COMMENTARY

by Bernard Dixon

CITROBACTER FOR CONTROL OF CADMIUM

Bernard Dixon, Ph.D.

It's quite an achievement to evolve a scientific theory that is both erroneous and highly influential. Within modern microbiology, one outstanding example comes to mind. It is immortalized in two books: C. N. Hinshelwood's *The Chemical Kinetics of the Bacterial Cell*, published in 1946, and A. R. C. Dean and Sir Cyril Hinshelwood's *Growth, Function and Regulation in Bacterial*

Cells, which appeared 20 years later. A man of encyclopaedic learning, Hinshelwood was knighted in 1948 and later enjoyed the unique distinction of being simultaneously president of the Royal Society and of the Classical Association. But in the first of those two books he made a conspicuously heterodox step, attacking the then emerging view that bacterial variation could be interpreted in terms of mutation and selection. In its place he offered what critics greeted as a neo-Lamarckian account of microbial "training."

Although the theory proved to be incorrect, it served a historically crucial purpose—not least as expressed in the later book, written with Alastair Campbell Ross Deari, which appeared just a year before Hinshelwood's death in 1967. While some saw this as a final defense of the defenseless, today we can appreciate two great merits in the work. First, the authors argued so powerfully and elegantly that they compelled opponents to marshal all the more carefully their evidence based on mutation and selection. Second, they emphasized the importance of seeking unifying principles behind microbial behavior as well as studying reactions in isolation. That injunction remains valid even today.

But to focus more directly on the subject of this month's essay, what actually happens to a distinguished scientist who is universally remembered among generations of researchers as co-founder of a theory which, however eruditely expressed, was at heart erroneous? In the case of A. R. C. Dean, the answer is that he has maintained a lively interest in the kinetics of bacterial growth while establishing a considerable reputation in another, more specific field—one of abundant interest to biotechnologists. Still active at the age of 65 in his laboratory at the University of Oxford, he is now concerned with the uptake of heavy metals by microorganisms.

During recent years Dean has been studying how various microbes accumulate metals such as copper and lead. One of his current preoccupations is peculiarly interesting, however, because it concerns an element so inimical to life of all varieties that Mary Ellen Curtin did not even mention it last year in her excellent review of metal recovery in *BIO/TECHNOLOGY* (1:228). He and his colleague Lynne Macaskie are studying the uptake of cadmium, a metal that has no known biological function and is extraordinarily toxic to both microbes and macrobes.

Industry certainly requires efficient techniques for removing this pervasive pollutant from wastes and effluents.

Cadmium is employed in a wide range of manufacturing processes, including electroplating and smelting as well as mining, all of which generate hazardous leftovers. While occupational inhalation is the route through which acute poisoning has caused most known deaths in the past, chronic intoxication via food and water now excites greater concern. During 1972, the World Health Organization reduced its standard for cadmium in drinking water from 0.01 ppm to 0.005 ppm, and set the tolerable human weekly intake at 400–500 micrograms per person. Yet even these vanishingly tiny figures are now being reviewed again, as we learn more about the dangers of human exposure through food chains in contaminated areas.

Previous researchers have described the accumulation of cadmium from solution (despite its universal toxicity) by microbes ranging from staphylococci to *Saccharomyces cerevisiae*. But these studies were conducted using rich culture media containing substances that form complexes with heavy metals. This can mean enhanced uptake if the chelating ligand is a substrate for the organisms concerned. An alternative possibility is chelation to a non-substrate, thereby reducing the metal's toxicity and uptake. What Dean and Macaskie decided to investigate was cadmium extraction in minimal media lacking chelating agents of this sort. Clearly, if such a process is to be applied in industrial scavenging, it will need to be effective with nutritionally sparse effluents.

The organism employed in Oxford was a species of *Citrobacter*, isolated from metal-polluted soil, which combined high resistance to cadmium with high uptake. But even this strain was ultrasensitive to the metal when cultured in minimal media—which, of course, limited its accumulation. The breakthrough came when Dean and his colleague grew the bacterium initially in cadmium-free medium, before exposing resting state cells to the metal. These organisms then tolerated a much higher concentration and removed most of the cadmium from solution. As reported in the *Journal of General Microbiology* (130:53, 1984), cells retained this ability even when immobilized in polyacrylamide gel inside a glass column. Challenged with a flow of liquid-carrying dissolved cadmium, the organisms extracted 65 percent of the element—and remained active for a considerable time.

As yet, the Oxford duo cannot be certain about the mechanism by which specially pre-grown *Citrobacter* so avidly accumulates a heavy metal that is normally so noxious. A cell-bound phosphatase, induced during preliminary growth by the glycerol 2-phosphate provided as sole phosphorus source, could be responsible. Indeed, Dean and Macaskie's original search for a naturally occurring bacterium or fungus able to take up cadmium may well have given them a mutant capable of resisting the metal not by excluding it (the usual stratagem) but by producing a suitably altered phosphatase. What is clear is that their organism, appropriately manipulated, seems a highly promising candidate for a future process designed to rid industrial effluents of a potentially lethal pollutant.

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