COVERT ACTIVITIES

by Bernard Dixon

Over 30,000 of them have been identified, with a bewildering range of shapes and sizes and a rich variety of biological actions. Many more are being described week by week, in organisms both familiar and arcane. They have no known roles in the internal economies of their producing cells, and the genes encoding them can often be knocked out without let or hindrance. These same substances already have major effects on the health, nutrition, and economics of our society. The continuing discovery of new ones promises rich rewards for the bioindustries of tomorrow. Yet they remain shadowy molecules—not always what they seem, and refractory to clear definition. No less than 27 of the world's top experts on these substances met for two and a half days at London's Ciba Foundation recently without even agreeing on how they should be defined.

What are they? Answer: secondary metabolites, whose precise categorisation is giving biologists as much difficulty as anything since N.W. Pirie penned his magnificent essay on "The Meaninglessness of the Terms Life and Living" in Joseph Needham and David Green's historic *Perspectives in Biochemistry* some 55 years ago. As on that occasion, it is comparatively easy to agree on what we are *not* talking about. Other than on *Monty Python*, there's little dispute about the deadness of a dead parrot. Nor is there dissent from the proposition that the central pathways of respiration and fermentation are appropriately described as primary metabolism. But what is life? What is *secondary* metabolism?

The riddle begins with the best known, most easily recognized members of the secondary club—the antibiotics. Here the apparent neatness of our own operational definition, based on therapeutic utility, soon evaporates when we ask what antibiotics are doing in nature anyway. Opinions vary, from a well-ordered analysis of antagonism in the soil and the selective advantages of making anti-microbials to deter other microbial species, to deep scepticism, arising from our considerable ignorance of why, when, and how much of these substances are formed in the biosphere.

True, there are some plausible scenarios. Speaking at the Ciba Foundation Symposium, Keith Chater of the John Innes Institute in Norwich, U.K., speculated that the *Streptomyces* mycellum in the soil begins to die as spore-bearing aerial branches appear. Another family of so-called secondary metabolites, lactones with aliphatic side-chains, seem to act as pheromones in a wide variety of microorganisms, from marine vibrios to *Aspergillus nidulans*.

Such phenotypic evidence indicates that these substances heighten the survival fitness of the microbes producing them. Molecular genetics lends support to that idea. As Dudley Williams of Cambridge University pointed out, secondary metabolites are mostly complex structures whose biosynthesis, programmed by many kilobases of DNA, requires considerable energy. The genes for their production, regulation, and resistance are usually clustered together, suggesting past selection of mutants with favourable clusters, while their antagonistic effects can be "astonishingly sophisticated."

But now consider an awkward fact advanced by Louis Nisbet of Xenova Ltd. in Slough, U.K. Many antibiotics are being found to have other activities—and when they do, those activities are more potent than the actions that give them their names. Erythromycin, for example, is an antagonist of motilin, a small peptide found in the duodenum and in pituitary and pineal glands that stimulates intestinal motility. Why? And why do we assume that its significant role is to banish infections such as teenage acne? What of the "other" biological effects of other antibiotics? How to decide which of two activities reflects an antibiotic's true nature and which is a molecular accident? Do all antibiotics have additional, more powerful activities?

A similar density of fog surrounds the purpose of other secondary metabolites which, apart from sharing a common characteristic of low molecular weight, cover a weird portfolio of functionalities—from toxins and pigments to growth promoters of plants and animals, from pesticides and herbicides to anti-tumor agents and immune modulators. Very few of these activities, classified according to the preoccupations and limited knowledge of *Homo sapiens*, have been demonstrated to have biological significance.

Or consider the problem from the perspective of evolution. Here Julian Davies of the University of British Columbia entered the ingenious speculation that secondary metabolites furthered the early development of the machinery of translation, as effectors of various steps in the formation of peptide bonds. We certainly recognise some antibiotics as inhibitors of protein synthesis because they interact with present-day RNA. And some of these have proved to be potent inhibitors of ribozymes. Are they the descendants of "old" molecules that played their starring roles in the RNA world that presaged the emergence of DNA as the carrier of genetic information? It's an enticing argument, though one that ends in the primeval soup, which according to twentieth century reconstructions probably contained many of the amino acids now found in secondary metabolites.

The debate will continue, as will the screening of microbial (and plant?) pharmacophores for exploitable functions. Time for a speculation of my own. Many secondary metabolites, especially those found in marine organisms, have potentially valuable activities but are also impossibly toxic for therapeutic purposes. Rather than rejecting their producers out of hand, should we not be throwing in various enzyme inhibitors in the hope of encouraging the accumulation of precursors that may have useful actions and much lower toxicity?