SIR—Cairns *et al.* (*Nature* **335**, 142–145; 1988) have recently argued that specific mutations may be induced in response to selection. Their case was based in part on the excess of mutations arising in stationary phase and under selective conditions relative to those arising during exponential growth in the absence of selection.

These experiments may not provide definitive evidence for directed mutation, as it is well known that the mutation rate of *E. coli* varies with environmental conditions, and in a way that may vary depending on the DNA composition in the vicinity of the mutation. This makes the control experiment very important and it is unfortunate that the control used by Cairns *et al.* was a gene for valine resistance. Many of these are frameshift mutations and may therefore not be directly comparable to the mutations that revert a nonsense mutation used by Cairns *et al.* to test for mutations induced in response to selection.

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SIR—I would like to suggest an alternative explanation for the observations that led Cairns *et al.* (*Nature* **335**, 142–145; 1988) to suggest there may be a mechanism for a cell to mutate specifically in response to selection pressure. If the Lac^+ mutant bacteria have a slower growth rate than their Lac^- parents, then the contribution of mutations occurring early in the growth of a culture will be curtailed. This will have the effect of producing a distribution similar to that observed by Cairns *et al.*, as I have shown in simulations using an explicit model.

My colleagues S.-K.Liu and I-S. Hwang (personal communication) have examined the distribution of the number of Gal^+ revertants of Gal^- parents in *E. coli* and show a deviation from the prediction of Luria–Delbruck clonal theory similar to that seen by Cairns *et al.* In this case more than half the Gal^+ mutants do grow relatively slowly, which could account in part for the deviation from the expected distribution.

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CAIRNS REPLIES—Before responding to certain specific comments, it might be helpful to remind readers of the context of our work. Because most mutations are rare, the circumstances surrounding their occurrence can be studied only in very large populations, such as bacterial cultures. Some very elegant experiments performed in the 1940s and 1950s showed that certain kinds of mutation in bacteria arise spontaneously, before there has been any selection. That conclusion agreed so well with everyone's preconceptions that the question of the origin of mutants has been left virtually untouched since then. We resurrected the issue partly because of increasing discomfort with the conventional view of the origin of the genetic changes in cancer[1] and in part because we realized that the classical experiments had not been a fair test. In our paper[2], we describe a study of three types of mutation *E. coli*.

The first is the reversion of a nonsense mutation in *lacZ*. We discussed this case at some length, even though the results were not at all clear-cut, because it shows how the timing of mutational events can be deduced from the distribution of numbers and time of appearance of mutant colonies. We found that although many of the mutations occur during the prior growth of each culture (before there has been any selection), most mutants seem to arise later, after the bacteria were put on to selective lactose-minimal plates. As a result, the distribution of mutant numbers among different cultures is much narrower than expected.

Our critics have offered several explanations for this result. Van Valen suggests that mutants are being lost all the time through what is called "periodic selection" (namely, the loss of the accumulated mutants in a population due to takeover by a fitter variant, which is observed in an irregular fashion when cultures are maintained for long periods under suboptimal conditions); since we are observing a regular phenomenon in cultures following rapid growth for a relatively short period of time, this explanation is most implausible.

Several people have suggested that the Lac^+ mutants are growing more slowly or surviving less well than non-mutants (ref. 3; the letters from Charlesworth *et al.* and from Tessman; R.E. Lenski, M. Slatkin and F.J. Ayala, personal communication). We feel that the simple version of this hypothesis is not very likely because most of the mutants we detected behaved as if they were revertants to wild type and, by the time that we could test their growth rate, appeared to grow as well as non-mutants. But there is lurking in this suggestion the more complex idea that it may be a special property of all new mutants that they are temporarily at a great disadvantage in the absence of positive selection pressure, and that, of course, is just another way of describing the very anomalies we are seeking to explain.

Our experiments with *lacZ* had a second part, in which we looked at the late accumulation of Lac^+ revertants on lactose-minimal plates. We showed that extra revertants do not accumulate unless lactose is present, and that this effect is specific because it does not cause the accumulation of unselected mutations (to valine-resistance). From this we concluded that the *lacZ* mutation is not significantly 'leaky', our *lacZ* strain is not growing on lactose-minimal plates and lactose is not acting as an indiscriminate mutagen.

It is imaginable, however, that the frameshift mutations, which are commonly the cause of valine-resistance, may not respond to starvation in the same way as point mutations, as Danchin suggests above. This is a valid criticism, and perhaps we should have used as control the reversion of point mutations in other genes more like *lacZ*. (Note, however, that the issue is whether our *lacZ* mutation is leaky rather than what might possibly be the reasons for its leakiness, as Holliday and Rosenberger seem to think.) The experiment has also been criticized on the grounds that the steady decline in the number of Lac^+ and valine-resistant mutants in the absence of selection is addit ional evidence that all mutants are at a disadvantage (see ref. 3; the letter from Charlesworth *et al.*; R.E. Lenski, M. Slatkin and F.J. Ayala, personal communication). But an unpublished control, using deliberately introduced Lac^+ cells, showed that this decline is not due to death of cells in the absence of an energy source, but to a slight delay in colony formation by cells that have been deprived of energy for several days.

The *lacZ* experiments led us to the rather cautious conclusion that populations of bacteria in stationary phase seem to have "some way of producing (or selectively retaining) only the most appropriate mutations". But we too were worried about the background noise, due to the sizeable contribution from spontaneous mutation during prior growth of the cultures. Indeed, if these had been the only experiments, the paper would not have been written.

We did, however, discuss at length two examples of genetic events that apparently occur only under conditions of selection, and it was in connection with these examples that we suggested that bacteria may be able to determine which mutations occur. Since our paper was written, other such examples have come to light[4,5]. Perhaps these will stimulate a second round of correspondence, specifically directed to mutations that occur only when there is selection.

In the meantime, we should remember that the doctrine, so vehemently defended by our critics, is an essentially negative assertion: phenotype never comes before genotype, and so all mutations have to arise before there can be any intimation of their consequences. Yet, it is easy to imagine mechanisms that might test the utility of mutations before they become irrevocably fixed into the genome[6]. If this seems too heretical, the heresy can be softened by postulating that the licence to indulge in such games of trial-and-error