutants.

Results obtained in three different experimental systems thus indicate that chemotactic mechanisms can promote as well as inhibit the initial invasion of mucosal surfaces by bacteria. Attractive and possibly repellent substances produced by the tissue are obviously important factors in these processes, but chemotactically active substances such as PMS in the medium bathing the mucosal surface seem to be equally potent modifiers of bacterial invasion. It is tempting to speculate about the *in vivo* role of chemotaxis in the interaction between bacteria and their mammalian host. For example, in the initial stages of infection an intestinal pathogen is rapidly propelled along the small intestine in a bolus of digesta, and the duration of its contact with any given area of the mucosal surface may not be longer than a few seconds. The ability of chemotactically attracted bacteria to quickly approach and penetrate the mucus gel may well be of even greater importance in that situation than in the invasion of the statically incubated tissues studied in the present experiments. Indeed, Chet and Mitchell[1] quote data showing rapid chemotactic movement of bacteria across and against fluid flow planes in flowing streams, that is, in conditions which kinetically resemble those of the small intestine. Furthermore, intestinal contents almost certainly contain chemotactically active substances which may either promote or inhibit chemotactic attraction of bacteria into the mucosa. Such a phenomenon may relate to certain aspects of the long-recognised relationship between diet and susceptibility to infection, a phenomenon for which exact mechanisms are still to be delineated[9]. It is also possible that chemotactically active substances added to the diet may exert a prophylactic effect against infections such as traveller's diarrhoea. The association of indigenous bacterial flora with the mucus layer of the large intestine may also be governed in part by chemotactic mechanisms.

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Helical shape and wall synthesis in a bacterium

THE main skeletal components of most bacterial cell walls are complex polymers called peptidoglycans[1]. The primary chemical structures of these and other wall components are well understood but little is known about the arrangement of the molecules in the wall or how new wall material is incorporated during growth[1]. We report here that several factors cause cells of *Bacillus subtilis*, normally straight rods (Fig. 1*b–e*), to assume a regular helical shape. We suggest a model for the structure and synthesis of Gram positive cell walls which could explain these observations. Explanations suggested by Mendelson[2] for an apparently different type of helical growth in *B. subtilis* do not account for our results.

In the model which most satisfactorily explained his observations, Mendelson[2] suggested that the pattern of wall growth in his mutant, and perhaps other strains, results in the rotation of the two ends of a cell relative to one another. When, in his strain, both ends of a cell filament remain attached to the old spore case this rotation is prevented and the resulting torque is relieved by the whole filament distorting to a helix.

We have observed that helices predominate among the abnormally-shaped cell filaments that occur in cultures of *B. subtilis* in the following conditions (f = approximate frequency of helical filaments in the populations).

(1) In medium supplemented with thel ocal anaesthetics chlorpromazine (3×10^{-5} to 4×10^{-5} M; 37 C) or methochlorpromazine (5×10^{-5} to 6×10^{-5} M; 37 C); $f = 50\%$.

(2) In medium containing penicillin G (0.1–0.25 U ml^{-1}; 30 C); $f = 1\%$ (Fig. 1*b–e*).

(3) Certain mutants of strain 168 (ref. 3) resistant to the detergent Triton X-100 (unpublished work) grow predominantly in a helical form, especially near the end of exponential growth. $f > 95\%$ (Fig. 1*a* and *f*).

(4) In cultures restarting growth after subculturing from stationary phase. $f < 1\%$.

(For (1) and (2) exponentially growing cells were subcultured, to an initial density of $2 \cdot 10^6$ ml^{-1}, into media containing the drugs. Strain 168*ind* (ref. 3) and Penassy broth (Difco) were used in (2), (3) and (4) but wild-type Marburg strain and nutrient broth (Difco) in (1).)

Our bacteria were not grown from spores, did not appear as closed loops (Fig. 1) and were grown in liquid media. Therefore, unlike Mendelson's example, anchorage of cell ends could not have contributed to their morphogenesis. In addition, the helices we observed were usually much more tightly coiled (Fig. 1).

Mendelson considered it improbable that helical shape could result from unequal growth rates at different points around the cell circumference because of the complexity of the necessary growth pattern[2]. This argument also applies to our observations — perhaps even more so because of the variety of circumstances involved.

Highton and Hobbs[1] described penicillin-induced helical growth, very similar to that reported here, in a penicillin-resistant mutant of *B. licheniformis*. They found abnormal wall thickening at the inside curvatures of bent cells. Similar thicken-