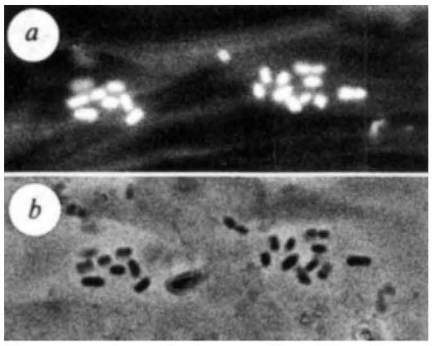
Actin in cell attachment

SIR — In their News and Views article[1] discussing the potential of polycationic beads to model bacterial infection, Joseph and Jean Sanger ask whether the filaments found in intestinal cells infected with enteropathogenic *Escherichia coli* could be actin. The answer is definitely "yes". Since the first description of this effect[2], a considerable amount of work has clarified both the composition of this lesion and the mechanism by which it forms.

That the dense fibrous pad formed beneath enteropathogenic *E. coli* (EPEC) when they attach to various cell types is indeed predominantly actin was shown by staining with an actin-specific phallotoxin conjugated with fluorescein



Human embryonic lung cells with adherent 'attaching effacing' EPEC. Dense plaques of actin visualized by fluorescence staining with phalloidin (*a*) correspond exactly with each adherent bacterium seen by phase contrast (*b*). Magnification, approx. × 700.

(see figure). We have proposed this fluorescence actin staining test[3,4] for rapid detection of EPEC, which were previously identified simply on the basis of serotyping. With regard to mechanism, we have also demonstrated a localized elevation of intracellular free calcium in EPEC-infected cells of various types[5], and proposed that this activates the actin-severing function of cytoskeletal villin, leading to effacement of the brush border microvilli[6]. Subsequent dissipation of local increases in calcium concentration by natural sequestration into intracellular storage would then allow dissociation of villin, generating nucleation sites for an explosive burst of actin polymerization. This would deform the plasma membrane (now denuded of its microvilli) into the characteristic pedestal structure seen in natural disease.

As Sanger and Sanger point out, the possible involvement of nucleating proteins in this process is, as yet, unclear, but obviously we need to identify a bacterial potentiator of actin polymerization and the signal pathways in target cells. But our model does answer another question posed by Sanger and Sanger, this time in the negative. If, as they speculate, EPEC were able to cruise the brush border, destroying microvilli by mopping up the actin supply, we would expect to see regions of effacement devoid of bacteria. In fact, effacement is only ever seen at sites of bacterial attachment. A more likely explanation for gross effacement of microvilli is that EPEC infection is a dynamic process in which bacteria grow and attached microcolonies of bacteria continually enlarge, so that eventually microvilli are lost from the entire apical surface of an infected cell.

The EPEC pedestal also contains other cytoskeletal proteins in addition to actin[7,8]. Of particular interest is myosin, in which the light-chain component has undergone phosphorylation by the calcium-dependent enzyme myosin light-chain kinase[8], possibly as a result of a kinase cascade. Indeed, we believe that the success of EPEC as a pathogen resides in its ability to hijack the natural processes of cytoskeletal protein recruitment that are central to the healthy functioning of a cell.

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SANGER AND SANGER REPLY — The evidence of Knutton, Baldwin and Williams that filamentous actin is a major component of the dense fibrous pad localized in cells where EPEC attach is quite convincing. The electron micrographs of the fibrous material[4] also resemble images of cells in the process of phagocytosing bacteria or particles. It was the short columns of fibrous material extending into the cytoplasm beneath attached EPEC in other cells[2,9] that attracted our attention and reminded us of the columns of actin beneath the beads on *Aplysia*[10]. Assuming that these short columns are also made of actin, perhaps they represent an earlier stage in infection than that observed by Knutton *et al.*

As microvilli can reform rapidly under experimental conditions, it would be most interesting to observe EPEC on the surface of living cells. For example, Goligorsky *et al.*[11] have demonstrated that a one-minute exposure of cultured kidney cells to parathyroid hormone initially reduces the number of microvilli, but in five minutes the microvilli reform completely. Thus, bacteria might move on the surface of the host cell and microvilli could reform in their wake. On the other hand, Knutton *et al.* may be correct in proposing that attachment itself leads to effacement. The ability of bacteria[12] and beads[10] to "hijack. . . cytoskeletal protein recruitment" will enable systems to be devised that give valuable insight into both normal and pathogenic processes in cells.

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Early nanotubes?

SIR — *Nature* has recently published several reports of the synthesis and structure of carbon 'nanotubes'[1–4]. The authors of these reports seem to consider the study of this form of carbon — a development since the discovery of Buckminsterfullerene[3]. But there was a report in *Nature* as long ago as 1953 by Davis *et al.*[5] describing very similar thread-like structures obtained from the reaction of CO and Fe_3O_4 and 450 °C on the surfaces of firebricks exhibiting 'iron spots'. The structures were described as layered threads, varying in thickness from 10 to 200 nm. The thicker strands appeared to be composed of many finer threads twisted into helical structures. The size and form of the threads appear to be similar, if not identical, to those described more recently[1–4].

As fullerenes are now known to be produced in sooting flames[6,7], it is not unreasonable to think that the structures produced by Davis et al. might have been nanotubes, although one certainly cannot tell this from their photograph.

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