FACTORS INVOLVED IN THE DEVELOPMENT OF RESISTANCE TO INSECTICIDES AND SOME MEASURES TO REDUCE ITS EFFECT

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The basic phenomenon in insecticide resistance is a change in the susceptibility of a population to the toxic effects of the chemical concerned. This change is not shown in the exposed generation but in succeeding ones. Attempts to demonstrate that the change is due to mutation(s), either caused by the chemical or occurring independently, have been uniformly unsuccessful so it may be concluded that with the materials at present in wide use, selection of a previously existing phenotype is the predominant mechanism. The development of resistance is thus an example of evolution in progress at a comparatively enormous rate with a chemical as the selecting agent.

The results of a selection usually are expressed in terms of the behavior of a population, as represented by the sample or samples tested, although the response is by individual insects. Therefore it is necessary to express the reaction of a group to a chemical in such terms as dosage required to knock down a given portion within a specified time, or to kill all individuals, or the time for death of a fraction to result from application of a certain dose, etc., etc. The simplest criterion to handle is mortality resulting from an increasing series of dosages, time being given for the full effect to develop but normal mortality being still low.

No group of individuals is entirely homogeneous and in the case of exposure to a chemical, the differences in susceptibility are represented by a dosage-mortality curve whose characteristic form is an elongated sigmoid. For different populations, these may vary greatly both in position and shape. Hence they are difficult to use in comparisons, especially quantitative ones. It is common practice to change to a linear form by a double transformation, giving the so-called ld-p lines, i.e., log dosage-probit or standard deviation lines. Not all dosage-mortality lines can be rectified, but most can, at least in the region of 50 percent mortality. Hence it has become the custom to compare populations or strains of insects in terms referring to 50 percent mortality, e.g., the LD 50, LC 50 or LT 50, in which the criterion is dosage, concentration, or time of exposure.

Figure 1, line A, shows a typical example. LD 50 = 0.42 and slope of line = 3.73, as measured by the mortality expressed in probits or standard deviations caused by a tenfold change in dosage (i.e., per one log unit of dosage). Now suppose 95 percent of the population are killed by a dosage of one unit of insecticide and the survivors give rise to a new generation. What will be its resistance? This is the problem with which plant and animal breeders have worked for many years in their selective breeding experiments and no simple answer is possible. It is helpful to show the ld-p line for the survivors of the treated generation. Since in the present case only one in twenty survived, the survivors at each dosage above the LD 95 will contribute twentyfold to the breeding population. Thus at dose 1.2, the mortality would be (97.7–95) x 20 = 55 percent. Other points on the resulting line B are calculated similarly. This ld-p line *for the survivors* will hold for their progeny only under the special condition that all genetic factors for susceptibility have been eliminated from the population and the breeding group is homozygous for resistance.

In any actual case, genes for resistance will occur in both heterozygous and homozygous individuals and when they are rare the great majority will be in the heterozygotes. In the next generation these will give rise to some individuals homozygous for susceptibility as well as others heterozygous and homozygous for resistance. Numerical calculation is possible only when the composition of the original population and of the survivors is known in terms of frequency of the genetic factors. It will be clear, however, that if all individuals lie on the ld-p line A, then the line for the next generation must lie between A and B, perhaps as shown for line C. If selection had been made at the 99 percent level, the line for the next generation would have been steeper and slightly more to the right. Repetition of selection would result in successively smaller changes and the population would become highly homogeneous.

It is obvious that from a population originally relatively homogeneous, as indicated by a steep ld-p line, and *whose members all fall on the line,* it is not possible to obtain a highly resistant strain. This situation appears to be the case with numerous species which fail to develop resistance under even intensive selection.

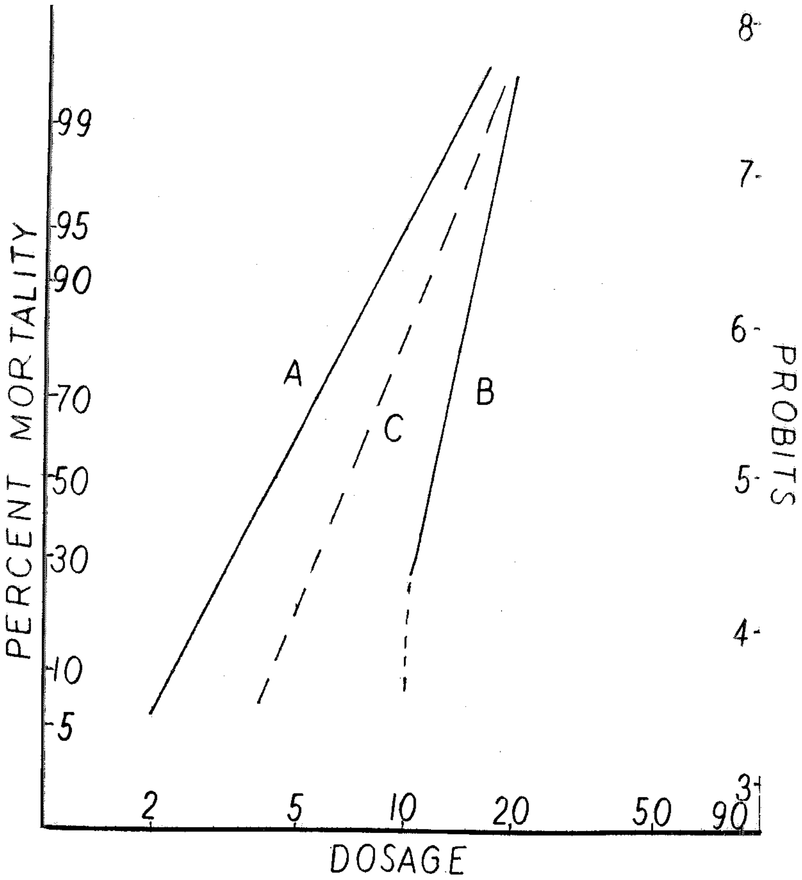

FIG. 1.—Dosage-mortality lines for a population before selection, A; of the survivors from 95 percent selection, B; of next generation, C.

The relatively small increase in resistance achieved may be caused by exposure to any unfavorable condition, for only the weaker members are lost. Hence this type of resistance has been called vigor tolerance. Many instances have been recorded in the literature. A typical one is the finding by Hawkins (1956) that in a colony of *Anopheles quadrimaculatus* the larval LD 50 changed during selection with DDT from 2.6 ppb to 7.0 ppb in five generations. During this time the upper limit of the more tolerant individuals did not change appreciably but the frequency of the very susceptible ones decreased notably.

In contrast, suppose that at the upper end of the ld-p line there are individuals more resistant than corresponds to any position on the line, as represented by points along the line a′-b in Figure 2. Let selection now be made at the 95 percent level and the composition of the survivors calculated as before. The result is line A-B which is often no longer straight. For the same reasons discussed in case of Figure 1, the generation coming from these survivors will give an ld-p line lying be-

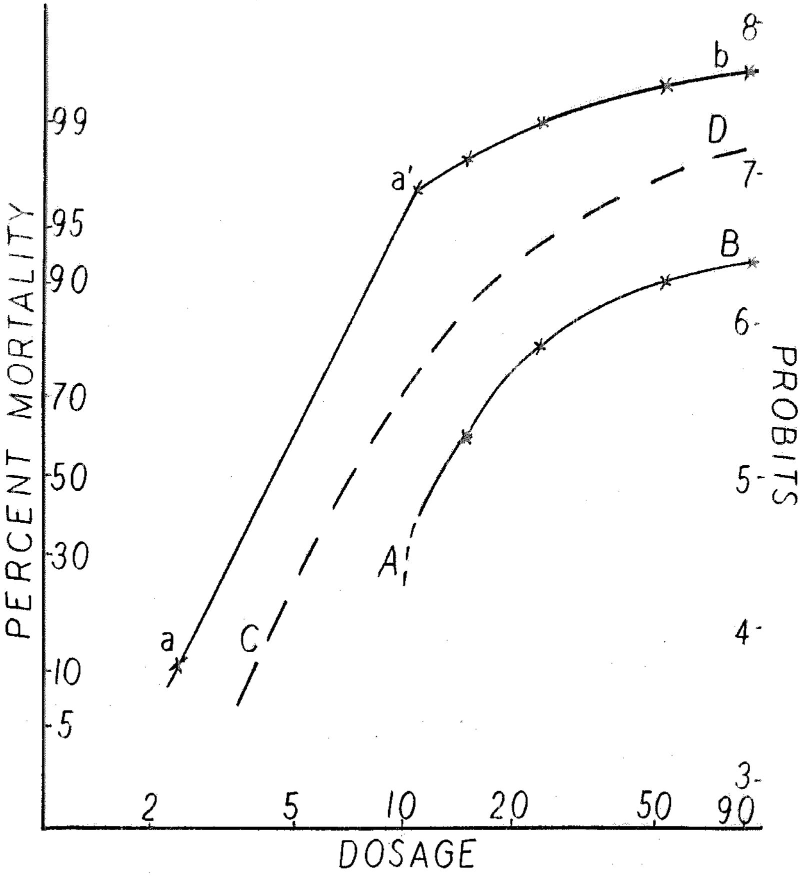

FIG. 2.—Dosage-mortality lines for a population having individuals off the straight ld-p line, a-a′-b; of survivors from 95 percent selection, A-B; of next generation, C-D.

tween lines a-b and A-B, e.g., such as C-D. This situation differs from the former one, however, in the very important point that since the original population was not restricted to a *straight* ld-p line, the succeeding generations will move increasingly to the right and reach a level of resistance determined chiefly by the most resistant individuals in the population before selection began.

It will be obvious from the foregoing discussion that the prime prerequisite for development of specific resistance under pressure from an insecticide is the presence in the population of certain individuals carrying in their genetic makeup the character or characters that cause resistance. These may be an enzyme that detoxifies the insecticide, abnormally impermeable integument or gut wall, extra fat that acts as a trap for absorbed insecticide, etc., etc. In any species controllable by a given chemical such individuals and their peculiar genetic characteristics are rare. The problem of determining if resistance can arise, before there is any practical evidence of it, is then to detect a rare property in the population.

If insecticide resistance should be associated with some visible character such as darkening of the integument, even close examination of many individuals would be feasible in order to detect the rare ones having this peculiarity. Unfortunately no such simple associated marker has been found except in special cases, in which the association appears to be by chance (Wiesmann, 1947; Bigelow and LeRoux, 1954). As yet, the only method for detecting resistant individuals is use of increasing dosages. If they are very rare it will be impossible to be certain that any are present, for no testing method is completely efficient. It cannot be emphasized too strongly that large populations must be used. In small groups the rare resistant individuals may be totally absent though heterozygous individuals carrying recessive genes for resistance may be present. For similar reasons, laboratory strains derived from a few ancestors are certain to lack many characters present in field populations because a small group cannot typify the whole species.

Several situations may be distinguished. If a population before use of a certain insecticide has a steep ld-p line, e.g., 3.5 or greater, and no individuals lying off the ld-p line at high dosages are found in groups of several hundred individuals, it is safe to conclude that at the worst, resistance will be very slow to develop and the effects of vigor tolerance will not make use of the chemical impractical. If the original ld-p line is rather flat, say having a slope of 2, but again all individuals seem to be upon it, the effect of control measures will be to raise the LD 50 a few fold only. On the other hand, if even a few individuals lie to the right of the ld-p line, that is sure evidence that genuine resistance can be developed, the degree depending upon how resistant these aberrant individuals are.

With a population under pressure from an insecticide, the customary procedure is to look for changes in the LD 50, or better, in the LD 90 or 95, as use of the chemical continues. If the population shifts toward a higher proportion of less susceptible individuals, because of removal of the more susceptible ones, the result is a decrease in slope. There is an increase in LD 50 and a greater increase in LD 90. It must be noted that this can result only from individuals lying to the right of the original ld-p line, for as noted earlier, in the absence of such individuals, elimination of the weaker ones *increases* the slope of the line. This is the reason for regarding a flattening of the ld-p line as sound evidence that resistance is developing. (Hoskins & Gordon, 1956.)

When resistance has developed to the level that an appreciable number of the treated insects are surviving and the damage characteristic of the species is occurring, the question arises of what units should be used to express the resistance. No single answer is possible. To take an extreme case, with red scale and hydrogen cyanide, control of at least 99 percent was needed in order to forestall serious damage to citrus. Hence the dosage (expressed in various terms) necessary to secure 99 percent mortality of scales at the optimum stage for control procedures was used as a measure of the resistance. For most agricultural pests and for insects of medical importance such high control is not required. Also very large numbers must be available for this level to be determined with accuracy. The LD 50 is not a good measure, for ordinarily considerably over half the population must be killed if the control program is to be useful. Consequently it is common practice to use the dosage for 90 or 95 percent mortality. Since the ld-p line steadily becomes flatter in the early stages of resistance, the dosage for this high mortality increases rapidly and significant differences are obvious by mere inspection. The slope should be included in reporting on tests for resistance since the whole ld-p line is determined by the slope and one dosage-mortality point. Even slight differences in the test method used often cause marked changes in the apparent relative susceptibilities of different strains. Comparisons then are meaningless. For this reason the work of the WHO in introducing and popularizing standard techniques for use with mosquitoes, lice and other insects of medical importance is extremely valuable. Unfortunately, standardization of testing methods for agricultural pests has made little progress.

It sometimes happens that severe selection brings the proportion of highly resistant insects to such a high level that mortality cannot be driven beyond a certain percent regardless of the dosage, i.e., the ld-p line tends to become flat. In such cases it is not possible to determine the LD 90 or 95 and the best expression of the resistance is the dosage beyond which further mortality is slight.

While detection and measurement of resistance are important, for purposes of long-range planning, prediction of the effect of proposed control programs is of prime interest. Some typical questions are:

(1) Can a satisfactory level of control be obtained without resistance soon making it impractical?

(2) Can sources of infestation from untreated areas offset the selection process?

(3) If use of a chemical is stopped before it becomes useless, will it be of value again after a waiting period?

(4) In case control may be applied at different stages of the insect's life cycle, how can a choice be made to minimize development of resistance?

(5) Will use of combined chemicals bring on resistance to the combination more rapidly than if they are used alternately or in sequence?

(6) Should chemicals that are highly toxic to predators and parasites be avoided no matter how effective they are against a pest species?

Doubtless many other similar questions will come to mind.

Satisfactory answers can be given to few such questions since the circumstances in particular cases often have decisive effects. One general guide is that the more severe the selection in the field, the more rapidly resistance will come on and the higher it will reach. This is not necessarily true with populations of limited size such as laboratory colonies, for if only a very few survivors are left to propagate the next generation, these may lack various genetic factors making for normal rate of growth and fertility. Whenever resistance is a rare property it is associated with some biochemical or physiological behavior that is unfavorable, such as the longer larval life found in houseflies. When natural tolerance is marked, the factor(s) for resistance is either neutral or even favorable for growth and reproduction. Natural tolerance is by no means rare for it occurs in case of any species which cannot be killed by the dosages used in control of other species. Thus grasshoppers in general are tolerant toward DDT and among mosquitoes, *Culex fatigans* is considerably less susceptible than related species (Wharton, 1951).

From the facts that severity of selection controls the rate at which resistant individuals become concentrated in the population and that resistance is a biological handicap, it follows that at least in theory a level of control can be maintained if properly set in the first place. Whether or not this is satisfactory from an agricultural or medical standpoint is determined by other factors. It might mean that the public would have to be satisfied with a lower quality of foodstuffs and learn to tolerate a few mosquito bites. The present attitude of the Food and Drug Administration against even a few insect parts in or on foods is incompatible with development of this idea.

The point just discussed is related closely to the matter of how intrusion of insects from untreated areas will affect development of resistance. In any practical control program some insects escape contact with the insecticide or receive a dose insufficient to prevent normal breeding. These then dilute the selected group and if further dilution occurs from outside, the breeding stock will contain a considerable fraction of susceptible individuals. To put this semi-mathematically, consider that the 5 percent survivors shown by curve A-B in Figure 3 (repeated from Figure 2) are joined by an equal number from outside having the same susceptibility pattern as the original population in the treated area. By adding the contributions from the two groups the discontinuous solid line DEF is obtained as the ld-p line for the parents of the next generation. With individuals homozygous for susceptibility now present in the breeding group it is obvious that the ld-p line, c-d, for the next generation will move farther to the left, largely overcoming the effect of selection. This is especially likely to occur with nonpersistent insecticides. It is possible to calculate the fraction that must move into a treated area (or simply escape contact with the chemical) in order to maintain a level of susceptibility but little different from that prevailing in the absence of treatment. If this happens to be within the tolerable control range, a situation very favorable for long-continued use of the insecticide is at hand. It often has been suggested that untreated areas be left in order to prevent or delay the development of resistance. The equivalent of this is spot treatment instead of area-wide application, as sometimes is done in fly or mosquito control. Even the rearing of a susceptible strain and liberation at a time when they would do least harm is not beyond reality in some cases.

All long range programs for insect control through chemicals should have as a basic consideration, the tendency of practical populations to revert toward their original condition whenever the pressure from an insecticide is lessened or withdrawn. It may happen in laboratory strains that heterozygotes are eliminated by strenuous selection so there are left only individuals homozygous for resistance. These are characterized by a steep ld-p line and inability to regress unless more susceptible individuals get into the colony from outside or, more rarely, an appropriate mutation occurs. But field populations will always contain individuals carrying genes for susceptibility and it is of practical interest to inquire if cessation of insecticide treatment for a time or use of a chemical having an entirely different mode of action, will enable a genuinely fresh start to be made.

Perhaps the best studied example of reversion in a large field population are house flies in Denmark where DDT was discontinued in 1949 when it was no longer effective. After six years in which chlorinated hydrocarbons were not employed, only a few percent of the flies were still resistant to DDT or chlordane (Wichmand, 1956). A similar sequence occurred with house flies in Southern California after intensive use of DDT and other chlorinated hydrocarbons was stopped in 1952. A very disturbing phenomenon has been observed upon reapplication of an insecticide to field and laboratory populations which had regressed from resistance. Thus in Denmark it was found that after two applications of DDT the field population of house flies once more was nearly all resistant. At first sight this would seem to mean that the resistance came about the second time through a different mechanism than that operating during the first use of DDT. However, the Danish flies separated sharply into susceptible and resistant individuals as shown by the ld-p line which had an almost horizontal portion in the central range of dosage. This probably signifies that many resistant flies were homozygous. Since the fraction of resistant individuals decreased during relaxation of DDT use only to 5–10 percent of the population, it is to be expected that upon reapplication, the homozygous individuals producing only resistant offspring would very quickly predominate. If the initial low fraction of resistant flies had been fully reached there is no reason to doubt that as long a period of use would have been possible as during the first years of control. The moral seems to be: use of a chemical must be stopped when resistance is still at a low level or an excessive period of recovery will be required to restore the original susceptible condition.

One further point may be mentioned in

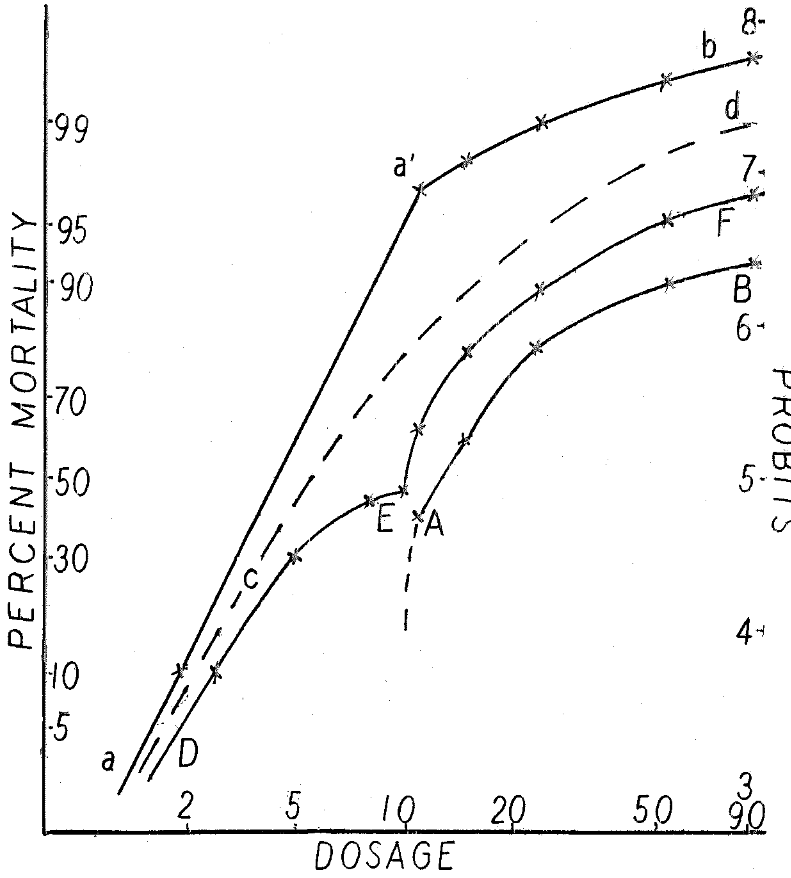

Fig. 3.—Dosage-mortality lines for a population having individuals off the straight ld-p line, a-a'-b; of survivors from 95 percent selection, A-B; of these survivors plus an equal number from original untreated population, D-E-F; of next generation, c-d.

connection with this matter. In the previous discussion there was no occasion to inquire whether resistance is due to one or to several factors. When one, such as the dehydrochlorinating enzyme of house flies, is the controlling factor, the genetic situation is relatively simple and the classical crossing tests may be made with susceptible and resistant strains to test for dominance, sex-linking, etc. And if the factor is either strongly dominant or recessive, it is possible to separate a population into two groups by use of a discriminating dose which kills all susceptibles and leaves all resistant ones. This is the case with the house flies studied by Keiding in Denmark (Wichmand, 1956). But if a number of biochemical or physiological processes are concerned to a significant degree in resistance, their effects enter to varying extents in different individuals and sharp separation into groups is impossible. Another point is that in the original susceptible population individuals will have a mild resistance because of any one of the factors, but no one insect includes them all. During selection, as resistant individuals contribute genetic material to their offspring, these isolated factors tend to congregate and eventually individuals are produced containing several of them. When this linking of resistance factors has once occurred, the level will be higher than in the original colony. Under this situation it is no longer true that resistance only approaches that of the most resistant individual in the original colony. Also such highly resistant individuals will reproduce their kind and some will survive during periods of pressure relaxation. Under renewed exposure they can very quickly make up a significant part of the population since the long delay associated with bringing the various factors together is not required. This doubtless is the reason why resistance is quickly restored in some insects, e.g., roaches (Fisk & Isart, 1953).

The success of insects as a form of life is due in large measure to their enormous reproductive potential. Dr. L. O. Howard once estimated that from a single pair of house flies starting in the springtime a total of 1.9×10^{20} progeny might be produced by fall—sufficient to cover the earth to a depth of nearly fifty feet. It is obvious that in this as in other cases an enormous natural mortality occurs. Chemicals make only a slight further reduction in numbers. It follows that development of resistance may be lessened if they are in such a manner and at such a stage that the *chemical selection* is at a minimum. As an example, take flies or mosquitoes which may be controlled either in the larval or adult stage. If 95 percent larval mortality is secured by a chemical, the survivors after undergoing natural mortality will give a certain permissible adult population. On the other hand, a certain treatment of adults will reduce the population to the same level. In either case the parents of the next generation will have survived exposure to the chemical and it might appear that the situations are identical with respect to development of resistance. But there are at least two differences. With no application to the adults there is more chance for intrusion of outsiders and consequent diminution in the rate of resistance development as discussed earlier. On the other hand, in early larval treatment there is no sexual discrimination and hence surviving males and females will in general have equal resistance factors. But adult treatment eliminates all but the most resistant males which then transmit this strong factor to their progeny. The two effects seem to work in opposite directions. It is unfortunate that this and similar theories have not been checked as yet by quantitative study of larval versus adult selection with recognition and proper control of the numerous conditions affecting the results.

Any consideration of the relative roles of natural and chemical control should take account of parasitic and predatory arthropods which in many cases are the chief destructive agents. These are subject to the action of insecticides to varying degrees, but some classes such as Hymenoptera and Diptera are very susceptible to most insecticides. In numerous instances destruction of the beneficial species by a chemical of wide effectiveness, such as parathion, has resulted in a regular outburst of pests after the effects of the chemical have worn off. The moral here is to use chemicals in such a manner and at such a time that the beneficial species are damaged as little as possible. Fortunately this principle is receiving increasing attention in modern economic entomology. A good example is the cooperative program between agricultural entomologists and biological control specialists in suppression of the spotted alfalfa aphid in California. Chemicals are considered as emergency weapons to be used only when natural control is inadequate, as early in the growing season or in any region before enemies of the aphid have become established. There doubtless are numerous situations in medical entomology in which the same principles are applicable.

The foregoing discussion has been concerned with what might be called a negative approach to resistance, i.e., methods for delaying its onset. It is of interest to consider the positive aspect—how to reverse the development without abandoning the responsible insecticide. The most obvious method is use of a second chemical. Synergists are materials of little effect by themselves but potent in restoring the effectiveness of an insecticide to which resistance has developed. A considerable number have been found for DDT. Most are structurally related to DDT but the first one, piperonyl cyclonene (Perry and Hoskins, 1950), is also a pyrethrin synergist of no obvious relation to DDT. Experience to date is that resistance to the combination develops after a period of use when it is employed widely and there seems to be no reason to believe that synergists can have more than a transient role in the resistance problem.

Simultaneous use of two insecticides having different modes of action has been suggested on the theory that resistance to one would not lessen the effectiveness of the other and hence resistant individuals would continue to be killed by the combination. This can at best only delay development of resistance and it is of interest to inquire whether the period of usefulness of the two materials used together would differ from the sum of their two periods if used in sequence. Crow (1952) has given calculations indicating an advantage for use in sequence. The calculations are based upon the assumption that the populations remain normally distributed in respect to susceptibility. The fact that the ld-p line becomes far from straight, as discussed earlier, is a consequence of the population departing widely from normality and hence these calculations cannot be valid for actual cases. At present there is no answer to the question and in any event, the final result is a condition of multiresistance which already is characteristic of insects in many parts of the world.

Addition of a second insecticide on the theory that what one misses the other will kill, is sound theoretically only if the second material is more toxic to resistant individuals than to susceptible ones. This idea has prompted several laboratory investigations aimed at effecting this "inverse selection." Mitlin *et al.*, (1956) found that among a series of chlorinated hydrocarbons of the general formula $(RO)_2P(O)OC(HCl)CCl_3$, the isopropyl compound was more toxic to DDT-resistant house flies than to sensitive ones by nearly threefold. Later Kearns (1957) exposed a fly population originally containing only 5 percent DDT-susceptible individuals to this chemical and in three generations obtained a susceptible population.

Following the discovery (Ascher and Kocher, 1954) that a Swiss strain of highly DDT-resistant house flies was knocked down more quickly than a susceptible one by alkali bromides administered orally, Ascher (1957a) tested bromo-, chloro- and iodo-acetic acid and certain esters by tarsal contact against DDT-resistant and -susceptible strains of *M. domestica* and *M. vicina.* The R strains were knocked down more quickly by cetyl bromoacetate and certain other compounds showed a more rapid toxic action to the R *vicina* than to the S strain. This work was further elaborated with tests against house fly strains selected by exposure to diazinon, chlordane or chloroacetic acid (Ascher, 1958). In all cases the knockdown by cetyl bromoacetate was about 50 percent more rapid with the resistant strain.

Another example of reverse selection was discovered by Ogita (1958a) who found that addition of phenylthiourea at the rate of 5 millimolar solution to the larval diet of several races of *D. melanogaster* suppressed the emergence of DDT-, BHC- and parathion-resistant strains but not of susceptible strains. With phenylurea the opposite result was obtained since emergence of susceptibles was completely suppressed by 50 millimolar solution which had little effect upon the resistant strains. For practical application Ogita (1958b) made a mixture of DDT and phenylthiourea in which the DDT was just effective against PTU-resistant individuals and the PTU was just effective against DDT-resistant individuals resulting from a cross of a DDT-resistant and a DDT-susceptible strains.

Cole and Clark (1959) have attempted to reduce the resistance of a DDT-resistant strain of body lice by selecting for breeding those individuals which were knocked down first by the compound 3-*p*-chlorophenoxy-1,2-propane oxide. After 20 selected generations there was no diminution of the resistance to DDT. However, it was not shown that this compound has a greater effect upon R than upon S individuals so this work really was not based upon the principle of inverse selection.

Instead of attempting to find a chemical effective in killing resistant insects, the same purpose may be served by one which lessens reproduction. Thus Ascher (1957a) found that female house flies, both susceptible and resistant, laid fewer eggs if they were allowed to walk on a deposit of di(*p*-chlorophenyl)-trifluoro methylcarbinol or the corresponding pentafluoro ethyl compound before being given milk to drink. Since live spermatozoa were found in the spermatheca and the ovaries were developed, this appears to be a case of forced retention of eggs. Similar experiments with female *Aedes aegypti* mosquitoes also resulted in lowered egg deposition. In this case contact was with the compound on filter paper for 30 minutes followed by 24 hours without food before a blood meal (Ascher, 1957a). There was no effect on longevity or feeding.

In these experiments of Ascher with house flies and mosquitoes no greater effect was found upon resistant than upon susceptible strains and hence the effect has no tendency to undo the resistance. But in certain experiments of Hunter *et al.*, (1958) DDT-susceptible and -resistant female flies were treated with sublethal amounts of diazinon to which each strain was susceptible. The estimated potential adult offspring per female (based on number of egg clutches, number of eggs per clutch, percent fertility, survival to adult stage and percent of resulting females laying eggs) was 78.5 percent of normal in the resistant strain and 101 in the susceptible strain, which therefore was favored to increase in an exposed mixed population.

It should be noted that none of the various procedures to reverse selection have been tried with populations under natural conditions and hence their practical value cannot be even estimated.

This brief review of insecticide resistance has ignored many important subjects and passed over others too lightly. It has not been directed especially toward mosquito control problems since these are being discussed by experts in the subject. If any special lesson is to be drawn, perhaps it is this: all living organisms are naturally in a dynamic balance of incredible delicacy. Any act that disturbs this balance will bring a reaction, for Nature has many resources. Man's alterations had best be made with a pointed spear which touches the desired area as lightly as possible rather than with the broadsword which cuts a heavy swath through the whole vicinity.

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