L. E. CHADWICK

MEDICAL LABORATORIES, ARMY CHEMICAL CENTER, MARYLAND, U.S.A.

RECENT ADVANCES IN BASIC STUDIES ON INSECT PHYSIOLOGY IN RELATION TO MECHANISMS OF RESISTANCE TO INSECTICIDES

Riassunto. — Attualmente le nostre conoscenze sul meccanismo fisiologico della resistenza agli insetticidi sono poco soddisfacenti. Come introduzione all'esame dei problemi che si pongono a questo proposito, l'Autore riassume gli ultimi risultati ottenuti in tre campi particolari e cioè:

1) disintegrazione biochimica del DDT nell'organismo;

2) osservazioni recentemente effettuate sul modo di azione del DDT;

3) esami di laboratorio sulla resistenza al di-isopropilfluorofosfato (DFP).

La trasformazione del DDT in DDE o in altri prodotti inoffensivi costituisce un fattore importante della resistenza di certi ceppi di *Musca* e di altre specie. Non v'è dubbio che altri fattori come la capacità di accumulare senza pericolo del DDT non disintegrato e la diminuzione del tasso di assorbimento dell'insetticida, hanno una parte importantissima. Tutti questi processi sono complessi e il dettaglio dei loro meccanismi può variare d'un ceppo e d'una specie all'altra. Inoltre è probabile che i tessuti nervosi delle mosche resistenti presentano una sensibilità al DDT inferiore al normale.

Il DDT agisce sul sistema nervoso sensorio degli insetti scemandone la stabilità e facendolo reagire in maniera anormale alle diverse eccitazioni. Ogni eccitazione di sufficiente intensità provoca allora nel tessuto nervoso avvelenato una reazione anormale di forma caratteristica.

L'analisi della resistenza al DFP d'un ceppo di *Musca* scelto in laboratorio non è ancora terminata; ciò nonostante, le osservazioni

indicano che i principali fattori responsabili di questa resistenza devono essere modificazioni che diminuiscono il tasso della penetrazione del veleno nell'enzima colinesterasi del sistema nervoso dell'insetto. I tegumenti (compresi probabilmente la parete della trachea) formano una buona parte di questo ostacolo, ma vi sono pure ostacoli interni.

In lavori di ricerca fisiologica concernenti la resistenza agli insetticidi, è opportuno soffermarsi specialmente sui seguenti punti:

1) allargare le nostre conoscenze fondamentali delle funzioni fisiologiche degli insetti normali;

2) chiarire il dettaglio dei meccanismi d'intossicazione non solo da DDT ma ancora da tutti gli insetticidi utilizzati su grande scala;

3) mettere al punto metodi soddisfacenti per la microanalisi di questi insetticidi e dei loro derivati o dei prodotti di disintegrazione nei tessuti degli insetti;

4) determinare il metabolismo di questi insetticidi nell'organismo degli insetti normalmente resistenti e normalmente sensibili;

5) identificare e spiegare in dettaglio tutti i meccanismi fisiologici che contribuiscono alla resistenza.

Résumé. — A l'heure actuelle, nos connaissances sur le mécanisme physiologique de la résistance aux insecticides sont fort peu satisfaisantes. Comme introduction à l'examen des problèmes qui se posent à ce propos, l'auteur résume les résultats les plus récents obtenus dans trois champs particuliers, c'est-à-dire:

1) désintégration biochimique du DDT dans l'organisme;

2) observations récemment effectuées sur le mode d'action du DDT;

3) examens de laboratoire sur la résistance au di-isopropil-fluorophosphate (DFP).

La transformation du DDT en DDE et en d'autres produits inoffensifs représente un facteur important de la résistance de certaines souches de *Musca* et d'autres espèces. Sans aucun doute, d'autres facteurs, comme la capacité d'accumuler sans danger du DDT non-désintégré et la diminution du taux d'absorption de l'insecticide, ont un rôle très important. Toutes ces réactions sont complexes et le détail de leurs mécanismes peut varier d'une souche et d'une espèce à l'autre. En outre

il est probable que les tissus nerveux des mouches résistantes aient une sensibilité au DDT inférieure à la sensibilité ordinaire.

Le DDT agit sur le système nerveux sensoriel des insectes en en diminuant la stabilité et en le faisant réagir d'une manière anormale aux diverses excitations. Toute excitation d'intensité suffisante provoque alors dans le tissu nerveux empoisonné une réaction anormale d'un type caractéristique.

L'analyse de la résistance au DFP d'une souche de *Musca* choisie dans le laboratoire n'est pas encore terminée; malgré cela, les observations indiquent que les facteurs responsables principaux de cette résistance doivent consister en modifications qui abaissent le taux de pénétration du poison dans l'enzyme cholinesterase du système nerveux de l'insecte. Les téguments (y compris probablement la paroi de la trachée) forment une bonne partie de cet obstacle, mais il y a aussi des obstacles intérieurs.

Dans les travaux de recherche physiologique concernant la résistance aux insecticides, il est important de s'arrêter sur les points suivants:

1) Aprofondir nos connaissances fondamentales des fonctions physiologiques des insectes normaux;

2) Préciser le détail des mécanismes d'intoxication due non seulement au DDT mais encore aux autres insecticides employés sur grande échelle;

3) Mettre au point des méthodes satisfaisantes de micro-analyse de ces insecticides et de leurs dérivés ou des produits de désintégration dans les tissus des insectes;

4) Déterminer le métabolisme de ces insecticides dans l'organisme des insectes ordinairement résistants et ordinairement sensibles;

5) Identifier et expliquer en détails tous les mécanismes physiologiques qui contribuent à la résistance.

Summary. — Current knowledge of the physiological mechanisms involved in resistance to insecticides is unsatisfactory. As an introduction to the problems concerned, recent data in three limited areas are summarized. These are: 1) The biochemical degradation of DDT in the body; 2) New observations on the mode of action of DDT; and 3) Laboratory studies of resistance to di-isopropyl fluorophosphate (DFP).

Metabolism of DDT to DDE or other harmless products is a significant

factor in the resistance of certain strains of *Musca* and other species. Other factors are also definitely involved. These include the capacity for harmless storage of unchanged DDT and a reduction in the rate at which the insecticide is absorbed. Each of these processes is complex, and the detailed mechanisms may vary in different strains and species. It is likely also that the nervous tissue of resistant flies is subnormal in sensitivity to DDT.

The action of DDT on the sensory nervous system of insects is to render this system unstable and abnormally responsive to stimuli of characteristic form in poisoned nerve.

Analysis of DFP resistance in a strain of *Musca* selected in the laboratory is still incomplete, but the observations indicate that the principal factors in this resistance must be changes that reduce the rate of access of the poison to the enzyme cholinesterase in the nervous system. A considerable part of the barrier is in the integument (presumably including the tracheal lining), but there are internal barriers also.

In physiological research related to insecticide resistance, further emphasis is particularly needed on:

1. Expansion of basic information concerning the physiological functions of normal insects.

2. Elucidation of the detailed mechanisms of poisoning, not merely for DDT, but for all insecticides in widespread use.

3. Development of satisfactory methods of microanalysis for these insecticides and their derivatives or metabolites in insect tissues.

4. Determination of the metabolic fate of these insecticides in the insect body, in normally susceptible and resistant forms.

5. Identification and detailed explanation of all physiological mechanisms that contribute to resistance.

Zusammenfassung. — Unsere heutige Kentnisse über das physiologische Mechanismus der Resistenz gegen Insektiziden sind nicht ganz befriedigend. Als Einführung zur Duchforschung der Problemen, die sich diesbezüglich stellen, fasst der V. kurz die letzten Ergebnisse die in 3 besonderen Feldern erhalten worden sind auf, und zwar: 1) biochemische Zersetzung des DDT im Organismus; 2) neuesten Beobachtungen über die Wirksamkeit des DDT; 3) Laboratorium-Untersuchungen über die Resistenz gegen DFP (di-isopropylfluorphosphat).

Die Umwandlung des DDT in DDE oder in anderen unschädlichen Stoffen stellt, bei einigen Stämmen von *Musca* und bei anderen Arten,

einen wichtigen Resistenzfaktor dar. Zweifellos spielen andere Faktoren, wie die gefahrlosen Ansammlungsfähigkeit von unzersetzten DDT und die verminderte Absorptionsmenge des Insektiziden eine sehr wichtige Rolle. Sämtliche genannten Prozesse sind kompliziert und die Einzelheiten dieser Mechanismen können, bei den einzelnen Stämmen und Arten, wechseln. Möglich ist es ausserdem dass die Nervengewebe der Resistenten-Fliegen eine Empfindlichkeit gegen DDT aufweisen die niedriger als die der Norm ist.

DDT wirkt auf dem Nervensystem der Insekten indem es dessen Stabilität beschränkt und dadurch bei den verschiedenen Erregungen anormal reagiert. Jede genügend starke Erregung übt auf den vergifteten Nervengewebe eine charakteristische anormale Reaktion.

Die Nachforschung der Resistenz gegen DFP bei einigen von *Musca* im Laboratorium gewählten Stämme ist noch nicht zu Ende gebracht worden. Nicht destowenig weisen die Beobachtungen darauf hin dass die wichtigsten Faktoren die die Verantwortlichkeit dieser Resistenz tragen wohl aus Änderungen bestehen die die Eindringungsfähigkeit des Giftes in dem Enzym der Cholinesterase des Nervensystems des Insekten vermindern. Die Tegumentbildungen (einschliesslich die Wände der Trachea) bilden einen wichtigen Teil dieses Hindernisses, doch inwendig befinden sich auch andere Hindernisse.

Bei physiologischen Nachforschungen über Resistenz gegen Insektiziden wäre es zweckmässig besonders folgende Punkte in Anbetracht zu nehmen:

1) Unsere grundlegende Kentnisse der normalen physiologischen Funktionen der Insekten zu erweitern.

2) Die Einzelheiten der Vergiftungsmechanismen die nicht nur von DDT, sondern auch von sämtlichen anderen in grossen Umfang gebrauchten Insektiziden hervorgerufen werden zu erklären.

3) Befriedigende mikroanalyse-Methoden dieser Insektiziden und deren Derivaten oder der Zersetzungsprodukten in den Insektengeweben zu bestimmen.

4) Das Metabolismus dieser Insektiziden in dem Organismus der Insekten die normalweise resistent und normalweise empfindlich sind festzustellen.

5) Im Einzeln alle physiologischen Mechanismen die zur Resistenz beiwirken zu identifizieren und erklären.

All of us will agree, I am sure, that we know far too little about the physiological responses of insects to poisons, and that this deficiency is being emphasized daily by the numerous problems that accompany the manifestation by insects of resistance to insecticides. It is fitting, therefore, that we should now take stock of our position, both that we may make intelligent use of the physiological information we possess and also so that major gaps in our knowledge may be brought to the attention of interested workers everywhere. We are truly grateful that the World Health Organization of the United Nations has provided an opportunity for such a survey under auspices so favorable to the aims just stated.

Despite the unsatisfactory state of our knowledge, the physiology of resistance to insecticides has in late years become too broad a topic for brief summarization. I shall therefore merely introduce the subject, by outlining data that bear on three relatively limited aspects of it. The observations concern:

1. The significance, in DDT resistance, of the biochemical degradation of the insecticide in the insect body;

2. Some recent extensions of our knowledge of the mode of action of DDT; and

3. Laboratory studies of resistance to an organic phosphonate.

DDT METABOLISM.

Rapid breakdown of DDT to non-toxic metabolites as a mechanism of resistance was first encountered, I believe, in a study by FERGUSON and KEARNS (1949) of the fate of DDT absorbed by the milkweed bug, *Oncopeltis*. This paper preceded by only a little other reports from the same and other laboratories (STERNBURG et al., 1950-a, 1950-b; PERRY and HOSKINS, 1950) that DDT resistant strains of *Musca* are able to convert relatively large amounts of DDT to the ethylenic derivative, DDE, which is harmless to flies, whereas susceptible strains produce little or none of this metabolite.

A second significant finding in these and subsequent studies is that the lethality of DDT to resistant flies may be enhanced by exposing the insects to various other chemicals that of themselves do not produce apparent poisoning; and that these activating agents reduce the conversion of DDT more or less in proportion to the increase in effectiveness they confer (cf. PERRY et al., 1953). Such observations obviously give support to the hypothesis that detoxification is a factor in resistance.

Still more important from the practical point of view, however, is the fact that these same studies demonstrate that ability to dispose metabolically of DDT cannot be the *sole* physiological mechanism involveld in resistance to the insecticide.

The need for attention to other physiological and biochemical processes is intimated first by the unfortunate truth that no combination of DDT and activator yet discovered is as effective in controlling resistant flies as was DDT alone with susceptible strains. Either the activators have been unable to block the detoxification process completely, in which case one may suspect that metabolism follows more than one pathway, or there must be some residue of resistance that does not depend on ability to metabolize the insecticide. There is evidence for both alternatives. Reserving for the moment further comment on the metabolic fate of DDT, one may mention the tolerance of resistant flies for large amounts of unchanged DDT in the internal tissues and the imperfect correlation between degree of resistance and ability to metabolize DDT (PERRY et al., 1952 and personal communication; KEARNS, personal communication). Thus, strains of approximately equal resistance may show very different capacities for degrading the poison, and therefore some fraction of resistance must be sought elsewhere than in the destruction of the agent.

Recent work has shown that, in certain of these instances, resistance is correlated with a relatively slow absorption of the insecticide. Thus we return to the early suggestion of WIESMANN (1946), that a reduced rate of penetration is a factor in DDT resistance; and it becomes apparent that those who, like myself (1952), were inclined to dismiss this suggestion were too hasty in our judgment. The overall picture is more complex than we had realized.

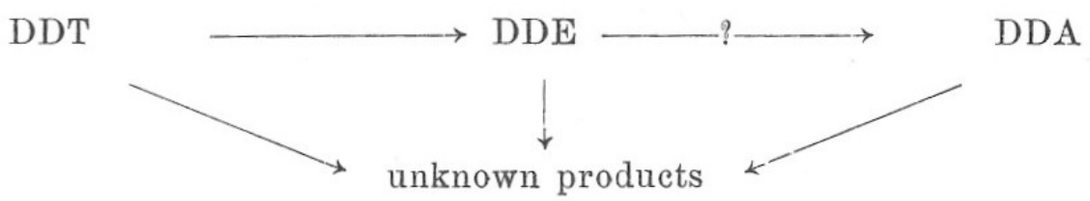
Currently, then, we are confronted with at least three distinct types of physiological mechanism, each of which contributes in some instances to DDT resistance. One is an enhancement, in the resistant insect, of the ability to metabolize DDT to non-toxic products. The second mechanism appears as the capacity for storing harmlessly in the tissues large amounts of unchanged DDT, although actually this mechanism may involve processes quite different from simple sequestration of DDT in, for instance, the fat depots. The third proven mechanism expresses itself in a reduced rate of absorption of DDT into the body.

There is at the present time no reason to presume that the rate of uptake of DDT is identically regulated in all cases; in fact, it is probably safer to assume the opposite. For if, as proposed by WIESMANN

(1946) and some other workers (cf. D'ALESSANDRO et al., 1949), such relatively gross structural features as differences in thickness of tarsal cuticle are concerned, we may be certain that still other factors are involved in changed absorption rates elsewhere, inasmuch as visible differences in thickness of cuticle are not found in some comparisons of normal and resistant flies (MARCH and LEWALLEN, 1950), which nevertheless absorb DDT at different rates.

Our knowledge of the biochemical breakdown of DDT, though far ahead of similar information for any other insecticide, is today in a rudimentary state. Lack of adequate methods for measuring the extremely small amounts of insecticides and their metabolites that are present in the tissues of poisoned specimens has been and still is a real obstacle to progress toward the definition and characterization of mechanisms of intoxication and resistance. Some highly significant groundwork is now being done in this area. Every encouragement should be given to those attacking these difficult problems, for their success is prerequisite to our understanding of an important class of resistance mechanisms and hence to the rational development of effective biochemical countermeasures.

Existing knowledge (or the deficiency thereof) about the metabolism of DDT may be summarized briefly in the accompanying diagram:



The conversion of DDT to DDE is enzymic. STERNBURG et al. (1953) have extracted and partially purified the responsible enzyme from several strains of resistant flies. The more highly purified preparations permit nearly quantitative recovery of added DDT as the sum of unchanged compound and DDE; i.e., no other metabolite is produced in significant amounts. With cruder extracts, there is some loss of added substrate (DDT), but it is not yet clear whether this is the result of breakdown of DDT to unidentified materials by other systems that may be present or of inadequate extraction procedures. However, there seems to be little doubt that in other circumstances (as, for example, with *Oncopeltus*) DDT disappears through some unknown chain of reactions that does not involve the production of DDE. Similarly, both injected DDE and DDA are degraded to unknown products by the milkweed bug (FERGUSON and KEARNS, 1949). Other well authenticated instances of the formation from both DDT and DDE of metabolites of

unknown identity could be cited (e.g., Tahori and Hoskins, 1953), so that the evidence for a diversity of metabolic pathways seems clear, not only in comparisons of different strains and species, but even in the individual insect. There is a strong suspicion, however, that the DDA reported in certain experiments may have been an artifact of the analytical procedure.

As shown by Perry et al. (1953), the intact fly is capable of dehydrating DMC [bis-(p-chlorophenyl)-methyl carbinol] by a process analogous to the dehydrochlorination of DDT; and it is supposed that the activating effect of DMC on DDT against resistant flies depends on a greater affinity of DMC for a common enzyme concerned in the metabolism of the two compounds. However, it is also known that DDT resistant flies will develop resistance to the DDT-DMC combination in a few generations of selection (Perry et al., 1952); and it is not easy to fit this fact into the relatively simple scheme that envisions DDT as the « normal » substrate of the dehydrochlorinating system and DMC as a metabolic antagonist.

Enough has been said to give some notion of the scope of unfinished business that faces us in connection with the metabolic breakdown of DDT in the insect and the relationship of these questions to the problem of resistance. Considering that we are also confronted with similar problems for each of a variety of other insecticides, that the list is growing almost daily, and that to date little has been attempted and almost nothing accomplished toward the analysis of the problem with these other agents, it is clear that, in spite of the real progress that is being made with DDT, the physiologist is far from keeping pace with adverse developments.

Mode of action of DDT.

Leaving further consideration of these biochemical problems for later general discussion, I am now forced to remind you that we are still largely in the dark as to details of the *intoxication mechanism* for DDT or any of the other organic insect control agents to which significant resistance has developed. It is unfortunate that there is still so little to be added to the picture that was already available two or three years ago, since we can hardly expect to cope successfully with the physiology of resistance while we continue ignorant of the antecedent mechanisms of poisoning.

Agreement seems general that DDT interferes rather directly with normal nervous function, though there is still some difference of opinion

as to what portions of the nervous system are the more significant sites of action. But just how DDT produces the observed effects remains obscure.

Of interest in this connection is the extraction, by FERNANDO and KEARNS (personal communication) from the nerve cords of DDT poisoned cockroaches, of an unidentified substance that is highly toxic to flies and that has some of the pharmacological activity of acetylcholine. The material is definitely not DDT nor any known DDT derivative. These observations suggest the possiblity that DDT itself may not be the active neurotoxin, but that it may instead induce the production by the tissues of some quite different material that is able to disrupt the normal properties of insect nerve. Little more is known about this substance, except that it is not acetylcholine either, since (1) it fails to respond to Hestrin's (1949) chemical test and (2) it induces contraction of cockroach leg muscle, on which acetylcholine is without effect. Here is more unfinished business, of sweeping fundamental significance.

More immediately related to the problem of DDT resistance is some of the work that has been done in the laboratory of K. D. ROEDER (personal communication) during the past two years. These investigators have spent considerable effort in developing preparations that will allow electrical recording of potentials from various portions of the nervous system of house flies, in order to be able to make such observations with the species in which insecticide resistance has been most conspicuous. Several such preparations are now available, and through their use, ROEDER and coworkers have been able to demonstrate in susceptible *Musca* the same type of sensory response to DDT that they had discerned previously in the American cockroach; that is to say, a replacement of discrete spikes in the ascending neurones of the leg by short bursts or trains of impulses. Thus we have learned that this characteristic symptom of DDT poisoning is not species-specific. Additionally, it has been observed that the effect does not occur in specimens of *Musca* from a DDT resistant strain, though there is still doubt as to whether the failure of the resistant flies to respond in the typical manner is due to poor penetration of the insecticide or to some more fundamental difference in the sensitivity of the nervous elements.

In other experiments from the same laboratory, Dr. T. SMYTH discovered that allowing susceptible flies to walk for a few minutes on a DDT deposit caused a significant reduction in the threshold of the feeding response to tarsal contact with solutions of sugar. Similar treatment of resistant flies was without effect on their chemosensory

threshold. While these experiments were in progress, Dr. Roys, another member of the group, observed that exposing the cockroach leg to toluene vapor produces a large volley of sensory discharge in the leg nerve. In view of Smyth's observations, it was a natural extension of Roy's experiments to examine the result of combining exposure to toluene with pretreatment of the cockroach leg with DDT. The outcome of such tests revealed that it was possible first to sensitize the leg with a dose of DDT which of itself was insufficient to alter the normal pattern of activity, and then to produce the typical symptoms of DDT poisoning by means of a subsequent exposure to toluene vapor. That is, the so-called DDT trains could be caused to appear and disappear by alternately supplying and withdrawing a toluene stimulus.

These new observations have led to the conception that the effect of DDT is not that of stimulating the discharge of the sense organs or sensory nerve, as had been supposed from the earlier studies with the cockroach and the fly, but that DDT acts merely to make the nerves unstable, so that their response, however provoked, is a multiple discharge of typical form. This scheme is basically quite similar to the interpretation advanced several years ago by Welsh and Gordon (1947).

If it can be shown with resistant flies that the absence of sensitization by DDT is not due to failure of DDT to penetrate the integument, it will then be necessary to conclude that at least a portion of resistance depends on changes at the level of the neurone or of the sensory cell, alterations that render these structures capable of maintaining their normal properties in the presence of DDT. Such factors, as well as improvements in storage capacity in a narrow sense, may well contribute to the ability of resistant flies to preserve normal behavior in spite of the presence within them of what would be several lethal doses for a normally susceptible individual. Recent evidence of high rates of absorption in certain resistant strains (Perry, personal communication) does indeed suggest that some fraction of resistance will have to be attributed to such changes in the properties of the nervous tissue. Other provocative data in support of this hypothesis have been published by Pratt and Babers (1953), who found evidence for greater responsiveness of normal than resistant flies to DDT applied directly to the exposed thoracic ganglion.

As already noted, the precise mechanism whereby DDT produces the observed sensitization of the nerves is unknown; nor do we yet know whether the nervous symptoms, conspicuous though they are, are causally related to the death of the insect, which often occurs only

long after all the neuromuscular hyperactivity has subsided. Fresh approaches to these problems should receive high priority in any investigative program.

Resistance to DFP.

The third topic I wish to recapitulate concerns studies done in our own laboratory on the resistance of flies to di-isopropyl fluorophosphate (DFP). Selection of *Musca* for tolerance of the vapor of this anticholinesterase was begun in 1948, for two principal reasons. One was that we wished to know something of the capacity of flies to develop resistance to compounds of this type, which it appeared might eventually be called on to fill the gap left by the spreading development of resistance to DDT and other chlorinated hydrocarbons. Another reason was that we felt we already had a clearer notion of the primary process in intoxication for this kind of compound than we had for any of the other insecticides; and that we would therefore be in a better position to assess the nature of such resistance mechanisms as might come into being during the selection process.

After selection at about the 75 to 90 per cent level of mortality for something over 100 generations, we estimate the derived tolerance for DFP vapor at 10 to 20 times normal, depending on the criteria of comparison. We ask then, « What physiological or other changes have accompanied the development of this resistance? ».

In gross appearance and structure the resistant flies are not noticeably different from the controls, except for the presence among them of an exceptionally high proportion of specimens with abnormalities in the wing venation. The developmental period is longer than normal by about 1 day in 8. The resistant flies are clearly more placid in their behavior. From time to time in the course of selection, the reproductive capacity of the selected strain has become so deficient as to force a temporary relaxation of the selection procedure. Our impression is that these changes, except for the high incidence of venational abnormalities, are unrelated to resistance. The aberrant groups in both strains are significantly more tolerant of DFP than are their sibs with normal wings, so that there seems to be a certain amount of linkage between the two phenomena.

Turning to a biochemical comparison, there has been no significant change over a period of several years in the cholinesterase activity of either strain. The level of this enzymic activity is approximately the same in both strains; and, *in vitro*, DFP reacts with the crude

suspension in accordance with the kinetics of a simple bimolecular reaction, the rate of which is identical for both strains. Therefore, we conclude that the resistance observed cannot depend on either quantitative or qualitative differences in the vital acceptor for the toxicant. Nor have we seen any other suggestion that a marked shift in the biochemistry of poisoning has occurred as a result of the selection.

The rate of uptake of DFP vapor does not differ in the two strains; but, unfortunately, the amounts of DFP required for kill, even of the resistant flies, are too small to furnish any useful information in regard to the possibility that the accumulated DFP might be differently localized in or on the body by the two strains. Attempts to demonstrate any difference in the ability of the two strains to destroy the inhibitor have also given negative results, which may signify either that there is no difference or that our methods were inadequate. No evidence of enzymic degradation of DFP was found with either strain.

The only conclusion to be drawn at present from these various lines of information is that access of the inhibitor to the enzyme is somehow impeded in the resistant flies. The hindrance might consist in an alteration of the properties of the integument, including presumably the tracheal lining, or of some more centrally located barrier, such as the nerve surface itself. Since administering the inhibitor by injection directly into the thoracic hemocoele reduces the factor of resistance from 10 to about 2 or 3, it seems that some considerable fraction of resistance must be provided by a less permeable integument in the resistant flies, but that there is also a remainder that must be ascribed to other barriers or processes that further reduce the amount of agent that actually comes into contact with the enzyme. The overall picture is thus quite reminiscent of the situation with DDT resistance, in that it seems to involve a complex of different mechanisms, at least one of which suggests a change in permeability, as well as in the unhappy fact that in spite of a good deal of work we are still on a very insecure footing with both problems.

Practical conclusions.

What practical guidance may be drawn from observations of the sort I have described? In the first place, there would seem to be little that one can do immediately towards overcoming resistance that depends on a decrease in permeability of the organism for the insecticide, other than to shift to chemicals with different penetration cha-

racteristics. There is, of course, a chance that, given sufficient knowledge of the limiting factors in penetration, one could provide formulations that would nullify the protective changes that selection has produced. With such objectives in view, studies of the fine structure and properties of the integument and other barriers should be encouraged; but it must be recognized that this is slow and difficult work and that a quick solution of the practical problems is not likely.

The prospect of competing by chemical means with the metabolic processes involved in detoxification offers definite promise, in spite of the fact that current developments in this direction have so far fallen short of providing a complete cure. As knowledge of the biochemistry of poisoning and detoxification increases, improvement in our ability to protect the insecticide against metabolic breakdown is certain to occur. What can be accomplished along these lines will however be limited by the relative importance of detoxification among the several mechanisms whose sum constitutes the resistance that must be dealt with by the practical entomologist in the field. Since we know already that resistance includes other types of mechanism, we cannot logically demand a complete solution of the problem *via* the discovery of more effective inhibitors of detoxification, but neither can we afford to despise the substantial gains in this area to which we may look forward if the necessary basic studies continue to receive adequate support. As I have intimated previously (1952), it is not too much to hope that we may eventually be able to give a suicidal twist to the detoxification mechanism, by providing it with a toxic substrate that will yeld other poisonous products when the agent is metabolized.

Even with our presently limited knowledge of physiological mechanisms, it is obvious that the capacity of insects to become resistant varies greatly with poisons of different types. Thus we are granted the opportunity of by-passing the resistance problem, by devoting a portion of our effort to development of agents from those classes of compounds to which resistance is less readily acquired. This course involves us in difficulties of other kinds, such as the improvement of residual quality, reduction of mammalian toxicity with retention of insecticidal effectiveness, etc., but there is reason for confidence that such problems can be solved. While we shall continue to feel the need for all information that frontal attacks on the problem of resistance can provide, we must also exploit the potentialities of such flanking maneuvers as fully as possible.

The principal practical lesson to be learned from current information

on the physiology of resistance is, however, that resistance is a many-sided phenomenon. I have enumerated several distinct types of resistance mechanism, each of which seems to be attested by valid data. But it is probable also not only that there exist other types of physiological resistance, as yet undocumented, but also that each of the types already described includes multiple mechanisms, similar in a general way yet differing in detail. Certainly there is more than one metabolic pathway for the biochemical degradation of DDT; while penetration of the insecticide may be impeded by numerous different barriers, and in a variety of ways at each. Although we know little about how unchanged DDT is stored or excreted without gaining access to the vital sites of action, the possibilities are obviously manifold.

A cool appraisal of the situation warns us, therefore, that we cannot expect any single new measure to provide the solution for all these problems simultaneously. For the time being, at least, we shall have to be content with partial success here and with some gain there. Consequently, it would seem of the greatest importance to obtain as prompt and complete a definition as possible of the physiological aspects of the problem, in each situation where resistance may arise, so that countereffforts may be directed intelligently toward specific goals that are known to be pertinent to the difficulties at hand. The likelihood that these goals will differ somewhat from one situation to another should also be recognized and accepted

Until we have learned more about the varied manifestations of the problem, physiological research concerning resistance will have to proceed on a very broad front. For this reason, the list given below should in no sense be considered exclusive; it includes merely those areas to which I believe the greatest effort should be given at this time.

1. Expansion of basic information concerning the *physiological functions* of normal insects.

2. Elucidation of the detailed *mechanisms of poisoning*, not merely for DDT, but for all insecticides in widespread use.

3. Development of satisfactory *methods of analysis* for these insecticides and their derivates or metabolites in insect tissues.

4. Determination of the metabolic fate of these insecticides in the insect body, in normally susceptible and in resistant forms.

5. Identification and detailed explanation of *other physiological mechanisms* that contribute to resistance.

The future success of chemical control measures for insects of medical and economic importance will depend greatly on how well we are able to meet the challenge of these broad and difficult questions and to integrate the resulting data with other equally pertinent fundamental observations. One hears too frequently of a distinction between practical and fundamental research in the field of insect control. The resistant fly has at least one good deed to its credit: it has convinced many that it is no longer practical to be ignorant of fundamentals.

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