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Physiological Resistance and Development of Resistance in Field Populations ⁴⁶⁷

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ABSTRACT

The report of physiologic resistance to DDT in *Anopheles sacharovi* Favre in 1951 was the first indication of resistance in a malaria vector. Today more than 35 species are resistant to DDT or dieldrin. Fourteen species are resistant to both compounds. A loss of susceptibility to both insecticides is found in several major vectors, such as *A. sacharovi* in Europe, *A. albimanus* Wiedemann in Central America, *A. stephensi* Liston in the Middle East, *A. sudaicus* (Rodewaldt) in Indonesia and *A. gambiae* Giles in Africa. Resistance to DDT and dieldrin relates to 2 independent genetic factors. In some instances the resistance factor is dominant; in others, the hybrid individuals may also be as

susceptible as the susceptible parent. The latter is more characteristic of DDT resistance, which fact partially explains its slow development as contrasted to the explosive nature of dieldrin resistance. On the whole, physiologic resistance has not been a major handicap to the overall malaria eradication efforts except in relatively restricted situations such as *A. stephensi* in the Middle East. Resistance to DDT and dieldrin can be overcome by resorting to organophosphorus compounds, but the latter course not only augments the cost of malaria eradication but also involves a higher level of technical competency and administration than necessary heretofore.

In 1951 *Anopheles sacharovi* Favre was reported to be resistant to DDT following 4 to 5 years' use of this insecticide in a nationwide campaign to control malaria in Greece (Livadas and Georgopoulos 1953). This report marked the 1st confirmed instance of a malaria vector developing resistance to an insecticide, a fact somewhat disheartening to countries where the fight against malaria had not met with the degree of success characteristic of the eradication effort in Greece. Since that time there has been a steady increase in the number of vector species that have become resistant to DDT, dieldrin and/or lindane (Busvine 1956; Brown 1958, 1960; Quarterman and Schoof 1958; Schoof 1959). Such resistance has now occurred to some degree in the major vectors of malaria of every global area. In certain instances, a species has become resistant to one or more of the insecticides used in its control in one endemic area, while remaining fully susceptible to the same compound in another locale. Thus, in Central America the vector *A. albimanus* Wiedemann is resistant to DDT in several countries, yet in many parts of these countries susceptible populations prevail despite the widespread use for many years of this chemical in house spraying and agriculture. To date no less than 35 anopheline species have shown resistance to one or more insecticides. Resistance to dieldrin and lindane is present in 34 species, and DDT has induced this type of response in 14 species.

had been susceptible to a given toxicant. Some authors may consider such populations as naturally resistant or refractory to the insecticide involved. Physiologic resistance is distinguished from behavioristic resistance by the fact that in the former the population can survive contact with and absorption of the toxicant, whereas in the latter the behavior of the population is such that its contact with the toxicant is limited. Whether behavioristic resistance is a result of a response change caused by exposure to the insecticide or is a natural pattern of the species is a moot question, principally because methods for its detection and measurement have not been standardized. Fortunately, the same does not apply to physiologic resistance for which standardized procedures and test kits have been devised, not only for mosquitoes but for a host of other arthropods.

Inasmuch as the term "resistance" is interpreted variously, let me define it at the onset of this discussion. Although several definitions have been proposed for physiologic resistance, the term essentially indicates that an insect population has developed the ability to survive exposure to dosages of an insecticide which previously had caused the death of the greater majority of the specimens exposed to it. This definition excludes a population or species which never

The significance of physiologic resistance in vector control led to an early search for methods to determine its presence. The initial technique for resistance determinations in mosquitoes was that developed by Busvine and Nash (1953). Another method devised by Fay et al. (1953) was subsequently revised (Mathis et al. 1959). The components of these techniques served as guidelines for the standard procedure that was adopted by WHO to measure resistance in adult mosquitoes (Anonymous 1960). The prime breakthrough with the WHO technique was the preparation of papers uniformly impregnated with Risella oil solutions of DDT or dieldrin that remain usable for 4 to 6 years. Concurrent with the appearance of a standard technique to determine resistance in mosquito adults was a procedure to evaluate larval susceptibility. Such techniques have been instrumental in detecting and confirming resistance whenever this phenomenon has developed in field populations of mosquitoes.

The appearance of resistance in species apparently is governed by numerous factors including the type of insecticide, the frequency of exposure to the compound, the genetic and physiologic makeup of the species, the stage of insect exposed, and its biotic potential and behavior. The relative significance of these elements is obscure in many instances. However, as to the relative tendency of anopheline populations to develop resistance to DDT or to dieldrin, it is quite apparent that most anopheline populations lose their susceptibility to dieldrin much more rapidly than they do to DDT. Such a response can, in part, be attributed to irritancy of DDT to the adult mosquito and in part to the genetic factors involved. Dieldrin resistance appears to be more absolute than that of DDT, which sometimes can be thwarted by more frequent insecticidal treatments or by increased dosages.

Experience has shown that the genes for resistance may or may not be present in a given population, a fact that has been a source of confusion, since attempts are frequently made to ascribe the resistance problem on a species rather than population basis. An example of this aspect is *A. gambiae* Giles in Africa. In West Africa this species has developed a high level of resistance to dieldrin, in some cases after only 18 months of its use in house spraying. However, in East Africa (Tanzania) such resistance has not been detected despite the routine application of dieldrin to native huts over a 3000- to 4000-mile² area for a 3-year period, during which time *Culex pipiens fatigans* Wiedemann (Smith 1958a), and other arthropods such as bedbugs *Cimex hemipterus* F. (Smith 1958b), and lice (Anonymous 1959) became resistant to dieldrin. Recently though, distinct evidence of dieldrin resistance in *A. gambiae* has been found in the Sudan and Madagascar (Hamon, personal communication) and Kenya (Bruce-Chwatt 1970).

A second example of the discontinuous aspect of resistance in a species has occurred with *A. quadrimaculatus* Say in the United States. Despite the widespread use of DDT in treating more than 2.5 million houses over the southeastern United States for malaria eradication in 1945-47 (Bradley 1966), *A. quadrimaculatus* never displayed any loss of susceptibility to DDT, even though the concurrent and greater use of the same chemical in agriculture placed the species under additional selective pressure. However, in 1952, Kruse et al. reported that the species was less susceptible than it had been previously to DDT larvicides used in the Tennessee Valley Authority malaria-control program, a dosage of 0.1 lb/acre now being required vs. that of 0.05 lb/acre previously. Subsequent work (Anonymous 1955) revealed that the assumed resistance was due to other causes, and Hawkins et al. (1958) reported that DDT remained the insecticide of choice in the Tennessee Valley Authority area after 13 years of repeated applications.

In 1954 studies were undertaken at the Technical Development Laboratories, Savannah, Ga., to select *A. quadrimaculatus* for resistance to DDT. Because the Tennessee Valley Authority had reported a loss of effectiveness in its DDT larvicide (Kruse et al. 1952), mosquitoes were obtained from TVA, colonized, and exposed to various types of DDT pressure as adults and/or larvae. Despite selection at a 99% level for 12 months, no loss of susceptibility could be demonstrated that was not eliminated after 1 generation without selection. After 2 years of negative results, the project was abandoned on the assumption that *A. quadrimaculatus* might well be a species incapable of developing resistance to DDT.

In 1956 Mathis et al. reported a dieldrin-resistant population of *A. quadrimaculatus* from Bolivar County, Miss., in areas where the principal exposure of the mosquito to dieldrin presumably occurred from agricultural use of this and related pesticides. However, both DDT and DDT-chlordane house treatments had been made in the area for many years. The adults of the Mississippi populations showed no mortality after 30-min exposure to 2 g of dieldrin/m², but their susceptibility to DDT was the same as that of a laboratory strain.

The fallacy of the premise that *A. quadrimaculatus* could develop resistance to dieldrin but not to DDT was clearly demonstrated 4 years later, when the Corps of Engineers, U. S. Army, at Savannah reported that it was having difficulty in controlling *A. quadrimaculatus* with DDT larvicide application at the Clark Hill Reservoir near Augusta, Ga. (Mathis et al. 1960). At a key inspection site adult counts of this species, after ranging from 4 to 40/inspection during the period 1954-58, had risen to more than 200/inspection in 1959 despite continued larvicidal treatments of DDT. From 1952 to 1959 the annual application of DDT was from 4,000 to 10,000 lb; the amounts for the years 1957, 1958, and 1959 were 9,600, 10,600, and 10,800 lb, respectively. Tests of the DDT used showed it to be fully toxic to laboratory strains of *A. quadrimaculatus*. However, parallel exposure of larvae from the Clark Hill Reservoir and those of a laboratory strain showed that 0.2 ppm of DDT produced 100% kill of the laboratory strain, whereas 2.5 ppm killed less than 50% of the Clark Hill strain. Evidence of resistance to dieldrin was apparent also from the complete kill of the laboratory strain at 0.004 ppm as compared with the 2.5 ppm required for 100% mortality of the Clark Hill strain. Exposure of the adults to 4% DDT papers revealed no kill of the Clark Hill strain vs. 100% mortality of the laboratory strain. With papers containing 0.4, 0.8, and 1.6% dieldrin, the kills of the Clark Hill strain ranged from 28 to 36%, whereas 96% kill of the Savannah strain was produced with the 0.8% dieldrin paper. In the absence of any dieldrin larvicidal applications, the only source of contact with this compound was from agricultural runoff.

The failure of DDT to control the larvae and the confirmed resistance to this chemical in both adults

and larvae resulted in the replacement of DDT with a malathion-lethane formulation. Satisfactory control has been obtained at the Clark Hill Reservoir with this treatment for the past 9 years. Another instance of DDT and dieldrin resistance in Georgia was later reported at Hartwell Reservoir 25 miles north of the Clark Hill impoundment (Mathis et al. 1965). Of interest are the tests made at that time at Clark Hill, which showed that after 2 years of exposure to the malathion larvicides, field-collected adults had little resistance to DDT, but the dieldrin resistance was the same as that in 1959 (Table 1), thus strengthening the premise that agricultural runoff was the source of resistance to this compound.

Numerous other examples of delayed development of resistant anopheline populations or a dispersed pattern of their occurrence are found in the literature. Resistance to dieldrin was first detected in *A. albimanus* in various parts of Central America in 1958, and in several countries populations resistant to both DDT and dieldrin were found. However, in Costa Rica no DDT-resistant populations of *A. albimanus* have been detected to date, despite the almost continuous DDT-house-spraying program that has been in effect since 1950. *A. pseudopunctipennis* Theobald in Mexico, *A. fluviatilis* James in India and Ceylon, and *A. minimus flavirostris* Ludlow, while resistant to dieldrin, still remain susceptible to DDT. This situation prevailed also with *A. gambiae* in West Africa until recently when Hamon et al. (1968) demonstrated DDT resistance in *A. gambiae* species A in the Upper Volta. In Greece, Turkey, Lebanon, and Iran, *A. sacharovi* has shown resistance to DDT (Brown 1960); yet in Italy and Romania the species remained susceptible to this compound despite more than 10 years of its use (Zulueta 1959). Table 2 gives a summary of species resistant to dieldrin and/or DDT.

Thirteen species have displayed resistance to both DDT and dieldrin, the principal examples of this phenomenon being *A. pharoensis* Theobald and *A. stephensi* Liston in the Middle East, *A. sacharovi* in Europe, *A. albimanus* in Central America, and *A. sundaicus* (Rodenwaldt) and *A. aconitus* Dönitz in Java. The double resistance problem with *A. stephensi* in the Middle East was well summarized by Zulueta et al. (1968). The initial suspicion of DDT resistance in this species in Saudi Arabia in 1953 was confirmed in 1955 by Davidson (1958). The sub-

Table 2.—Insecticide resistance in *Anopheles* mosquitoes. Species without an asterisk resistant to dieldrin; with single asterisk (*) resistant to DDT and dieldrin; with double asterisk (**) to DDT only. (From Brown (1968) with modifications.)

<i>aconitus</i> *	<i>neomaculipalpus</i> Curry
<i>albimanus</i> *	<i>munetozari</i> Galbaldo**
<i>albiparvus</i> Lynch	<i>pharoensis</i> *
Arribálzaga*	<i>philippinensis</i> Ludlow
<i>annularis</i> Van der Wulp*	<i>pseudopunctipennis</i>
<i>aquasalis</i> Curry	<i>pulcherrimus</i> Theobald
<i>barbiparvus</i> Van der Wulp	<i>quadrifasciatus</i> *
<i>costani</i> Laveran	<i>rangeli</i> Galbaldo, Covas-
<i>crucians</i> Wiedemann	Garcia, & Lopez
<i>culicifacies</i> *	<i>sacharovi</i> *
<i>flavipes</i> Manalang	<i>sergenti</i> (Theobald)†
<i>fluviatilis</i>	<i>splendidus</i> Koizumi
<i>funestus</i> Giles	<i>stephensi</i> *
<i>gambiae</i> *	<i>strodei</i> Root
<i>labranthiae</i> Falleroni	<i>subpictus</i> Grassi*
<i>l. atroparvus</i> Van Thiel	<i>sundaicus</i> *
<i>maculipennis</i> Meigen	<i>triannulatus</i> (Neiva & Pinto)
<i>minimus flavirostris</i>	<i>vagus</i> Dönitz
<i>m. messiae</i> Falleroni	

sequent spread of DDT resistance in Iran and Iraq led to the replacement of DDT with dieldrin in 1957. However, by 1959, resistance to dieldrin appeared in southeast Iran and soon was common in south Iran and Iraq. Subsequent outbreaks of malaria in Iraq in 1963 resulted in the use of DDT again, since the resistance to this compound was much less than that to dieldrin. Successful results with DDT house spraying extended to its general application in Iraq, and the picture of resistance remained unchanged for several years. However, in 1966, a sharp upswing occurred in the DDT-resistance level which coincided with a marked increase in *A. stephensi* densities and an extension in the range of the species toward the north.

Similar documentation for *A. aconitus* and *A. sundaicus* is found in the reports of Soerono et al. (1965a, b). Resistance to dieldrin first occurred in *A. aconitus* in 1959 after 3 years of dieldrin applications in central Java. Such resistance was evident throughout Java by 1962. In an area of Jogjakarta, central Java, where DDT had replaced dieldrin after 1957, *A. aconitus* populations suddenly displayed a high level of resistance to DDT in 1962 that was coupled with dieldrin resistance. Subsequent surveys in central Java revealed such double resistance characterized *A. aconitus* populations wherever they were checked. However, in the Jogjakarta area, no increase in DDT resistance occurred from 1962 to 1963. The assumption was that the tendency of *A. aconitus* to feed on cattle resulted in a large number of mosquitoes not contacting sprayed surfaces, thereby reducing the selection pressure of the insecticide. In *A. sundaicus* the initial resistance detected was to DDT in western Java in 1955. Dieldrin proved highly effective against such DDT-resistant populations for 3 years, at which time the program was temporarily halted. Two years later susceptibility tests revealed no resistance to either compound in the Semarang area. None-

Table 1.—Percent mortalities of *A. quadrifasciatus* adults collected at the Clark Hill Reservoir, Ga., 1959 and 1962, and exposed to DDT/dieldrin Risella oil papers.

Toxicant (%)	1959	1962
DDT	1.0	0
	2.0	1
	4.0	0
Dieldrin	0.4	28
	.8	31
	1.6	36
		46

theless, subsequent studies disclosed DDT-dieldrin-resistant strains in central and eastern Java, southern Sumatra, and North Borneo. The agricultural use of dieldrin and DDT played a role in the development of such resistance in these species; in 1 instance the resistance of *A. aconitus* to dieldrin was solely a result of the use of agricultural pesticides.

Although the development of suitable field techniques for detecting resistance in field populations made it possible to detect this phenomenon throughout the world, the value of the methodology lies in the interpretation of the data obtained, particularly in regard to the application of such findings to the problem of controlling the vector. Some workers have tended to use the data as the criterion on which to base the control approach, without giving proper attention to the fact that there are numerous factors that frequently cause the failure of control measures. Difficulties have arisen in deciding the relative importance of slight changes in response, the effect of season on the susceptibility of a vector, the significance of the LC_{50} vs. the LC_{95} or LC_{100} , and the interpretation of a reduced response in relation to the need to change insecticides. Unfortunately, time has not provided all the answers to these queries. However, today we have a much better understanding of resistance and its development.

The general concept of the development of resistance is that in an insect population a small number of individuals possess the genes that produce physiologic resistance. In most instances such individuals would not be apparent in a population, but the exposure of the population to the selective action of an insecticide eliminates or reduces the number of susceptible individuals and results in an increase in the number of resistant individuals present. Whether, and how rapidly, this type of mosquito becomes the dominant element of the population depends on the insecticide, how general the treatment is, the genetic makeup of the population, and upon other factors. When only a segment of the adult population is exposed, the unexposed and largely susceptible portion may dilute and keep the resistance level of the population as a whole at a low level. The composition of a mixed population of resistant and susceptible individuals is reflected in the shape of the dosage-mortality regression line. Susceptible homogenous populations show a linear response, whereas populations containing a resistant element show a flattening or plateau response. The level at which this plateau effect occurs reflects the proportion of resistant individuals present. Laboratory studies of mixtures containing different percentages of susceptible and resistant individuals from which samples were drawn at random and exposed to various dosages of dieldrin showed this effect (Mathis et al. 1959). When the resistant individuals represented 20% of the caged populations, the dosage-mortality line showed a plateau effect at the 80% mortality level (Fig. 1). With 40% of the population resistant, the plateau occurred at the 60% mortality level. Thus the ab-

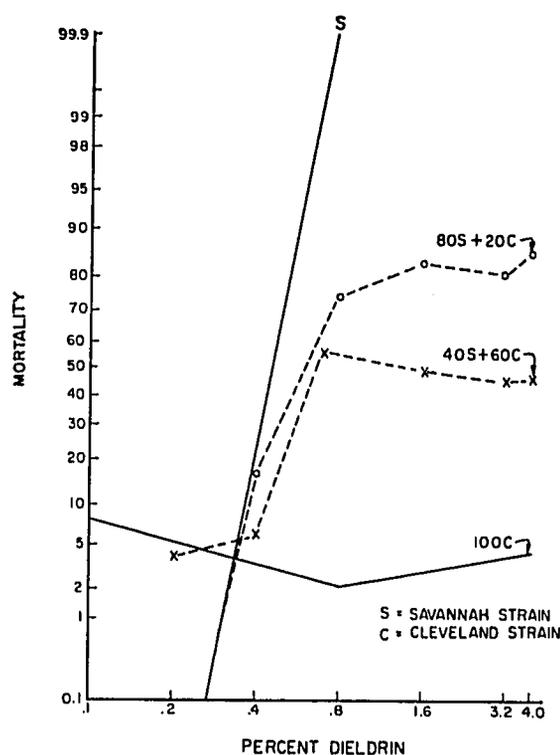


FIG. 1.—*A. quadrimaculatus* mixed population-dieldrin.

sence of any increase in kill with the increase in dosage is indicative of a resistant element in the population. The wholly susceptible strain had a typical linear response, whereas the highly resistant strain (Fig. 1, Cleveland) showed less than 10% kill despite the 20-fold increase in the percentage of dieldrin.

To answer the question of how important a change in response is to the control effort, one must look first at the purpose and reliability of the technique involved. The method for determining susceptibility was devised to give us a tool whereby we could determine if difficulties in the control program were due to the resistance of the vector to the insecticide in use. Essentially, the control failure is a symptom; the susceptibility test is one means of determining its cause.

On any program periodic susceptibility determinations will enable a close check on the response of the mosquito population to the insecticide. Reduced kills at dosages that formerly were lethal to 90-100% of the population indicate the need for further testing to see whether the results are due to normal variations or to an actual change in response. The season of the year, temperature, number of test specimens, nutritional state, vigor, and other factors (Shalaby 1968) may be responsible for the change in response that occurs. Where slight resistance to DDT is apparent, discriminating dosages can be used to determine if the proportion of susceptible vs. resistant individuals in the population is changing.

Although the susceptibility test gives information on one possible cause, obviously there are others, any one of which could be equal to or greater in significance than resistance. Inadequate treatment, poor suspensibility of the formulation, exceptionally high densities of the vector, breakdown of the insecticide on the surface material treated, changes in mosquito behavior, all represent other possible causes of control failures. Thus, the results of the susceptibility test must be weighed against the other possibilities before any specific decision is made on the course of action. *In no case should a change be made on such test data per se.*

To stress the periodic assessment of the physiologic response of the anopheline mosquito and not give consideration to other characteristics of the insect would be both misleading and erroneous. The genetic variability that brings about the development of physiologic resistance may also affect other facets of mosquito biology so the selection pressure leads to populations that may differ in their biology from their forebearers. In *A. pseudopunctipennis* in Mexico changes that have occurred in a population are reported to have caused an increase in the number of specimens entering a treated hut and in the number of escapees (Zulueta 1964). Such changes, coupled with a low level of physiologic resistance, might well exert a deleterious effect on the efficacy of an eradication program, whereas the modified susceptibility response in itself might be unimportant.

Considerable progress has been made on the genetic background of resistance, since dieldrin resistance in *A. gambiae* was reported by Davidson (1956) as simple Mendelian inheritance. Studies on several species (*A. sudaicus*, *albimanus*, *gambiae*, *stephensi*) have revealed that the resistance to DDT and to dieldrin, and related cyclodiene compounds, relates to 2 independent genetic factors. In most strains of *A. gambiae* resistance to dieldrin involves incomplete dominance of the resistance factor so offspring from a cross of susceptible and resistant parents are intermediate in their response to the insecticide. Davidson (1956) reported that 0.33%¹ and 4.0% dieldrin-impregnated papers could serve as discriminating dosages to separate the susceptible, intermediate, and resistant groups. While this type of semidominance for dieldrin resistance is general, a strain of *A. gambiae* from the Ivory Coast contains a dieldrin-resistant gene that is completely dominant (Davidson and Hamon 1962). With this strain, any cross of susceptible and homozygous-resistant individuals results in individuals that survive exposure to both 0.4 and 4.0% dieldrin papers.

The single genes responsible for DDT resistance usually are recessive, and consequently DDT is lethal to heterozygous individuals. Thus, resistance to DDT presents less of a field problem, because the susceptible individuals form a large segment of the popula-

tion. The reverse would be true for dieldrin resistance. Field experience with these 2 compounds supports this viewpoint, dieldrin resistance being relatively explosive in its appearance, whereas resistance to DDT is slow to develop to the point where control failures occur.

Much has been written on the impact of resistance on the chemical control of arthropods, and without question there are numerous instances where this factor has led to a revamping of the chemical control approach. However, when we look at the effect of resistance on malaria-eradication programs, it is apparent that to date this problem has been of a critical nature in only a few instances.

In many cases the question resolves itself into whether the level of resistance is such as seriously to hamper the control program. The initial resistance of *A. stephensi* to DDT in the Middle East was associated with the presence of adults in sprayed houses and an increase in malaria incidence. When dieldrin replaced DDT, the vector practically disappeared in the treated areas. However, within 18 months after dieldrin was substituted for DDT in Iraq, resistance to it also was apparent and at a level much more critical than that previously evident for DDT. As a result, DDT was again used to curb an outbreak in Iraq in 1963 (Zulueta et al. 1968), and the spraying markedly reduced the densities of *A. stephensi* in sleeping quarters. Later in 1964 the use of DDT was extended to the southern and central regions of that country. However, in late 1966, the number of *A. stephensi* surviving in treated bedrooms increased about 25 times that observed the previous year. This augmentation coincided with a decrease in the susceptibility of the vector and a rise in the malaria incidence (Anonymous 1967).

In Central America, the failure to achieve eradication apparently is due to a combination of factors that may or may not include physiologic resistance, since the disease persists in areas where the principal vector, *A. albimanus*, is susceptible to DDT. After the substitution of dieldrin for DDT stimulated a rapid and widespread onset of dieldrin resistance in 1958, DDT was reintroduced. Despite its use alone or supported by drug therapy, high rates of malaria continue to occur in many areas. Since the failure to eradicate malaria in the areas where *albimanus* is the vector arises essentially from the behavior of *albimanus* and not in its resistance to DDT, one must look at the latter as possibly a contributing, but not as a critical factor in the persistence of malaria in those countries. In such situations the question arises also as to the feasibility of the residual house spraying technique alone as a means of disrupting malaria transmission in this region.

In Indonesia, Soerono et al. (1965a) reported that DDT resistance in *A. aconitus* did not cause any increase in malaria transmission principally because of the depletion in the parasite reservoir brought about by the effective eradication program that preceded

¹The standard 0.4 and 4.0% dieldrin papers in the World Health Organization kit serve for this purpose.

the development of resistance in the vector populations.

Aside from these examples, there are numerous other reports of resistance in important vectors (e.g., *A. sacharovi* and *A. culicifacies* Giles) which had an effect on their control but did not materially influence the progress of the malaria eradication effort.

Thus, when one considers the overall impact of physiologic resistance upon the progress of malaria eradication, the obvious conclusion is that in itself resistance (particularly that associated with DDT) has not been a major stumbling block to malaria eradication efforts. This viewpoint does not differ greatly from that expressed by Muirhead-Thomson and Bruce-Chwatt (1964) who stated "In general the areas in which this (physiologic) resistance has developed represent only a small part of the total geographic distribution of each vector species. There are several major vectors of malaria which remain completely susceptible to DDT despite many years' exposure to insecticide pressure." The basic assumption that residual treatments of homes in malarious areas would interrupt transmission has proved valid with various vectors in different countries but, at the same time, serious questions have been raised against it in countries where the habits of the vectors and/or of the people are such that house spraying exerts only a slight effect on the transmission cycle. Even in Ceylon and India, where the disease apparently capitulated to the vector control attack, recent resurgences of malaria have emphasized that constant vigilance is required to eradicate the disease.

Nonetheless, it must be remembered that in several instances, resistance has proved to be a critical barrier to the success of a program. Such a situation now prevails in parts of the Middle East where DDT resistance in *A. stephensi* has reached such a level that malaria transmission has increased despite good spray coverage, an additional spray cycle, and 2 chemotherapeutic applications (Anonymous 1967). Of interest is the inference that the 3rd round of DDT treatment in the Fao area of Iraq may have favored the development of a highly resistant population of *A. stephensi* since, in other areas where only 2 spray cycles were used, the resistance levels remained the same as in the previous years. However, as was pointed out in the report (Anonymous 1967), factors other than resistance were involved in the Fao developments. The 2nd area where the use of DDT might be considered of limited value is Java, where *A. sundaiensis* and *A. aconitus* have developed resistance to it and to dieldrin in several localities. However, as previously mentioned, Soerono et al. (1965a) felt that such resistance had not seriously influenced the progress of the program up to that time. The termination of the eradication program sponsored by the Agency for International Development in 1964 has forestalled further evaluation of the influence of this resistance.

However, the fact that resistance has thwarted con-

trol of several mosquito species, including the anophelines previously mentioned, makes this phenomenon a potential and dangerous threat to any program dependent for success on chemical control methods. Thus, one must seek ways of preventing or retarding resistance development or of overcoming it. To date, only a few suggestions appear valid. One is to restrict the use of insecticides to adult stages wherever feasible, since the application of larvicides tends to hasten the development of resistance, inasmuch as a greater proportion of the population in the area of treatment is exposed to selection pressure. Where larvicides must be used, and apparently this may be the case in some areas, the compound used against the larvae should be of a type different from that employed against the adult (i.e., organophosphorus vs. chlorinated hydrocarbon). A reduced number of cycles may also be of benefit if the situation in Iraq with *A. stephensi* can be interpreted in that light. Consideration might be given also to the use of 1 rather than 2 g of DDT/m² where multiple cycles are desirable. It would appear logical that in situations where the heterozygotes are similar to the susceptibles in response to DDT, the selection pressure should be kept at a level that would prevent the recessive homozygous-resistant specimens from assuming dominance in the population.

All the previously suggested approaches are based on the premise of lowering the pressure of the selective measures, thereby lessening the chances of resistance occurring or of it reaching a critical level. However, it must be recognized that careful regulation of the regime of anopheline control practices may not accomplish the prevention anticipated because of the agricultural use of insecticides. The absence of DDT resistance in Costa Rica as contrasted to other countries in Central America is ascribed by some workers as being due to the limited amount of agricultural pesticides used in that country, particularly by aerial application. In Java the resistance of *A. aconitus* and of *A. sundaiensis* can be attributed in part to agricultural pesticides, and the same is true for *A. quadrimaculatus* in the United States.

To overcome resistance to a specific chemical, the obvious solution is to switch to another type. At present the 2 alternatives are principally DDT and dieldrin or benzene hexachloride. Other materials that have been found effective against DDT-dieldrin resistant strains are malathion, fenthion,² fenitrothion, and propoxur (Baygon®). Unfortunately, each of these compounds has a shorter residual life than the chlorinated hydrocarbon toxicants, and each is much more costly. Malathion is considered to be promising against DDT-dieldrin resistant *A. sundaiensis* and *A. aconitus* in Java; in Uganda it has been shown to interrupt malaria. The other compounds are still in the experimental stage of field

²In the 16th Report of the WHO Expert Committee on Insecticides on the Safe Use of Pesticides, fenthion was not considered suitable for routine indoor spraying because of the possible adverse effect on spraymen.

evaluation. As yet, none of these compounds have been used sufficiently to know what changes in response would occur in the vector populations. However, several culicine mosquitoes have developed resistance to organophosphorus compounds, and the absence of such resistance among the anophelines can be assumed to be due largely to their minimum amount of exposure to such pesticides. Some reports from Central America (Bruce-Chwatt 1970) have indicated a lowered response to malathion in *A. albimanus* which could well have emanated from the large-scale use of organophosphorus compounds in agricultural spraying.

From an overall viewpoint, the conquest of physiologic resistance appears to depend upon the adoption of measures that will prevent the development of super-resistant populations. Such tactics would be combined with an integrated type of control effort whereby biological agents, genetic mechanisms, therapeutic measures, and common sense would be brought into play to reduce the dependence that is now placed on residual house spraying as a means of decimating the disease. It likewise would lessen the chance of resistance development and decrease the amount of insecticidal contamination. Unfortunately, the shift to such an approach will not only increase the cost of malaria eradication but will also involve a higher level of technical competency and administration than heretofore required, the lack of which constitutes already important factors in malaria eradication in developing countries.

In summation it can be stated:

1. Physiologic resistance to DDT or dieldrin occurs in more than 35 anopheline species, 14 of which are resistant to both compounds. Dieldrin resistance develops much more rapidly than DDT resistance, and the latter frequently is not at a high enough level to hamper eradication efforts. Two independent genetic factors are involved; one confers resistance to DDT, the other to dieldrin and lindane. In many areas anopheline vectors have remained susceptible to DDT despite its use for periods of 10 years or greater.
2. Physiologic resistance in itself has not disrupted the progress of the overall global malaria eradication program except in a few specific areas such as in the Middle East, where the vectors are resistant to DDT and dieldrin.
3. In many areas physiologic resistance may contribute to the failure or the slow progress of malaria eradication, but its role is comparatively minor in relation to other critical problems that exist in the administrative and technical phases of program operation and to the lacunae in our knowledge of vector biology.
4. Insecticide resistance poses a constant threat to programs that depend for their success on chemical control measures. With increased emphasis on other types of chemical treatment, particularly those that place the mosquito population under greater selection pressure, the future significance of resistance may augment considerably.
5. At the present time the principal remedy for resistance is the use of alternate insecticides. Compounds that are effective against dieldrin- and DDT-resistant populations include malathion, propxur, and fenitrothion, each of which is far more costly and has a shorter period of residual activity than either DDT or dieldrin.
6. The application of insecticides for the control of agricultural pests has been an important factor in the development of resistant vector populations. In some instances such treatments apparently have been the principal cause of resistance to certain compounds occurring in anopheline mosquitoes.

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Discussion of Paper by Dr. H. F. Schoof

DR. DAVIDSON.—Dr. Schoof has not mentioned anything about the possible use of mixtures of insecticides to prevent the appearance of resistance. In the past many people have dismissed the idea of the use of mixtures because of their experience with house flies which developed resistance so readily to a whole range of chemicals, though as far as I can find out, this has been through their consecutive use and not through their immediate use as mixtures. It is some 7 years since I was involved in trying mixtures in Java, and we did some preliminary trials using mixtures of benzene hexachloride (BHC), DDT, dieldrin, and malathion. We were working in an area where the population of *A. sundaicus* was already resistant to dieldrin. We did find that mixtures of BHC and malathion, and dieldrin and malathion, did select against dieldrin resistance, that is to say that mosquitoes that escaped from a hut treated with a mixture showed a lower level of dieldrin resistance than in the general population. I do understand that very recently WHO is showing a renewed interest in the use of mixtures, and I would very strongly support this, not just a mixture of 2 insecticides, but perhaps 3 or even 4. Mixtures of prophylactics have been used with considerable success, and I was hoping that Dr. Clyde might say something about that this morning. I gather that some of the French workers had considerable success with the use of mixtures of prophylactic drugs in the prevention of the appearance of resistance.

DR. SMITH.—Several years ago we made some selection experiments with house flies, *Musca domestica* L.; bedbugs, and cockroaches, in which we exposed nonresistant colonies to a chlorinated hydrocarbon and an organophosphorus insecticide in mixtures in each generation and alternately in alternate generations. In each instance we developed resistance to both kinds of insecticide, at a speed which was just about proportional to the selection rate at which they were used. I question whether some of the drug combinations are based on experimental evidence that they prevented the development of resistance under conditions in which resistance developed to the components when used separately. As you know, the Food and Drug Administration has recently withdrawn registration of several drug combinations on the basis of invalid claims. I think in the absence of some experimental indication that susceptibility to 2 specific insecticides is negatively correlated, their use in mixtures in the hope that the combination will prevent the development of resistance will probably be futile and possibly wasteful. Through development of resistance to both at the same time, the usefulness of the 2 might be lost more quickly than if they had been used consecutively. This is, of course, in complete contradistinction to a situation in which one insecticide is needed to control one species and a different insecticide to control a different species, and both species must be controlled with the same application, or a combination of a quick knockdown chemical and another which gives long residual action.

Just a random mixture of insecticides doesn't appear to me to be very promising.

DR. SCHOOF.—Dr. Davidson was quite correct in that WHO was giving further consideration to the potential of mixtures at the Laboratory Director's meeting in its last 2 sessions. But I might say also that the idea has been received with mixed emotions. The difficulty is in trying to evaluate this procedure. At the last gathering it was decided that there ought to be some work done, not only with mosquitoes, but also with some other insect that's more plastic, such as the house fly. I can't quite agree with Dr. Davidson on use of 4 compounds. We have enough problems right now with one. If we ever get four in the field it certainly is going to complicate some aspects, and I think it will also increase the costs, and that is one of the major stumbling blocks to the use of compounds other than DDT.

DR. HAMON.—The use of mixtures of insecticides, at least against adult *Anopheles*, presents some difficulties, because the residual efficacy of the various components of the mixture will be very different. Also, in some places in the world, such as West Africa, we have had very little selection of resistant *Anopheles* populations by health department application of insecticide. Most, if not all, of our resistant populations came from BHC dusting of cocoa and coffee in the south, DDT and endrin dusting of cotton in the middle belt, and DDT-BHC dusting of the peanuts in the north. And now in the whole of West Africa, dieldrin-resistant *gambiae* populations occur with a very high frequency of the resistant gene. For example, in Mali Republic, where I made a survey some months ago, we have *A. gambiae* resistant to DDT and dieldrin, as well as *A. funestus* dieldrin resistance. In this area no insecticide has ever been used inside houses against anophelines, or against any other pest of humans. Everything comes from cotton dusting, and now the cotton pests are becoming DDT- and dieldrin-resistant, and the agriculture department is beginning to use organophosphates and carbamates. On cocoa I heard they are expecting to use propoxur soon. And it is probable that at least in Africa, where malaria-control operations have been very much delayed, we shall select resistant populations of the vector before any application of the insecticides by the health departments.

MR. JOHNSON.—Another point that Dr. Schoof might want to include in his list is to have a more accurate delimitation in malarious areas followed by selective residual DDT treatment of the houses in areas where malaria is found and no treatment of the areas where there is no malaria. There have been many countries that have sprayed everything whether it had been shown that malaria was present or not. In some countries, for example, they say "all houses up to the altitude of 500 m shall be sprayed with DDT." Just recently one of our men came back from Ethiopia and mentioned that there they are doing this now, although in many areas there is no

evidence that malaria is present. If spraying such areas can be avoided you will not only save money but will delay development of resistance.

MR. GARRETT-JONES.—I think the point just made by Mr. Johnson is a very good one. But if national authorities when planning their spray operations try to play safe by spraying, let us say all houses up to 500 m or whatever is the arbitrary altitude chosen, I feel that this decision must many times be dictated by the impossibility with which they find themselves faced of making adequate surveys everywhere beforehand. So they have to adopt some rule of thumb as a means of economizing on their personnel, if you like, or of avoiding a lot of delay before beginning to spray at all. This links up with a point made by Dr. Schoof in his closing remarks, where he pointed out the possible increased importance of the anopheline resistance problem in the future would entail more technically competent personnel to detect and assess such resistance. I don't know, Mr. Chairman, if it is within the scope of the recommendations you wish to make here, to deal with matters of personnel. But, I should like to give my personal, strong support to any plea for training and placing in the field in malarious countries a larger number of entomologists and entomology technicians than we have seen since the inception of the global malaria-eradication campaign. I believe a great weakness all along has been the lack of provision for training sufficient numbers of men and for orienting the training epidemiologically so that we can have something different from the "straight" medical entomologist whose training, if it was anything like mine, laid the major emphasis on comparative morphology and systematics. Of course, not only do we require more attention to the training of such men, but also to their employment. It is no good training men unless there is some authority to see that those men are then deployed into the field areas where their skill is needed.

MR. CONWAY.—I should like to support Mr. Garrett-Jones in his remarks. It seems to me that so many of the comments that have been made today have pointed out the necessity of seeing the malaria control and the *Anopheles* situation in a total systems framework. What is needed is the training of entomologists from this point of view, training entomologists with a broad ecological knowledge not only of their own eventual speciality, i.e., anopheline mosquitoes, but also of crop pests and the interactions of spraying against crop pests with their own activities against malaria; a knowledge of sociology; a knowledge of agricultural practices; irrigation practices, etc. I think some moves along these lines are being made in some universities in this country, in Canada, and in England. Training at the graduate level in what is called "Systems Ecology." As a former crop entomologist, I was very interested in hearing of the effects of crop spraying on insecticide resistance in *Anopheles*.

Also, throughout the world, resistance of cotton insects to insecticides is becoming so great that very

soon spraying of cotton with insecticides will have to be abandoned or cotton growing will have to be abandoned. The practice now is to spray cotton about 20-25 times season. There have been several major disasters, including a classic one in Peru, where all the major pests became resistant, and even after 25 sprays, no control was achieved and the cotton crop was completely lost. There is now intensive pressure to change to integrated control for both tropical crops and temperate crops—use of biological control agents, use of various other sophisticated techniques—to integrate these methods into a total system of crop pest control. It will be important to integrate control of both crop and medical pests so there is a complete system over which one has control and knowledge. At any rate means must be found to prevent the many scientists in different disciplines working against one another.

DR. GOULD.—One of the questions which was raised by Mr. Garrett-Jones was the lack of competent technical personnel in malariology throughout the world. As we discussed proceeding from the classical insecticide measures of control into more sophisticated approaches, such as genetic manipulation and biological control, it occurs to me that we may be even worse off as far as supply of adequately trained technical help. I don't know how we are going to resolve this problem. Does anybody have any suggestions?

DR. MULLA.—I think malaria-eradication programs were primarily based on knowledge and notions which were developed 20 or 25 years ago. The development of new information has not kept up with the need for more technically trained people, especially people who are doubly trained in biology and ecology. It is extremely difficult to take a particular species of mosquito out of its total environment and deal with the organism without considering the other biotic and abiotic factors. I was surprised to hear Dr. Hamon mention that in the Colombian-Venezuelan area they found *A. nuneztovari* to be the vector at least 20 years ago. This species is exophilic, it rests outside and bites outside, yet residual house spraying has continued for 15-20 years without regard to this biological fact. It seems that most of our programs are stereotyped, and once we get into a rut we cannot get out of it. We need flexibility, where we can change policies, where we can change the approaches, according to new information as it develops.

DR. HAMON.—Mr. Chairman, I want to second Mr. Conway's remarks and not only in malaria eradication. I know a very important program to be developed in West Africa soon in which one of the major obstacles of the program will be the lack of specialized entomologists. It is a vector-borne disease problem which is very important for economic development of the country, and requires 18 specially trained medical entomologists, whereas no more than 4 or 5 are available at the present time.

DR. PLETSCH.—Speaking to the point brought up by Mr. Johnson and Mr. Garrett-Jones in reference to dangers inherent in spraying nonmalarious areas when this possibly might be avoided, the point raised by Mr. Garrett-Jones I believe is untenable. I don't believe that rules of thumb should be applied in initiating programs if the basic epidemiological base lines have not been established. The Taiwan program was mentioned this morning by Dr. Davidson as an exceptional and successful one in the Western Pacific area. It took only 3 months to define the malarious areas of Taiwan with sufficient accuracy to proceed on epidemiological rather than completely administrative grounds. A Rockefeller-type of spleen and parasite survey was accomplished using school children throughout the island, in all the schools throughout the island. In other words, there are quite a number of techniques that are known but not judiciously employed. Subsequently we fall back on rules of thumb, when the flexibility mentioned by the other speakers, if employed, might greatly minimize the extent to which such rules of thumb would have to be applied.

DR. SCITTOF.—From these comments concerning the availability of manpower; if we go back 10 or 15 years, we were talking about the same thing then. I think we must recognize the fact that any program of the magnitude of malaria eradication can't be sold on the basis that we have to study all these different factors before we come up with an answer. We also must recognize that residual spraying did work very effectively in many areas. Unfortunately, we now are left with the hard core of real problem areas and really don't know how to cope with them. We started out with residual spraying, then brought in drugs, and now we are bringing in larvicides. Pretty soon we'll be bringing in approaches such as biological control and genetic mechanisms. In essence, what we are now going back to is a mosquito-control approach in which we use all the tools that are available to control the insects. My only concern now is where will we get the funds to subsidize such an activity, since even now the cost of propoxur, which works better than DDT in areas where DDT resistance is a problem, may prevent its use in such areas.

DR. MILLER.—I am not an entomologist, and I'm a little confused about some of the terminology. Naturally I'm not up on your literature, but when you speak of physiological resistance, your definition reminds me of the treatment of gonorrhoea. It takes a lot higher blood level, but still under practical therapy we can attain that blood level so we do not say it is resistant, we say it is less susceptible. Now with mosquitoes, you can normally expect a 95% kill with a 1% insecticide, and you eventually work up to 10%. Is this physiological resistance or is this less susceptibility? Can you still get your kill with a practical, usable dosage? Second question: with the new test system you described briefly, with the carbamates and organophosphates, where you have a 10-fold

difference in dilution and vary the time, is this method sensitive enough for an operational unit to alter its program, or is this only a screen to indicate that you need a more sensitive titration by a laboratory before you alter your program?

DR. SCHOOF.—Acceptance of the time method was based on the fact that they had enough evidence to show that the time series would give you essentially the same type of information as the dosage series. Now the reason for not switching both test systems was the fact that all our information on DDT and dieldrin is now based on dosage, and there is no reason to change a procedure that is already well accepted and completely satisfactory. With the carbamates and organophosphates, misinformation was being gathered, because the papers did not have any substantial residual life as compared with DDT and dieldrin. As far as switching insecticides goes, on the basis of a resistance test alone you should have additional information, because in any determination of susceptibility a slight change in response can be termed resistance. The point is, what does it mean in practical terms? For example, some species show more than 25-fold resistance based on larvicide tests, but actually when you go out in the field you can kill them just as easily as you did before. Therefore you must base any change of insecticides on field observations and laboratory test data, not on the latter alone. Now to your 1st question about increasing dosage, I think with DDT, for example, you can increase dosage or time and you can kill the mosquito readily with the increased dosage or time period. Most tests are based on a limited time exposure. I mentioned mosquitoes from Mississippi which you cannot kill with a 1-hr exposure to dieldrin. Yet, if you sprayed an experimental hut with dieldrin and left the anopheline adults in the hut overnight, you would kill 50% of the specimens. You can also increase dosage, but when you do, you increase other parameters like cost, application problems, hazards, and other factors. In the areas where we have put on 2 g of DDT year after year, I wonder just how many grams of DDT have accumulated on the wall surface?

DR. ROBERTS.—There is one thing that hasn't been mentioned here that to me as an outsider is a little curious, not having worked with mosquitoes very much. That's the fact that all of this DDT has been applied in the system, and there is a tremendous

amount of complaint with the United States. Yet it seems that on the worldwide scene this same concern doesn't exist. Is this observation correct?

COLONEL ALTMAN.—I would have to refer to someone else for the concern throughout many parts of the world. I know that in certain parts of the world there is even more concern than there is in the United States. It has been banned completely in Sweden, and many other countries certainly are concerned with the problems caused by the persistence of DDT. As far as the hazard to the environment from a residual spray program is concerned, this is certainly less clearly defined. It is my opinion that DDT residual sprays applied inside buildings do not materially contribute to the contamination of the environment.

DR. JEFFREY.—In recent years there have been suggestions of possible biological advantages possessed by strains of parasites which are resistant to anti-malarial compounds, particularly in infectivity to the vectors, etc. I would like to know if there are biological advantages in the anophelines which are resistant to insecticides but which are not directly related to the association of the insecticide and the vector.

DR. SCHOOF.—If you hadn't said that last part, I could give you some examples. I think with *gambiae* resistant strains have certain biological advantages over the nonresistant strains. But without the contact with insecticides? Maybe I'd better ask Dr. Davidson to comment.

DR. DAVIDSON.—I think that dieldrin resistance imparts at least a balanced effect and possibly a slight advantage, because as Dr. Hamon will tell you, in many parts of West Africa, populations of *gambiae* show well over 50% dieldrin-resistant individuals, even without having experienced any insecticides whatsoever. I think that the development to the extent of a large proportion of resistant individuals in the absence of insecticidal selection implies that there are some advantageous characteristics of dieldrin resistance.

DR. SCHOOF.—I think that with almost any strain you maintain in a laboratory, removal of the insecticide as a selecting agent will force the strain to become susceptible. Obviously the susceptible strain in these instances must have some biological advantages.