of cells from young rats, straight or angular processes of length equivalent to from one-quarter to two times the cell body diameter could be seen in about a quarter to a third of the cells in each preparation (Fig. 2). Growth cones could sometimes be seen either at the tips of the processes or where they changed direction.

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Metabolism of Gammexane in Flies, Ticks and Locusts

THE insecticide gammexane is known to be converted into water-soluble compounds in a variety of invertebrates[1], but the nature of these metabolites is not known. Some indication of their structure has been given by Bradbury and Standen[2], who obtained a mixture of dichlorothiophenols after the alkaline hydrolysis of excreta from gammexane poisoned flies. It is also known that homogenates of flies require glutathione if enzymatic conversion of gammexane to water-soluble derivatives is to occur[2]. These results have suggested that a compound like *S*-(pentachloro*cyclo*hexyl)glutathione might be a metabolite, and this hypothesis is supported by the presence of highly active enzymes in a variety of insects which catalyse the condensation of other chloro-compounds with glutathione[3].

We have attempted to identify such a metabolite in ticks, houseflies or locusts dosed with gammexane or in enzyme extracts fortified with glutathione, but have found the major metabolic product in each case to be an aromatized molecule which appears identical with *S*-(2,4-dichlorophenyl)glutathione.

The synthesis of this compound was achieved by a variation of the general method of preparing *S*-aryl cysteines[4] in which the cuprous mercaptide of glutathione was treated with diazotized 2,4-dichloraniline. The product was isolated by isoelectric precipitation at *p*H 3 and crystallized from ethanol–water. The *S*-(2,4-dichlorophenyl)glutathione had m.p. 215° C decomp. and $[\alpha]_D^{20} = 0 \pm 5$ ($c = 1$ in 0·1 N sodium hydroxide). Found: C, 42·5; H, 4·6; N, 8·4; Cl, 16·0; S, 7·0 per cent. $C_{16}H_{19}N_3O_6Cl_2S$ requires: C, 42·5; H, 4·2; N, 9·3; Cl, 15·7; S, 7·1 per cent. This compound was also isolated in good yield from unsuccessful attempts to prepare *S*-(pentachloro*cyclo*hexyl)glutathione from gammexane and glutathione in liquid ammonia as described for other glutathione derivatives[5]. The *S*-(2,4-dichlorophenyl)glutathione had an absorption spectrum similar to that of the analogous cysteine derivative with λ_{max} 258 mμ, ε_{max} 6,600 in 0·1 N sodium hydroxide and λ_{max} 257 mμ, ε_{max} 5,600 in 0·1 N hydrochloric acid. After hydrolysis at 100° C with 2 N hydrochloric acid for 4 h, 2,4-dichlorophenylcysteine was isolated identical with a sample prepared according to Parke[4].

Gas chromatographic analyses of cattle ticks (*Boophilus decoloratus*) kept at 35° C overnight showed that only 77 per cent (64–96 per cent) of a 10 μg topical dose of gammexane was recoverable unchanged. In similar experiments with ^{14}C-gammexane 28 per cent (6–30 per cent) of the dose was found in the water-soluble fraction after partitioning the homogenized ticks between water and xylene.

Enzymatic experiments were carried out with homogenates of cattle ticks, houseflies, or locust fat body containing 5 mM glutathione at *p*H values between 5 and 8 and with sufficient ^{14}C-gammexane to give a concentration of 5 mM. Water-soluble radioactive products were only formed when both glutathione and enzyme were present. When the reaction mixtures were examined chromatographically (Table 1) or by ionophoresis at *p*H 2, 3, 5, 7 and 8, a major part of the metabolized material was identical in behaviour with reference 2,4-dichlorophenylglutathione.

Table 1. R_F VALUES OF POSSIBLE GAMMEXANE METABOLITES

	S-(2,4-dichlorophenyl)-cysteine	*S*-(2,4-dichlorophenyl)-glutathione
Collidine–lutidine–H_2O, 1:1:1	0·75	0·50
n-Butanol–acetic acid–H_2O, 4:1:5	0·70	0·60
Pyridine–*n*-butanol–H_2O, 1:1:1	0·80	0·70
n-Propanol–ammonia sg. 88, 7:3	0·80	0·65
2-Butanone–H_2O, 1:1	0·05	0·05

The characteristics of this enzymatic reaction differed from those associated with the same enzyme sources when 2,4-dinitrochlorobenzene or *p*-nitrobenzyl chloride were used as substrates[3]. While the reaction rate with these substrates was constant over several hours, the reaction rate with gammexane fell off to a negligible value in 2–5 min. Moreover, the conversion of gammexane to a water-soluble derivative was not significantly affected by 0·1 mM bromsulphonphthalein, which completely inhibits the enzyme-catalysed reaction between *p*-nitrobenzyl chloride or 2,4-dinitrochlorobenzene and glutathione[3].

The formation of an aromatic derivative from gammexane is in general agreement with Bradbury's observation[1] that 4–6 atoms of chlorine are lost from gammexane when it forms water-soluble metabolites. On the other hand, it is difficult to reconcile the formation of a single major metabolite with Bradbury and Standen's isolation[2] of substantial amounts of 2,5- as well as 2,4-dichlorothiophenols from hydrolysed fly excreta unless the corresponding isomeric *S*-arylglutathiones are identical in all our chromatographic and ionophoretic systems or unless 2,4-dichlorophenylglutathione is an artefact formed from a very labile pentachloro*cyclo*hexylglutathione.

It has been suggested[6], on the basis of a colorimetric estimation procedure, that pentachloro*cyclo*hexene is a major but transient metabolite of gammexane in resistant flies, though this has not been confirmed by isotopic techniques. We have therefore applied the Schechter-Hornstein[7] colorimetric procedure to 2,4-dichlorophenylglutathione to see if it would give a false indication of pentachloro*cyclo*hexene. The metabolite did not interfere significantly in the reaction, but it would be desirable to investigate its probable precursor, pentachloro*cyclo*hexylglutathione, as the 'apparent' pentachloro*cyclo*hexene found by Sternberg and Kearns[6].

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