In this context is should be mentioned that as many as 77 out of a total of 220 commercial and experimental organic insecticides contain chlorine and fluorine.

One has to be as careful in the case of toxicological considerations. From the toxicological point of view, β-dihydroheptachlor, although having insecticidal activity similar to that of DDT, has merely an LD_{50} value for animals in the same range as has sodium chloride. Therefore we should avoid all kinds of generalization and investigate the influence of every individual compound of a substance class on the environmental quality separately.

In view of the considerable amounts of environmental compounds, it is obviously most important to determine the priorities of the substances to be examined. The choice of our programme was determined by the following properties of a compound: (1) production; (2) chemical and metabolic persistence; (3) tendency of worldwide distribution; (4) expectation or proof that the residues have toxicological effects on man and also unfavourable effects on the animal environment.

I would like to discuss briefly some results (with references). For the above reasons, we concentrated our efforts during the last years on investigations of chlorinated hydrocarbons like aldrin, dieldrin, telodrin, heptachlor, chlordane, endrin, dihydroheptachlor, lindane and DDT, and the most important results are the following:

(1) It was shown for the first time experimentally that all chlorinated hydrocarbons are metabolized in micro-organisms and insects (Korte, Ludwig & Vogel, 1962, first publication), mammals (Mörsdorf, Ludwig, Nogel & Korte, 1963, first publication) and higher plants (Klein *et al*, 1967, first publication). Hitherto, chlorinated hydrocarbons had been considered to be metabolically inert.

(2) We succeeded in proving by feeding experiments, e.g. with aldrin and endrin, that mammals do not accumulate those compounds *ad infinitum* when a constant dose is administered daily. However, a steady state of storage was reached depending on the daily doses, the sex of the animal and the chemical structure of the compound.

(3) Several metabolites could be identified and their toxicological properties characterized.

(4) In the case of heptachlor we succeeded in identifying the first terminal residue in mammals, namely 1-hydroxychlordene 2,3-exoepoxide.

(5) Although it was assumed until 1967 that compounds of this class did not penetrate into higher plants and therefore were not metabolized, we have been able to prove since then that all of the compounds examined did penetrate into the plants after foliar and soil application and were in fact metabolized by them. We also found that, for instance, after foliar application of endrin on white cabbage, more than 95% of the applied quantity escaped into the atmosphere by evaporation and transpiration within 4 weeks.

(6) Although the presence of many such substances in the air has been increasingly substantiated, so far hardly any reactions have been examined under atmospheric conditions. We found merely that under controlled conditions photoreactions resulting in rearrangement products are possible. Recently we were able to determine the reaction mechanism of these rearrangements. Only for vinyl phosphates did we obtain first results under atmospheric conditions.

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Membrane-Active Antibacterial Compounds

By W. A. Hamilton. (*Department of Biochemistry, University of Aberdeen, Aberdeen AB9 1AS, U.K.*)

By virtue of the structural organization of the procaryotic cell, the bacterial plasma membrane is the site of many of the biochemical activities within the cell. In addition to the permeability properties demonstrated by all plasma membranes, the procaryotic plasma membrane is also the site of activities otherwise associated with the mitochondrial, endoplasmic reticular and nuclear membranes. Further, the syntheses of cell wall, capsule and extracellular enzymes are membrane-associated phenomena in bacteria. Clearly therefore any compound affecting this structure is a potential antibacterial agent and its biochemical mechanism of action may be expressed in the inhibition or destruction of any one of several of the cell's vital functions.

It is unfortunate therefore that the earliest studies on synthetic detergents and antibiotics, such as the quaternary ammonium salts and tyrocidine, which have their effect at the cell membrane, should have been carried out with compounds whose action is to destroy the permeability barrier of the membrane in an apparently non-specific manner. Since then the demonstration of leakage of intracellular contents has often been taken as full and sufficient proof of the mechanism of cell death being the destruction of the membrane permeability barrier. More often than not this conclusion is incorrect, and even when it is justified it is now pertinent to enquire further into the nature and mechanism of this alteration in the semipermeable character of the membrane.

In this discussion the term 'membrane-active' is used to describe only those antibacterial compounds active at the plasma membrane and known to affect its permeability and transport functions. Within these terms of reference there exist a large number of membrane-active antibacterial compounds. They include certain organic solvents, the alcohols, the phenols and bisphenols, detergents, various substituted salicylanilides, carbanilides and guanidines, and the polypeptide and macrotetralide antibiotics, such as valinomycin, gramicidin, polymyxin, the actins and nigericin. This group of compounds has been the subject of two recent reviews (Hamilton, 1970; Harold, 1970).

On the basis of binding studies and certain of their chemical and physical properties, we have proposed (Hamilton, 1968) that a common feature of the mode of action of these membrane-active antibacterial compounds is their initial adsorption on the sensitive membrane. This adsorption is a physicochemical event and non-specific in any biochemical sense. Although it seems certain that, once adsorbed, the action of organic solvents, quaternary ammonium salts, tyrocidine and polymyxin is in fact to destroy non-specifically the membrane's permeability barrier to small-molecular-weight substances (Hotchkiss, 1944; Newton, 1958; Salton, 1968), the salicylanilides, carbanilides and guanidines and other antibiotics listed above demonstrate a considerable degree of specificity in their action on the bacterial plasma membrane.

Many of these compounds have been found to act as uncouplers of oxidative phosphorylation in both bacterial and mitochondrial systems. In recent years therefore they have been studied extensively, but more from consideration of their effect on mitochondrial function than as antibacterial agents. The most striking result of these studies has been the finding that these synthetic antibacterial compounds and antibiotics can render the mitochondrial, bacterial and even lipid bilayer membranes specifically permeable to various monovalent cations. In particular, valinomycin at physiological pH values is almost absolute in its specificity toward K^+, whereas gramicidin increases membrane permeability to K^+ and Na^+, Rb^+, Cs^+ and H^+ (Mueller & Rubin, 1967). It has also been shown that the uncouplers dinitrophenol and carbonyl cyanide *m*-chlorophenylhydrazone render the membrane permeable to H^+ (Mitchell & Moyle, 1967). Whether these changes in ion permeability are the primary event in the action of these uncouplers is one of the crucial points in the argument between the chemical and chemiosmotic hypotheses of oxidative phosphorylation.

Our own work, and that of Harold and his coworkers in Denver, has been directed towards the study of the action of these compounds as agents affecting ion permeability and energy transformations in bacterial systems, and the attempt to explain their antibacterial properties in terms of these activities.

In their elegant studies with *Streptococcus faecalis*, which does not carry out oxidative phosphorylation, Harold & Baarda (1967) showed that the increases in ion permeability caused by valinomycin and gramicidin could explain the bacteriostatic action of these antibiotics. They also demonstrated the increased permeability to protons induced in this organism by tetrachlorosalicylanilide and carbonyl cyanide *m*-chlorophenylhydrazone (Harold & Baarda, 1968), and it was concluded that these compounds affected neither glycolysis nor ATP synthesis or even the utilization of ATP in macromolecular synthesis; only active transport processes were inhibited. Further to this, Pavlasovà & Harold (1969) have found that, whereas the ATP-dependent accumulation of β-galactosides in anaerobically grown *Escherichia coli* is sensitive to uncouplers such as tetrachlorosalicylanilide, the transport of α-methyl glucoside by the phosphotransferase system in which phosphoenolpyruvate is the energy donor (Simoni *et al.* 1967) is unaffected.

In our own work we have been examining the effects of tetrachlorosalicylanilide, trichlorocarbanilide, carbonyl cyanide *m*-chlorophenylhydrazone, valinomycin and gramicidin in three bacterial systems: (*a*) amino acid transport in *Staphylococcus aureus*; (*b*) ion permeability in whole cells and protoplasts of *Bacillus megaterium*; (*c*) phosphorylating activity in membrane vesicle preparations from *B. megaterium*.

We shall report on our most recent findings from these studies and discuss them in relation to the mechanism of action of membrane-active antibacterial compounds.

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