Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1964, **30**, 57-64

The Conversion of DDT to DDE by Some Anophelines

H. LIPKE [1, 2] & JILL CHALKLEY [1]

The metabolism of DDT has been followed in pure lines of laboratory-reared resistant and susceptible anophelines using gas-liquid chromatography. Relatively large amounts of DDE were formed in vivo *by susceptible strains of* Anopheles stephensi, A. quadrimaculatus *and* A. gambiae, *and by resistant strains of* A. stephensi *and* A. quadrimaculatus. *Resistant and susceptible* A. albimanus *showed no difference in the rate of DDE production. Volatile metabolites of DDT other than DDE were not observed in most of the strains chromatographed. A procedure for the measurement of DDT-dehydrochlorinase in small numbers of mosquitos was devised based on conditions developed for the glutathione-dependent DDT-dehydrochlorinase present in resistant houseflies. This method has shown the enzyme to occur in highest titre in DDT-susceptible* A. albimanus *and* A. stephensi, *indicating little or no correlation with resistance to DDT. Major differences in dehydrochlorination rates and in patterns of resistance between anopheline species have been observed. In* A. stephensi, *these differences extend to the level of strains.*

In the housefly, the appearance of high levels of resistance to DDT is accompanied by the concomitant development of increased titres of DDT-dehydrochlorinase (Lipke & Kearns, 1960). Auxiliary mechanisms of resistance in houseflies and other species have been proposed (Bradbury & Winteringham, 1958; Brown, 1960), but conclusive evidence concerning the significance of these mechanisms has not been forthcoming. The contribution of detoxicating mechanisms to the etiology of resistance in resistant (R) strains may be assessed by measurement of the conversion of insecticide to innocuous products *in vivo* and *in vitro*, followed by comparison of these same properties in susceptible (S) strains.[3] Only where significant differences in the rate of degradation are demonstrable between the R and the S can detoxication be assigned a major role. The enhanced rate of detoxication in the treated strains must in addition be detectable by comparison of appropriate enzyme preparations from both populations to minimize behavioural or mortality differences. The degree to which anophelines fulfil these conditions has not been established and its determination would appear to be a necessary adjunct to the investigation of other biochemical or behavioural phenomena that may be operative in this genus (Micks, 1960; Vargas, 1960; Garms, 1960).

Resistance exhibited by anophelines to DDT, although of considerable practical importance, is generally of a lower order than that observed in houseflies, and is less amenable to detailed biochemical investigation. The first report of DDE formation by DDT-treated anophelines (*A. quadrimaculatus*), was by Gartrell & Ludvik (1954). This metabolite appears in adult *A. sacharovi*, *A. maculipennis*, *A. labranchiae*, *A. stephensi* and *A. claviger* (Perry, 1960). Similar results were obtained with *A. atroparvus* (Frontali & Carta, 1959) and *A. subpictus* (Kalra & Pal, 1959). Other metabolically derived products of DDT have not been reported in anophelines, nor has the successful conversion of DDT and DDE *in vitro*. This contribution describes the extent of conversion by certain anophelines of DDT to DDE as measured by gas-liquid chromatography. The conversion of DDT to DDE by tissue extracts using the conditions known to favour this conversion in the housefly is also

[1] Ross Institute of Tropical Hygiene, London School of Hygiene and Tropical Medicine, London, England.

[2] Permanent address: Physiology Division, Edgewood Arsenal, Maryland, USA.

[3] Throughout this paper R is used to designate DDT-resistance and S to designate DDT-susceptibility. Other abbreviations used are: DDT, 1,1,1-trichloro-bis-(*p*-chlorophenyl) ethane; DDE, 1,1-dichloro-bis-(*p*-chlorophenyl) ethylene; TDE, 1,1-dichloro-bis-(*p*-chlorophenyl) ethane; DTB, 4,4′-dichloro-*alpha*-trichloromethylbenzhydrol; EDTA, ethylene diamine tetraacetic acid; GSH, reduced glutathione.

reported for some populations. The results show DDE to be the major metabolite of DDT, but fail to establish any correlation between insecticide metabolism in the living animal and resistance or the ability to degrade DDT to DDE *in vitro*.

EXPERIMENTAL METHODS AND MATERIALS

Organisms

The S and R strains of *Musca domestica* were kindly furnished by Professor Busvine and Dr Fine of the London School of Hygiene and Tropical Medicine. This species was reared according to the technique of Busvine (1959) and represents normal and Swedish strains previously described (Fine, 1961; Lipke & Chalkley, 1962). Anophelines were reared and tested for susceptibility according to the techniques described by Davidson (1958) and Davidson & Jackson (1961) and represent genetically pure lines isolated at the Ross Institute over a period of years (Davidson, 1958; Davidson & Mason, 1963). Susceptibility was also measured under the conditions described below with no apparent discrepancies between the various modes of treatment. Some characteristics of the strains studied are presented in Table 1, where the treatment periods conducive to the most favourable demonstration of differences in detoxication rates are presented.

DDT treatment

Unsexed mosquitos in groups of 50-200, or 10-20 houseflies, were subdued at 5°C and transferred to round stainless steel cages 13 cm high and 7 cm in diameter. The sides of the cages were lined with a double layer of Whatman No. 1 filter-paper, the innermost of which was treated with a 4% solution of DDT (Geigy, 99.9% *p,p'*) in Risella oil. The DDT concentration was 40 mg/100 cm.[2] The base of the cage rested on a pad of moist cotton-wool. Control groups were similarly handled, but for the absence of toxicant. Following recovery of half the population from cold anaesthesia, usually after 5-10 minutes, exposure proceeded for the intervals described in Table 1. The filter-paper was then withdrawn by a slight displacement of the cage lid and the subjects held at −20°C for a few minutes until quiescent. Transfer was effected to Potter-Elvehjem grinders equipped with loose-fitting pestles and containing spectral grade 2,2,4,-trimethyl pentane (1 ml/20 mosquitos) and Pyrex glass ground to 200-400 mesh. After homogenization for 2 minutes, initially at 300 r.p.m. and finally at 800 r.p.m., the extract was filtered through No. 1 paper containing 4 g anhydrous Na_2SO_4 and stored at −20°C following the addition of one drop of refined liquid paraffin to reduce volatilization of the insecticide and metabolites. Housefly extracts contained 5 flies/ml trimethyl pentane. External DDT and DDE were removed from the flies prior to grinding by rapid rinsing in acetone.

TABLE 1

RESISTANCE TO DDT OF ANOPHELINES EXPOSED TO FILTER-PAPER IMPREGNATED WITH 4% DDT IN RISELLA OIL

Species and strains [a]	Origin	Nature of resistance	Exposure time (minutes)	Percentage prostrate
A. albimanus				
S(PALB)	Panama		60	85
S(ITALB)	Panama		30	70
R(LLTT)	El Salvador	DDT, dieldrin	60	3
A. quadrimaculatus				
S(QC)	S. Carolina, USA		50	85
R(QMY-1)	Maryland, USA	DDT, dieldrin	270	10
A. gambiae				
S(TAV)	Kenya		60	80
S(Bobo)	Upper Volta	Dieldrin	50	50
A. stephensi				
S(SS)	India		60	50
S(YF)	Iran	Dieldrin [b]	60	75
R(IQ)	Iraq	DDT	120	1
M. domestica				
R(Swedish)	Sweden	DDT, pyrethrin	720	5

[a] Resistant strains prefixed by R, susceptible by S.
[b] Formerly R to DDT and dieldrin, reverted to S for DDT.

Gas-liquid chromatography

We are indebted to the staff of the Shell Woodstock Agricultural Research Centre, Sittingbourne, Kent, for all the gas chromatographic analyses as well as for the development of this method of revealing

submicrogram quantities of insecticides and metabolites rapidly and definitively. The procedure has been described in detail by Goodwin et al. (1961). In brief, the extracts are chromatographed on 24-inch columns, using both polar and non-polar stationary phases, and detected by an electron-capture ionization detector. The polar column is formed of 1% polyethylene glycol 4000 on 100/120-mesh Celite. The non-polar column consists of 2.5% silicone elastomer E301 plus 0.25% Epikote resin 1001 on 100/120-mesh Celite. Both columns were operated at 163°C with O_2-free N_2 as carrier gas.

The retention times of DDT and the various analogues examined are shown in Table 2. It can be seen that the peaks for DDT and DDE and 4,4′ - dichloro - *alpha* - (trichloromethyl) benzhydrol (DTB) (Agosin et al., 1961) are well separated on both columns. The experimental error is ±5% and ±10% for DDT and DDE respectively. The presence of interfering material in mosquito extracts at 4.3 minutes on the non-polar column prevents any positive identification of DTB.

All the insecticides and derivatives with the exception of DDT were gifts from the Rohm & Haas Corporation, Philadelphia, Pa., and were recrystallized from ethanol before use. Good agreement was obtained between the results for DDT and DDE obtained from chromatography on the polar and non-polar stationary phases with all the extracts subjected to chromatography. The results with gas-liquid chromatography were confirmed in the case of the housefly by reverse-phase paper chromatography of dehydrochlorinase reaction media (Bridges, Harrison & Winteringham, 1956; Mitchell, 1957) and showed only DDT and DDE to be present.

TABLE 2

MEAN RETENTION TIMES (IN MINUTES) OF DDT AND ITS ANALOGUES FOR GAS-LIQUID CHROMATOGRAPHY [a]

Compounds	Column [b]	
	Non-polar	Polar
p,p′ DDT	14.3	12.9
o,p DDT	10.3	7.3
DDE	7.8	4.1
TDE	7.8	4.1
Dehydrochlorinated TDE	4.1 (major) 2.7, 7.8 (minor)	3.2 (major) 1.3 (minor)
4,4′-dichloro-*alpha*-(trichloromethyl)-benzhydrol (DBT)	4.3 (major) 7.8 (minor)	3.3
Mosquito extracts [c] (Risella oil only)	1.0 (major) 4.3 (minor)	3.2 (minor)

[a] Minor components represent impurities or decomposition products comprising 2%-10% of the total.

[b] See the text and Goodwin et al. (1961) for explanation of polar and non-polar columns.

[c] Mosquitos treated as described in text in absence of DDT.

Assay of DDT dehydrochlorinase

Initial studies using the glass-bead technique of DDT-dehydrochlorinase assay described by Sternburg et al. (1954) showed a rate of DDE formation in the R flies of 39 mμmoles/mg protein/90 minutes. No dehydrochlorination was evident with the S strain of flies or with any of the mosquitos tested by this method. It appeared that a more sensitive assay was required if low levels of enzyme were to be measured. A technique for the estimation of DDT-dehydrochlorinase using small amounts of lipoprotein to solubilize DDT has been described in which the dehydrochlorination proceeded to completion (Lipke & Kearns, 1959); the reliability of this method has been confirmed by radiometric analysis.[1] The lipoprotein method has been modified for mosquito extracts although the extent of reaction is incomplete, probably due to the presence of an inhibitor in the concentrated unclarified lipoprotein preparations. The dependability of the modification used with anophelines was established using enzyme from *Musca domestica* (R) prepared according to procedure B of Sternburg et al. (1954) and was also verified with the high-speed supernate of housefly homogenates, which exhibit lower dehydrochlorinase activity.

Preparation of enzyme extracts

From 600 to 1000 anopheline adults were ground with the aid of glass powder in pre-chilled mortars containing 5 ml of homogenization medium. The composition of the medium was as follows: Na and K phosphate buffer, 0.05 M; EDTA, 1.5×10^{-3}M; GSH, 3×10^{-3}M; bovine serum albumin 0.5%; the mixture was adjusted to pH 7.0. The mash was filtered through muslin (previously boiled) and centrifuged at 5000 *g* for 15 minutes at 1°C and assayed immediately.

[1] Personal communication from Dr R. D. Elliott, Communicable Disease Center, Savannah, Ga., USA.

Incubation of extracts

For DDT-dehydrochlorinase analysis, lipoprotein was prepared by the method of Lipke & Kearns (1959, 1960) and dialysed against 4% Na_3 citrate containing 0.05% EDTA for 24 hours. After saturation with DDT, the material was diluted with an equal volume of citrate and stored at 5°C. For assay, 0.09 ml of lipoprotein-DDT was added to 0.45 ml of enzyme and 0.10 ml of 3×10^{-3}M GSH, all held at 0°C under N_2. The reaction was initiated by placing the mixture in a water-bath at 37°C with agitation while gassing was continued. After 120 minutes, the reaction was stopped with 0.70 ml of concentrated H_2SO_4 and the products were analysed as described below. Routine controls included lipoprotein without DDT as well as a replicate where the reaction was stopped at time zero.

DDT and DDE analysis

In the preparation of alumina for chromatography, Al_2O_3 (BDH Ltd., Alumina for chromatography) was heated for three hours at 380°C and suspended in 4 litres of 0.12 N-HCl. The absorbent was washed free of Cl^- with water, suspended three times in 4 litres of methanol and heated to 70°C for 12 hours. After washing with petroleum ether (60°C boiling point) the alumina was activated by heating to 180°C for 4 hours. For chromatography, columns of 0.9 cm internal diameter containing 5 g of absorbent were prepared immediately before use. A 1-cm layer of anhydrous Na_2SO_4 was placed above the alumina on the columns. No degradation of DDT or DDE was mediated by alumina prepared in this manner.

Analysis of enzyme preparations

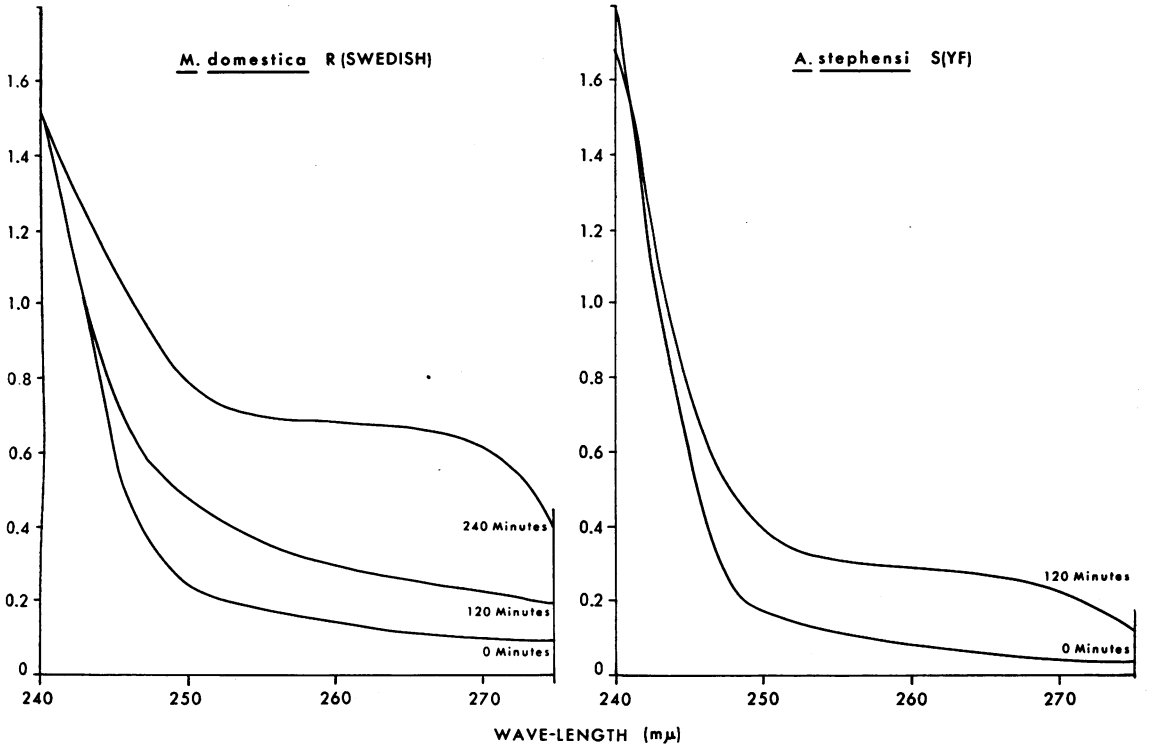
To the sulfuric-acid-treated reaction mixture were added 10 ml of water; the tube was stoppered tightly and held at 100°C for 15 minutes. The digest was diluted to 50 ml with water and extracted in ungreased separatory funnels with 50 ml of petroleum ether (40°C boiling point) which had previously been passed through an alumina column. The aqueous phase was discarded and the ether washed once with water, once with 1% NaOH and again with water until neutral to phenolphthalein. The ether extract was washed with saturated NaCl solution and filtered through No. 1 paper containing 10 g of anhydrous Na_2SO_4. After the addition of one drop of refined paraffin the extract was taken to dryness with a gentle stream of air at room temperature.

The sample was transferred to the column with 5 ml of petroleum ether followed by 30 ml of petroleum ether containing 0.5% redistilled ethyl ether. The first 5 ml of effluent were discarded and the remainder of the effluent containing essentially all of the DDT and DDE was collected and taken to dryness after the addition of a drop of paraffin. Each of the preparations was dissolved in 4 ml of ethanol or trimethyl pentane and the absorbance recorded in a Hilger Uvispek spectrophotometer at 230, 241, 245, 250, 260, and 275 mμ. Blank values from the zero time samples or those devoid of DDT were substracted, the values were adjusted for equal absorbance at the isosbestic point for DDT and DDE (241 mμ), and the differences due to DDE at 260 mμ were taken as evidence of DDE formation. The shapes of the absorption spectra were identical with those obtained using this procedure with various mixtures of lipoprotein-DDT and lipoprotein-DDE to which enzyme extracts had been added immediately following addition of the sulfuric acid. These procedures are modifications of methods reported by Sternburg & Kearns (1952) and Sternburg et al. (1954). Data treated in this manner are presented in the figure, which shows the spectral changes accompanying DDE formation. It can be seen that the absorbance at 260 mμ due to DDE formation is a function of the incubation time. Evidence that the 260-mμ-absorbing material observed in the experiments with mosquitos is DDE is restricted to the general shape of the absorption spectrum, which is identical with that obtained with authentic lipoprotein-DDE similarly processed, and to the chromatographic mobility of the reaction product on alumina when compared with authentic DDE.

RESULTS

In addition to detecting DDT and congeners, gas-liquid chromatography can reveal the presence of the large number of volatile ether-soluble organic halides which can be formed by mild or extensive degradation of the parent compound. Table 3 illustrates that detectable amounts of DDE are formed in all the groups analysed, no correlation with resistance being evident. In the case of *A. stephensi*, strains S(SS) and R(IQ) form approximately equivalent amounts of DDE, whereas the reverted strain S(YF) produces relatively little DDE. The DDE formed by R(IQ) may be even less than that formed by S(SS) during the first 60 minutes of treatment. A similar pattern was observed with

ABSORPTION SPECTRA OF REACTION PRODUCTS OF DDT-DEHYDROCHLORINASE[a]



[a] The system contains: Na and K phosphates, 0.05 M (pH 7.0); glutathione, 3×10^{-3} M; bovine serum albumin, 0.5 %, lipoprotein-DDT, 0.09 ml; enzyme extract 0.45 ml, volume 0.65 ml. Incubated at 37°C under N_2 for indicated interval. Sample preparation described in text.

A. quadrimaculatus S(QC) and R(QMY-1), where the disparity between DDE formation and time of treatment is even more pronounced. The ability of 97% of the R(LLTT) *A. albimanus* to withstand 60 minutes of exposure to DDT (Table 1) is not accompanied by a corresponding increase in DDE production; no significant difference in this capacity is evident relative to the S(ITALB) individuals, 70% of which become prostrate in 30 minutes. The widespread occurrence of the DDE-forming mechanism in anophelines is made more evident by the identification of this olefin in the three S strains of *A. gambiae* as well as in a culicine (*Culex fatigans*) which was also included in this series. With respect to the R housefly, about 25% of the dose is dehydrochlorinated during the interval of treatment with essentially complete recovery.

The occurrence of significant amounts of metabolites other than DDE appears unlikely in most of the strains examined, since complete removal of the chlorine from the parent molecule would be required to escape detection in the gas chromatogram. One exception may be *A. albimanus* R(LLTT), where a peak in the region of DTB was observed. Exposure of *A. stephensi* S(YF) for 30 minutes occasionally produced a component with mobility similar to DTB. This was lacking in populations treated for longer periods, e.g., of 60-minutes' duration (Table 3). Further characterization of this benzhydrol-like substance requires the elimination of an interfering material in the ether extracts which sporadically obscured the benzhydrol region of the chromatograms. Efforts to this end were not successful. Resistance to dieldrin in *A. gambiae* and *M. domestica* is not accompanied by extensive metabolism of the toxicant (Winteringham, Harrison & Davidson; [1]

[1] Winteringham, F. P. W., Harrison, A. & Davidson, G. (1960) *Absorption and metabolism of ^{14}C-labelled aldrin by susceptible and resistant mosquito larvae.* In: *WHO Information Circular on Insecticide Resistance*, No. 21 (unpublished).

TABLE 3

GAS CHROMATOGRAPHIC ANALYSIS OF FLY AND MOSQUITO EXTRACTS FOLLOWING TREATMENT WITH DDT OR DIELDRIN [a]

Species and strain	Treatmen		Total amount recovered (μg)		
	Insecti-cide	Time (mi-nutes)	DDT	DDE	Un-iden-tified
A. stephensi					
S(SS)	DDT	60	3.9	0.20	None
S(YF)	DDT	60	9.0	0.02	None
R(IQ)	DDT	120	6.5	0.20	None
A. quadrimaculatus					
S(QC)	DDT	50	3.0	0.20	None
R(QMY-1)	DDT	270	9.4	0.17	None
A. albimanus					
S(ITALB)	DDT	30	1.9	0.02	None
R(LLTT)	DDT	60	2.4	0.03	Hydrol-like
A. gambiae					
S(TAV)	DDT	60	12.0	0.30	None
S(Bobo)	DDT	50	2.5	0.20	None
S(Bobo) [b]	Dieldrin	60	5.0 [d]		None
C. fatigans					
(Lagos)	DDT	60	4.0	0.14	None
M. domestica [c]					
R(Swedish)					
Internal	DDT	1 440	6.0	1.8	None
External	DDT	1 440	2.0	0.20	None

[a] Susceptible (S) insects were sacrificed when prostrate, resistant (R) insects at incipient prostration.

[b] S(Bobo) strain resistant to dieldrin but not to DDT.

[c] *M. domestica* recoveries per fly following topical application of 10 μg DDT.

[d] Value represents μg dieldrin recovered; no metabolites evident on chromatograms.

TABLE 4

DDT METABOLISM BY ANOPHELINE EXTRACTS AND IN THE INTACT MOSQUITO

Species and strain	DDE formed *in vivo* [a]	DDE formed *in vitro* [b] (μmole)
A. stephensi		
S(SS)	+++	None
S(YF)	+	0.056
R(IQ)	+++	None
A. quadrimaculatus		
S(QC)	+++	None
R(QMY-1)	++	None
A. albimanus		
S(ITALB)	– [c]	0.046
S(PAL)	+	0.022
R(TTLL)	+	0.002
A. gambiae		
S(TAV)	+++	None
S(Bobo)	+++	– [c]

[a] Data from Table 3; + = < 0.05 μg DDE recovered by gas chromatogram; ++ = 0.05-0.19 μg; +++ = > 0.20 μg.

[b] Assay conditions as in the figure; incubation period 120 minutes. Quantities of DDE less than 0.002 μmole are reported as "None".

[c] No test run.

Brooks, 1960). These observations are confirmed and have been extended to the dieldrin-resistant strain of *A. gambiae* S(Bobo), where only dieldrin was observed in the volatile fraction which registered on the detector (Table 3).

Table 4 presents the information regarding DDT-dehydrochlorinase. It can be seen that little or no relation is evident between the presence of a GSH-dependent dehydrochlorinase in cell-free extracts and the degree of resistance. A marked lack of correlation between the ability to dehydrochlorinate DDT *in vivo* and the dehydrochlorinase activity of the enzyme extracts was obtained. For example, the greatest titre of dehydrochlorinase, 0.056 μmole/120 minutes, was observed in *A. stephensi* S(YF), which formed DDE relatively slowly *in vivo*. The S(SS) *A. stephensi*, although capable of DDE formation *in vivo*, produced little or no DDE enzymatically. The results with a resistant *A. stephensi*, strain R(IQ), emphasized the diversity of response in this genus, there being little dehydrochlorinase *in vitro* but significant dehydrochlorination in the intact mosquito. No dehydrochlorinase could be detected in the *A. quadrimaculatus* or *A. gambiae* strains, even though significant levels were observed *in vivo*. In *A. albimanus* high levels of dehydrochlorinase were associated with susceptibility rather than with resistance. The S *A. albimanus* strains appear somewhat similar to *A. stephensi* S(YF) with respect to moderate to high dehydrochlorinase activity coupled to limited dehydrochlorination *in vivo*.

DISCUSSION

The evidence for different mechanisms of resistance within a genus, first suggested by Bradbury & Standen (1960), constitutes the major trend of the findings (Table 4); the demonstration of a dehydro-

chlorinase in mosquitos is of minor importance. Most of the S strains produced significant amounts of DDE following treatment. In two cases, *A. stephensi* and *A. albimanus*, higher titres of DDT-dehydrochlorinase characterized the S strain rather than the R strain. The most resistant of the species studied, *A. quadrimaculatus*, showed no demonstrable DDT-dehydrochlorinase and no relation of resistance to detoxication *in vivo*. Table 4 presents evidence that strains can produce low, intermediate, or high levels of DDE and still be classified as resistant. Diversity of the same type is observed in the S strains. These anomalies can in fact be viewed to the effect that dehydrochlorination is without major significance in these members of this genus as a mechanism of resistance.

The conversion of DDT to DTB has been reported in *Culex quinquefasciatus* by Agosin et al. (1961) and Dinnamarca et al. (1962). Some decomposition of DTB occurs on the polar and non-polar columns as well as occasional interference from the mosquito extracts (Table 2). It appears, however (Table 3), that large accumulations of this metabolite do not occur in any of the strains tested, although traces might be present in *A. stephensi* S(YF) and *A. albimanus* R(LLTT).

Consideration of the evidence concerning the anophelines must be preceded by considerable qualification. (1) The chromatographic method evolved makes no distinction between absorbed and unabsorbed toxicant, and does not allow for differences in pick-up rates. (2) The water-soluble fraction of the mosquitos was discarded for the analyses. (3) Some strains may degrade DDT or DDE to compounds not visible on the chromatograms which would be overlooked in the absence of data accounting for the entire dose received by the insect. (4) Marked differences (Table 3) in exposure times were required in order to accentuate metabolic differences with respect to DDT degradation. (5) No detailed investigation of the optimum conditions for the enzymic reactions were undertaken. (6) Strain S(YF) of *A. stephensi*, whose performance in the various trials was particularly unusual, was originally a DDT- and dieldrin-resistant strain which had reverted to S for DDT, but whose dieldrin-resistance was unimpaired. (7) Definitive identification of all the metabolites was not attempted. (8) Finally, the method of exposure to a DDT residue did not allow behavioural patterns to come into play (Trapido, 1954).

The sensitivity of the gas chromatographic procedure (about 10^{-10} g of organic halide) and the simplicity of sample preparation makes possible the determination of the levels of insecticide pick-up and degradation in very small numbers of field-caught anophelines. No effort was made in these studies to ascertain the minimum number of mosquitos that could be analysed by the procedure described, but it would appear that as few as 10 mosquitos can be homogenized and chromatographed with significant results.

One of the objects of this study was to judge the suitability for field stations of gas chromatography for the rapid detection of DDT metabolism in mosquitos from treated areas. Consequently, relatively crude petroleum-ether extracts were employed, with the intentional avoidance of clean-up procedures. The procedure described appears adequate for the detection of DDE, but the possible occurrence of the benzhydrol in some strains indicates that some elimination of interfering substances is necessary. The method is capable of further refinement for the analyses of enzyme incubation mixtures. In this respect, Berger (1961) has recently described a modification of the direct spectrophotometric assay for DDT-dehydrochlorinase that may eliminate the shortcomings of the lipoprotein assay.

With the appearance of resistance in anophelines, considerable discussion has been devoted to similarities and differences of resistance patterns characteristic of this genus in relation to other resistant genera. The present data indicate that modes of resistance may in fact diverge at the level of strains within a species.

ACKNOWLEDGEMENTS

To his colleagues in the Ross Institute the senior author is especially indebted for their counsel and assistance. Special gratitude is expressed to Professor G. Macdonald and Mr G. Davidson, who presented the opportunity to take up a US Public Health Service Fellowship and to utilize the facilities of the Institute. Dr R. A. E. Galley, Research Director of Shell Woodstock Agricultural Research Centre, kindly offered the co-operation of that organization. Mr Kenneth Elgar and staff of the Analytical Section provided the knowledge and effort to make

possible the presentation of the chromatographic information. Much apparatus was made available through the kindness of Dr W. E. Ormerod, Dr E. Stickings, Dr J. Busvine, Dr B. Fine, and Miss W. Wall of the London School of Hygiene and Tropical Medicine. The United States Army Chemical Corps granted leave to the senior author in support of the anti-malaria campaign of the World Health Organization.

RÉSUMÉ

Chez la mouche domestique, on a observé que l'apparition d'une résistance élevée au DDT s'accompagnait de titres accrus de déhydrochlorase du DDT, en présence de laquelle le DDT est transformé en DDE. On a proposé, pour préciser le rôle de la détoxication dans les mécanismes de résistance, de comparer le taux de cet enzyme chez les souches sensibles et les souches résistantes. Dans le cas d'une différence significative, on pourrait considérer la détoxication comme le mécanisme principal de la résistance. Jusqu'ici, les recherches n'avaient pas porté sur les anophèles. Les auteurs comblent cette lacune.

Le métabolisme du DDT a été étudié par la méthode chromatographique gaz-liquide sur des lignées pures d'anophèles sensibles ou résistants, élevés en laboratoire. Des quantités relativement élevées de DDE sont formées *in vivo* par des souches sensibles de *A. stephensi*, *A. quadrimaculatus* et *A. gambiae*, et par des souches résistantes de *A. stephensi* et *A. quadrimaculatus*. Les *A. albimanus*, résistants ou sensibles, ne présentaient pas de différences dans le taux de production du DDE. Des métabolites volatils du DDT autres que le DDE n'ont pas été observés dans la plupart des souches soumises à la chromatographie. Un procédé de mesure de la déhydrochlorase du DDT a été mis au point, sur le modèle de celui que l'on a utilisé pour le dosage de la déhydrochlorase du DDT dépendante du glutathion, chez les mouches domestiques résistantes. L'enzyme existait à un titre maximum chez les *albimanus* et les *stephensi* sensibles, donc sans relation évidente avec la résistance au DDT. On a observé d'importantes différences dans le taux de résistance et dans les schémas de résistance au sein des genres étudiés. Chez *A. stephensi*, ces différences se manifestent au niveau de la souche.

REFERENCES

Agosin, M., Michaeli, D., Miskus, R., Nagsawa, S. & Hoskins, W. M. (1961) *J. econ. Ent.*, **54**, 340

Berger, R. S. (1961) *The determination of DDT-dehydrochlorinase*, Ithaca, N.Y. (Thesis, Cornell University)

Bradbury, F. R. & Standen, H. (1960) *J. Sci. Food Agric.*, **11**, 92

Bradbury, F. R. & Winteringham, F. P. W. (1958) *Rep. Progr. appl. Chem.*, **43**, 607

Bridges, A. G., Harrison, A., & Winteringham, F. P. W. (1956) *Nature (Lond.)*, **177**, 86

Brooks, G. T. (1960) *Nature (Lond.)*, **186**, 96

Brown, A. W. A. (1960) *Misc. Publ. ent. Soc. Amer.*, **2**, 59

Busvine, J. R. (1959) *Ent. exp. appl.*, **2**, 58

Davidson, G. (1958) *Bull. Wld Hlth Org.*, **18**, 579

Davidson, G. & Mason, G. F. (1963) *Ann. Rev. Ent.*, **8**, 177

Davidson, G. & Jackson, C. E. (1961) *Bull. Wld Hlth Org.*, **25**, 209

Dinamarca, M. L., Agosin, M., & Neghme, A. (1962) *Exp. Parasit.*, **12**, 61

Fine, B. (1961) *Nature (Lond.)*, **191**, 884

Frontali, N. & Carta, S. (1959) *Riv. Parassit.*, **20**, 107

Garms, R. (1960) *Z. Tropenmed. Parasit.*, **2**, 353

Gartrell, F. E. & Ludvik, G. F. (1954) *Amer. J. trop. Med. Hyg.*, **3**, 817

Goodwin, E. S., Goulden, R. & Reynolds, J. G. (1961) *Analyst*, **86**, 697

Kalra, R. L. & Pal, R. (1959) *Bull. nat. Soc. India Malar.*, **7**, 123

Lipke, H. & Chalkley, J. (1962) *Biochem. J.*, **85**, 104

Lipke, H. & Kearns, C. W. (1959) *J. biol. Chem.*, **234**, 2123

Lipke, H. & Kearns, C. W. (1960) *Advanc. Pest Control Res.*, **3**, 253

Micks, D. W. (1960) *Bull. Wld Hlth Org.*, **22**, 519

Mitchell, L. C. (1957) *J. Ass. off. agric. Chem.*, **40**, 294

Perry, A. S. (1960) *Bull. Wld Hlth Org.*, **22**, 743

Sternburg, J. G. & Kearns, C. W. (1952) *J. econ. Ent.*, **45**, 505

Sternburg, J. G., Kearns, C. W. & Moorefield, H. (1954) *J. Agric. Food. Chem.*, **2**, 1125

Trapido, H. (1954) *Bull. Wld Hlth Org.*, **11**, 885

Vargas, L. (1960) *Mosquito News*, **20**, 386