

CLOSING REMARKS

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I would like to thank Dr Rakhit for giving me the opportunity of offering some closing remarks at the end of this interesting symposium. As a result of these three days of excellent talks and the subsequent discussions, I know that we microbiologists can now better appreciate the triumphs, as well as the problems, of the chemists; and I would hope that the chemists can now communicate somewhat more easily with the biologists. I think that all of us who work on antibiotics can point with pride to our collective accomplishments in the alleviation of human suffering, especially in these days when the general public appears so suspicious of the results and relevance of current scientific research.

As Dr Vezina pointed out this morning, the use of antibiotics is not limited to human health but also is important in animal production, plant protection, food preservation and in providing tools for solving the problems of molecular biology. Although the quality of life has no doubt been markedly enhanced by antibiotics, we cannot feel complacent for our greatest challenges lie ahead.

Several of our speakers beautifully described the wealth of structures elaborated by presently known antibiotic producing organisms. Dr Celmer's discussion of over 50 macrolides and the variety of microbial hydroxamates described by Dr Maehr were quite impressive in this respect. Yet despite the large number of structures already known and exploited, our major objective remains the preparation of new and improved antibiotics. The reasons follow:

(1) Effective control of gram-negative infections is yet to be accomplished. Although the kanamycin-gentamicin series, reviewed by Dr Cooper yesterday, extended the antibacterial spectrum of the aminoglycosides to *Proteus* and *Pseudomonas*, these drugs are not without toxicity. In the area of penicillins and cephalosporins with gram-negative activity, greater potency and improved resistance to enzyme inactivation are still needed.

(2) Systematic anti-fungal agents which are non-toxic are needed.

(3) Antiviral and antitumour agents are desperately in need. The progress in the antiviral field, as summarized by Dr Haff on Monday, has been painfully slow and relatively unproductive. However, the possible use of high-molecular weight interferon inducers by intranasal administration, the discovery of low molecular weight interferon inducers and the activity of rifamycin derivatives against certain viruses at least constitute some hope in this area. Our performance in the antitumour field has also been unimpressive, but we now await the results of clinical trials of mycophenolic acid, an agent with activity against solid tumours as described by Dr Gerzon, and

of the bleomycins which are active against squamous cell carcinoma as reported by Dr Umezawa. It will be interesting to see whether the above antiviral and antitumour candidates are any more successful in the clinic than the nucleoside antibiotics, the chemistry of which was so well described by Drs Fox, Gerzon and Haff.

(4) Antiprotozoal agents are needed.

(5) Antibiotics not used for human therapy are needed as growth stimulants in animals and possibly for food preservation.

(6) Development of resistance to known antibiotics is now an accepted fact of nature, and new antibiotics must replace the old ones. I believe Dr Smith's discussion of resistance mechanisms leaves no doubt about this. Dr Cooper's remark that a new R factor mediating resistance to gentamicin has now been found should remove any feeling of complacency concerning aminoglycoside therapy of gram-negative infections. It will be of interest to see whether the new nebramycin factor VI is also affected by this new resistance factor.

The continuous need for new antibiotics has led to the establishment of gigantic screening programmes in most of the pharmaceutical houses and institutes throughout the world. Much money is spent annually screening soil microorganisms for their ability to produce new and useful entities. Although this effort was quite rewarding for twenty years, the number of unique molecules discovered has become vanishingly small. I believe the day of randomly examining soils for activity against *B. subtilis* or *E. coli* is over. What can be done to obtain new modified antibiotics?

(1) *New groups of producing organisms.* This approach using *Micromonospora* has yielded the currently important gentamicin and still could be quite important.

(2) *Directed biosynthesis.* Whenever a new molecule of some possible utility is found, attempts to modify it by nutritional supplementation should be carried out. Although this is an old technique, it is still very useful. Its applicability was exemplified in the descriptions by Dr Umezawa of over 40 new bleomycins, some more active than natural bleomycins, obtained by feeding different polyamines, and by Dr Katz with respect to polypeptide antibiotics. The value of this technique is limited, however, by the permeability and toxicity of the additive and its susceptibility to degradation and to utilization in other pathways of the producing organism.

(3) *Chemical modification of known antibiotics.* This technique is receiving much attention now due to recent successes in improving the characteristics of antibiotics in the following respects: (a) broadening antibacterial spectra (penicillin G→carbenicillin, ampicillin); (b) increasing activity versus resistant organisms (penicillin G→methicillin, cloxacillin); (c) increasing potency (cephalosporin C→cephalothin, cephaloridine); and (d) increasing acid-stability for oral administration (cephalosporin C→cephaloglycin, cephalixin).

After Dr Fox's and Dr Keil's elaborate descriptions of chemical synthesis and modification of antibiotics, there should be no worry about the ability of the chemists to modify structures at will, but I must say that the chemist has been limited by the structures produced by the microorganism.

(4) *Bioconversion.* One way to present the chemist with new structures for

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modification is to biologically convert the antibiotic to new derivatives, as described by Dr Perlman yesterday. Although there have been limited attempts to produce more active antibiotics by bioconversion, the combined bioconversion—chemical approach to semisynthetics has not been extensively pursued despite the success of this procedure in the penicillin field. The paper which Dr Keil just delivered emphasizes the usefulness of a similar approach in which the 'transformation' is carried out chemically, i.e. coumermycin A1 → biologically inactive PNC-amine → new coumermycins.

Another means of producing new structures for chemical modification is the accumulation of intermediates and shunt products by genetically-blocked mutants.

(5) *Biochemical mechanisms of resistance.* The study of the biochemical basis of resistance development *in the clinic*, as described by Dr Smith, has revealed unexpected and novel antibiotic inactivation mechanisms such as phosphorylation, adenylation and acetylation. These findings are extremely important in the design of new drugs which retain antibacterial activity yet are not substrates for R-factor mediated enzymes. Certainly the existing aminoglycosides will be so modified. However, it is surprising that the mechanism of resistance development to the albomycins, the trihydroxamate antibiotics described by Dr Maehr, is still unknown. Since the main drawback of this otherwise excellent antibiotic appears to be rapid resistance development, it would be profitable to uncover the mechanism involved in resistance.

(6) *Cell-free synthesis.* The papers of Dr Katz and Dr Dhar on cell-free synthesis of antibiotics show us that we no longer have to depend on intact cells to produce antibiotics. There is no doubt that such enzymatic systems are valuable not only in elucidating biosynthetic pathways and in understanding of the regulation of antibiotic synthesis, but could be important in discovering new antibiotics. Directed biosynthesis in cell-free systems, for example, is not limited by permeability, toxicity, degradation, or utilization of the potential precursor for other cell processes. The cell-free studies of Dr Abraham were very exciting to those of us interested in penicillins and cephalosporins. The activity of isopenicillin N, but not of penicillin N, as a substrate for penicillin acyl transferase of *P. chrysogenum* has been a long-awaited result establishing isopenicillin N as a true precursor of penicillin G. Further, the announcements of the cell-free synthesis by *Cephalosporium* extracts of the tripeptide, L- α -aminoadipyl-L-cysteinyl-D-valine, and the tetrapeptide, AAA-cys-val-gly were other highlights of this meeting, as was the finding of β -hydroxyvaline instead of valine in an *in vivo* produced tetrapeptide. These findings will help considerably in our attempts to unravel the cephalosporin biosynthetic pathway.

Let me conclude on a note of optimism derived from Dr Friedman's talk on the mode of action of antibiotics. During the early days of molecular biology, it was felt that targets such as ribosomal protein synthesis or DNA-directed RNA polymerase would be useless, i.e. antibiotics acting at these sites would be toxic because both man and microbe use these macromolecules for growth unless, of course, differential permeability existed. However the selective action of antibiotics which affect bacterial ribosomes but not mammalian ribosomes or that of rifampicin which inhibits bacterial

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RNA polymerase but not the mammalian enzyme offers great hope that a host of new and improved antibiotics will be discussed at the next Symposium on Antibiotics. I hope to see you all again then.