The importance of this discovery lies in the fact that copper-arsenic alloy ornaments are very unusual. The trace-element content shows that copper sulf-arsenate ores must have been deliberately used for the easier manufacture of the ornaments. These ores were not in general use in the Middle East and even today no copper sulf-arsenate ore bodies are described in the geological literature other than those in Armenia (*5*). If the raw material for these ornaments did indeed come from Armenia, it must have traveled a supply route which existed in Chalcolithic times.

C. A. KEY

Geochemistry Division, Geological Survey of Israel, Jerusalem

References and Note

1. A. Lucas, *Ancient Egyptian Materials and Industries* (Arnold, London, ed. 3, 1962).
2. R. F. Tylecote, *Metallurgy in Archeology* (Arnold, London, 1963).
3. P. Bar-Adon, *Archeology* **16**, No. 4, 251 (1963).
4. R. J. Forbes, *Metallurgy in Antiquity* (Brill, Leiden, 1959).
5. G. O. Grigoryan, *Geochemistry USSR English trans. No. 4* (1962), p. 388.
6. Published by permission of the director, Geological Survey of Israel.

21 October 1964

DDT Antagonism to Dieldrin Storage in Adipose Tissue of Rats

Abstract. *Storage of dieldrin in the adipose tissue of female rats was markedly depressed when DDT and dieldrin were fed simultaneously. The amount of dieldrin present in the tissues of rats fed 1 and 10 parts of dieldrin per million was significantly reduced by the addition of 5 ppm DDT to the feed. The addition of 50 ppm DDT to the feed caused a 15-fold reduction in the amount of dieldrin stored in rats fed 1 ppm dieldrin, and a 6-fold reduction in rats fed 10 ppm dieldrin. This antagonistic effect of DDT suggests that the criteria used in predicting the pharmacological effects of combined residues of related insecticides need some revision.*

Members of the chlorinated hydrocarbon group of insecticides tend to accumulate in the tissue lipids of animals. When ingested orally or absorbed through the dermis, such compounds are retained for extended periods with only a slow rate of dissipation. Both DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane] and its metabolite DDE [1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethylene] are present in the tissue lipids of people in the general population of the United States (*1*). More recently, dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,-5,6,7,8,8a-octahydro-1,4-*endo,exo*-5,8-dimethanonaphthalene) and benzene hexachloride (1,2,3,4,5,6-hexachlorocyclohexane) were found in the body fat of United States residents (*2*).

Some factors that affect the accumulation rates of such compounds in the body fat of animals have been studied, but with only one compound being tested at a time. This report concerns the amount of dieldrin stored in adipose tissue of rats after they were fed DDT and dieldrin simultaneously. Dieldrin storage was markedly depressed under the experimental conditions.

Seventy-two female rats weighing 100 g each were fed a basal diet of Purina laboratory chow (*3*). Recrystallized dieldrin was added at a concentration of 1 part per million or 10 ppm and recrystallized DDT at a concentration of 5 or 50 ppm. Cottonseed oil was incorporated into the diets at the rate of 1 percent to facilitate uniform dispersal of the insecticides. To the control diets, only 1 ppm or 10 ppm dieldrin was added. Each diet was fed to 12 rats. The rats were individually housed and fed for 10 weeks.

Methoxychlor [1,1,1-trichloro-2,2-bis-(*p*-methoxyphenyl)-ethane] was added to the diets of many individuals as a third variable. The methoxychlor did not affect the dieldrin storage pattern, however, and it is therefore not considered in this report.

All rats survived the feeding period. They appeared normal, and no significant differences in weight gains or food consumption were observed among them. A sample of adipose tissue was taken from the abdomen of each rat at the time of killing. This tissue was analyzed for lipid content and insecticide concentrations.

For anaylsis, 0.5 g of each tissue sample was ground in a mortar together with sodium sulfate, transferred to 100-ml centrifuge bottles and extracted five times with *n*-hexane. The five extracts were combined and concentrated to a 50-ml volume. A 1-percent portion was removed for the estimation of lipid by the dichromate oxidation procedure of Bragdon (*4*). The remaining extract was chromatographed on deactivated Florisil to separate the dieldrin present from the DDT and DDE (*5*). The dieldrin fraction was analyzed by gas chromatography with electron capture detection, a 5 percent Dow 11-Chromosorb W column operated at 180°C being used.

Table 1. Effect of DDT in the diet on the accumulation of dieldrin in adipose tissue of rats. Results are expressed as the mean values for groups of 12 rats ± standard error.

DDT added to diet (ppm)	Dieldrin found in tissue lipid (ppm)	
	1 ppm fed	10 ppm fed
0	15.1 ± 0.94	67.5 ± 2.7
5	5.4 ± 0.46	43.9 ± 2.4
50	1.0 ± 0.06	11.2 ± 0.62

The accuracy of the analytical procedure was checked by the following observations on representative samples. Duplicate analyses agreed closely. The standard deviation of the differences between duplicate samples was 0.5 percent of the mean dieldrin value (17.5 ppm) for a group of low dieldrin samples, and 7.4 percent of the mean dieldrin value (66 ppm) for a high dieldrin group. When dieldrin was added to tissue samples prior to analysis, recovery averaged 103 percent. When DDT was added prior to analysis of tissues containing dieldrin, no effect on dieldrin values was noted.

The concentrations of dieldrin found in the extractable lipids of the samples of adipose tissue are listed in Table 1 together with the various amounts of DDT administered.

Both concentrations of DDT in the diet markedly reduced the amount of dieldrin stored in the tissue. The incorporation of 50 ppm DDT in the diet caused a 15-fold reduction in the amount of dieldrin stored in rats fed 1 ppm dieldrin, and a 6-fold reduction in rats fed 10 ppm dieldrin. The DDT appeared to antagonize directly the storage of dieldrin. Both DDT and DDE accumulated additively in the adipose tissue according to the dosage of DDT. Data on this and other phases of the experiment will be published in detail elsewhere.

No research of this type has been reported previously, although pairs of chlorinated hydrocarbon insecticides have been tested for evidence of possible combined effects in insects. Storrs and Burchfield studied the nature of the joint action of several pairs of

chlorinated hydrocarbon insecticides in an assay method with mosquito larvae (*6*). They found no instances in which the joint action could be classified as either antagonistic or synergistic. Aldrin, which is chemically related to dieldrin, was reported to act independently of DDT. Turner, however, showed that dieldrin plus DDT resulted in increased toxicity toward adult milkweed bugs and found evidence of "interaction" (*7*). The pairing of other compounds was also reported to give enhanced toxicity. The results reported in this paper might appear to contradict the studies of toxicity in insects because the amount of dieldrin stored was reduced, not enhanced, by the presence of DDT. The reduced accumulation of dieldrin in the tissues, however, might mean that greater quantities were circulating and available for specific toxic action. In that case, the different reports might be reconcilable. It remains to be determined whether the toxic effects of dieldrin are enhanced by the presence of DDT in mammals, as was reported by Turner (*7*) for an insect species.

Alternatively, the effect of DDT on dieldrin storage may be related to the rat's ability to transform dieldrin into readily excreted hydrophilic products (*8*). Such a mechanism would be of potential significance in dealing with hazards arising from the toxicity of dieldrin.

Clearly, data obtained by studying single compounds in mammals and pairs of compounds in insects may not be indicative of the behavior of mixtures of compounds in mammals or their pharmacological effects.

JOSEPH C. STREET

Department of Animal Husbandry, Utah State University, Logan

References and Notes

1. W. J. Hayes, Jr., G. E. Quinby, K. C. Walker, J. W. Elliott, W. M. Upholt, *A.M.A. Arch. Ind. Health* **18**, 398 (1958).
2. W. E. Dale and G. E. Quinby, *Science* **142**, 593 (1963).
3. Rats were obtained from the Holtzman Co., Madison, Wis.
4. J. H. Bragdon, *J. Biol. Chem.* **190**, 513 (1951). All lipid values were based on stearic acid calibration data.
5. W. A. Moats, *J. Assoc. Offic. Agr. Chemists* **46**, 172 (1963).
6. E. E. Storrs and H. D. Burchfield, *Contrib. Boyce Thompson Inst.* **18**, 69 (1954).
7. N. Turner, *Conn. Agr. Expt. Sta. New Haven Bull. No. 594* (1954).
8. F. Korte, G. Ludwig, J. Vogel, *Ann. Chem.* **656**, 135 (1962).

Supported in part by USDA regional research funds, project W-45, and by USPHS research grant EF-00543. I thank Adrian Blau for his contribution.

October 1964

Sequential Gene Action in the Establishment of Lysogeny

Abstract. *A study of temperature sensitive clear-plaque-forming mutants of bacteriophage P22 demonstrates that the* c_1 *and* c_2 *loci must function in a temporal sequence in the establishment of lysogeny in* Salmonella typhimurium. *The* c_1 *locus acts for only a 4-minute interval between the 7th to 11th minute of the infection. The* c_2 *locus begins to function some minutes later, and its continued activity is necessary for perpetuation of the lysogenic state.*

The establishment of the lysogenic condition on infection of *Salmonella typhimurium* strain LT2 with bacteriophage P22 requires two sequential repressions of phage DNA synthesis. These conclusions derive from studies on the rates of DNA synthesis as measured by incorporation of H^3-thymidine into acid-insoluble material during 1-minute pulses in complexes of bacterial cells infected with the wild-type, c^+ phage and its clear-plaque-forming mutants, c_1 and c_2 (*1*). Under appropriate conditions, infections with c^+ phage result in almost 100 percent lysogenic responses; in contrast, infections with either c_1 or c_2 produce 100 percent lytic responses. These mutants complement to give high frequencies of lysogeny (*2*). At 37°C, phage-associated DNA synthesis (*1*) begins rather early in c^+ infected cells and continues until the 6th minute of the infection, when a sharp repression in the overall rate of synthesis sets in. This early repression persists until the 16th minute, at which time host-specific DNA synthesis is released from inhibition. The rate of synthesis then increases until it reaches the same rate of increase and parallels the rate of synthesis in uninfected control cells at about 45 minutes. At this time the infected cells begin to divide and produce lysogenic progeny.

Striking alterations in this pattern of DNA synthesis are seen on infection with either of the two clear-plaque-forming mutants. Cells infected with c_1 mutants do not exhibit the repression of synthesis at 6 minutes. The rate continues to increase until about 25 minutes have elapsed, when the cells start to lyse and liberate phage. Cells infected with mutant c_2 do exhibit the early inhibition, but a sharp rise in the rate of DNA synthesis occurs at 16 minutes which greatly exceeds control rates; the synthesis reaches a peak at 50 minutes, when cell lysis begins. These mutants complement one another in mixed infection, and the pattern of DNA synthesis is similar to that of c^+ infected complexes. The functions of the c_1 and c_2 loci are to control phage DNA synthesis in the establishment of lysogeny. The c_1 locus represses phage DNA synthesis at the 6th minute of the infection, and the c_2 locus maintains the repressed state from the 16th minute onward as cellular replication resumes. We now report studies with temperature-sensitive c_1 and c_2 mutants which confirm and extend the conclusions reached from the studies on the rates of DNA synthesis. By shifting infected cells between low (31°C) and high (42°C) temperature it is possible to pinpoint the time of onset and duration of action of these loci in the establishment of lysogeny.

A lysate of phage c^+, produced in the presence of 100 γ of bromodeoxyuridine per milliliter of M-9 medium supplemented with casamino acid (*1*), was used as the source for selection of temperature-sensitive, clear-plaque-forming mutants. These mutants were detected by plating large amounts of treated phage on nutrient agar and incubating at 42°C. Phage from clear plaques was isolated and tested by plating for each mutant at both 31° and 42°C. Most of these isolates formed clear plaques at both temperatures and

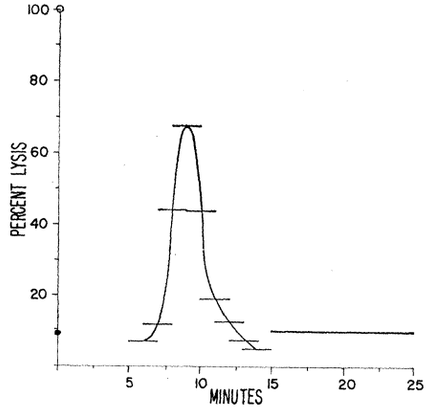

Fig. 1. The effect of 2-minute heat pulses at various times after the onset of the infection on the lysogenic responses of cells infected with temperature-sensitive c_1 mutants of bacteriophage P 22. ●, Frequency of lysis by control infections at 31°C; ○, frequency of lysis by control infections at 42°C; short dash, frequencies of lysis after a heat-pulse at indicated intervals; long dash, frequency of lysis after a high-temperature treatment from the 15th to the the 25th minute.