- McIlwain, H. (1959). *Biochemistry and the Central Nervous System*. London: J. and A. Churchill.
- Overton, E. (1902). *Pflüg. Arch. ges. Physiol.* **92**, 346.
- Pappius, H. M., Rosenfeld, M., Johnson, D. M. & Elliott, K. A. C. (1958). *Canad. J. Biochem. Physiol.* **36**, 217.
- Post, R. L., Merritt, C. R., Kinsolving, C. R. & Albright, C. D. (1960). *J. biol. Chem.* **235**, 1796.
- Rybová, R. (1959). *J. Neurochem.* **4**, 304.
- Schou, M. (1957). *Pharmacol. Rev.* **9**, 17.
- Schou, M. (1959). *Psychopharmacologia*, **1**, 65.
- Skou, J. C. (1957). *Biochim. biophys. Acta*, **23**, 394.
- Skou, J. C. (1960). *Biochim. biophys. Acta*, **42**, 6.
- Skou, J. C. (1961). In *Symposium on Membrane Transport and Metabolism*, p. 228. Prague: Czechoslovak Academy of Sciences.
- Stadie, W. C. & Riggs, B. C. (1944). *J. biol. Chem.* **154**, 687.
- Takagaki, G. & Tsukada, Y. (1957). *J. Neurochem.* **2**, 21.
- Tsukada, Y. & Takagaki, G. (1955). *Nature, Lond.*, **175**, 725.
- Tsukada, Y., Takagaki, G. & Hirano, S. (1958). *J. Biochem. Tokyo*, **45**, 489.
- Varon, S. & McIlwain, H. (1962). *J. Neurochem.* **8**, 262.
- Wallgren, H. & Kulonen, E. (1960). *Biochem. J.* **75**, 150.
- Weil-Malherbe, H. (1938). *Biochem. J.* **32**, 2257.

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Glutathione, Oxidized and Reduced, in some Dipterans Treated with 1,1,1-Trichloro-2,2-di-(*p*-chlorophenyl)ethane

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(Received 26 February 1962)

It has been shown that DDT-resistant houseflies (*Musca domestica*) are capable of dehydrochlorinating massive doses of 1,1,1-trichloro-2,2-di-(*p*-chlorophenyl)ethane (DDT) to the innocuous olefin DDE, 1,1-dichloro-2,2-di-(*p*-chlorophenyl)ethylene. In susceptible strains this detoxicating mechanism operates to a very limited extent (Lipke & Kearns, 1960). Extracts of resistant flies catalyse this conversion to a degree several thousand-fold that of preparations from susceptible strains when fortified with glutathione, indicating that DDT-dehydrochlorinase activity may play a major role in the survival of resistant populations. With the partial exception of cysteinylglycine, other cofactors or thiols do not function as activators of the dehydrochlorinase. Under the conditions of the dehydrochlorinase assay *in vitro*, oxidation of GSH cannot be detected (Lipke & Kearns, 1959*a, b*), and in the intact animal measurements of GSH oxidation during DDT treatment have not been reported. Cotty & Henry (1958) found minor differences in rates of incorporation of [³⁵S]cysteine into GSH in resistant and susceptible strains of flies; the effect of DDT treatment was not investigated.

DDT poisoning is characterized by a striking increase in the respiratory rate of susceptible strains. A variety of substrates have been reported for the DDT-dependent increment of respiration (Winteringham & Barnes, 1955; Winteringham, 1958), but oxidation of thiols has not been investigated. In vertebrates, GSH apparently does not serve as a

major substrate or carrier in electron transport although some involvement has been proposed in special cases (Waley, 1959; Mapson, 1959). GSH concentrations in DDT-poisoned insects are of added interest since dipterans are provided with high activities of quinone-forming oxidases (Ohnishi, 1959), whose action may interfere with thiol metabolism (Barron, 1951).

A NADPH-dependent mixed-function oxidase that hydroxylates DDT has been described in resistant and susceptible flies by Agosin, Michaeli, Miskus, Nagasawa & Hoskins (1961). These workers suggest that utilization of NADPH by the hydroxylase may limit the supply of GSH available to the dehydrochlorinase. This proposal assumes that GSH is limiting in the fly and is metabolized during dehydrochlorination. This situation would be reflected by appropriate changes in GSH and GSSG content or major differences in GSSG-reductase activity in resistant and susceptible flies. The relation of concentrations of GSH to the metabolic changes occurring during DDT poisoning and detoxication have consequently been studied in the housefly and a DDT-resistant malaria vector, *Anopheles albimanus*.

EXPERIMENTAL

Resistant and susceptible strains of flies and mosquitoes are referred to throughout as R and S respectively. Quantities and concentrations of oxidized glutathione are described in terms of the equivalents of glutathione which they would yield on reduction. The insecticide 1,1,1-tri-

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chloro-2,2-di-(*p*-chlorophenyl)ethane is written as DDT, the dehydrochlorination product 1,1-dichloro-2,2-di-(*p*-chlorophenyl)ethylene as DDE.

Preparation of extracts

Organisms. The housefly (*Musca domestica*) was reared by standard methods (Busvine, 1959) and the adults were maintained on a diet of sugar and skim milk. Concentrations of GSH and GSSG were the same in animals fed on the sugar and milk diet and those receiving water alone. These concentrations were not affected by aging in these insects. No individuals of the S strain survived a dose of 0.7 μ g. of DDT applied topically. No measurable DDT-dehydrochlorinase activity was observed with the bead technique and procedure B of Sternburg, Kearns & Moorefield (1954). The R strain of housefly of Swedish origin (Fine, 1961) showed a 95% survival 24 hr. after topical treatment with 10 μ g. of DDT. Dehydrochlorinase assay of this strain showed DDE formation at a rate of 39 μ m-moles/mg. of protein/90 min. Reversed-phase chromatography on paraffin-treated paper (Bridges, Harrison & Winteringham, 1956; Mitchell, 1957) revealed only DDT and DDE in the reaction mixture. Gas-liquid chromatography of extracts of the internal tissues of the R strain on E-301 silicone-Epikote (9:1) revealed a 25% conversion of DDT into DDE in 24 hr. after topical application. Recoveries were quantitative and no evidence of additional metabolites of DDT was obtained. This procedure detects 1 μ mg. of organic halide when 50–500 μ mg. is chromatographed (Goodwin, Goulden & Reynolds, 1961).

Anopheles albimanus was cultured as described by Davidson (1958) and maintained on 1% glucose solutions. The S strains, PAL and ITALB, were identical except for viability. These two susceptible strains exhibited 100% mortality after exposure of the adults for 1 hr. to a filter-paper surface treated with 4% of DDT in risella oil. The R strain, TTLL, could withstand exposure for 4 hr. with 80% survival. With both flies and mosquitoes analyses were run on different generations of animals as more material became available. Additional information is available on the toxicology and genetics of the housefly strains (Fine, 1961).

DDT treatment. About 250 mosquitoes or 25 flies were anaesthetized by cooling to 5° for 15 min., weighed and transferred to cages 7 cm. $\times$ 13 cm. made of steel screening and provided with a tight-fitting cardboard lid impregnated with insecticide. The sides of the cage were lined with a double layer of Whatman no. 1 paper; the innermost sheet was impregnated with 4% of DDT in risella oil applied at a dispersion of 1 ml./100 cm.². Controls were placed on risella oil alone. Moisture was provided by wet cotton wool placed against the bottom of the cage. Weight changes of the insects were not considered during prolonged treatment; these amounted to about 15% during prostration. All experiments with houseflies were run at least in triplicate; experiments with mosquitoes in duplicate. In a study of the effects of cold anaesthesia, no difference in GSH/GSSG ratio was observed in untreated animals anaesthetized by chilling zero, two or six times. Insecticide treatment was carried out at 23°. Zero time was taken when half the population had recovered from anaesthesia.

Fixation and extraction. After exposure, the papers were withdrawn by a slight displacement of the lid and the cages were immediately plunged into liquid N₂ for 5 min. The cages were drained and the animals transferred immediately

by means of funnels pre-chilled in liquid N₂ to homogenizer tubes held in ice-water. The homogenizer tubes contained 1.0 ml. of 3% sulphosalicylic acid/100 mg. of tissue. With mosquitoes, 100 mg. of acid-washed ground Pyrex glass was added to the tubes and a looser-fitting homogenizer pestle was used than with the softer-bodied flies. The animals were ground with 60 strokes for 1 min. with a chilled motor-driven pestle at 0°. The tubes were held in ice-water for 30 min. and centrifuged at 10 000g. The clear supernatant, free of the fatty layer at the meniscus, was assayed immediately since rapid deterioration of the frozen extracts was observed. Further grinding or aging of the preparations was ineffective in raising the titres of GSH or GSSG. Repeated extraction of the precipitate with sulphosalicylic acid or 0.01 N-HCl did not increase titres of GSH or GSSG. Extensive oxidation of GSH was observed in flies ground with the omission of the treatment with liquid N₂ or when macerated in powdered solid CO₂ after cooling of the flies and glassware in liquid N₂ (Fowler & Lewis, 1958).

Estimations

Oxidized and reduced glutathione. GSH and GSSG were determined manometrically with yeast apoglyoxalase, essentially as described by Martin & McIlwain (1959). In this method, the washed yeast suspension used as a source of apoglyoxalase serves also as a reducing system for the conversion of GSSG into GSH during preincubation in the absence of methylglyoxal. Standards consisting of 0.1 and 0.2 μ equiv. of GSH and GSSG were included in each run and blank values were subtracted. Acid formation from GSSG preincubated with methylglyoxal was negligible. With 140 mg. of apoglyoxalase and 0.2 μ equiv. of GSH in a total volume of 2.70 ml., CO₂ was produced at a rate of approx. 25 μ moles/vessel/hr. Recoveries of GSH and GSSG added to sulphosalicylic acid extracts of flies averaged 92 and 90% respectively. Reduction of GSSG by the yeast reductase averaged 88 $\pm$ 7%, as determined by subsequent analysis with apoglyoxalase. Variation in the period of reduction or the addition of cysteine or serum albumin to the GSSG system failed to produce rates of CO₂ evolution from GSSG as high as those obtained with GSH. This was not due to its water content since prolonged drying of GSSG over P₂O₅ *in vacuo* did not improve the response. Only GSSG could be detected in the standard by paper chromatography. The rate of acid formation from GSH was reproducible with quantitative recoveries of standard GSH and GSSG in mixtures. A method of calculation was developed based on these observations that permitted the estimation of the GSSG titres from the slopes of the curves for (1) standard GSSG, (2) standard GSH, (3) the total GSH plus GSSG curve of the sample and (4) the GSH curve of the sample. Since the contribution of GSSG to the slope of curve (3) can be described as the slope of curve (3) minus the slope of curve (4), the concentration of the oxidized peptide can be obtained by reference of this difference to curve (1).

Preparation of housefly glutathione reductase. R and S flies and mosquitoes were frozen in liquid N₂ and stored at -20°. A portion (1 g.) of tissue was homogenized while frozen in 4 ml. of a medium containing 50 mM-sodium phosphate-potassium phosphate, pH 7.0, and 660 μ M-EDTA. The material was centrifuged at 15 000g, and the supernatant dialysed against 300 vol. of the same medium for 8 hr. with stirring. Measurements were made in a Hilger Uvispek spectrophotometer at 25°. The change in extinction

at 340 m μ on oxidizing NADPH was taken as 6.22 cm.²/ μ mole (Horecker & Kornberg, 1948). In cuvettes of 3 ml. capacity, the final concentration of reactants included 175 μ M-NADPH, mM-GSSG, phosphate and EDTA, as described above, and a preparation equivalent to 50–200 mg. of tissue/ml. Higher concentrations of nucleotide and GSSG did not stimulate the rate of NADPH oxidation for the reaction intervals chosen. Protein concentration was 1–3 mg./ml., as determined by the biuret test (Robinson & Hogden, 1940). A general increase in extinction over the range 240–420 m μ was observed when GSSG was added to dialysed fly extracts in the absence of NADPH (Fig. 1). This effect occurred over a wide range of GSSG concentration, but was absent in preparations fractionated with (NH₄)₂SO₄ and was not investigated further.

Paper chromatography. Animals were frozen in liquid N₂, ground as described for GSH analysis and the extract was stored at –20°. After thawing, *N*-ethylmaleimide was added to the chilled extracts to a final concentration of 5 μ moles/ml., followed by neutralization with cold *N*-Na₂CO₃ (Hanes, Hird & Isherwood, 1950). The samples were held at room temperature overnight, adjusted to pH 1.5 with *n*-HCl and extracted twice with equal volumes of CHCl₃. The layer of CHCl₃ as well as the flocculent material at the interface was discarded and the samples were evaporated to a syrup over a steam bath with the aid of a stream of N₂. Desalting was performed as described by Smith & Tuller (1955) on 12 cm. $\times$ 1 cm. columns of Zeo-Karb 225 (H⁺ form; X8) with aq. *m*-NH₃ soln. as the eluent. Ammonia was removed with a stream of washed N₂ and the samples were concentrated to a volume suitable for chromatography. Chromatographic standards were subject to the same procedure.

One-dimensional ascending methods were used with propan-1-ol-water (7:3) and butan-1-ol-formic acid-water (7:2:1) as the solvents. This combination was recommended by Gutcho & Laufer (1954) for the identification of a variety of S-containing peptides. The solvent was permitted to ascend the paper three times. Thiol-*N*-ethylmaleimide adducts were revealed by the method of Benesch, Benesch, Gutcho & Laufer (1956) and with ninhydrin. Disulphides were identified by the nitroprusside dip of Toennies & Kolb (1951). Analysis of the extracts for thiol was made according to Grunert & Phillips (1951).

Reagents. Methylglyoxal was synthesized by the method of Neuberg as modified by Patterson & Lazarow (1955).

Other substrates and standards were of commercial origin and dried over P₂O₅ before use. Pyridine nucleotides and GSH were obtained from the Sigma Chemical Co., *N*-ethylmaleimide and GSSG from L. Light and Co. Ltd., and DDT (99.9%, *pp'*-DDT) from the Geigy Co. Carbon dioxide-free glass-distilled water was used for glyoxalase and GSSG-reductase measurements.

RESULTS

Concentrations of glutathione in flies and mosquitoes. Table 1 shows that the doses, strains and intervals chosen for the demonstration of resistance and susceptibility are suitable for revealing the effect of DDT on GSH metabolism. Most of the R individuals survived the treatment, most of the S became prostrate. With respect to the S strain, recovery was not observed when groups treated with DDT were transferred to cages free of DDT. The data show that DDT poisoning does not affect the GSH/GSSG ratio significantly. The proportion of GSSG differs only 1 % between DDT-treated S flies and untreated controls. The results with S mosquitoes were similar to those with flies. The data also indicate that the operation of the mechanism or mechanisms responsible for resistance in either species do not appear to involve the oxidation of GSH or gross changes in total GSH content. In both flies and mosquitoes, the R strains subjected to DDT stress differed in the proportion of GSSG in the tissues by about 1 % from the untreated animals. The variation in the total GSH of the populations may reflect ecological or nutritional differences resulting from the uncontrolled nature of the larval population density during rearing, or differences in food intake in this stage. Neither of the species studied is adaptable to the precise control of these variables in succeeding generations.

Glutathione reductase. Measurement of reductase activities of flies revealed a number of factors that complicate the analysis. Preparations of acetone-dried powders (Sternburg *et al.* 1954), with subse-

Table 1. *Glutathione of resistant and susceptible houseflies and anophelines treated with DDT*

Values for the housefly (*M. domestica*) are given $\pm$ S.D. Mosquito values (*A. albimanus*) are the averages of two or more determinations.

more determinations.		Number prostrate (% of population treated)	Glutathione			Percentage oxidized
Organism	Time of DDT treatment (hr.)		Total	GSH	GSSG	
			(μequiv. of GSH/g. fresh wt.)			
<i>M. domestica</i>						
Susceptible	0	0	2.35 ± 0.01	2.05 ± 0.09	0.32 ± 0.08	13.6
	2	100	2.48 ± 0.17	2.13 ± 0.17	0.36 ± 0.24	14.0
Resistant	0	0	1.56 ± 0.02	1.42 ± 0.14	0.14 ± 0.09	9.0
	24	10	1.86 ± 0.12	1.67 ± 0.33	0.19 ± 0.18	10.2
<i>A. albimanus</i>						
Susceptible	0	0	1.88	1.71	0.17	9.1
	1	80	1.21	1.11	0.10	8.3
Resistant	0	0	1.70	1.45	0.25	14.4
	3	5	1.28	1.11	0.18	13.3

quent extraction with phosphate as recommended by Martin & McIlwain (1959), proved ineffectual, as did a procedure involving the removal of pigment and ultraviolet-absorbing material by isoelectric precipitation at pH 4.5. Reproducible results were obtained with the high-speed supernatant of homogenates followed by carefully controlled dialysis, Fig. 1 shows the dependence of such preparations on the presence of added NADPH and GSSG. No reduction of GSSG was observed with the substitution of NADH for NADPH, and the addition of KCN, AMP or albumin failed to improve the system. Fig. 2 shows that the close agreement in GSH/GSSG ratio in R and S flies is matched by a similarity in the activities of GSSG reductase in these strains. The high proportion of inert cuticular material present in small insects contributes to the relatively low value of 3 μ moles of NADPH oxidized/g. of insect/hr. at 25°, compared with a value of 250 for brain extracts assayed at 37° (McIlwain & Tresize, 1957). When the rates are expressed on the basis of NADPH oxidized/mg. of soluble protein the values exceed those reported for crude extracts of other insects (Powning & Irzykiewicz, 1960). A partially purified reductase of improved stability was precipitable from extracts of either strain with (NH₄)₂SO₄ between 0.40 and 0.65 saturation. Specific activity increased about sevenfold, but this procedure was not convenient for measurement of

differences in reductase activities between populations. Attempts to demonstrate the presence of this enzyme in several species of anophelines were unsuccessful, probably because of technical difficulties rather than a deficiency in the reductase. The grinding of large numbers of smooth, hard-bodied minute organisms such as mosquitoes in the frozen state meets with many difficulties, made evident by a low yield of soluble protein.

Paper chromatography. Only two substances capable of forming an adduct with *N*-ethylmaleimide were observed in R and S flies (Table 2). The predominant material in chromatograms of both strains exhibited a mobility identical with that of *S*-(*N*-ethylsuccinimido)glutathione. Traces of the cysteine derivative were also present. The disulphide reagent of Toennies & Kolb (1951),

Fig. 1. Glutathione reduction by fly extracts with reduced NADPH. The system contained 50 mM-sodium phosphate and potassium phosphate, pH 7.0; 660 μ M-EDTA; fly extract, 2 mg. of protein/ml. (A) 100 μ M-NADPH present initially; GSSG, 3 μ moles, added where indicated. (B) 1 mM-GSSG present initially; NADPH added as indicated (μ m-moles).

Fig. 2. Glutathione reduction by extracts of resistant and susceptible houseflies. The system contained phosphates and EDTA as described in Fig. 1; 175 μ M-NADPH was added as indicated by arrow; GSSG, 1 mM. Fly extract from the strains indicated: 2.1 mg. of protein/ml. Values are corrected for NADPH oxidation in the absence of added GSSG. Δ , Resistant strain; $\bullet$, susceptible strain.

Table 2. *Relative mobilities of N-ethylmaleimide adducts present in resistant and susceptible houseflies*

Spots received desalted extract equivalent to 40 flies. Solvent A, propan-1-ol-water (7:3); solvent B, butan-1-ol-formic acid-water (7:2:1).

<i>N</i> -Ethylmaleimide adducts of	Solvent A	Solvent B
GSH	1.00	1.00
Cysteine	1.60	2.77
Resistant flies	1.03; 1.62	1.00; 2.64
Susceptible flies	1.02; 1.65	1.05; 2.95

which reacts with *N*-ethylmaleimide addition products, also showed these substances to be present in the extracts as well as small amounts of GSSG, but no other disulphides. The nitroprusside method of Grunert & Phillips (1951), which shows moderate specificity for thiols, was used to compare the total thiol with GSH, as determined with glyoxalase. Analysis of crude sulphosalicylic acid extracts indicated that at least 80 % of the nitroprusside-positive material in the extracts was GSH, confirming the results obtained by chromatography. Chromatograms of mosquito extracts were of necessity loaded with lesser amounts of extract and showed only *S*-(*N*-ethylsuccinimido)glutathione.

DISCUSSION

In isolated systems which are readily auto-oxidizable or are fortified with the appropriate cofactors, GSH undergoes rapid oxidation to the disulphide form and is subject to a variety of transfer and degradative reactions (Waley, 1959). The data show, however, that in the intact animal GSH constitutes one of the more stable tissue components. No gross changes in total GSH or the GSH/GSSG ratio were observed when *S* flies and mosquitoes were poisoned with DDT, an agent that induces a prolonged increase in oxygen consumption as well as a derangement of neuromuscular coordination. The stability of GSH in tissues subject to DDT stress is thus worthy of note and serves as evidence against a role in biological oxidations.

The precautions taken against post-mortem changes suggested by Martin & McIlwain (1959) have been shown to be fully justified in this work with the further necessity of immediate analysis of the sulphosalicylic acid extracts. This procedure reveals that, just as in cerebral tissues of higher forms, GSH is present in flies and mosquitoes in predominantly the reduced state, with a GSH/GSSG ratio of 7:1 and a total GSH content of 1.5–2.5 μ equiv./g. of tissue. It has been reported that GSH added to larval diets of the yellow-fever mosquito, *Aedes aegypti*, but not of the housefly (Brookes & Fraenkel, 1958), results in a stimulation of growth and increased survival (Singh & Brown, 1957), which suggests that dietary GSH may affect concentrations in the tissues of the adult mosquito and may bring about a variation in the total GSH of different generations. The absence of GSH synthesis in the pupae and adult mosquito has not been demonstrated, but the findings of Cotty & Henry (1958) clearly indicate new synthesis of GSH in the pupae and adult housefly. In the adult therefore concentrations of GSH may be more or less independent of larval nutrition.

Glutathione-reductase activities were virtually identical in the two strains of flies examined; resis-

tance to DDT does not involve large differences in titre of this enzyme between the two strains. The presence of the reductase probably contributes to the maintenance of concentrations of GSH in the tissues, and, since these concentrations are apparently unaffected by DDT, it would seem unlikely that NADPH stores are depleted because of any involvement of GSH in processes of intoxication or insecticide degradation as suggested by Agosin *et al.* (1961). With respect to a possible binding of thiols by DDT during intoxication, F. R. Bradbury (personal communication) has calculated that the average toxic dose of DDT to *S* flies is about 10^{-2} μ moles/g., whereas the thiol content (calculated as GSH) is at least 100 times this concentration, not considering the contribution of protein thiol groups. These figures indicate that little or no binding of thiol by DDT would be detected by the methods conventionally used for thiol analysis, if such binding occurs on a mole for mole basis as postulated by Gessner & Smith (1960). Our work appears to support Bradbury's calculations, but does not eliminate thiol-DDT interaction at localized sites of action or the formation of unstable complexes. The failure to observe large differences in the GSH content of treated and untreated flies would also stand against the possibility that DDT poisoning liberates large quantities of quinones or other oxidizing agents capable of reacting with GSH as well as interfering with normal metabolic processes.

The absence of major differences between R and S strains of flies and mosquitoes with respect to tissue GSH, glutathione reductase and chromatographically identifiable thiols leaves unanswered the question of metabolic differences other than the enhanced rates of detoxication observed in R flies. The mechanisms responsible for the survival of R populations do not apparently deplete the GSH supply, the results with intact animals supporting those obtained with the purified dehydrochlorinase (Lipke & Kearns, 1959*b*). Competition for NADPH between the reductase and DDT oxidase, as suggested by Agosin *et al.* (1961), would thus require more experimental evidence. The same reasoning would serve as evidence against the scheme suggested by Gessner & Smith (1960), who postulate a decysteinylation of GSH in DDT-treated R animals, leading eventually to DDE. In the experiments involving long periods of exposure of R flies to DDT, with a correspondingly prolonged operation of the dehydrochlorinase system, no significant loss in apoglyoxalase-positive material was apparent. In the cockroach, an organism relatively unaffected by DDT and incapable of converting DDT into DDE *in vitro*, treatment with DDT did not affect concentrations of GSH measured with glyoxalase (Forgash, 1951).

In the presence of GSH, the addition of catalytic quantities of cysteinylglycine results in an increased rate of DDE formation with purified dehydrochlorinase. The near correspondence of GSH concentrations as measured by glyoxalase and thiol as measured by the less-specific nitroprusside reagent, as well as the chromatographic data, which showed only one thiol-containing peptide, negates the supposition that the tissues have sulphur-containing peptides, other than GSH, with an action analogous to that of cysteinylglycine. Cotty, Henry & Hilchey (1958) reported traces of unidentified materials in flies injected with various [35 S]amino acids but do not describe the oxidation state of the sulphur in the compounds in question. By an independent method, these workers observed concentrations of GSH in flies about 125 % of those reported here (V. F. Cotty, personal communication), clarifying an ambiguity in an earlier report which suggested that concentrations of GSH in flies are unusually low (Cotty & Henry, 1958). The differences in GSH content observed between strains and between batches of flies from a seemingly homogeneous population present a major and unsolved difficulty in comparative studies on the composition of insects.

SUMMARY

1. In the housefly, *Musca domestica*, the content of reduced plus oxidized glutathione is about 2 μ equiv./g. of tissue, with a GSH/GSSG ratio approximating to 7:1. The same values hold for the mosquito, *Anopheles albimanus*.

2. Insignificant differences were observed in the GSH/GSSG ratio between DDT-treated or untreated animals or between DDT-susceptible and DDT-resistant strains of the two species. Minor differences in total GSH content between strains may be due to factors other than resistance.

3. No differences were observed in the NADPH-glutathione-reductase activities of resistant and susceptible strains of houseflies: an average rate of NADPH oxidation of approximately 3 μ moles/g./hr. was obtained in both cases.

4. Paper chromatography of fly extracts showed GSH to constitute the principal free thiol to be present in the tissues.

H.L. wishes to express his gratitude to his colleagues of the London School of Hygiene and Tropical Medicine and the Ross Institute for their hospitality and assistance during his stay in the School; special thanks are indicated to Dr W. E. Ormerod, Mr B. Fine, Miss W. Wall and to the members of the Department of Biochemistry. This work was financed in part by a grant to the Ross Institute, Professor G. MacDonald, Director, from the U.S. Public Health Service in support of the Malaria Eradication Program of

the World Health Organization. Leave to take up this Fellowship was generously granted by the U.S. Army Chemical Corps Research and Development Command.

REFERENCES

- Agosin, J., Michaeli, D., Miskus, R., Nagasawa, S. & Hoskins, W. M. (1961). *J. econ. Entomol.* **54**, 340.
 Barron, E. S. G. (1951). *Advanc. Enzymol.* **11**, 218.
 Benesch, R., Benesch, R. E., Gutcho, M. & Laufer, L. (1956). *Science*, **123**, 981.
 Bridges, R. G., Harrison, A. & Winteringham, F. P. W. (1956). *Nature, Lond.*, **177**, 86.
 Brookes, V. J. & Fraenkel, G. (1958). *Physiol. Zool.* **31**, 208.
 Busvine, J. R. (1959). *Entomol. exp. appl.* **2**, 58.
 Cotty, V. F. & Henry, S. M. (1958). *Contr. Boyce Thompson Inst.* **19**, 393.
 Cotty, V. F., Henry, S. M. & Hilchey, J. D. (1958). *Contr. Boyce Thompson Inst.* **19**, 379.
 Davidson, G. (1958). *Bull. World Hlth Org.* **18**, 579.
 Fine, B. C. (1961). *Nature, Lond.*, **191**, 884.
 Forgash, A. (1951). *J. econ. Entomol.* **44**, 870.
 Fowler, K. S. & Lewis, S. E. (1958). *J. Physiol.* **142**, 165.
 Gessner, T. & Smith, J. N. (1960). *Biochem. J.* **75**, 165.
 Goodwin, E. S., Goulden, R. & Reynolds, J. G. (1961). *Analyst*, **86**, 697.
 Grunert, R. R. & Phillips, P. H. (1951). *Arch. Biochem.* **30**, 217.
 Gutcho, M. & Laufer, L. (1954). In *Glutathione: A Symposium*, p. 79. Ed. by Colowick, S. P. et al. New York: Academic Press Inc.
 Hanes, C. S., Hird, F. J. R. & Isherwood, F. A. (1950). *Nature, Lond.*, **166**, 288.
 Horecker, B. L. & Kornberg, A. (1948). *J. biol. Chem.* **175**, 385.
 Lipke, H. & Kearns, C. W. (1959a). *J. biol. Chem.* **234**, 2123.
 Lipke, H. & Kearns, C. W. (1959b). *J. biol. Chem.* **234**, 2129.
 Lipke, H. & Kearns, C. W. (1960). *Advanc. Pest Control Res.* **3**, 253.
 McIlwain, H. & Tresize, M. A. (1957). *Biochem. J.* **65**, 288.
 Mapson, L. W. (1959). *Symp. biochem. Soc.* **17**, 28.
 Martin, H. & McIlwain, H. (1959). *Biochem. J.* **71**, 275.
 Mitchell, L. C. (1957). *J. Ass. off. agric. Chem.* **40**, 294.
 Ohnishi, E. (1959). *J. Insect Physiol.* **3**, 219.
 Patterson, J. W. & Lazarow, A. (1955). *Meth. biochem. Anal.* **2**, 259.
 Powning, R. F. & Irzykiewicz, H. (1960). *Aust. J. biol. Sci.* **13**, 59.
 Robinson, H. & Hogden, C. G. (1940). *J. biol. Chem.* **135**, 707.
 Singh, K. R. P. & Brown, A. W. A. (1957). *J. Insect Physiol.* **1**, 199.
 Smith, E. L. & Tuller, D. F. (1955). *Arch. Biochem. Biophys.* **54**, 114.
 Sternburg, J. G., Kearns, C. W. & Moorefield, H. (1954). *J. agric. Fd Chem.* **2**, 1125.
 Toennies, G. & Kolb, J. J. (1951). *Analyt. Chem.* **23**, 823.
 Waley, S. G. (1959). *Symp. biochem. Soc.* **17**, 85.
 Winteringham, F. P. W. (1958). *Proc. 4th int. Congr. Biochem., Vienna*, **12**, 208.
 Winteringham, F. P. W. & Barnes, J. M. (1955). *Physiol. Rev.* **35**, 701.