the corresponding 5β substances (Anderson & Haslewood, 1962), and we therefore think that the rate of movement on paper of anhydrochimaerol supports the opinion that it is (III). This opinion receives further strong support from the infrared spectrum of anhydrochimaerol (see the Experimental section).

Biological. The structure (I) is exactly what would be expected for a biochemical precursor of scymnol, for C-terminal hydroxylation would lead directly to this compound. Hence the principal finding of this work is that the chemistry of *C. monstrosa* bile salts does support the view that the living chimaeras are survivors of a group ancestral to those elasmobranchs that contain scymnol. Okuda, Enomoto, Morimoto & Kazuno (1962) have isolated from the bile of the sting ray, *Dasyatis (Dasybatis) akajei*, a (non-crystalline) substance which is an artifact of the alkaline hydrolysis used and to which they assign the structure (III). This may (depending on configuration at C-24 and C-25) be anhydrochimaerol, made from chimaerol sulphate in the bile salts by the alkaline hydrolysis. Thus *D. akajei* may also contain chimaerol sulphate, and if this is so it emphasizes even more strongly the close relationship between the chimaeras and the other elasmobranch fishes. Anhydroscymnol and cholic acid have also been isolated from the bile of *D. akajei* (Ashikari, 1939).

SUMMARY

1. Bile salts of *Chimaera monstrosa*, when treated with the dioxan-trichloroacetic acid reagent used by Bridgwater *et al.* (1962a) for the isolation of scymnol from its sulphate, yielded a

new bile alcohol, chimaerol, which is believed to be $3\alpha,7\alpha,12\alpha,24\epsilon,26$ -pentahydroxy- 25β -coprostane (II). Paper chromatography suggested that scymnol was also present.

2. Alkaline hydrolysis of the bile salts gave anhydrochimaerol (III), an artifact.

3. The structure of chimaerol supports the view that the chimaeras belong to a group which included the ancestors of those elasmobranch fishes that contain scymnol.

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The Metabolism of Glycerol 1-Phosphate in Resistant and Susceptible Houseflies (*Musca domestica* L.) and the Effect of Dieldrin

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Winteringham (1960) found that the amount of glycerol 1-phosphate in the thoracic tissues of adult houseflies was higher under conditions of cyclopropane anaesthesia than under conditions of normal activity. It was suggested that this difference was associated with the change from activity to rest. Previous poisoning with dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,-

8a-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene) or DDT [1,1,1-trichloro-2,2-bis-(p-chlorophenyl)ethane] abolished or reduced this effect of cyclopropane anaesthesia (Winteringham, Hellyer & McKay, 1960).

Anoxia also increases the concentration of glycerol 1-phosphate in many insects, e.g. in *Periplaneta americana* thoracic muscle (Kubista, 1957),

in *Locusta migratoria* flight muscle (Bücher, Klingenberg & Zebe, 1958) and in *Musca domestica* thorax (Winteringham, 1960; Heslop, Price & Ray, 1963), and this effect of anoxia is inhibited by dieldrin. The large body of knowledge on the effects of anoxia in various species makes it a useful experimental tool for the study of insecticidal action on glycerophosphate metabolism.

MATERIALS AND METHODS

Experimental animals. Adult male houseflies, *Musca domestica* L., were bred at this Laboratory. They were kept at 27° on a diet of glucose and water and used 3–6 days after emergence from pupae. Four strains were used. The P.I.L. strain was susceptible to dieldrin (LD_{50} for females 0.03 µg. per individual on topical application in acetone). The other three were derived from some *Musca domestica* var. *vicina* (provided originally by Dr J. R. Busvine) that had a history of dieldrin-resistance but proved on receipt to be susceptible (the S strain). This strain was heterogeneous in its response to dieldrin with LD_{50} between 0.02 and 0.25 µg. per individual female. By incorporating increasing amounts of dieldrin up to 150 p.p.m. in the larval food the level of resistance was raised to LD_{50} greater than 30 µg. per individual female; the stock was maintained under dieldrin pressure as the R strain. The RND strain was obtained by breeding R flies under dieldrin-free conditions. The LD_{50} declined under such conditions to 9 µg. per female through about 12 generations, but when this level of resistance was reached the stock was discarded and replaced by fresh R flies. Adult female R flies contain 8.4 µg. of dieldrin per fly 1 day after emergence, falling to 1.8 µg. per fly at 15 days old (Bridges & Cox, 1959).

Dieldrin was applied topically in 2 µl. of acetone and controls were treated with acetone alone while under mild chill-coma.

Determination of glycerol 1-phosphate. The method used for the determination of thoracic glycerol 1-phosphate in normal or anoxic flies is described by Heslop *et al.* (1963).

Determination of glycerol 1-phosphate dehydrogenase. For the determination of cytoplasmic glycerol 1-phosphate-dehydrogenase activity 50 flies were chilled in ice and the heads and abdomina removed. The thoraces were gently crushed in 1 ml. of ice-cold sarcosome-isolation medium [as by Lewis & Fowler, 1960, but with only 1% (w/v) of serum albumin], and squeezed through six layers of muslin. A further 2 ml. of isolation medium was added to the crushed thoraces, which were again squeezed, and the combined filtrate was centrifuged at 23 000g for 20 min. at 0°. The final sarcosome-free supernatant was made up to 5 ml. with isolation medium and diluted to 100 ml. with 0.01M-EDTA, pH 7.3, before assay. The conversion of dihydroxyacetone phosphate into glycerol 1-phosphate was followed by observing the decrease in ultraviolet absorption at 340 mµ as $NADH_2$ was oxidized, and the enzyme concentration was successively decreased until the reaction rate depended on it alone. The final concentrations in the 1 cm. cuvettes in which the reaction was carried out were: dihydroxyacetone phosphate (33 µM), glyceraldehyde phosphate (33 µM), $NADH_2$ (330 µM), phosphate buffer, pH 7.3 (33 mM), EDTA (5 mM) and fructose diphosphate (1 µM); the temperature was 20°.

Determination of glycerol 1-phosphate oxidase. For the determination of the glycerol 1-phosphate-oxidase system of sarcosomes the combined filtrate after squeezing through muslin was centrifuged at 150g for 3 min. at 0° to remove coarse suspended fragments and the sarcosomes were then sedimented by centrifuging at 2000g for 10 min. at 0°. The pellet was resuspended in 3 ml. of albumin-free isolation medium and again centrifuged. The sarcosomes were finally suspended in 0.95 ml. of isolation medium containing 5% (w/v) of albumin. Glycerol 1-phosphate-oxidase activity was determined manometrically at 26.5° in 1 ml. of reaction medium of the following final composition (recommended by Mr S. E. Lewis, personal communication): sucrose (166 mM), albumin (0.5%, w/v), EDTA (1.0 mM), ADP (3.5 mM), $MgCl_2$ (6.6 mM), KH_2PO_4 (10 mM), tris, pH 7.3 (20 mM), and sarcosomes equivalent to five thoraces. Glycerophosphate, which contained 40% of L-glycerol 1-phosphate by enzymic determination, was added from the side arm to give a final concentration of 30 mM at the start of the reaction. The centre well of each flask contained 0.1 ml. of 10% (w/v) NaOH and a roll of filter paper. Protein determinations on samples of the sarcosome suspension were made by the biuret method of Gornall, Bardawill & David (1949) as modified by Cleland & Slater (1953).

RESULTS

Weighed groups of 10 S flies were used. The flies were individually treated with 0.2 µg. of dieldrin and frozen in liquid nitrogen after various times. The concentrations of thoracic glycerol 1-phosphate were expressed as µmoles/g. wet wt. of thorax based on 59% of the weights of whole flies before application of the insecticide. The results with details of symptoms are given in Table 1. The fall in the concentration of glycerol 1-phosphate $3\frac{1}{2}$ hr. after treatment with dieldrin was significant by the *t* test (*P* less than 0.01). Starvation for periods up to 5 hr. has no effect on the amount of thoracic glycerol 1-phosphate (Winteringham, 1960; J. P. Heslop & J. W. Ray, unpublished work).

Flies of the P.I.L. strain were similarly treated with dieldrin and frozen in liquid nitrogen after different times. Some groups of flies were subjected to 30 min. of anoxia before freezing and the results are summarized in Table 2. They confirm that the thoracic glycerol 1-phosphate concentration is decreased during dieldrin poisoning and show that this effect is reflected in the glycerol 1-phosphate concentrations of flies made anoxic at various stages of poisoning.

To compare this effect in the S, RND and R flies a slightly different procedure was adopted. Though S flies can be 'knocked down' in 2 hr. at a dose of 0.4 µg. per individual, it may take 15–30 hr. to reach the same stage of poisoning in RND and R flies with much higher doses. About 150 flies of each strain were individually treated with 0.4 (S strain), 20 (RND strain) or 75 µg. (R strain) of dieldrin in acetone or with acetone alone and kept

Table 1. *Effect of dieldrin poisoning in thoracic glycerol 1-phosphate in houseflies*

The results are expressed as μ moles of glycerol 1-phosphate/g. wet wt. of thorax $\pm$ s.d. (with the numbers of observations in parentheses). Experiments were performed at 20–22° on batches of 10 male S strain houseflies. Experimental details are given in the text.

Topically applied dose	Time after treatment (hr.)	Thoracic glycerol 1-phosphate (μ moles/g.)	Symptoms
Acetone (2 μ l.)	$\frac{1}{2}$ –1	1.3 $\pm$ 0.28 (14)	Normal
Dieldrin (0.2 μ g.) in acetone (2 μ l.)	1	1.2 $\pm$ 0.41 (5)	All affected; hyperactive with locomotor disturbance; few showing flight convulsions
Dieldrin (0.2 μ g.) in acetone (2 μ l.)	3 $\frac{1}{2}$	0.9 $\pm$ 0.46 (3)	Most insects knocked down but still active; few still at hyperactive or flight-convulsion stage of poisoning

Table 2. *Effect of dieldrin poisoning with and without anoxia on thoracic glycerol 1-phosphate in houseflies*

Each value, which is expressed as μ moles/g. wet wt. of thorax, is the mean of two determinations on batches of 10 flies (except where indicated by the numbers in parentheses). The time after dieldrin treatment includes any period of anoxia. Experimental details are given in the text.

Time after treatment with 0.2 μ g. of dieldrin (min.)	Thoracic glycerol 1-phosphate (μ moles/g.)	
	Without anoxia	With anoxia (30 min.)
Untreated control	2.4 $\pm$ 0.48 (3)	14.2
145	2.0	9.3
195	1.0	6.7
240	0.8	4.1
300	0.8 (1)	3.8

glycerol 1-phosphate in the absence of anoxia in S and R strains (P less than 0.001) and in the RND strain (P less than 0.05). In the anoxic dieldrin-treated flies the concentration of glycerol 1-phosphate in the R strain was higher than that in the S (P less than 0.001) and RND (P less than 0.01) strains; this was probably a reflexion of the longer time-course of poisoning in the resistant flies. The thoracic concentration of glycerol 1-phosphate in untreated P.I.L. flies (cf. Table 2) was significantly higher than that in the S, RND and R flies (P less than 0.001 in all cases).

Four batches of 50 P.I.L. flies were taken, two after 5 hr. of starvation and two 5 hr. after treatment with 0.2 μ g. of dieldrin. The thoracic cytoplasmic glycerol 1-phosphate-dehydrogenase activities were: control, 97 μ moles of dihydroxyacetone phosphate reduced/g. wet wt. of thorax/min.; treated, 94 μ moles/g. wet wt. of thorax/min. In similar batches of flies the thoracic mitochondrial glycerol 1-phosphate-oxidase activities at 26.5° were: control, 827 μ l. of oxygen/mg. of protein/hr.; treated, 902 μ l. of oxygen/mg. of protein/hr.

DISCUSSION

The amount of glycerol 1-phosphate in housefly thoracic tissue varies from one strain of flies to another. In the P.I.L. strain it is higher than in any of the other strains studied. Such a difference need not, however, indicate metabolic disabilities or even differences in the actual pathways available. Very high glycerol 1-phosphate concentrations are found in thoraces of flies which, to all outward appearance, have completely recovered after exposure to anoxic conditions (Ray & Heslop, 1963). A comparison of the LD₅₀ values to dieldrin with the thoracic glycerol 1-phosphate concentrations of these strains does not show them to be related. In all four strains tested the effect of anoxia was to raise the concentration of thoracic glycerol 1-phosphate to 14–16 μ moles/g. The differences between the concentrations of glycerol

at 20°. From 5 hr. after treatment to the time of 'knock down' they were provided with aq. 10% (w/v) glucose. Groups of 10 were selected at 'knock down', i.e. when they were just unable to stand, and kept for a further 2 $\frac{1}{2}$ hr., placed in oxygen-free nitrogen for 30 min. and then immediately frozen in liquid nitrogen. Other groups were frozen at comparable times after treatment with acetone or 3 hr. after 'knock down' as appropriate. The thorax weights used for calculating the values in Table 3 were computed from the mean weight of 50 flies from the culture used for each experiment multiplied by 0.55 (P.I.L. strain), 0.59 (S strain), 0.56 (RND strain) or 0.46 (R strain).

The results (Table 3) show that anoxia increases the amount of thoracic glycerol 1-phosphate in all three strains (P less than 0.001) and that in dieldrin-poisoned flies anoxia has the same effect (P less than 0.001 in S and R flies, P less than 0.01 in RND flies). The concentrations of glycerol 1-phosphate in dieldrin-treated anoxic flies are less than those of untreated anoxic flies (P less than 0.001 in all cases). Dieldrin poisoning decreases the amount of

Table 3. *Effect of dieldrin poisoning with and without anoxia on thoracic glycerol 1-phosphate in different strains of houseflies*

Three strains of fly are compared. Glycerol 1-phosphate, expressed as $\mu\text{moles/g. wet wt. of thorax} \pm \text{s.d.}$ (with the numbers of observations in parentheses) was determined in groups of 10 adult male flies. The time after 'knock down' includes any period of anoxia. Experimental details are given in the text.

Strain	Thoracic glycerol 1-phosphate ($\mu\text{moles/g.}$)			
	Treated with 2 $\mu\text{l.}$ of acetone		Treated with 0.4 (S strain), 20 (RND strain) or 75 $\mu\text{g.}$ (R strain) of dieldrin in 2 $\mu\text{l.}$ of acetone and killed 3 hr. after 'knock down'	
	Without anoxia	With anoxia (30 min.)	Without anoxia	With anoxia (30 min.)
S	1.3 ± 0.28 (14)	14.7 ± 0.97 (3)	1.0 ± 0.64 (6)	4.6 ± 2.84 (8)
RND	1.2 ± 0.15 (9)	14.6 ± 0.99 (3)	0.8 (1)	5.8 ± 1.54 (5)
R	1.3 ± 0.17 (4)	15.8 ± 0.13 (3)	0.7 ± 0.22 (3)	7.7 ± 1.01 (5)

1-phosphate in different strains kept in aerobic conditions were not reproduced by those in flies that had been exposed to anoxic conditions. This suggests that the cause of these differences in the normal amount of glycerol 1-phosphate lies in those sections of metabolism that are inhibited by 30 min. of anoxia (Heslop *et al.* 1963). In all strains tested, dieldrin poisoning to a comparable stage, judged by symptoms, lowered the glycerol 1-phosphate concentrations of both the flies kept in aerobic conditions and those subjected to 30 min. of anoxia; it is therefore probable that this effect of dieldrin is produced by interference with glycolytic processes which, it is suggested, continue during the first 20–30 min. of anoxia. The R flies retain in their tissues considerable amounts of dieldrin without showing any sign of poisoning (Bridges & Cox, 1959). The effect of dieldrin on the concentrations of glycerol 1-phosphate of both the flies kept in aerobic conditions and those subjected to anoxia seems, therefore, to be connected with the appearance of symptoms and not with the presence of the insecticide.

The action of DDT in cockroaches can be clearly divided into two parts: a 'trigger reaction' which is specific to the poison, and a stress syndrome which is superimposed on the trigger reaction and consists of a large number of secondary effects. The latter can equally well be produced by other insecticides or by bodily stress alone (Heslop & Ray, 1959). It is suggested that the effect of dieldrin discussed above is a secondary effect of poisoning analogous to the stress syndrome in cockroaches. This view is supported by the finding that a similar effect on housefly thoracic glycerol 1-phosphate is produced by DDT (J. P. Heslop & J. W. Ray, unpublished work).

The mechanism by which dieldrin affects glycerol 1-phosphate is still obscure. It is not associated with inhibition of glycerol 1-phosphate oxidase

or the cytoplasmic glycerol 1-phosphate dehydrogenase, two of the enzyme systems responsible for glycerol 1-phosphate turnover *in vivo*.

SUMMARY

1. As the symptoms became more advanced dieldrin poisoning progressively lowered the amount of thoracic glycerol 1-phosphate in resistant and susceptible houseflies whether or not they were examined under anaerobic conditions.

2. This effect was not associated with the normal differences in glycerol 1-phosphate concentration between strains nor with the presence of dieldrin alone.

3. Neither the sarcosomal glycerol 1-phosphate-oxidase system nor the cytoplasmic glycerol 1-phosphate dehydrogenase was inhibited in thoracic preparations made from houseflies poisoned with dieldrin.

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