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BIOCHEMISTRY AND PHYSIOLOGY OF RESISTANCE BY INSECTS TO INSECTICIDES.

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PRIOR to the reports of the development of resistance by houseflies and mosquitoes to D.D.T. in 1947, resistance had generally been considered a minor entomological problem affecting only a few agricultural pests. Indeed, there were many entomologists who did not believe the early reports of resistance and attributed the insecticide failures to poor application, adulterated insecticide, the weather, or ignorance on the part of the operator. Today, resistance is almost universally considered the most serious problem confronting the entomologist interested in the control of agricultural pests and arthropod vectors of disease.

Dr. George Decker (1958) quotes S. A. Forbes as saying "The struggle between man and insects began long before the dawn of civilization, We commonly think of ourselves as the lords and conquerors of nature, but insects had thoroughly mastered the world and taken full possession of it long before man began the attempt. we can scarcely flatter ourselves that we have gained any very important advantage over them. and since the world began we have never yet exterminated — and we probably never shall exterminate — so much as a single insect species." Many entomologists will undoubtedly disagree with the attitude expressed but the present state of our knowledge of how to control insects and related species, certainly indicates the need for serious thinking on the problem. It seems obvious that relaxation of our efforts even for a short time may have far-reaching consequences.

At the present time, there are only three genera of insects of public health importance in which resistance has not been found, *Simulium*, *Phlebotomus*, and *Glossina*. Resistance has appeared and has been authenticated to all the synthetic and natural insecticides now in widespread use. To date, the only apparent sure way to prevent the development of resistance is to stop the use of insecticides. This obviously is not a satisfactory solution. The literature on resistance is now most voluminous. However, most of the publications pertain to reports of new species becoming resistant, determining the build-up of resistant species, and to

ascertaining the efficacy of substitute insecticides in controlling species resistant to those materials in previous use. Only a small fraction of the publications deal with the biochemistry, physiology, ecology or genetics of resistance. Much genetical data relating to resistance that was obtained with *Drosophila melanogaster* Meig. has been used as the basis for conclusions on the inheritance of resistance in general. Whether or not such conclusions are always valid remains to be determined. The resistance developed by the housefly, *Musca domestica* L. to D.D.T. has been spectacular and widespread and for these reasons the housefly has been the insect of choice by many investigators who are attempting to elucidate the fundamental aspects of the resistance problem. D.D.T. has been the insecticide most frequently used, not only because of its importance but because it can be readily obtained in pure form and microanalytical methods are available both for D.D.T. and some of its common metabolic products.

It is the purpose of this paper to briefly summarize the present state of knowledge of the physiological and biochemical aspects of arthropod resistance to insecticides. For more extensive coverage, the reader is referred to the reviews by Brown (1958), Metcalf (1955), Winteringham and Barnes (1955), Hoskins and Gordon (1956), Crow (1957), Busvine (1957) or Lhoste (1955).

Much of the published evidence continues to be somewhat contradictory in nature. However, many of the differences are probably more apparent than real and are attributable to results indicating normal strain variation rather than being directly related to resistance. To date, none of the differences reported between the susceptible and resistant strains have universal applicability to all strains tested nor do any of the reported differences seem to offer a solution to the serious problems of how to control insecticide resistant arthropods once resistance is acquired, or how to control insects with insecticides and insure that resistance will not develop.

The physiology of resistance to the insecticides in general use prior to World War II is not discussed at this time. For a summary of the situation up to 1951, the review by Babers and Pratt (1951) is suggested.

Drug tolerance in humans has been known for many years. It has one aspect in common with arthropod resistance to insecticides — neither process is at all understood — but there most of the similarity ends. In man, the individual to whom the drug is administered is the one that develops tolerance but in arthropods it is the progeny of those that are treated that exhibit increased resistance.

Lindquist and Wilson (1948) were apparently the first to develop, from a normal laboratory colony, insects resistant to the post World War II insecticides. Since their report, many colonies, resistant to many insecticides and mixtures of insecticides, have been established. The general procedure has been to treat a large susceptible population with such a dosage of insecticide that about 90 per cent mortality will result. Eggs are obtained from the survivors and the treatment is repeated for succeeding generations. For the chlorinated hydrocarbons such as D.D.T., appreciable resistance is usually evident in about ten generations. Increase is then rapid and after about 25 - 30 generations, the colony may be virtually immune. With the pyrethrins and organophosphorus insecticides, resistance develops more slowly and generally to a lesser extent.

When the normal colony is treated with lower doses, a somewhat different effect is obtained. Hoffman *et al.* (1951) treated resistant and susceptible flies with sub-lethal doses of D.D.T. six times in three days. These flies were then more susceptible than unexposed insects. Hadaway (1956) obtained similar results with gamma isomer hexachlorocyclohexane (gamma-B.H.C.) on houseflies as did Beard (1952) working with D.D.T., nicotine, or pyrethrum treated *Galleria mellonella*. Harrison (1952) failed to produce a resistant strain of houseflies by treating susceptible insects with sub-lethal doses of D.D.T. but Eddy *et al.* (1955) produced highly resistant body lice, *Pediculus humanus corporis*, by treatment of a susceptible colony with D.D.T. that at first produced only about 5 per cent mortality. However, Cole *et al.* (1957) obtained only two-fold resistance in a louse colony after 26 generations exposure to a D.D.T. dosage that produced no mortality.

According to Brown (1958) insecticides do not produce mutations. Luers (1953) found no increase in the mutation rate of D.D.T.-treated *Drosophila*. It is doubtful, however, whether valid conclusions on the genetics of the whole resistance problem can be drawn from work with *Drosophila*. If one accepts that mutations may be caused by such chemicals as the nitrogen mustards, then the possibility must certainly exist that certain insecticides under favourable conditions may induce mutations. Crow (1957) concludes that "all the evidence still supports the original view: Insecticide resistance is an example of evolutionary change, the insecticide acting as a powerful selective sieve for concentrating resistant mutants that were present in low frequencies in the original population".

Reports have frequently been made of strains of insects being from a few to several thousand times as resistant as normal strains. However, extreme care must be used in attempting to correlate or evaluate such figures with results from other laboratories since the results vary greatly with the method used to determine the degree of resistance. Busvine (1951) for example, found that when D.D.T. in acetone was applied topically to adult houseflies, an LD50 of 36 gamma per fly was obtained but, when a non-volatile mineral oil was used as a solvent, the LD50 was only 7.2 gamma/fly. When exposures are made to increasingly heavy residual deposits of an insecticide, one must question the effect of the layers of insecticide below the exposed surface. Considerable variation also occurs when different generations are tested under identical situations. If comparisons then are to be made, rigid adherence to standardized conditions must be the rule and conclusions should be drawn only after careful consideration of all facets of the data.

Many papers have been published in which attempts were made to establish morphological or biological differences between susceptible and resistant strains. Darker pigmentation, stiffer tarsal bristles, wider tarsal segments, thicker cuticle, and intersegmental membranes of the pulvilli, smaller diameter pulvilli, and differences in the ratio of the width to the length of the second abdominal sternum have all been reported as relating to resistance in houseflies but none seem to have universal applicability.

It has been reported from various laboratories (Brown, 1958) that houseflies emerging later were more resistant to D.D.T. than those emerging early and a resistant strain was developed by such selection. However, other reports indicate the

opposite to be true and it must be considered as not proven that length of larval period is directly related to resistance.

Other ratios determined between susceptible and resistant strains have shown no difference in pupal weights, egg production, viability of eggs, pupal or adult weights, sex variation, or length of egg stage. Sokal and Hunter (1955) made a detailed morphometric analysis of 16 body measurements of five resistant and four susceptible strains of houseflies and found no relationship to resistance.

That reduced rate of insecticide penetration is not the answer to resistance is clear from the fact that similar resistance is evident when the insecticide is applied by injection.

As pointed out by Metcalf (1955) "modern toxicological theory almost exclusively relates the mode of action of poisons to specific interference with biochemical systems, largely enzyme in nature. It is most probable that the secrets of insecticide resistance are to be found within this realm of biochemistry and enzymology.....".

At the present time the mode of action of no insecticide has been completely elucidated. Hence, it is not surprising that the biochemical and physiological nature of resistance have not been determined. Much progress, however, has been made. Evidence has been presented that at least in some cases, detoxification systems have been developed by the insect at the same time that resistance builds up so that the insect can cope with doses of toxicants that otherwise would be fatal.

In humans, according to Williams (1947), besides oxidation and reduction reactions, there are nine synthesis reactions or conjugation processes by which detoxication is accomplished. In a review on detoxication processes in insects, Smith (1955) lists 13 known processes by which poisons are eliminated but not all have been related to resistance. Detoxication processes are considered to be "all chemical changes which foreign organic compounds undergo in the animal body". The definition, of course, includes the change of non-toxic materials to toxic compounds as is the case with some of the phosphorus-containing insecticides.

Enzyme systems in arthropods are undoubtedly as numerous and as complicated as those in other living things. Only a few of these systems have been studied and none are completely understood.

The role of acetylcholine and cholinesterase in insects and related species has been frequently studied but is still not clear. However, the large amount of cholinesterase present in some tissues undoubtedly is there for a purpose. The housefly brain is one of the richest known sources of cholinesterase type enzymes but the amount present and the specific nature of the enzyme varies greatly between arthropod species. Spider mites apparently have little or no cholinesterase. Pratt and Babers (unpublished results), using about two milliliters of parathion resistant or susceptible *Tetranychus bimaculatus* Harvey could not demonstrate the presence of a cholinesterase in either strain. The insecticidal organophosphates generally are very active cholinesterase inhibitors, or through transformations in the animal or plant become so. It is frequently stated in the literature that in insects, the toxicity of the organophosphates is due to their anti-cholinesterase effect. Kearns (1956) in his review brings out the pros and cons of this theory and presents

weighty evidence that other enzyme systems are also involved. One can only conclude that the mode of action of the organophosphates is presently not known.

Houseflies, honeybees and the American roach contain other esterases in addition to cholinesterase. One esterase, not inhibited by metal salts, hydrolyzed a number of organophosphate insecticides such as paraoxon and parathion (Metcalf *et al.*, 1956).

Both an organophosphorus-resistant strain of houseflies and a susceptible strain metabolized paraoxon at a similar rate according to Lord and Solly (1956). No difference, other than variation in susceptibility to paraoxon, was noted between the strains. However, the LD50 for the resistant strain was only about twice that of the susceptible and for this reason the similarity in metabolic rates may not be meaningful.

Sternburg *et al.* (1950) and Perry and Hoskins (1950) and later numerous others showed that D.D.T.-resistant houseflies could much more rapidly metabolize D.D.T. than could susceptible strains. While the main metabolic product has not yet been isolated and its identity confirmed, excellent presumptive evidence indicates that the principal degradation product in houseflies is the relatively non-toxic 2,2 bis (p-chlorophenyl)-1,1 dichloroethylene commonly called D.D.E. Later work by Kearns and his co-workers has shown that the degradation process is enzymatic in nature, and they have called the responsible enzyme D.D.T.-dehydrochlorinase. Small amounts of D.D.E. have also been reported as being found in the tissues of D.D.T. treated susceptible strains. Hoskins and Gordon (1956) hypothesize that the small amount of D.D.E. in susceptible flies is due to the slow metabolism of D.D.T. by enzymes specific for other reactions or possibly by non-enzymatic chemical reactions.

Sternburg and Kearns and co-workers have been unable to demonstrate D.D.T.-dehydrochlorinase in susceptible insects and have concluded that the ability of resistant flies to degrade D.D.T. to D.D.E. is a major factor in the survival of poisoned flies. Apparently, only D.D.E. appears in the tissues as a metabolic product soon after resistant flies are treated with D.D.T., but after several days other metabolites are found according to Terriere and Schonbrod (1955).

Kerr *et al.* (1957), starting with a colony that had been established from wild houseflies in 1939 and had never been exposed to insecticides, developed two strains with resistance specific to D.D.T. One strain, "D", was selected by exposing the larvæ to D.D.T. and the other, "L", by selecting the late emerging adults from D.D.T. free media. When compared to the parent unselected strain, "U", both "D" and "L" showed resistance and when acetone-ether extracted tissues were tested for D.D.T.-dehydrochlorinase activity, the same order of enzyme activity was present. The males were consistently somewhat less resistant than the females but exhibited slightly more D.D.T.-dehydrochlorinase activity. Thus "production of D.D.T.-dehydrochlorinase is not necessarily dependent on a stimulus from D.D.T.". The function of the enzyme in flies that had never been exposed to an insecticide, is not known. According to the authors, the enzyme activity of the unselected strain was negligible. However, the females of this strain produced 11.8 per cent of the amount of D.D.E. produced by females of the "D" strain and 9.4 per cent of that produced by females of the "L" strain. The significance of these amounts has yet to be determined.

Perry and Sacktor (1955) compared the absorption and metabolism of D.D.T. by three susceptible strains of houseflies with seven resistant strains and attempted to relate cytochrome oxidase activity with degradation of D.D.T. They reported that the susceptible strains metabolized little D.D.T. whereas the resistant strains rapidly metabolized the absorbed D.D.T. to D.D.E. The relative significance of this factor varied with each strain. Cytochrome oxidase activity varied between strains but there was no distinction due to resistance and no direct relationship between cytochrome oxidase activity and degradation of D.D.T. The authors conclude that "D.D.T. resistance among all strains cannot be characterized by a single common factor" and suggest that "each strain possesses a combination of attributes for resistance which may be different from that found in other strains". Miyake *et al.* (1957) consider that the internal tissues in houseflies have sufficient dehydrochlorinating ability to protect the vital site from those doses of D.D.T. brought to the site by the transport system.

In numerous cases, the amount of D.D.T. and metabolites recovered did not account for the D.D.T. applied to houseflies and the existence of other metabolites than D.D.E. has been suggested. In certain other strains, D.D.E. appears to be the only product since D.D.E. plus D.D.T. accounts for the D.D.T. applied. It is possible that the inability to account for all of the applied D.D.T. is a question of poor analytical technique as indicated by the work of Perry *et al.* (1955) who conclude that D.D.E. is the principal degradation product of D.D.T. by houseflies. It seems more probable that some strains of flies or the same strains under different conditions metabolize D.D.T. by different processes and to different end products.

In resistant *Musca domestica*, Sternburg and Kearns (1950) found that important sites of D.D.T. metabolism appear to be the hypodermal cells of the integument following topical application and the wall of the gut following ingestion. Miyake *et al.* (1957) found D.D.T.-dehydrochlorinase listed in order of decreasing abundance in the fat body, brain, cuticle, hæmolymph muscle, ovary, and intestinal tract.

Although most of the research with houseflies has been done with the adult insect, sufficient work has been done to indicate that the overall picture of D.D.T. metabolism in the immature stages is similar to that in the adult (Metcalf, 1955).

The presence of D.D.T.-dehydrochlorinase has not been demonstrated in susceptible houseflies. When resistant strains are developed from susceptible strains in which no dehydrochlorinase is seemingly present, apparently no attempt has been made to determine when the enzyme first appears nor whether it first appears in small quantities and then increases regularly with increased resistance.

It seems probable that the enzyme is present in susceptible insects in quantities too minute to be demonstrable by available assay methods and increases as resistance increases.

The effect of suspensions of D.D.T., D.D.E. and related compounds on the *in vitro* activity of the succinoxidase system of resistant and susceptible flies, was determined by Anderson *et al.* (1954). No pronounced differences were noted in the sensitivity to D.D.T. of enzyme preparations from the two strains. The inhibition of succinoxidase and its components by D.D.T., is not considered a primary

factor in the mode of action of D.D.T. or in the mechanism of D.D.T. resistance in the housefly.

Triosephosphate dehydrogenase in D.D.T. susceptible and resistant strains of houseflies is inhibited by iodo- and chloro-acetic acid according to Bettini and Boccacci (1956). There was little difference between the strains in enzyme activity or inhibition by the chemicals.

Synergists to enhance the activity of D.D.T. against resistant flies have been suggested and in some cases have at first shown promise. However, resistance soon develops to the combination and this line of attack has largely been abandoned. The more active synergistic compounds found so far are close structural relatives of D.D.T. The most effective seemed to be D.M.C. [1, 1-bis(para chlorophenyl) methyl carbinol] but at no time was the lethal dose of D.D.T. reduced to that of the susceptible strain. D.M.C. is rapidly metabolized by living flies and is excreted principally as a compound tentatively identified as D.D.A. [bis-(para chlorophenyl) acetic acid]. D.M.C. irreversibly inhibits D.D.T.-dehydrochlorinase and Moorefield and Kearns (1955) attribute the failure of D.M.C. to show a synergistic effect on susceptible flies to the lack of the enzyme in these strains. The dehydrochlorinase is also inhibited by other D.D.T. synergists.

Ability to rapidly metabolize an insecticide apparently is not always related to increased resistance. The American cockroach *Periplaneta americana* is normally susceptible to D.D.T. and as yet, no confirmed report of the development of resistance by this species has been noted. However, Vinson and Kearns (1952) report that at 15°C., 12 hours after treatment with D.D.T., 47 per cent of the D.D.T. absorbed is metabolized and this increases to 78.7 per cent, 96 hours after treatment. When the roaches were held at 35°C., the percentages metabolized increased to 60.8 per cent after 12 hours and 83.6 per cent after 96 hours. Although the weight of evidence indicates otherwise, Weisman and Reiff (1956) concluded that D.D.T. metabolism by houseflies had no bearing on resistance. The tarsi of their susceptible houseflies secreted twice as much lipoid onto the walking surface as did resistant insects and consequently picked up more D.D.T. They concluded that tissue lipoids provide a protective factor in resistance to insecticides. Continuing this line of investigation, Weisman (1957) reports that the epicuticle of resistant flies has 30 - 40 per cent more lipoids than that of susceptible flies. Resistant flies have inclusions of small fatty drops in the epidermal cells at the base of the setæ on the abdominal wall. The inclusions act as a barrier against the penetration of the active ingredient of D.D.T. because the substance entering through the papillæ of the setæ is dissolved in the lipoids and thus blocked. Butts *et al.* (1953) treated *Periplaneta* with C¹⁴ labelled D.D.T. and found that the main metabolic product was a conjugate not extractable from water by organic solvents. The conjugate was thought to be carbohydrate in nature and this would, according to Smith (1955), suggest a preliminary hydroxylation of D.D.T. followed by conjugation or else conversion of D.D.T. to the acid D.D.A. and conjugation of the carboxyl group. Neither oxidation of D.D.T. nor conversion to D.D.A. has been shown to occur in insects. Robbins and Dahm (1955) also used radioactive D.D.T. and found that about 75 per cent of the applied D.D.T. was excreted in the feces within 24 days. Little of the excretory product (less than 10 per cent) was D.D.T., D.D.E. or D.D.A.

The blood of D.D.T.-poisoned *Periplaneta* contains an ether insoluble toxin that is equally toxic to resistant and susceptible flies on injection. (Sternburg and Kearns, 1952).

Pratt and Babers (1953) applied D.D.T. directly to the thoracic ganglion of houseflies and found that leg tremors in resistant insects were produced less frequently than in susceptible ones and in the resistant insects, the induced tremors were of shorter duration. These authors (Babers and Pratt, 1953) also found that when massive doses of D.D.T. were injected into resistant insects, little or no metabolism of the insecticide occurred and the insects seemed unaffected. The argument has been advanced that, when large doses of insecticides are injected, the insecticide does not reach a site of action. The fact remains that large doses injected into susceptible insects are invariably fatal so at least some transportation of injected heavy doses of D.D.T. to vital sites does occur.

Weiant (1955) found that the sensory nerves of resistant flies are less sensitive to the direct action of D.D.T. than the nerves of susceptible insects, and the D.D.T.-resistant flies were less sensitive than susceptible flies to toluol vapour when this chemical was used as the evocative agent in electro-physiological studies.

According to Bradbury and Standen (1955 : 1956a), a resistant strain of flies metabolized gamma B.H.C. more rapidly than a susceptible strain with the formation of water soluble metabolites. Later, using radioactive insecticide, Bradbury (1957) detected eleven different metabolites by paper chromatography but radioactive CO_2 was not produced.

Oppenoorth (1956) also reported that gamma B.H.C. is rapidly broken down to a non-toxic derivative by a lindane (pure gamma B.H.C.) resistant strain of houseflies. The susceptible strain metabolized the material more slowly, and eventually metabolism ceases completely. This might be caused by toxic action of B.H.C. on the flies. Oppenoorth concludes that resistance to B.H.C. is due to a decreased rate of absorption and an increased ability to detoxify the material.

Sternburg and Kearns (1956) did not detect D.D.T. dehydrochlorinase in a B.H.C.-resistant strain of houseflies. However, both B.H.C. resistant and susceptible insects metabolized lindane and pentachlorocyclohexene was one of the intermediates in the metabolism.

At the Pest Infestation Laboratory in England [Anonymous (1958)] dieldrin-resistant flies *Musca domestica Vicina* were shown to excrete the Sulphur-35 analogue of dieldrin faster than do susceptible flies, especially during the first few critical hours following topical application of the insecticide. The dieldrin analogue appeared to undergo no extensive breakdown within the tissues and resistance was not due to greater impermeability of the cuticle. Since the dieldrin-resistant flies were also resistant to gamma B.H.C., carbon 14 labelled alpha- and delta-isomers were used to study the metabolism of these materials. In agreement with the work in other laboratories, both resistant and susceptible flies metabolized the materials to pentachlorocyclohexene and similar quantities of metabolite were found in each strain three hours after application. Significant quantities of a water soluble metabolite were found in the excreta. To the observers it seemed unlikely that either metabolism or a faster rate of excretion by resistant flies can account for the resistance.

Topically applied heptachlor is readily absorbed by heptachlor-resistant houseflies and is metabolized to heptachlor epoxide (Perry *et al.*, 1958). The epoxide is also toxic to susceptible houseflies. However, the susceptible insects also rapidly metabolize heptachlor, also to the epoxide. After 24 hours, the entire topically applied dose had been absorbed by both strains. About 53 per cent was recovered from the resistant insects and of this, 93 per cent was the epoxide. With the susceptible strains, 62 per cent of the applied dose was recovered and of this, 78 per cent was the epoxide. The authors conclude that "housefly resistance to heptachlor does not specifically involve a detoxication mechanism".

Reiser *et al.* (1953) noted a direct correlation between fat content and survival of cotton boll weevils (*Anthonomus grandis* Boh.) exposed to toxaphene, dieldrin, E.P.N., methyl parathion, and calcium arsenate, but the resistance was of the seasonal variety rather than true insecticide resistance. Hoffman and Lindquist (1952), using a bioassay technique, showed that toxaphene and chlordane were absorbed and metabolized by resistant houseflies but did not attempt to identify the metabolic products.

Alexander *et al.* (1958) attempted, without success, to relate phosphatase activity to resistance in houseflies and German roaches. March and Lewallen (1956) examined the fresh tissue extracts of four susceptible and five insecticide resistant strains of houseflies by means of paper chromatography. A number of differences in chromatographic patterns between the strains were noted but none could be directly correlated to susceptibility or resistance. Rather, origin of the strain seemed to be the important factor since a resistant strain showed the same pattern as the susceptible strain from which it was developed.

Resistant mosquitoes also metabolize D.D.T. to D.D.E. as shown by Brown and Perry (1956). Resistant *Aedes teniorhynchus*, admixed with *Aedes sollicitans*, and *Aedes aegyptii* were compared with susceptible insects to determine their relative ability to produce D.D.E. Larvæ from each strain were exposed to D.D.T. and their D.D.T. uptake and *in vivo* formation of D.D.E. determined. One hundred and forty (140) susceptible *Aedes teniorhynchus* larvæ took up 5.6 micrograms and produced 2.4 micrograms D.D.E. The same number of resistant larvæ took up 73.3 micrograms D.D.T. and produced 15.9 micrograms of D.D.E. These correspond to about 21 and 24 per cent conversion respectively. For *Aedes aegyptii*, two micrograms and 1,553 micrograms were absorbed by susceptible and resistant mosquitoes and 72 per cent and 11 per cent of that absorbed was converted to D.D.E. The authors conclude "Both normal strains produced an insignificant amount of D.D.E., their uptake of D.D.T. being small" but to the reviewer both the uptake and amount metabolized seem significant. Tissue homogenates of neither strain converted D.D.T. to D.D.E. under conditions suitable for housefly material. Dieldrin and gamma B.H.C. resistant *Anopheles gambiae* absorbed no less B.H.C. than did susceptible insects. Only 11 per cent of absorbed B.H.C. was metabolized according to Bradbury and Standen (1956b).

In the German roach *Blattella germanica*, resistance is a serious problem in the United States. While the importance of cockroaches, as vectors of disease, has generally received little attention, a recent publication by Roth and Willis (1957) indicates that the medical importance of cockroaches may have been greatly underestimated. Chlordane-resistant German roaches more rapidly converted

D.D.T. and D.D.E. than did a susceptible strain (Babers and Roan, 1953) but most of the applied D.D.T. was converted to metabolites not appearing in analyses by the Schechter Haller procedure.

Certain chemicals have proven to be somewhat more toxic to resistant flies than to susceptible insects (Ascher, 1957; Mitlin *et al.*, 1956). While this interesting line of approach has not been fully explored, present indications are that probability for successful application to actual control procedures is low.

When the biological habits of the insect make it feasible, the release of large numbers of sterilized male insects to compete with the wild males offers interesting possibilities. Knipling (1955) has shown that with the screwworm, *Callitroga americana* (C. & P.), this method is indeed practical. None of the resistant species so far studied have the requisite biological habits, particularly single matings by the female, to permit the use of this procedure.

Insecticide failures, generally with mosquitoes, because of "behavioristic resistance" have also been reported (Brown, 1958). Little or no evidence has been published that relates these failures to a physiological response but such relationship must exist. Whether or not the insects' avoidance habits are due to increased sensitivity, or whether the insecticide acts as a repellent, is not clear. It does seem established that certain resting habits of the mosquitoes have changed so that control is not now accomplished by methods that were formerly successful. Yet, when the insecticide is applied directly to the insect, no increased tolerance is evident.

As indicated earlier, resistance by most arthropod vectors of disease, and by many agricultural and household pests, has already developed. However, little or no physiological or biochemical work with post-war insecticides has been done on other species than those already mentioned in this brief summary.

Experts, and the word is used loosely, have offered much advice on how to prevent the development of resistance. They have advised that space spraying will prevent development of resistance while residual treatments cause its development: only sufficient insecticide should be applied to effect prevention of disease transmission (this level, of course, is unknown); thorough coverage should be attained; treatment should be aimed at eradication; treatment should be aimed at achieving complete kill of a population etc. etc. As a matter of fact, insufficient experimental work has been done to determine what treatment process will minimize development of resistance and none so far proposed seems to offer a permanent solution to the problem.

The most effective procedure for controlling resistant insects has been to change to more toxic insecticides. Generally, the control pattern has been to first use D.D.T. or chlordane and when resistance develops, change to dieldrin, aldrin or B.H.C. Within a few generations, a switch to the organophosphates is necessary. At the present time, no new class of insecticides is available for use when the organophosphates become ineffective. It is for that reason that large scale investigation of all possible control measures is urgently indicated.

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