When the *ompF* gene is deleted, alterations to a similar outer membrane porin, OmpC, are obtained. Why these *ompC* mutations are not obtained when the cell is *ompF*$^+$ remains a mystery since the alterations in both *ompF* and *ompC* are analogous at the DNA and protein levels and reconstruction experiments show the *ompC* mutations are sufficient to allow colony formation in a lawn of *ompF*$^+$ cells (S.A.B. *et al. J. molec. Biol.* in the press). I believe that we are seeing a form of directed mutagenesis in this selection, though we have no data on how this directed response might occur. One explanation is that the *ompF* gene, due to its chromosomal position, is preferentially mutated when the cell senses a need to increase its outer membrane permeability during starvation. As pointed out by F. Stahl (*Nature* **335**, 112–113; 1988) one could envision a number of possible molecular mechanisms to account for how cells might, under certain circumstances, direct or influence mutational events. Further tests of the notion of a directed mutagenic response during non-lethal selections are needed. We owe a debt of gratitude to the Cairns group for pointing out the limitation of the evolutionary dogma formulated in the 1940s and 1950s and for helping to pave the way for studies that re-address the question of how bacteria evolve and respond to evolutionary stress.

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SIR—J. Cairns *et al.* (*Nature* **335**, 142–145; 1988) have presented experimental evidence and circumstantial information which they claim supports the occurrence of "directed mutation" in bacteria in stationary (non-growing) phase. Their crucial evidence for this assertion came from an experiment in which *Lac*$^-$ *Escherichia coli* were plated on a medium (M9) which supposedly kept them in stationary phase. After various times in this condition, they were exposed to a lactose-containing medium which supposedly also prevented growth, and on which revertants to *Lac*$^+$ genotype could be scored by colony growth. The authors argue that if the mutations producing revertants were occurring before lactose was added, the number of colonies growing on the lactose medium should have increased with the length of time spent in the minimal medium (M9) before the lactose was added. If the mutations did not occur until the lactose was added, then the number of colonies using lactose should be a function only of the time since lactose addition. The latter was claimed to be the case, apparently supporting the occurrence of directed mutation rather than the considerably more conventional random mutation model.

It is crucial to the argument that the detection of mutations occurring before and after exposure to lactose was the same; Cairns *et al.* imply that all mutations occurring in both conditions were detected by the methods used. However, the logic of this relies on a number of unwarranted assumptions underlying the interpretation of the experiment, the most important of which is that neither death nor growth was occurring.

During culture on the M9 plates before lactose exposure, death of some revertants may have occurred. Death of revertants would tend to lead to a negative correlation between time spent on the minimal medium and subsequent absolute number of colonies growing on exposure to lactose. This negative correlation could act to reduce any positive correlation arising from the accumulation of mutants. If death outweighed the accumulation of mutants then the net number of mutants would decrease as a function of time. This is what Fig. 3 of Cairns *et al.* shows; the growth curves in the presence of lactose do not superimpose, but start off at a lower level in the late-exposed cultures. The authors themselves note that the number of *Lac*$^+$ colonies detected actually goes down with time on M9 plates, supporting this argument. It is not necessary for the argument that the death rate of *Lac*$^+$ and *Lac*$^-$ cells differed; any death of *Lac*$^+$ cells would lead to an underestimate of mutation rate. The estimate of random mutation rate before lactose exposure may therefore have been too low.

To overcome the death problem one could plot the number of *Lac*$^+$ colonies as a proportion of the total number of cells still alive, rather than as the absolute number of colonies which Cairns *et al.* measured. Even then, however, there would be a further difficulty if cells of the two genotypes differed in survival probability in the absence of lactose.

The second major difficulty is that the mutation rate after exposure to lactose may well have been elevated by growth. Cairns *et al.* themselves note that there would be a problem if the *Lac*$^-$ cells were better able to grow in the presence of lactose than on M9 alone, either because the mutant was leaky or because the lactose was impure. If lactose medium supported slow growth, and if the resulting extra DNA replication allowed the accumulation of more mutations than in culture on M9, then the number of mutant colonies detected would be a function of time since the lactose was added for this reason alone, without any need for the occurrence of directed mutation. Cairns *et al.* argue against this possibility by showing that a different mutation (conferring valine resistance) does not accumulate during the time that the colonies are exposed to lactose; presumably, then, they are not accumulating merely random mutations as a function of growth. But this argument is flawed. It crucially assumes that the mutation conferring valine resistance is selectively neutral in the absence of valine. Such an assumption is unwarranted, because the mutation may reduce survival rate under these culture conditions. This, in addition to random cell death, could explain the finding that the number of Val(R) colonies actually decreased as a function of time spent in the lactose medium before the valine challenge. We can conclude, then, that there may be selection against Val(R) in the absence of valine, and the logic of the crucial control experiment collapses.

The design of the experiment was complex, and the interpretation hinges critically on the assumptions that there was no death and no replication. It would seem preferable to search for a design that avoids these assumptions since it is hard to see how they could be directly validated.

In a second experiment Cairns *et al.* describe further evidence based on reversions to *Lac*$^+$ in an *E.coli* strain with the lacZ region under the control of an arabinose positive regulator, but rendered *Lac*$^-$ by the presence of a bacteriophage Mu DNA insertion between them. Deletion of this sequence causes reversion, and apparently occurred at a higher rate on minimal medium to which lactose and arabinose had been added than in rich cultures deficient in these two sugars. However, there was no control experiment to measure the deletion rate of this bacteriophage sequence from other parts of the *E. coli* genome under these conditions, so that it is not clear if the mutations were genuinely directed. Furthermore, the interpretation relies on the untested assumption that the survival of revertants in the absence of lactose and arabinose was as good as that of the original strain; the point that we raised earlier in connection with the valine-resistance study.

Finally, even if the Cairns *et al.* interpretation of the *Lac(Ara)*$^+$ experiment was correct, it is in their own words "rather a special case", because it appears to involve a specific mechanism for the movement of a foreign DNA fragment, rather than a mutation in the ordinarily accepted sense of the word. We submit that conceptual disarray might result from trying to solve the problem of random versus directed mutation by studying the complex rules of warfare between bacteria and their parasites.

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