History) were examined. None is preserved, but we were able to infer that some had once been polyp-balls because of their small size and lack of epitheca. Some of the more mature specimens, larger in size and with epitheca, showed none of the irregularities seen in our own adult colonies. These probably did not bear polyp-balls. In other respects these corals were all very similar.

10. The corals were observed and collected while one of us (J.D.T.) was engaged in ecological studies on the atoll of Aldabra in the western Indian Ocean (46°24′E, 9°24′S). These investigations are part of the Royal Society Expedition to Aldabra. The specimens which were collected are now in the British Museum (Natural History). We thank J. W. Wells for commenting on the manuscript and G. R. Adams for the photographs.

5 June 1969 ■

Adenosine Triphosphatase Sensitive to DDT in Synapses of Rat Brain

Abstract. *The insecticide DDT selectively inhibits the action of a Na^+,K^+, Mg^{2+}-adenosine triphosphatase found in the nerve ending fraction of the rat brain. As judged by the concentrations of inhibitors that give 50 percent of enzyme inhibition, DDT was approximately 1000 times more toxic than its non-insecticidal analog, 1,1-dichloro-2,2-*bis(p-*chlorophenyl)ethylene. The degrees of inhibition of this enzyme system by various toxic and nontoxic DDT analogs were closely related to a general toxicity in vivo of these compounds. Moreover, the extents of inhibition of this enzyme system by DDT were much higher at low temperatures, an indication of a causal relation between poisoning in vivo by DDT and the inhibition in vitro of the Na^+,K^+,Mg^{2+}-adenosine triphosphatase system.*

The toxicity of DDT may result from its ability to disrupt the transport mechanisms of sodium and potassium ions in the nervous system (*1*). However, the biochemical mechanisms by which DDT causes such disruptions are unknown.

Chlordane and other chlorinated hydrocarbon insecticides partially inhibit the adenosine triphosphatases in rabbit brain (*2*), and also there is an adenosine triphosphatase that is particularly sensitive to DDT in the rat brain (*3*).

There are at least three different groups of adenosine triphosphatases existing in the nervous system: Na^+,K^+, Mg^{2+}-adenosine triphosphatases (*4*), which are particularly sensitive to ouabain; Mg^{2+},Ca^{2+}- or contractile adenosine triphosphatase, which is sensitive to Mersalyl (*5*); and other Mg^{2+}-adenosine triphosphatases (*4*, *6*). The former two enzymes may take part in the mechanisms of ion transport in the nervous system.

In view of the possibility that a causal relation might exist between the enzymatic system involving active ion transport mechanisms and the sites of DDT attack, we have expanded our earlier study (*3*) to partially characterize the DDT-sensitive adenosine triphosphatase and to study the effects of other DDT analogs on the enzyme system.

The enzyme source was the acetone powder preparation of the nerve ending fraction (*3*). The preparations were either suspended in 0.05*M* tris(hydroxymethyl)aminomethane - hydrochloride (tris-HCl) buffer (*p*H 7), or in buffer similar to the one developed by Skou (*7*) (*p*H 7.6), containing 0.25 mole/liter of sucrose, 0.03 mole/liter of imidazole, 1 mmole/liter of ethylenediaminetetraacetate, and 0.1 percent deoxycholic acid. The preparations were homogenized at 0°C for 3 minutes with a small Potter-Elvehjem homogenizer. The homogenate was centrifuged for 30 minutes at 20,000*g*. The precipitate was resuspended in the same medium to contain 0.1 to 0.2 mg/ml of protein. A portion (0.2 ml) of the enzyme preparation was added to 1.6 ml of a standard incubation mixture containing 0.1*M* NaCl, 20 m*M* KCl, and 6 m*M* $MgCl_2$ in 0.03*M* tris-HCl buffer (*p*H 7.6) (*8*) or in 1.6 ml of a standard assay mixture at *p*H 7 as described (*3*). In all cases the system was first incubated at 24°C with Mersalyl (10^{-6} mole/liter, Sigma) for 10 minutes (added with 18 μl of distilled-deionized water). The DDT and its analogs (*9*) were added to the system with 18 μl of ethanol (95 percent); the system was maintained for an additional 10 minutes. To assay the adenosine triphosphatase activity, 1 μmole of adenosine triphosphate (ATP) (disodium salt) was added with 0.2 ml of tris-HCl buffer, and the system was maintained for 30 minutes, normally at 24°C. The total scale of assay procedure for inorganic phosphorus was 15 ml (*10*). The enzyme activity (24°C) of average preparations was in the order of 15 nmole per milligram of protein per minute of ATP hydrolyzed at *p*H 7.6. In all cases, an identical set of tests was made with the same enzyme sources, except that they were first incubated with $10^{-4}M$ ouabain (Nutritional Biochemicals). This portion of the enzyme activity was subtracted from the total activity to estimate the Na^+,K^+,Mg^{2+}-adenosine triphosphatase activity. On average, 37 percent of the total activity of these enzyme preparations was due to the adenosine triphosphatases sensitive to ouabain at *p*H 7.6 and 24°C.

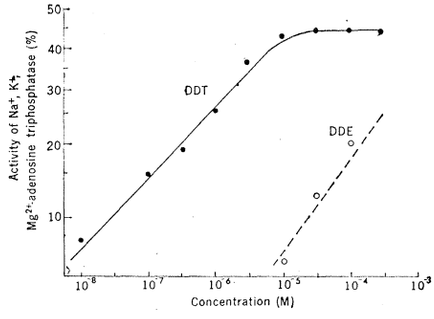

Fig. 1. Relation between the concentration of DDT and DDE and the degrees of inhibition of Na^+,K^+,Mg^{2+}-adenosine triphosphatases. Degrees of inhibition of the enzyme was plotted in probability units to give a linear relation against log-concentration of inhibitors (*16*).

To establish the general identity of the adenosine triphosphatase sensitive to DDT, (i) the Mg^{2+},Ca^{2+}-adenosine triphosphatase was isolated from the rat brain homogenates at *p*H 7 by the method of Puszkin *et al.* (*5*), and (ii) the Na^+,K^+,Mg^{2+}-adenosine triphosphatases of the acetone powder preparation (*3*) from the nerve ending fraction of the rat brain were partially activated by the enzyme solution and the assay buffer mixture of Skou at *p*H 7.6 in the presence of $10^{-6}M$ Mersalyl (*5*). The Mg^{2+},Ca^{2+}-adenosine triphosphatase was more sensitive toward DDE than to DDT (inhibition of $10^{-5}M$ DDT and DDE was 33.7 percent and 47.1 percent, respectively). It cannot, therefore, be the DDT-specific, target enzyme. This was in agreement with the previous observation that $2.5 \times 10^{-4}M$ Mersalyl mainly inhibited Mg^{2+},Ca^{2+}-adenosine triphosphatase so that the remaining enzyme became more sensitive to DDT than to DDE (*3*).

When the Na^+,K^+,Mg^{2+}-adenosine triphosphatase was tested against DDT and DDE (Fig. 1) it became evident that a portion (approximately 50 percent) of this enzyme system was particularly sensitive to DDT but not to DDE. Since the DDT-inhibition curve showed a sharp break at 50 percent, it appeared likely that there were more than one Na^+,K^+,Mg^{2+}-adenosine tri-

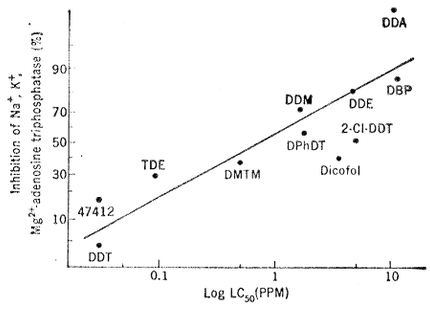

Fig. 2. Relation between the toxicities in vivo of DDT analogs (*9*) toward mosquito larvae (*Aedes aegypti*) and the amount of the Na^+,K^+,Mg^{2+}-adenosine triphosphatase activity remaining after inhibition by $10^{-5}M$ of these analogs [expressed in percent activity of the enzyme remaining in probability units (*16*)]. The toxicities are expressed in terms of insecticide concentration which gives 50 percent of mortality (LC_{50}) in the mosquito populations as tested by the World Health Organization standard method. The LC_{50} value for DDA could not be determined because of its low toxicity to mosquito larvae.

phosphatase in this preparation and that one of them was particularly sensitive to DDT. The inhibitor concentrations that resulted in 50 percent inhibition (I_{50}) of Na^+,K^+,Mg^{2+}-adenosine triphosphatase was for DDT approximately $3 \times 10^{-7}M$ as opposed to $3 \times 10^{-4}M$ for DDE.

The same enzyme source was also tested against DDT and DDE in the K^+,Mg^{2+} buffer at *p*H 7 (*3*). The inhibition curves obtained for K^+,Mg^{2+}-adenosine triphosphatases were almost identical to those in Fig. 1.

To study the relation between the actual toxicities in vivo and the extent of adenosine triphosphatase inhibition, several DDT analogs (final concentration, $10^{-5}M$) were incubated with the same enzyme preparation. The results (Fig. 2) indicated that the degrees of inhibition of the Na^+,K^+,Mg^{2+}-adenosine triphosphatases were closely related to the actual toxicities in vivo against mosquito larvae of those DDT analogs.

The insecticide DDT is much more toxic at lower temperature (negative temperature correlation). Experiments, at 37°C, indicated that the activity of Na^+,K^+,Mg^{2+}-adenosine triphosphatase in relation to the total adenosine triphosphatase activity (mainly Mg^{2+}-adenosine triphosphatases) was much less at 37°C (less than 10 percent). Accurate assessment of toxicity of DDT to this small portion of Na^+,K^+,Mg^{2+}-adenosine triphosphatase activity was, therefore, difficult. The I_{50} value appeared to be, however, higher than $10^{-5}M$. When the incubation temperature was lowered to 13°C, the activity of the Na^+,K^+,Mg^{2+}-adeonosine triphosphatase increased to 50 percent of the total adenosine triphosphatase activity and the I_{50} value toward DDT decreased to approximately $5 \times 10^{-8}M$. Moreover at this temperature, 100 percent of the Na^+,K^+,Mg^{2+}-adenosine triphosphatases were sensitive to DDT.

Electrophysiological studies (*11*) indicate that the minimum concentration for DDT to elicit the neurophysiological symptoms of DDT poisoning in the isolated abdominal nerve cord (central nervous system) of the German cockroach at 24°C is 1 to $3 \times 10^{-6}M$. Eaton and Sternburg (*12*) showed that when 1 μg (threshold or sublethal amount) of DDT was injected into a male American cockroach only a portion (25 percent) of given DDT (approximately $10^{-6}M$) reaches to the nerve cord itself, and the remainder stays with the lipid sheath. Our I_{50} value, therefore, is at least in the same order as the actual threshold concentration of DDT in the central nervous systems of the German and American cockroach.

Another piece of evidence supporting the view that this enzyme system is involved in the actual poisoning processes of DDT is the fact that the same enzyme preparation contains a component which specifically binds with C^{14}-DDT (*13*). The concentration of specific DDT-binding substances is also in the same magnitude at $3 \times 10^{-7}M$ (*13, 14*).

The Na^+,K^+,Mg^{2+}-adenosine triphosphatases are known to be present in many biological membranes and the evidence of their contribution to the process of active transport of Na^+ and K^+ is accumulating (*4*). Although the synapses are not known to be the most sensitive site for DDT attack (sensory endings are), they take part in the process of DDT poisoning (*15*) and contain significant amounts of Na^+,K^+, Mg^{2+}-adenosine triphosphatases. Therefore, the involvement of an adenosine triphosphatase or an adenosine triphosphate-utilizing system, or both, in DDT poisoning is a likely possibility.

FUMIO MATSUMURA
K. C. PATIL

Department of Entomology,
University of Wisconsin,
Madison 53706

References and Notes

1. T. Yamasaki and T. Narahashi, *Botyu-Kaguku* **22**, 296 (1957); W. E. Dale, T. B. Gaines, W. J. Haves, G. W. Pearce, *Science* **142**, 1474 (196→ T. Narahashi and H. G. Haas, *ibid.* **157**, 1438 (1967).
→ R. B. Koch, *J. Neurochem.* **16**, 269 (1969).
3. F. Matsumura, T. A. Bratkowski, K. C. Patil, *Bull. Environ. Contam. Toxicol.*, in press.
4. J. C. Skou, *Physiol. Rev.* **45**, 597 (1965).
5. S. Puszkin, S. Berl, E. Puszkin, D. D. Clarke, *Science* **161**, 179 (1968).
6. O. Gonda, A. Kaluszymer, Y. Avi-Dor, *Biochem. J.* **73**, 583 (1959).
7. J. C. Skou, *Biochim. Biophys. Acta* **58**, 314 (1962).
8. ———, *Biochem. Biophys. Res. Comm.* **10**, 79 (1963).
9. Abbreviations are: DDT, 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane; and DDE, 1,1-dichloro - 2,2 - *bis*(*p* - chlorophenyl)ethylene. Chemical names of uncommon DDT analogs are: DDM, *bis*(*p*-chlorophenyl)methane; DPhDT, 1,1 - diphenyl - 2,2,2 - trichloroethane; DBP, *p*-chlorophenyl-*p*-chlorobenzophenone; CP-47412, 1,1-*bis*(*p*-chlorophenyl)-2,3-dichlorocyclopropane; DMTM, 1,1-*bis*(*p*-methoxyphenyl)-2,2,2-trimethylethane; 1-Cl-DDT, 1,1-*bis*(*p*-chlorophenyl)-1-chloro-2,2,2-trichloroethane; DDA, 1,1-*bis*(*p*-chlorophenyl) acetic acid; TDE, 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethane; and dicofol, 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethanol.
→ B. B. Marsh, *Biochim. Biophys. Acta* **32**, 357 (1959).
11. C. M. Wang and F. Matsumura, unpublished data.
12. J. L. Eaton and J. G. Sternburg, *J. Econ. Entomol.* **60**, 1699 (1967).
13. H. Brunnert and F. Matsumura, *Biochem. J.*, in press.
→ F. Matsumura and R. D. O'Brien, *J. Agr. Food Chem.* **14**, 36 (1966).
15. J. L. Eaton and J. G. Sternburg. *J. Econ. Entomol.* **60**, 1358 (1967)
16. C. I. Bliss, *Science* **79**, 38 (1935).
17. Supported in part by a PHS research grant CC-00252 from the National Communicable Disease Center, Atlanta, Ga. We thank Dr. D. J. Hennessy for some of the analogs of DDT.

28 May 1969

Phosphonomycin, a New Antibiotic Produced by Strains of Streptomyces

Abstract. *Phosphonomycin is a newly discovered antibiotic produced by streptomycetes. It is effective, when administered by the oral route, to mice infected with Gram-positive or Gram-negative microorganisms. The antibiotic is bactericidal and inhibits cell-wall synthesis.*

A new antibiotic, phosphonomycin, was discovered during a search for organisms producing broad-spectrum antibiotics. We report here some of the biological properties of the agent.

Phosphonomycin is produced in aerated, submerged cultures of strains of *Streptomyces fradiae* (ATCC 21096), *Streptomyces viridochromogenes* (ATCC 21240), and *Streptomyces wedmorensis* (ATCC 21239) grown on a variety of media. One medium which produced activity and was employed for the isolation studies had the following composition: oatmeal, 20 g; tomato paste, 20 g; and water to 1 liter. Phosphonomycin was differentiated from