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COMMENTARY

by Bernard Dixon

RESURRECTING THE SUPERINFECTING PHAGE

Every so often, as a microbiology watcher, I feel a deep affinity with the commentators and pundits who turn an honest penny analyzing the exploits of the grandmasters of world chess. Of course, science entails far more than the relentless, focused computational skills that win on the chess board. Yet whenever I sense that an ingenious but neglected experimental method is about to come into its own, I suspect that my satisfaction is akin to that of a chess buff noting the growing popularity of a gambit whose value others have never fully recognized.

It was a special pleasure, therefore, during the recent American Society for Microbiology's symposium on "Engineered Organisms in the Environment," to hear Thomas Brock from the University of Wisconsin urging the merits of an elegant technique for which I have long had a soft spot. The ASM had gathered in Philadelphia to mull over the possible hazards of introducing genetically manipulated microbes and plants into natural environments for beneficial uses—in agriculture and other fields. Risk assessment, microcosm tests, environmental impact analyses, and *a priori* prediction based on basic biology—all received careful consideration. The one option upon which many participants expressed varying degrees of skepticism was the monitoring of microbes after release. Given their astronomical numbers, the difficulty in enumerating them, and the particular problem of deducing from population samples whether an organism was proliferating or demising, how could one possibly establish realistic machinery for surveillance?

It was Prof. Brock who reminded his audience of the clever tactic that English microbiologist Guy Meynell evolved several years ago to measure the true rates at which pathogenic bacteria divide in animal tissues. Although it was possible to count viable organisms at various times after infection, there was then considerable confusion—and not a little disagreement—about what these figures really meant. Such counts were clearly the resultants of multiplication and destruction—plus mechanical removal in the case of intestinal pathogens like *Salmonella typhii*. Meynell and his co-workers (first at the Lister Institute in London and later at the University of Kent, Canterbury) came up with the perfect answer. Called the "superinfecting phage technique," it provided genuine division rates, which in turn allowed calculation of actual death rates.

Meynell's secret was to introduce into bacteria genetic markers which did *not* integrate into the chromosome and did not replicate independently in the cytoplasm. He lysogenized his pathogens with a temperate bacteriophage and then introduced a related phage. This second, superinfecting, phage neither lysed the bacteria nor produced further copies of itself. When the cells divided, therefore, one particle went into one daughter cell only. As this continued to happen with each succeeding generation, a simple comparison of the proportion of cells containing the marker at the outset and after a particular period of time gave the number of divisions occurring between.

The technique is as simple in practice as it was elegant in conception. Ultraviolet irradiation releases both the temperate and superinfecting phages. So diseased tissue is taken, homogenized, and plated out on two different bacterial strains—one sensitive to both phages (giving plaques indicating the total number of viable cells) and one sensitive only to the superinfecting phage (giving plaques indicating the number of cells containing that marker).

An alternative approach, also pioneered by Meynell, is based on abortive transduction. A phage transfers a fragment of a donor bacterium's genome (carrying an identifiable biochemical marker) into a recipient pathogen. Because this piece of DNA is not incorporated into the chromosome, as in normal transduction, and cannot reproduce autonomously, it too passes into one daughter cell at each division, allowing comparative counts.

Used on *S. typhimurium* and *Escherichia coli*, these techniques have provided considerable insights into the course of infection. They have proved, for example, that bacterial multiplication and destruction can occur simultaneously, sometimes in near equilibrium (*British Journal of Experimental Pathology* **49**:597, 1968), and shown how sharply population size may grow when host defenses are impaired (*British Journal of Experimental Pathology* **54**:99, 1973). When Dr. Meynell first introduced them, their utility was limited by the narrow range of bacteria on which the requisite genetic manipulation was possible. Even today, with our much greater ability to fabricate organisms as we wish, they do not seem to have been taken up as vigorously as they might in studying other infections. And there is little evidence that they are being explored for the purpose outlined by Prof. Brock—monitoring the fates of microbes in the environment—although these methods seem beautifully appropriate.

Why? Is specialization the answer? Have soil microbiologists and plant pathologists not pursued these ideas because they have appeared mostly in the pages of journals concerned with medical microbiology? Tangential support for that proposition may come from the fact that their originator has long been one of the most versatile of investigators. It was he, for example, who discovered the cause of "foxing," the discoloration on the pages of antique books. A bibliophile himself, Meynell was puzzled by this phenomenon and asked experts in the book trade what it was. "Foxing," they said, and nothing more. But the microbiologist in Meynell was unsatisfied—and many fluorescence, scanning, and transmission micrographs later, he had demonstrated beyond cavil that fungal infection was to blame (*Nature* **274**:466, 1978). A cobbler shouldn't always stick to his last—biotechnologists please note.

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