CORRELATION BETWEEN THE POSSESSION OF A CHITINOUS CUTICLE AND SENSITIVITY TO DDT *

A. GLENN RICHARDS AND LAURENCE K. CUTKOMP [1]

Zoology Department, University of Pennsylvania; Marine Biological Laboratory, and Division of Entomology, University of Minnesota

Introduction

The remarkable insecticidal qualities of 2, 2-bis (p-chlorophenyl) 1, 1, 1, trichloroethane, more commonly called "dichloro-diphenyl-trichloroethane" or just "DDT," were discovered by Paul Müller of the J. R. Geigy Company of Switzerland a little more than five years ago (Lauger, Martin, and Müller, 1944). The first samples reached this country in August, 1942. Researches in Switzerland, Germany, England, and the United States, accelerated by war-time urgency, have developed a voluminous amount of data. Most of these data are purely practical and will not be reviewed here; [2] further, these economic reports and papers are appearing so rapidly that it is very difficult to separate fact from fancy in the confusing maze of seeming contradictions. Certainly numerous popular articles and stories are dangerously misleading (see, e.g., warning by Cox, 1945, and Anonymous, 1945). The fact remains, however, that DDT is a remarkably potent insecticide and that from the human point of view it can be used with relative safety for certain insect pests (flies, mosquitoes, lice, bedbugs, etc.).

Since this new compound is so extremely toxic to many insects, one is naturally led to inquire what its effects are on other animals. Both a desire to find more favorable animals for studying the physiological effects of DDT, and a need to evaluate the effects of the compound on the entire biota of natural waters, led to determinations of the toxicity of DDT to representatives of various animal phyla. During the course of making these determinations it was noted that animals with exoskeletons, especially chitinous exoskeletons, were notably susceptible. Experiments were accordingly performed to test the validity of this correlation. From these experiments it was concluded that the correlation is valid. A hypothesis is developed in this paper that chitinous cuticles selectively concentrate DDT from the bathing media by adsorption and result in a higher concentration of the toxin inside the animal. There is good evidence to indicate that the relation of the chitinous cuticle to the selective action of DDT is no more than that of a concentrating mechanism. The actual lethal action of DDT seems to be another problem (see, e.g., Yeager and Munson, 1945).

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[1] Present address of both authors: Division of Entomology and Economic Zoology, University Farm, St. Paul 8, Minn.

[2] Large numbers of papers can be found, especially in the February, 1944 and June, 1945 issues of the *Journal of Economic Entomology*. The review in *Soap* is a useful digest (Anonymous, 1945).

The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Pennsylvania, and, later, the University of Minnesota. Most of the data were incorporated in a paper presented at the meetings of the Entomological Society of America in New York City, December 14, 1944.

Materials and Methods

For the purpose of this study only aquatic species or aquatic stages were employed. The toxin was applied as what seems to be a collodial suspension in the bathing medium because the solubility of DDT in water is very low, at room temperatures too low to show significant toxicity to susceptible animals. Mechanically shaking DDT in distilled water (68 hours), filtering, and then centrifuging with an angle centrifuge to remove minute particles (15,000 G for one hour),[3] gave a solution that slowly killed about 50 per cent of the mosquito larvae tested at 15° C. but had no significant effect on larvae at 28° C. Several slightly different tests gave similar results. Comparing these data with data from bio-assay of known dilutions of collodial suspensions prepared from acetone solutions, we concluded that the solubility of DDT in water probably lies between 0.2 and 1.0 parts per billion when prepared by agitation in water.[4] Presumably suspensions from acetone solutions would give much higher solubility values, perhaps one hundred times as high (Lewis and Randall, 1923). The exact physical state, then, of the preparations we are calling "suspensions" is uncertain but at least the preparations are sufficiently homogeneous to dilute satisfactorily.

The suspensions were prepared by adding a known volume of an acetone solution of DDT beneath the surface of a known volume of water (distilled, fresh, or sea water). A suspension of one part per million that will give reproducible results and dilute satisfactorily can be prepared by adding one ml. of a 0.1 per cent DDT solution beneath the surface of a liter of water. Stronger suspensions were usually made from more concentrated DDT solutions. Suspensions as strong as one part per hundred thousand are not stable and do not dilute satisfactorily.

Suspensions of one part per million in sea water diluted with distilled water and tested by bio-assay with mosquito larvae indicated that the suspensions in sea water have essentially the same characteristics as those in fresh and distilled water.

Since all suspensions contained a small amount of acetone, at least two controls were always used. One was a control in the water indicated; the other, the same plus whatever was the highest per cent of acetone in any suspension. In general, most animals were indifferent to 0.1 per cent acetone. The few cases where 0.1 per cent acetone seemed to have a possibly deleterious effect (e.g., certain Coelenterata), tests were repeated with lower acetone concentrations. The more dilute suspensions, prepared by dilution of a one part per million suspension containing 0.1 per cent acetone, had only negligible amounts of acetone.

[3] This is a force sufficient to sediment bacteriophage particles of approximately 20 mμ diameter but should not affect molecules several times larger than the molecule of DDT.

[4] This is very much lower than the one part per million figure given by Neal *et al.* (1944). Our bio-assay data, as well as similar data from other laboratories, are not consistent with this high estimate.

The amount of fluid used varied with the size of the animals. Protozoa and certain other forms were tested in watch glasses containing 10 ml. of fluid. Other forms were tested in shell vials or finger bowls containing 25–200 ml. of fluid. Of course, single series and replicates were with uniform volumes. Continuously flowing suspensions were not feasible; the more difficultly cultured marine animals were changed to fresh suspensions twice daily. For species that did not require frequent changing of the medium, subsequent assays with mosquito larvae or hermit crabs were commonly employed to verify the continued toxicity of test fluids.

Specimens were obtained from various sources. Protozoa were from laboratory cultures and were identified by Dr. D. H. Wenrich. Marine forms were collected at Woods Hole, mostly by the Supply Department, and the names used are those current at that laboratory. Other forms were collected or purchased, and identified by the authors.

Chemical tests for the presence of chitin were routinely performed on exoskeletons using the methods given by Campbell (1929). Positive results were obtained with Arthropods and the perisarc of Colenterates. The Bryozoan used (*Bugula*) gave atypical results suggesting a skeleton composed of something similar to, but not, chitin.[5]

For adsorption tests cuticle was removed from Horseshoe Crabs (*Limulus polyphemus*), manually cleaned of cells, rinsed thoroughly with distilled water, and dried. Part of this material was treated with 5 per cent NaOH at 100° C. until the color was removed and the pieces were negative to protein tests. This latter material is referred to herein as "chitin" although tests showed that a small percentage of the material had been changed to chitosan (see Campbell, 1929). Smaller amounts of cuticle were prepared from cockroaches and used in a few experiments, but most of the adsorption work was done with the more available cuticle of *Limulus*.

All tests were performed in replicate. Many of them were repeated.

Results

The results obtained are summarized in the accompanying table. In all cases the toxin (DDT) was applied as a colloidal suspension in the bathing medium. The resistances or susceptibilities shown refer only to the indicated concentration in the surrounding medium. The figures are not comparable to median lethal doses and should not be interpreted as implying that "resistant" animals would be resistant to the injection of solutions or emulsions.

[5] The cuticle of *Bugula* is variously referred to in the literature as "chitinous" or "material akin to chitin." We applied the various chemical tests used by Campbell (1929) and others. The cuticle is not dissolved in hot concentrated KOH. After prolonged heating (which changed control pieces of known chitin to chitosan completely) it only crinkled in 3 per cent acetic acid instead of dissolving. The addition of one per cent H_2SO_4 gave no change and no precipitate. Another piece treated with I + KI in water turned brown but only very slowly. After the addition of one per cent H_2SO_4 it remained brown for several minutes and then after 5–10 minutes slowly changed to a *greenish-brown* (instead of the clear violet given by chitosan). It dissolved immediately in 75 per cent H_2SO_4 but after standing no crystals resembling chitosan sulfate crystals were found. These tests suggest a similarity to chitin but certainly, at least in this species, the bryozoan cuticle is not true chitin.

TABLE I

The effective toxic concentrations of DDT for various animal phyla

All tests were replicated at least once. Susceptible species were tested repeatedly. Except where indicated otherwise tests were continued for 7 days or longer. The fluid on species that do not survive well in stagnant water was renewed daily or more frequently.

Group	Species	Temperature, C.	Type of water	Concentrations of DDT suspension					
				1:1,000,000,000	1:100,000,000	1:10,000,000	1:1,000,000	1:100,000	1:10,000
Protozoa	*Ameba proteus*	room	fresh	—	—	—	No effect (14 da)	Slow effect?	Slowly killed (3–10 da.)
	Paramecium sp.	room	fresh	—	—	No effect	No effect	No effect (1 mo.)	—
	Stylonychia putrina	room	fresh	—	—	—	No effect	—	No effect (1 mo.)
	Euglena gracilis	room	fresh	—	—	—	No effect	—	No effect (1 mo.)
	Chilomonas sp.	room	fresh	—	—	—	No effect	—	—
	Peranema sp.	room	fresh	—	—	—	No effect	—	No effect (1 mo.)
Coelenterata	*Obelia* sp.[1]	15°	sea	—	Many killed	Killed (1–2 da.)	Killed (<1 da.)	—	—
	Campanularia sp.[1]	15°	sea						
	Tubularia sp.	15°	sea	—	No effect	Affected slightly [2]	Affected [2]	—	—
	Pennaria sp.	15°	sea	—	No effect	Killed slowly	Killed	Killed	—
	Hydractinia sp.	15°	sea	—	No effect	No effect	No effect	Affected [3]	—
	Hydra fusca	15°	fresh	—	No effect	No effect	No effect	Affected [3]	—
	Astrangia sp.	15°	sea	—	—	No effect	No effect	Affected [3]	—
Platyhelminthes	*Phagocata* sp.	room	fresh	—	—	No effect (1 mo.)	Killed (7–40 da.)	Killed (7–20 da.)	Killed (1–6 da.)
Nematoda	*Anguillula aceti*	room	fresh	—	No effect	No effect	No effect	No effect	No effect (1 mo.)
	Ascaris lumbricoides	35°	alkaline saline	—	—	—	No effect	No effect	No effect
Rotifera	*Philodina* sp.	room	fresh	—	No effect	No effect	No effect	No effect (1 mo.)	No effect
	Diplax sp.	room	fresh	—	—	—	No effect	No effect (1 mo.)	No effect
	Monostyla sp.	room	fresh	—	—	—	Some killed slowly (1–2 mo.)	Killed slowly (10–30 da.)	Killed slowly (7–10 da.)
Gastrotricha	*Chaetonotus* sp.[4]	room	fresh	—	—	No effect	No effect	—	—
Bryozoa	*Bugula* sp.	15°	sea	—	No effect (10 da.)	Killed slowly (4–6 da.)	Killed slowly (3–4 da.)	—	—

[1] At least two, probably three, species of *Obelia* and *Campanularia* were used in these tests but identification of species in this group is quite difficult.

[2] *Tubularia* stems selected by Dr. L. G. Barth were set-up as in regeneration studies. All specimens regenerated but polyps quickly died in stronger concentrations and almost without exception did not regenerate a second time. Controls regenerated repeatedly.

[3] At 1:100,000 the specimens of *Hydra*, *Hydractinia*, and *Astrangia* were rounded and slow in reacting at the end of six days but were still living.

[4] These gastrotrichs were not tested separately. They were present in one of the rotifer cultures, and data cover only those test dishes, in which they were found.

TABLE I—*Continued*

Group	Species	Temperature, C.	Type of water	Concentrations of DDT suspension					
				1:1,000,000,000	1:100,000,000	1:10,000,000	1:1,000,000	1:100,000	1:10,000
Mollusca	*Littorina* sp.	room	sea	——	No effect	No effect	No effect	No effect	Killed slowly (6–7 da.)
	Mytilus sp.[5]	15°	sea	——	——	——	No effect	——	——
	Helisoma trivolva	25°	fresh	——	——	——	——	No effect	No effect
	Physa sp.	25°	fresh	——	——	——	——	No effect	Killed slowly
Echinodermata	*Thyone* sp.	room	sea	——	——	No effect	No effect	No effect	No effect
	Asterias sp.	room	sea	——	——	No effect	No effect	Killed (4 da.)	Killed (2 da.)
Annelida	*Aeolosoma* sp.	room	fresh	——	——	No effect	No effect?	Effect? [6]	——
	Polydora sp.[5]	15°	sea	——	——	——	——	No effect (7 da.)	——
	Nereis virens	15°	sea	——	No effect	No effect	No effect	Killed (4 da.)	Killed (2–3 da.)
	Lumbricus terrestris	15°	fresh [7]	——	——	——	——	No effect	No effect
Crustacea	*Artemia salina*	15°	sea	Most killed 2–6 da.	Killed (2–6 da.)	Killed (1–5 da.)	Killed (1–5 da.)	Killed (2–5 da.)	Killed (1–5 da.)
	Gammarus sp.	15°	fresh	Most killed (1–6 da.)	Killed (1–3 da.)	Killed (1–3 da.)	——	——	——
		28°	fresh	<half-killed (6 da.)	Killed (1–3 da.)	Killed (1–2 da.)	Killed (1 da.)	Killed (<1 da.)	Killed (<1 da.)
	Daphnia sp.	15°	fresh	Effect? [8]	Most killed (2–7 da.)	Killed (1–5 da.)	——	——	——
	Cyclops sp.	room	fresh	——	No effect	No effect?	Killed (<3 da.)	Killed (<3 da.)	Killed (<3 da.)
	Pagurus sp.	15°	sea	——	——	Killed (1–3 da.)	Killed (1 da.)	——	——
	Emerita talpoida	15°	sea	——	——	Effect?	Killed (1–2 da.)	Killed (1–2 da.)	——
Insecta	*Aedes aegypti* larvae	15°	fresh	Half killed (7 da.)	Killed (2–7 da.)	Killed (2–4 da.)	Killed (2–4 da.)	——	——
		28°	fresh	No effect (7 da.)	Most killed (7 da.)	Killed (1–4 da.)	Killed (1–2 da.)	——	——
	Culex pipiens larvae	15°	fresh	——	——	Killed (<1 da.)	——	——	——
		30°	fresh	——	——	Killed (1–3 da.)	——	——	——
	Chironomus sp. larvae	room	fresh	——	——	Killed (1–2 da.)	——	——	——
	Chaoborus sp. larvae [9]	10°	fresh	Some killed (5 da.)	Killed (5 da.)	Killed	——	——	——
		30°	fresh	No effect (5 da.)	Most killed (5 da.)	Killed	——	——	——
Fungi	*Rhizopus nigricans*	room	fresh	——	——	——	——	——	No effect
	Penicillium sp.	room	fresh	——	——	——	——	——	No effect
	Saccharomyces cervisiae	room	fresh	——	——	——	No effect	No effect	——

[5] Only a few specimens (not a statistically significant number) were tested of *Mytilus* and *Polydora*. The negative results suggest that these are both resistant species, as are other members of their phyla.

[6] At 1:100,000 there was an initial decrease in numbers followed by an increase. It would seem that some are killed but that the survivors reproduce readily.

[7] The earthworms were tested in flat dishes with the fluid not sufficiently deep to cover the animals completely.

[8] Anderson (1945) records an effect on *Daphnia* at concentrations greater than one part per billion and no effect at concentrations lower than one part per billion. Apparently his cultures were slightly more susceptible to DDT than ours.

[9] Experiments on *Chaoborus* by Dr. Hsing Yun Fan.

Of the species tested, the Protozoa, Nematoda, Gastrotricha, Rotifera, Mollusca, Annelida, and Echinodermata were resistant to DDT suspensions or in the case of a few species, were slowly killed by very concentrated suspensions. The single species of the Platyhelminthes tested was comparatively resistant. The single species of Bryozoa was quite susceptible. And, the various species of Arthropoda and those Coelenterata with a complete perisarc were highly susceptible. The most interesting data come from the hydrozoan Coelenterata where those species with a complete chitinous perisarc (*Obelia* and *Campanularia*) are highly susceptible, those species with a chitinous perisarc over only the main stalk (*Tubularia* and *Pennaria*) were less susceptible, and those without a perisarc (*Hydra* and *Hydractinia*) were nearly resistant. The bryozoan, *Bugula,* is an intermediate which is almost as susceptible to DDT as the arthropods and sensitive colenterates. *Bugula* has a complete cuticle which is composed of some substance seemingly similar to but not identical with chitin.[5] Correlated with this similarity is the more or less similar relationship to DDT poisoning from dilute solutions. The most reasonable assumption at present is that the cuticle of *Bugula* behaves towards DDT similarly to chitinous cuticles but that it is less effective (or possibly the animals are really more resistant).

The data presented herein do not include vertebrates. The most nearly comparable data on fishes indicate an intermediate sensitivity (Eide, Deonier, and Burrell, 1945; Ginsburg, 1945) and that concentrations entirely adequate for mosquito control (i.e., well above the minimum lethal concentration) do not injure fish (Metcalf *et al.,* 1945). With terrestrial vertebrates truly comparable data are not available. The closest parallel is with aerosols and mists; very high concentrations of DDT applied in this manner cause little or no injury to mammals (Neal *et al.,* 1944). DDT in solution in oil can be absorbed through mammalian skin (Draize *et al.,* 1944) but the dosage needed to produce an effect is fairly high. Also, it is well known that DDT has been approved for use as a powder on vertebrates to kill ectoparasitic insects. The data, then, suggest that for external applications more or less comparable to our data, fish occupy an intermediate position in susceptibility—being some ten to one hundred times more resistant than mosquito larvae. Mammals seem to be much more resistant to external applications than either fish or insects.

The obvious correlation of the above data is to the chitinous exoskeleton. Actually there are a number of possibilities, notably (1) that some of the animal groups are truly more or even completely resistant to the toxin; (2) that certain animal groups may be able to detoxify or excrete DDT better than others; (3) that there may be a reaction between a chitinous cuticle and DDT to produce some other more toxic substance; or (4) that the susceptibility may be due solely to penetration and accumulation which is favored by a chitinous cuticle. The evidence at hand suggests that both the first and last named possibilities are partly correct. Considering the points in order:

Data derived from the feeding and injection of DDT solutions (not suspensions) and emulsions show that on a dosage/weight basis mammals have approximately the same order of susceptibility as cockroaches (Draize, *et al.,* 1944; Smith and Stohlman, 1945; Chadwick, 1945). While some variability is obtained among insects (Chadwick, 1945), the fact remains that internal applications do not

show the tremendous differences between vertebrates and insects that external applications show.

However, there must be a considerable amount of variability in true resistance among different groups on invertebrates. *Ascaris* is immune to external applications of DDT suspensions but the median lethal dose for injection of emulsions (on a dosage/weight basis) seems at least several times as high as the MLD for cockroaches.[6] And, injection of DDT emulsions into the common aquarium snail, *Helisoma trivolva,* was apparently without effect even when one mg. of DDT was injected into a snail whose weight (without the shell) was less than a gram. Unfortunately, we have no precise knowledge of the site of injection but at least it was into the interior of the animal and sometimes into the tissues. It seems clear from these data (1) that there must be considerable difference among various animals as to the effect of a given amount of DDT after it is *in* the animal, and (2) that relative absorbability also varies.

The second possibility, detoxification or excretion by certain groups, seems unlikely both because of the similarity of vertebrate and insectan median lethal doses on injection and because of the great dissimilitary in excretory organs. The role of detoxification may actually be considerable. One non-toxic breakdown product, dichlorodiphenylacetic acid, has already been positively identified in mammalian urine (White and Sweeney, 1945). Detoxification, however, does not seem to contain the full answer to the wide variations in susceptibility reported in this paper.

The third possibility, a new and more potent toxin arising from a reaction with chitinous cuticles, seems eliminated by several points. The above mentioned similarity of median lethal doses and symptoms following injection of emulsions is against it. Also pertinent is the fact that DDT suspensions have no visible effect on cultured tissues whether chitin particles are added to the medium or not (Lewis and Richards, 1945).

The above considerations led to two different types of tests on the fourth possibility; namely, that cuticles might facilitate the entry of DDT into animals. If chitinous cuticles facilitate the entry of DDT it might be possible to find aquatic invertebrates which are immune to external applications but killed by injections. We have data on one such case. *Acaris* does not have a chitinous cuticle and is completely resistant to immersion in the strongest DDT suspensions. It is killed by the injection of emulsions although requiring a somewhat higher dose than cockroaches and vertebrates.

While these data on *Ascaris* are suggestive, they, of course, do not prove anything about chitinous cuticles. More direct data were sought by trying to find a possible mechanism by means of which chitinous cuticles might facilitate the entry of DDT into an arthropod or coelenterate. The following data suggest that such a mechanism does, indeed, exist and that adsorption by chitin plays an important role in it.

Obviously, DDT can penetrate insect cuticles since external applications can kill even when the possibility of ingestion is eliminated. Tests showed that DDT could be adsorbed from water by activated charcoal. DDT suspensions in water

[6] We determined the median lethal dose for *Ascaris* only to a rough order of magnitude and so prefer not to cite a definite quantitative figure.

lost all toxicity to mosquito larvae after being stirred with charcoal and filtered. It follows from this that the DDT particles have been removed by the charcoal. Similarly, we found that DDT could be adsorbed from aqueous suspensions by arthropod cuticle and even better by purified chitin. The results are only qualitative; accordingly, tabular data are not presented. Cuticle and purified chitin from *Limulus* and from cockroaches were prepared as described in the section on methods. An excess of either material was added to a moderately dilute suspension of DDT in distilled water, equilibrated at 6° C., and filtered. The filtrate had its expected toxicity to mosquito larvae greatly reduced or abolished. From this bio-assay it follows that the cuticle or chitin had removed part or all of the DDT. Cuticle or chitin was then added to a more concentrated suspension, equilibrated at 6° C., filtered, and the pieces of cuticle or chitin were soaked twice in cold distilled water and filtered to wash off any DDT that might be present in the water on the surface or within the matrix of the pieces. The lots of cuticle and chitin were then soaked in hot distilled water (90° C.) adjusted to pH 3.8 with 0.1 molar H_2SO_4, filtered, and the filtrate tested by the usual bio-assay method with mosquito larvae. Symptoms and mortality showed that DDT had been recovered. Since DDT could be removed and recovered by these adsorption methods it follows that cuticle and purified chitin are capable of adsorbing DDT.

In addition to the controls accompanying the above adsorption tests, a number of other things were tested. As judged by subsequent bio-assay a small amount of DDT was adsorbed by cleaned dry spines of sea urchins, but none was adsorbed by snail shells, coral, a suspension of erythrocytes (cow), or chunks of muscle (*Limulus*).

Another point which is consistent with the idea that adsorption by chitin facilitates the entry of DDT, is that mosquito eggs are resistant to any concentration of DDT tested, whereas, the larvae on hatching are susceptible. This might represent differences in embryonic stages or a barrier between the egg shell and the enclosed embryo or larva but it could conceivably be due to the fact that larvae have a chitinous cuticle, whereas, the insect egg shell contains no chitin.

If adsorption phenomena are playing an important role, one would expect to find a negative temperature coefficient, i.e., that DDT is more toxic at lower temperatures than at higher temperatures. Solubility tests show that DDT has a positive temperature coefficient for solubility in both polar and apolar solvents. Accordingly, a negative temperature coefficient is good evidence in support of an adsorption hypothesis. Actually the temperature relationships are complex and not fully understood. Data obtained to date show that at low concentrations a negative temperature coefficient is, indeed, obtained but that at higher concentrations this changes to a positive temperature coefficient. However, it seems logical that a concentrating mechanism would be most dominant at lower concentrations. Accordingly, the temperature data support the hypothesis that adsorption of DDT by chitinous cuticles acts as a concentrating mechanism. The shift to a positive temperature coefficient at higher concentrations would seem to imply that other factors are involved and that the adsorption by chitinous cuticles is only for concentrating the toxin, the actual toxic action of DDT being something else.

Most of the temperature studies have been performed using mosquito larvae at 15° and 28° C., but similar results have been obtained with the fresh water shrimp

Gammarus.[7] Lindquist *et al.*, (1945) have independently found a negative temperature coefficient in spray work with adult flies, and during the past year it has come to be generally considered that DDT works better in insect control operations in cool climates than in hot climates. Attempts to obtain data with coelenterates at high and low temperatures failed because we were not successful in keeping the species available at Woods Hole alive in warm, non-running water.

Discussion

From the data presented here, we conclude that the sensitivity of certain animal groups to DDT applied externally as a colloidal suspension in the bathing medium is correlated with the presence of a chitinous or similar cuticle in addition to true differences in tissue susceptibility. This correlation is supported by the similarity of toxicity to certain animals when emulsions are injected in contrast to dissimilarity from external applications, and by direct demonstration of adsorption of DDT by chitin and chitinous cuticles, and non-adsorption by certain other types of exoskeletons and tissues. The idea that adsorption processes are important is further supported by the demonstration of a negative temperature coefficient for toxicity at lower concentrations. The data seem to concern only the differences in sensitivity and not to explain the toxic action itself. The exceptions recorded herein (e.g., snails) show that the action of DDT is more complex but they do not invalidate the cuticle relationship. Accordingly, we propose as a hypothesis that chitinous cuticles act to selectively concentrate DDT from the surrounding medium by adsorption processes.

This hypothesis is in some respects similar to the old idea of penetration due to partition coefficients. Several authors have invoked the idea of partition coefficients to explain insecticide penetration (Wigglesworth, 1941; Richards and Weygandt, 1945). The present hypothesis for concentrating DDT varies in substituting an active adsorbing interface of chitinous cuticle. On theoretical grounds Hurst (1943) has suggested that the insect cuticle may adsorb toxins, as work by Castle (1936) had already indicated. The data in the present paper, however, give the first direct demonstration that a chitinous cuticle can adsorb an insecticide and that the adsorption may play a role in selective toxicity to different groups of animals.

Since one is inclined to think of the arthropodan cuticle as a solid sheet of material, it is natural to ask where sufficient surface is available for the demonstrated adsorption. A possible clue is given by Castle's (1936) work on the "oriented imbedding" of organic substances in chitin. This is a kind of adsorption process that apparently utilizes the surfaces of known intermicellar spaces. Perhaps the same intermicellar surfaces may be available for the adsorption of DDT.

Obviously, adsorption by the cuticle can be only part of the story of the action of DDT. Some mechanism is needed to transport the DDT from the cuticle to its effective locus inside the animal. We have no direct evidence as to what this mechanism is. Perhaps one can interpret the data from distribution of the radio-

[7] More recently Dr. Hsing Yun Fan has made an intensive study of the negative temperature coefficient using both mosquito larvae and midge larvae (*Chaoborus* sp.). His data will be published subsequently.

active bromine analog (Hansen, Hansen, and Craig, 1944) to mean that DDT is more soluble inside insects than in water. Such a difference in solubility in connection with an actively adsorbing cuticle could account for a selective accumulation in insects and other groups with similar cuticles.

A careful consideration of reported exceptions is necessary. Many papers have been published on the use of DDT as an insecticide.[2] These papers, while showing a general susceptibility of insects to DDT, are mostly not comparable to the present paper. They are not primarily concerned with the resistance or susceptibility of the various species but with the practicability of controlling pest insects under natural conditions. A susceptible insect that is not reached by the insecticide in the field will not be killed. Also the thickness, dryness, nature of the epicuticle (external layer outside of chitinous cuticle), and other properties of the cuticle vary greatly from one group to another within the arthropods (Richards, 1944). One should expect variation in susceptibility with these variables. In some cases it seems clear that forms which are not satisfactorily controlled are nevertheless susceptible. For instance ticks and mites are said not to be well controlled by DDT (Cox, 1945) yet they are by no means resistant (e.g., Gouck and Smith, 1944; Smith and Gouck, 1944; Vargas and Iris, 1944, etc.), and at least one species (*Rhipicephalus sanguineus*) is readily controlled by DDT. In some other cases, notably certain beetles with thick, hard, dry cuticles, insects seem little or not at all affected. Since these are terrestrial species, the difficulty in obtaining data comparable to data presented in this paper is considerable. In the Crustacea there is a considerable range of variability; most species tested were highly susceptible but the copepods are much less susceptible (see also Anderson, 1945; Seagren, Smith, and Young, 1945). It seems certain that more and less resistant species do occur among arthropods even though available data refer principally to practical field tests. While it is possible to offer hypothetical explanations of these variations on the basis of differences in cuticle structure, variations in degree of effect seem to be a factor also (Chadwick, 1945). However, the known susceptibility of arthopods in general is so broad that we feel inclined to suggest that the variations will be explained without vitiating the hypothesis advanced in the present paper.

The fungi with chitinous cell walls (*Rhizopus,* etc.) are a true exception. They are apparently indifferent to DDT in the medium bathing them. This resistance cannot be evaluated until we are more certain as to the method by which DDT kills. One obvious possibility lies in the differences between plants and animals, particularly, the absence of a nervous system in plants.

Another line of exceptions concern the intermediate susceptibility of fishes (Eide, Deonier, and Burrell, 1945; Ginsburg, 1945, and others). No explanation of this is offered, but there is no reason to assume that intermediation of a chitinous cuticle is the only means by which DDT can enter an aquatic animal.

The most serious exceptions, however, are those invertebrates which are not affected by the injection of emulsions (snails). These are species representative of a group unaffected by external applications. No explanation of this exceptional lack of effect on injection is offered in the present paper, but obviously the snails demonstrate beyond question that more is involved in the whole story of DDT action than just the facilitation of entrance by an appropriate cuticle.

Summary

1. Throughout the animal phyla there is a correlation between the presence of a chitinous cuticle and susceptibility to external applications of DDT. Those aquatic animals with a chitinous cuticle (arthropods and certain coelenterates) are highly sensitive to external applications of DDT, other animals are not so susceptible although there is considerable variability.

2. The correlation to a chitinous cuticle is supported by studies on various coelenterates, on adsorption, and on temperature coefficients. Coelenterata with respectively a complete, partial, and no chitinous perisarc are respectively highly sensitive, somewhat sensitive, and nearly insensitive to DDT. DDT can be adsorbed by chitin and chitinous cuticles and at low concentrations shows a negative temperature coefficient for toxicity to arthropods.

3. From these data a hypothesis is proposed that chitinous cuticles facilitate the entry of DDT into the animal body by selectively concentrating the compound by adsorption phenomena.

4. This is the first demonstration that an insecticide can be adsorbed by chitinous cuticles and the first direct evidence that such adsorption actually can play a role in insecticide action.

5. The present paper does not consider the nature of the toxicity of DDT to protoplasm. The data given here refer only to the selective action of DDT as a function of penetration facilitated by chitinous exoskeletons. The shift to a positive temperature coefficient at higher concentrations, the lack of effect from injecting DDT emulsions into snails, and the variability in median lethal doses for injected DDT emulsions in different animals, all indicate that the selective adsorption of DDT by chitinous cuticles is only a part of the story of the toxic action of this compound.

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