DDT: A New Hypothesis of Its Mode of Action

Abstract. *It is suggested that DDT and perhaps other chlorinated hydrocarbon insecticides owe their activity to the formation of a charge-transfer complex with a component of the nerve axon, with consequent disturbance of function. Experimental evidence is provided for the formation of two complexes with components of cockroach nerve; the complexes have been partially purified. Their formation is accompanied by an absorption in the 245- to 270-millimicron range.*

Although DDT is the best known and most extensively used of the insecticides, its mechanism of action remains obscure. Because the symptoms of poisoning may include tremoring, convulsions, and paralysis (both in insects and vertebrates), the nervous system is the probable target, and indeed it is well known that the nervous system of DDT-poisoned insects is highly unstable, responding to a single stimulus with a train of high frequency impulses (*1*). In rats, it was shown recently that symptoms of poisoning are correlated with the concentration of DDT in the brain (*2*). Experiments on cockroaches with localized applications of DDT have shown that the sensory nerves are particularly sensitive (*3*). Intracellular recording techniques have shown that DDT prolongs the negative afterpotential, suggesting an interference with potassium permeability (*4*); it may be that this prolongation is the cause of the nervous instability. The question remains, what is the molecular basis for these effects upon nerve? It is of particular interest that, whereas the majority of agents affecting the nervous system have their effect upon the synapse, usually by an interference with a transmitter mechanism, DDT acts upon the axon itself, as do the veratrum alkaloids (*5*). In 1963, Allison (*6*) speculated about the possible participation of charge-transfer complexes in the action of a variety of pesticides, and referred us to the topic of these complexes reviewed by Szent-Györgyi (*7*). Recently, it occurred to us that the formation of such a charge-transfer complex in the case of DDT and perhaps other chlorinated hydrocarbon insecticides was compatible with two striking features of such compounds: (i) their high electron affinity, which is attested by the remarkable sensitivity of their detection in gas-chromatogram effluents by the electron-capture device, for example, 13 picograms for DDT and 0.1 picogram for lindane (*8*), and (ii) their extreme persistence in the environment, including soils, plants and animals, a property which has caused so much alarm recently. This persistence suggests that these compounds are generally of low biological reactivity and makes plausible the view that their potent effect upon nerves depends on a physical complex rather than a chemical reaction with some component.

It is known that membranes formed of alternate layers of two compounds, selected with appropriate electron affinity and ionization potential, respectively, such that they can be acceptor and donor in a charge-transfer complex, display the property of semiconductivity (*9*).

The above considerations suggested the hypothesis that DDT and related compounds act by forming a charge-transfer complex with a component of the axon, thus destabilizing the axon, perhaps by inducing localized semiconductivity. We have now obtained evidence in favor of such a hypothesis by showing that DDT does indeed form complexes with components of insect nerve and by an indication that charge-transfer is involved.

The first experiments consisted of equilibrating the whole or homogenized nerve cords of the cockroach, *Periplaneta americana* (L.), with various concentrations of C^{14}-labeled DDT and determining the quantity of DDT taken up from solution. A plot of uptake versus concentration showed two plateaus, suggesting sequential saturation of two components, whose dissociation constants (calculated from their half-saturation values) were calculated as 6.38×10^{-6} and $7 \times 10^{-7} M$.

In the second set of experiments, five cockroach nerve cords were homogenized in 1 ml of Ringer's solution and incubated with $10^{-5} M$ C^{14}-labeled DDT for 10 minutes at 25°C, after which they were fractionated. In addition to a Sephadex column and elution with 0.9 percent NaCl (Fig. 1), the bound DDT was eluted (along with a small fraction of organic matter) at about 10 ml elution volume; the bulk of the organic matter was eluted between 20 and 30 ml, and free C^{14}-labeled DDT could only be eluted by ethanol. The first radioactive fraction was then applied to a diethylaminoethyl cellulose column (Fig. 2), and two radioactive

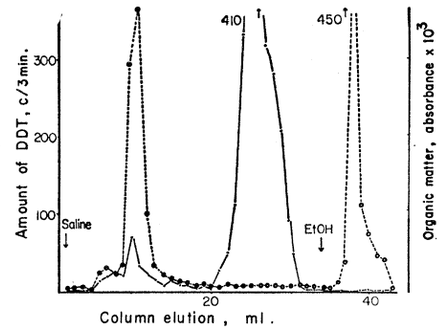

Fig. 1. Elution of bound and free C^{14}-labeled DDT (- - - -) and organic matter as measured at 280 mμ(——). The first peak represents bound DDT and the third peak, free DDT. (Amount of DDT shown as counts per 3 minutes; EtOH, addition of ethanol; organic matter scale as in Fig. 2.)

fractions were found, the first associated with little organic matter and the second associated with much.

These data show that a complex of DDT with components of cockroach nerve does occur. The only evidence so far that a charge-transfer complex may be formed is an observation that on incubation of $10^{-5} M$ DDT with whole nerve cord homogenates or the appropriate Sephadex fraction or the combined diethylaminoethyl cellulose fractions, a new shoulder of absorption was observed in the ultraviolet spectrum, in the 245- to 270-mμ range.

We have not yet attempted to show that formation of the above complexes is causally related to the symptoms of poisoning. If such a relation can be shown, the findings must be examined with respect to the views of Mullins (*10*), who suggested that toxic chlorinated hydrocarbons are those which fit precisely into a hypothetical intermolecular lattice; and of Gunther *et al.* (*11*), who have proposed that the symptoms

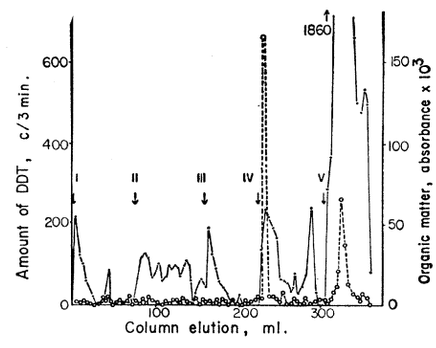

Fig. 2. Chromatographic separation of bound C^{14}-labeled DDT (- - - -) and of organic matter (——) on a diethylaminoethyl cellulose column. (Amount of DDT shown as counts per 3 minutes.)

of poisoning depend on the binding of DDT to a protein in nerve by Van der Waals' force.

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Endogenous Circadian Rhythm in Cytoplasm of Acetabularia: Influence of the Nucleus

Abstract. *It was shown by three different methods that in the unicellular and uninuclear green alga* Acetabularia *the nucleus is capable of determining the phase of the circadian rhythm of the oxygen balance in the cytoplasm.*

In the green alga, *Acetabularia*, there is a circadian rhythm of oxygen balance, and it has been demonstrated that this periodicity continues in the absence of an exogenous "Zeitgeber," or synchronizer (*1*, *2*). Even 40 days after removal of their nuclei, anucleate cells retained their rhythmic diurnal variations. These results seemed to indicate that the circadian rhythm under investigation was independent of the nucleus. On the basis of this evidence alone, however, involvement of the nucleus in the periodicity could not be definitely excluded.

In an attempt to solve this problem, we decided to combine the nucleus with cytoplasm in different phases, and thus to find out which of these two parts governs the rhythm under conditions of constant light.

The technique used for the determination of the oxygen balance was essentially the same as that described previously (*2*). The treatment of the plants and the experimental conditions were varied to achieve three types of experiments.

In experiment 1, the rhizoids were transplanted. At least 14 days before the experiments were started, plants of one culture were divided into two groups. One group was illuminated from 8 a.m. to 8 p.m. and the other from 8 p.m. to 8 a.m. At the beginning of each experiment, the rhizoids in the plants of one group were replaced by the rhizoids of plants of the other group (*3*). In addition, the tips of the acceptor stalks were amputated to remove the metabolically most active part of the plants (*4*). The transplantations resulted in combinations of rhizoids (containing the nuclei) and stalks with opposite phases of their periodicities. After transplantation, the plants were kept under constant conditions to avoid any exogenous zeitgeber. The oxygen balance was determined every 12 hours (at 8 a.m. and 8 p.m.), starting 3 days after transplantation.

In experiment 2 only the isolated nuclei were transferred (*5*). As in experiment 1, one group of plants was illuminated from 8 a.m. to 8 p.m., and the other from 8 p.m. to 8 a.m., for at least 14 days before implantation of the nuclei. The isolated nuclei were practically free of cytoplasm prior to implantation, so that cytoplasmic effects were presumably excluded.

In experiment 3, two different parts of the same plant were exposed to op-

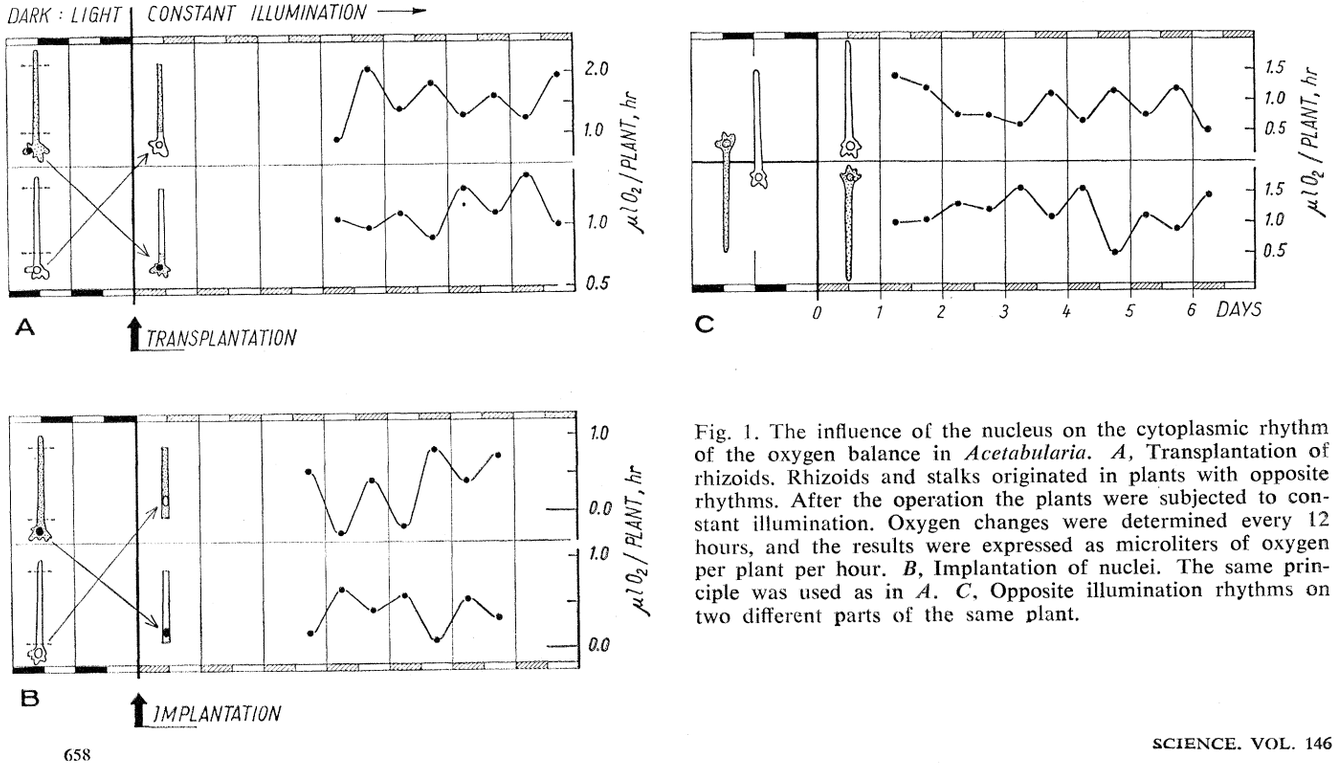

Fig. 1. The influence of the nucleus on the cytoplasmic rhythm of the oxygen balance in *Acetabularia*. *A*, Transplantation of rhizoids. Rhizoids and stalks originated in plants with opposite rhythms. After the operation the plants were subjected to constant illumination. Oxygen changes were determined every 12 hours, and the results were expressed as microliters of oxygen per plant per hour. *B*, Implantation of nuclei. The same principle was used as in *A*. *C*, Opposite illumination rhythms on two different parts of the same plant.