COMMENTARY

VALUABLE VIRUSES

by Bernard Dixon

One of the books that really fired my imagination as a student was *The Microbe's Contribution to Biology* by A. J. Kluyver and C. B. van Niel. I still have my copy today, the green dust jacket now severely etiolated but as familiar as a well-worn tie.

Although described on the cover as an American microbiologist, Cornelis Bernardus van Niel was born in Haarlem, The Netherlands in 1897. He began his microbiological career in 1922 at the Technological University of Delft where Jan Kluyver, some 11 years his senior, had recently become professor. The book, published in 1956, was the printed version of a series of lectures given at Harvard University two years earlier by what was obviously an inspired pairing. An account of "developments in microbiological research which...have significantly expanded our knowledge of the basic characteristics of living organisms," it was a work of rare intellectual enticement. "Microbes," the authors vouchsafed, "may play a crucial role in the eventual formation of fundamental biological concepts." In retrospect, the book was remarkable for doing rather more than recounting the facts of life as learned by humans (from Pasteur to Lederberg) as they probed captive populations of bacteria. The book also hinted at a future explosion in knowledge concerning the regulation of metabolism and its genetic machinery—although this was to happen even more quickly than Kluyver and van Niel imagined.

From time to time, subsequent authors have embellished the theme. Most recently Boris Magasanik of Massachusetts Institute of Technology, writing in *Science* (240:1435, 1988), recounted the massive contributions made by bacteria to the development of genetics, biochemistry, and physiology over the half century since Kluyver and van Niel wrote their book. Concurrently, two other MIT biologists, David Botstein and Gerald Fink, lauded the unique merits of yeasts as experimental organisms (*Science* 240:1439, 1988). Other authorities have recorded their indebtedness to slime moulds, penicillia, and further sects of the protista.

What we seldom hear about is the assistance that viruses can give to biologists studying higher organisms. The outstanding exception to this brash generalisation comes, of course, from the bacteriophages, which have found several useful applications, from the typing of food-poisoning *Salmonellae* to the ferrying of genes in the process of transduction. They were, indeed, the *raison d'être* of the phage school of the 1940s and early '50s, which was crucial to the emergence of molecular biology. But viruses proper, the sort that cause foot-and-mouth disease in cattle and spotted wilt disease in tomatoes, are still seen as wholly unhelpful and malevolent. It's no coincidence that the angriest of the anarchist magazines I have picked up recently is called *Virus*.

Hans Kuypers, the distinguished neuroscientist who died at the sadly early age of 64 last September, would

have rejected this notion of viral infamy. For it was Kuypers who, in the last few years of his life, had begun to develop the extraordinary technique of using viruses to trace the intricate labyrinths and cat's cradles of interconnections that comprise the mammalian nervous system. Working with Gabriella Ugolini at Cambridge University in England, he had already achieved some astonishing results, particularly with herpes viruses. A paper by the two anatomists, published recently in *Trends in Neurosciences* (13:71, 1990), shows both the strengths of this novel methodology and the degree to which its full potential is still to be realised.

Kuypers was particularly interested in phenomena such as the delicate finger manipulations that are unique to the primates. In trying to understand how these movements are controlled through descending pathways from the brain, he sought ever more powerful methods of visualising nerve fibers and tracing their interconnections. He was, for example, one of the first neuroscientists to exploit the capacity of living fibers to take up and transport proteins, such as the enzyme horseradish peroxidase, that can serve as markers. Another of his innovations was the so-called double-labelling technique for studying branching fibers, based on pairs of fluorescent dyes with the capacity for being picked up by separate branches.

But these approaches have limitations in charting entire networks of connected neurones. What researchers need for this purpose are "transneuronal tracers"—substances they can apply at one end of a chain of several neurones and then monitor as the label passes successfully to the other end. Possible candidates include wheat germ agglutinin and certain fragments of tetanus toxin, but only small quantities of these tracers are transferred. Labelling is correspondingly weak. What Kuypers, Ugolini, and colleagues have accomplished is to develop labels that are not diluted as they pass from cell to cell. On the contrary, they are actually amplified. As described last year in *Science* (243:89, 1989) and now reviewed and extended in *TINS*, the secret is to use live neurotropic viruses.

Herpes simplex virus type 1, pseudorabies virus, fixed rabies virus, and a vesicular stomatitis mutant have proved particularly effective. They are transported in both directions, forward to axon terminals and backwards to the parent cell bodies. Their antigens can be detected by well-established immunohistochemical methods. They work both *in vitro* and *in vivo*. And they are already beginning to win rich rewards when deployed in such formidable tasks as charting connections between peripheral nerves and neurones in the brain.

For decades, popularisers of microbiology have had nothing good to say of viruses other than that they are responsible for the colourful infections of Rembrandt tulips. Now, at last, they can praise them for their exquisite sensitivity as tools for solving some of the most intractable remaining problems of biological science.