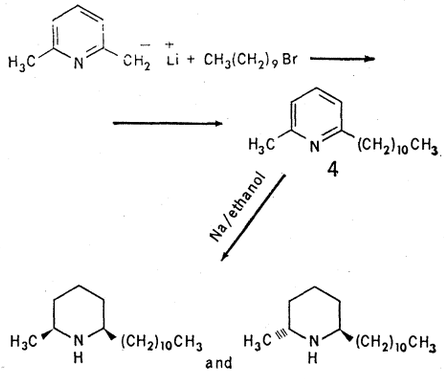
the six-membered ring structure (*10*). Also, the occurrence of the base peak at *m/e* 98 is taken in support of the piperidine structure (*11*). The structure arrived at, therefore, is an isomer of 2-methyl-6-*n*-undecylpiperidine.

2-Methyl-6-*n*-undecylpyridine (**4**) was synthesized by alkylation of the lithium salt of 2,6-lutidine with 1-bromodecane (*12*). The alkylated pyridine gave the following nuclear magnetic resonance spectrum ($CCl_4$, $\tau$) (*8*): 9.12 (distorted triplet, 3 H, terminal methyl group), 8.71 (singlet, 18 H, methylene groups), 7.55 (singlet, 3 H, methyl group on ring), 7.35 (triplet, $J = 7.2$ hz, 2 H, methylene on ring), 3.23 (doublet, 2 H, protons in positions 3 and 5 on ring), and 2.72 (triplet, $J = 8.0$ hz, 1 H, proton in position 4 on ring). This data is in full accord with the expected structure (**4**). High-resolution mass spectrometry established the formula as $C_{17}H_{29}N$ (found, *m/e* 247.2300; calculated, *m/e* 247.2299). Compound **4** was




then reduced with sodium metal in absolute ethanol (*13*). The identity of the major isomer was confirmed by comparison with the product of catalytic hydrogenation (presumably *cis*). The separation of the *cis* and *trans* isomers was achieved by chromatography over alumina, the *cis* eluting in hexane-ether mixtures, and the *trans* eluting only in ether containing 20 percent ethanol. Although the mass spectra of the two isomers were virtually indistinguishable, the infrared spectra allowed easy differentiation: the *cis* isomer exhibits relatively strong absorption near 7.6 $\mu$m, the *trans* absorbs only weakly in this region. Except for double-bond absorption showing in the spectrum of the venom (attributable to other components), the infrared spectra of the venom and the synthetic *trans*-2-methyl-6-*n*-undecylpiperidine are superimposable, as are the spectra of the corresponding *N*-acetates (C = O, about 6.1 $\mu$m). The amines are eluted with identical retention times from polymethylsiloxane: 3 percent OV-1 (180° C), and 3 percent SE-52 (180°C). The acetates were also indistinguishable on the SE-52 column (200°C). The mass spectra of solenopsin A and the synthetic *trans* compound are identical.

To our knowledge, alkylated piperidines have not been described from venoms of animal origin. The venoms of stinging ants are typically proteinaceous (*14*). 2-Methyl-6-alkylpiperidines occur in some plants, such as pinidine (*15*), cassine (*16*), and carpaine (*17*), but in each case the alkyl groups are *cis* to each other.

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20 February 1970

## Antagonism by DDT of the Effect of Valinomycin on a Synthetic Membrane

Abstract. *The potassium conductance which is induced by $10^{-6}$ molar valinomycin in a lecithin-decane membrane is reversed by $3 \times 10^{-6}$ molar DDT. Membranes not treated with valinomycin are not affected by DDT. This blockade of potassium conductance parallels the effect of DDT on axonic conduction. Dieldrin and lindane, whose physiological actions are in some ways like those of DDT, do not affect valinomycin-induced conductance of lecithin-decane membranes.*

The toxicity of DDT [2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane] is due to its excitatory effect on axons; it blocks the potassium flux associated with the falling phase of the action potential. It has been suggested that there is also an effect on the sodium flux associated with the rising phase (*1*). The prevailing view is that DDT accomplishes these effects by forming complexes in some way with the conducting membrane of the axon (*2*)—for example, perhaps by forming a charge transfer complex (*3*) with a hypothetical ion "gate."

Dieldrin and lindane are structurally quite unlike DDT, although all three are chlorinated hydrocarbons. Both dieldrin and lindane have excitatory effects on axons, and their toxicity is probably caused by such effects; but there are physiological differences in the relative rates of peripheral and central effects, in the form and sequence of excitatory axonic effects, and in temperature coefficients (*4*). There is evidence for complex formation between dieldrin and components of insect nerve (*5*).

We have been seeking ways to explore effects of DDT on membrane systems less complex than the whole axon. Valinomycin can render a synthetic lecithin membrane permeable to $K^+$ (*6*). We report here that DDT blocks this induced $K^+$ permeability, suggesting a parallel with its effect on the axon.

The apparatus was a modification of that used by Thompson (*7*) and del Castillo (*8*). Transmembrane resistance was determined by applying a square pulse (100 mv, 50 pulses per second), and by measuring the potential difference between each of two Ag-AgCl electrodes placed in each compartment. After amplification through

high-impedance operational amplifiers, both input and output signals were fed to a dual trace oscilloscope so that a direct comparison of signals could be made. The output trace showed the expected decay characteristics due to the capacitance of the system, and therefore both membrane resistance and capacitance could be determined simultaneously.

Lecithin was prepared by the method of Pangborn *et al.* (*9*) and chromatographed through Unisil. Lecithin (100 mg) was mixed with decane (100 mg), and the mixture was diluted with 0.25 ml of a solution of $CHCl_3$ and $CH_3OH$ (3:2). Membranes were made by brushing small amounts of this formulation over a hole (0.079 cm) drilled in a Plexiglas sheet separating the two compartments which contained a well-stirred buffer (*p*H 7.4) made up of equal volumes of 0.05*M* $KH_2PO_4$–$K_2HPO_4$ and 0.05*M* KCl. Because chloroform in the formulation might dissolve some of the Plexiglas and interfere with the experiments, both Teflon and Plexiglas sheets were tried, and Plexiglas was adopted. Both gave similar values for membrane resistance and capacitance. Advantages of Plexiglas were that the membranes bonded more easily to it and that, if the light beam were directed to one side of the membrane, an indirect lighting of the membrane was obtained. This latter effect showed islands of material and bubbles in the surface of imperfect membranes, defects which often were not apparent by reflected light alone. If sets of Fizeau fringes appeared superimposed on one another, a multilayer membrane had formed, since the chamfered hole was of finite thickness. Such a multilayer showed only a very slow or no response to valinomycin. About 1 out of 15 attempts produced a successful membrane which was bilayered and stable.

After the membrane had aged for 20 to 30 minutes, $10^{-6}M$ valinomycin was added to produce a desired increase in membrane conductance. Conductance increased rapidly over 30 seconds and reached a plateau in 5 to 15 minutes. Finally, $10^{-6}M$ quantities of a chlorinated hydrocarbon were added and the change in membrane conductance was observed. All compounds in methanol were added to each cell compartment to keep the concentration of the components equal on both sides of the membrane. Methanol at the concentrations used had no effect on a membrane.

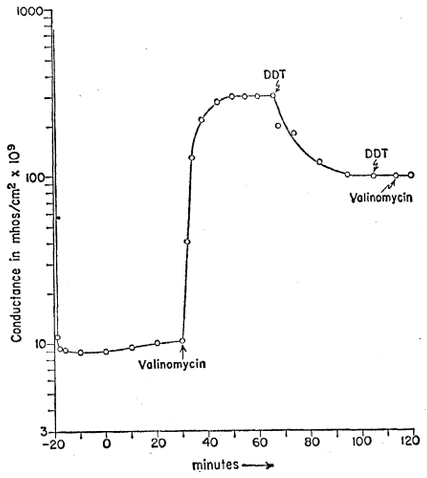


Fig. 1. The effect of $3 \times 10^{-6}M$ DDT after treatment of a membrane with $0.7 \times 10^{-6}M$ valinomycin.

An attempt was made to correlate change in membrane conductance with valinomycin concentration. Valinomycin was added in increments of 0.2, 0.4, up to 1.0 $\mu$g/ml (final concentration per cell side). This produced a stepwise curve as expected, but the change in conductance varied three- to sevenfold for each addition. Although a stepped curve could be produced from experiment to experiment, each curve differed from the next, so that the proper concentration of valinomycin to produce a desired membrane conductance could not be predicted easily. Time of addition of valinomycin to the membrane influenced the final membrane conductance considerably. For example, valinomycin added during the Fizeau fringe stage caused a much larger increase in conductance than if it were added after the membrane had aged. This may be due to the difficulty in incorporating valinomycin into the more ordered system of an aged membrane. The valinomycin effect was demonstrated in 89 experiments representing about 80 percent of the total number of cases in which true bilayers appeared to be formed. When $3 \times 10^{-6}M$ DDT was added to a membrane treated with valinomycine, the conductance decreased markedly (Fig. 1). The effect, though qualitative in that it varied three- to tenfold from experiment to experiment for a given concentration, was always in the direction of decreased membrane conductance. In 2 out of 42 experiments, further addition of DDT produced an additional slight decrease in conductance. Valinomycin, added after the decrease in conductance from DDT had become constant, showed no effect. By contrast, DDT added to an untreated membrane had no effect on conductance; when valinomycin was added to a membrane which had been treated with DDT only, there was an increase in conductance but the effect was much smaller and the response time was slower.

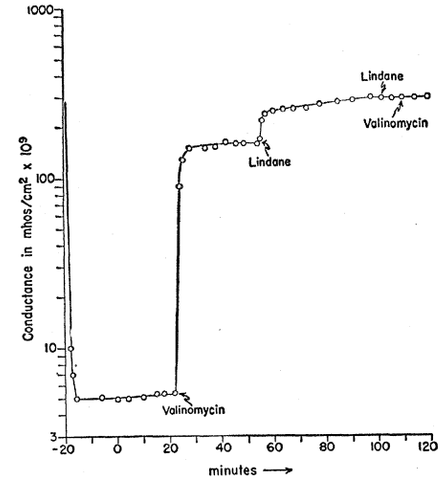


Fig. 2. The effect of $3 \times 10^{-6}M$ lindane added after treatment of a membrane with $0.7 \times 10^{-6}M$ valinomycin.

Dieldrin and lindane added at similar concentrations ($3 \times 10^{-6}M$) produced the opposite effect—that is, an increase in membrane conductance (Fig. 2). The change was not quite as marked as in the case of DDT in that it was only one- to twofold in magnitude and occurred only in 15 out of the 19 trials. Valinomycin added after the conductance had become constant produced no change (three out of four trials). As in the experiments with DDT, lindane and dieldrin added to untreated membranes had no effect and showed a much slower response and smaller increase in conductance with the subsequent addition of valinomycin in four out of five trials. Moreover, lindane or dieldrin, added after membrane conductance was decreased by the addition of DDT, caused no increase in conductance.

One can only speculate on the molecular basis of the action of DDT in these experiments. Valinomycin might act by forming a 4-Å hole in the membrane or, alternatively, by forming a $K^+$ complex which can migrate through the membrane (*6*). In either case, DDT may form a complex with the valinomycin in such a way as to prevent $K^+$ penetration or complex formation. Whatever the molecular basis, it seems well established that DDT antagonizes

the valinomycin-induced $K^+$ conductance in this model system. Is this phenomenon related to the effect of DDT on $K^+$ conductance in axons? The apparent parallel is attractive but will not be fully established until it can be shown that analogs of DDT which are inactive on axons are inactive on membranes treated with valinomycin. One would like also to reproduce the DDT effect on an experimental membrane derived from axonic material.

Dieldrin, lindane, and DDT are potent against an adenosine triphosphatase of nervous tissue commonly held to be associated with, and perhaps identical to, the energy-requiring "sodium-potassium pump" (*10*). Such an action is entirely distinct from the effects on ion "'gates." It appears unlikely that an effect on the pump could be the cause of neurotoxic actions of these compounds, because such an effect would cause depression and, ultimately, failure of axonic transmission [as occurs in poisoning of nerve by azide, dinitrophenol, or cyanide (*11*)], rather than the excitatory effect which is observed experimentally.

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## Mosaic Mutants: Absence in a Eucaryotic Organism

Abstract. *Exposure of procaryotic and eucaryotic cells to mutagenic agents generally gives both complete mutants and mosaic mutants. Irradiation of the eucaryotic multicellular alga* Ulva mutabilis *with ultraviolet light has given exclusively complete mutants.*

A cell in which a primary mutational event has taken place may give rise either to mutant cells only, or to both mutant and wild-type cells. In the former case, the mutation is said to be complete; in the latter it is referred to as a mosaic mutation. Exposure of procaryotic cells as well as eucaryotic cells to mutagenic agents usually gives both kinds of mutation. However, in a few experiments with *Escherichia coli* (*1*) the mutations seemed to be complete. In my experiments with a eucaryote no mosaic mutants were obtained.

The experimental object was the multicellular green alga *Ulva mutabilis* (*2*). Its life-cycle alternates between a haploid and a diploid phase, morphologically similar. The diploid sporophytes, developed from zygotes, form haploid zoospores by meiosis. The zoospores develop into haploid gametophytes which form haploid gametes through mitotic divisions. If the gametes are allowed to unite they form diploid zygotes; if not, the majority of them will develop parthenogenetically into haploid germlings which become diploid by chromosome doubling when they consist of more than 100 cells. The first cell division in a germling (gametophytic or sporophytic) occurs after 3 to 4 days of a development which starts when the reproductive units lose their motility.

The algae were grown in petri dishes with Erdschreiber medium (*3*) at 18°C at a diurnal cycle of 17 hours light and 7 hours dark. Zoospores—gametophytic germlings aged 0, 1, 2, 3, and 5 days respectively, swimming gametes, and 5-day-old germlings developed parthenogenetically from gametes, were irradiated with a 30 W Hanovia Chromatolite ultraviolet-lamp (*4*) and kept in the dark for 24 hours after exposure, to prevent photoreactivation. The plants were examined 15 to 20 days after irradiation, and specimens which differed morphologically from the wild type were isolated as presumptive mutants. In *Ulva* gametophytes may produce gametes and sporophytes may produce zoospores from almost the whole thallus. Progeny from any part of the thallus may thereby be examined. Only plants with mutant progeny or plants where parts of the thallus produced mutant progeny were finally considered to be mutants (Table 1). Mutants induced with ultraviolet irradiation were similar to spontaneous mutants collected during routine cultivation of the alga in the laboratory (*5*). All mutants, to date, show normal segregation.

Chimeras, which consist of two genetically different parts, developed among plants irradiated as 3- or 5-day-old germlings when the first cell divisions were observed in the cultures. These chimeras show that cells of different genotypes can express their own character phenotypically within the same plant. If mosaic mutations occur they could therefore result in chimeric plants and be detectable. However, chimeras were never detected among germlings irradiated at the single-cell stages (Table 1, *6*). Consequently, mosaic mutants do not occur or are extremely rare among mutants with a visible influence on the morphogenesis in *Ulva mutabilis*.

It is not known whether a eucaryotic chromosome is either structurally or functionally single- or multi-stranded, but even a single-stranded chromosome, as in the procaryotes, gives mosaic mutants, which is attributed to the

Table 1. Induction of mutants by ultraviolet irradiation in *Ulva mutabilis*. From random samples of approximately 150 plants it was estimated that 3-day-old zoospores have 1.34 cells per plant, 5-day-old zoospores have 4.88 cells per plant, and 5-day-old gametes have 4.27 cells per plant.

| Survival range (%) | Age (days) | Plants examined (No.) | Mutants (No.) | |
|---|---|---|---|---|
| | | | Whole thallus | Chimeras |
| | | *Zoospores* | | |
| Control | | 12588 | 3 | 0 |
| 80–90 | 0 | 31601 | 79 | 0 |
| 50–60 | 0 | 10453 | 43 | 0 |
| 10–20 | 0 | 2242 | 21 | 0 |
| 80–90 | 1 | 43773 | 66 | 0 |
| 80–90 | 2 | 58425 | 61 | 0 |
| 80–90 | 3 | 63272 | 49 | 14 |
| 80–90 | 5 | 56426 | 12* | 29 |
| | | *Gametes* | | |
| Control | | 34139 | 6 | 0 |
| 80–90 | 0 | 18490 | 32 | 0 |
| 50–60 | 0 | 9520 | 48 | 0 |
| 10–20 | 0 | 8749 | 89 | 0 |
| $<2$ | 0 | 1307 | 19 | 0 |
| 50–60 | 5 | 16382 | 4* | 13 |

* Expected, according to the control, to be spontaneous mutants.