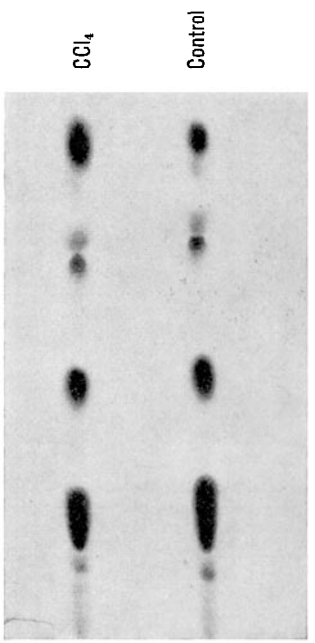
Liver Phospholipides after Carbon Tetrachloride Intoxication in Rats

Extensive studies have indicated that in the livers of rats given carbon tetrachloride there is an impairment in the synthesis of plasma lipoproteins, resulting in a decreased mobilization of lipids and fatty infiltration[1-5].

Investigations in our laboratory have revealed accumulation in the liver of newly synthesized phosphatide during the early stages of carbon tetrachloride intoxication, followed by a considerable decrease in the phospholipid content within 24 h[6]. This has been confirmed by observations by AIYAR et al.[7], who attributed the increase in liver lipids (triglycerides) primarily to an impaired synthesis of phospholipides, while a decreased oxidation of fatty acids would occur in addition in the later stages of the development of fatty liver.

In the present study, liver phospholipides from rats given carbon tetrachloride have been separated by thin-layer chromatography. The intoxication was produced by force-feeding under light ether anaesthesia a 1:1 mixture of carbon tetrachloride and mineral oil at a dose of 0.5 ml of the mixture per 100 g body weight. Control rats received 0.25 ml of mineral oil per 100 g body weight. 40 min after this, the rats were injected intraperitoneally with ^{32}P-orthophosphate (supplied from the Radiochemical Centre, Amersham) in NaCl solution, pH 7, and killed after 40 min by cervical dislocation. Samples of liver (approximately 1 g) were homogenized and extracted in chloroform-methanol (2:1, v/v) according to FOLCH et al.[8]. The extracts, dried at 45°C under nitrogen, were resuspended in chloroform and filtered; suitable samples were taken for counting, lipid-phosphorus estimation, and chromatographic analysis. Samples from chloroform solutions containing about 2 mg of total fat were plated directly on aluminium planchets, dried in air, and the radioactivity counted. Lipid phosphorus was determined after hydrolysis as previously described[6]. Thin layer chromatography was carried out on silica gel G plates activated at 110°C for 2 h, with chloroform:methanol:water (65:25:4), using tank saturation conditions. The spots coloured with iodine vapour (Figure) were circled and the silica gel removed from the plate for counting and phosphorus estimation[9].



A chromatogram on silica gel 'G' of phospholipides of rat liver after CCl_4 administration.

Counts were made by using an end-window Geiger tube (Tracerlab type TG-C-2) of window weight 1.6 mg/cm^2.

The results presented in the Table demonstrate that as early as 80 min after carbon tetrachloride administration ^{32}P-labelled lysolecithin is markedly increased in liver extracts, whereas the incorporation of ^{32}P into phosphatidic acid is strongly diminished. The specific activity of the latter is lowered, which indicates a block in the synthesis of the compound. The precociously increased incorporation of ^{32}P into lysolecithin corresponds to the initial stage of transiently increased specific activity of the liver phosphatide[6].

It should be mentioned that mitochondrial phospholipase A activity has been reported to increase in the fatty livers produced by hyperlipidic diet[10].

The prominent role of phospholipides in maintaining mitochondrial function is known[11-12] and the involvement of early mitochondrial alteration in the aetiology of the

Radioactivity of phospholipides labelled with ^{32}P in vivo in livers of rats given carbon tetrachloride

Phospholipide	Radioactivity Total cpm × 30 per 4 μg of total phospholipide-P	
	Controls	CCl_4 fed
Lysolecithin	735 ± 68	1844 ± 412
Sphingomyelin	329 ± 85	513 ± 191
Lecithin	2867 ± 741	3145 ± 994
Cephalin	4077 ± 1063	4451 ± 870
Phosphatidic acid	134 ± 48	38 ± 22
Cardiolipin	81 ± 53	41 ± 30
Total extract	9053 ± 722	11,600 ± 656

Results are given as means ± S. D. from eight experiments

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fatty livers caused by poisoning with carbon tetrachloride is under discussion[2,7,13–25].

Riassunto. Dopo avvelenamento acuto di ratti con CCl_4 somministrato con dose singola per via orale l'incorporazione in vivo di P^{32} nei fosfolipidi del fegato è alterata precocemente. A 80 min dalla somministrazione del tossico si osserva un forte aumento delle P^{32}-lisolecitine, mentre risulta bloccata la sintesi di acido fosfatidico.

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Ontogenetic Fate of the Neurosecretory Cells in the Larval Brain of *Sarcophaga ruficornis* (Fabricius, 1794) (Diptera: Cyclorrhapha)

Neurosecretory cells have been stained selectively in the whole brain of insects, diverse invertebrates and vertebrates by applying directly to the brain techniques used for revealing such neurons in sections[1]. Their study in situ in the brain of the post-embryonic developmental stages of *Sarcophaga ruficornis* disclosed the fate of the neurosecretory cells in ontogeny. This information, being presented here, is based on an examination of about 450 brains, stained with Cameron and Steele's modification of Gomori's paraldehyde-fuchsin technique, as adapted[1], and mounted in canada balsam, either as such (Figures 1, 3, 5, 6) or after appropriate dissections (Figures 2, 4, 7). (From the brains the neural lamella, also being PF-positive, was always cut away.) It was verified on sections, in the transverse, longitudinal and horizontal planes of about 400 brains, stained according to CAMERON and STEELE[2]. The flesh-flies were reared at 28°C (± 1°C) and 70% RH.

Each cerebral hemisphere of the brain of all the three larval instars contains four (1, 2, 3, and 4) groups of PF-positive neurosecretory cells, of which the disposition in the first-instar larva is shown in Figure 1. Their comparison with the neurosecretory cell groups of the third-instar larva of *Lucilia caesar*, in which six groups are present[2], shows the existence of close correspondence in the topography, staining reactions and number of neurosecretory cells comprising the groups (Table), as also of two differences. One difference is due to the absence in *S. ruficornis* larva of neurosecretory cells in the locus of the transient group 6 of *L. caesar* larva. The other difference stems from the splitting up of the cluster of neurosecretory cells at one locus into two groups (3 and 4), mainly on differences in their staining reactions in the diapausing, but not in the non-diapausing, larva of *L. caesar*[3]. But, one group (2) of the third-instar larva of *S. ruficornis*, which develops directly, corresponds to two groups (3 and 4) of the non-diapausing larva of *L. caesar*. As the larva of *S. ruficornis* differs from the diapausing larva only of *L. caesar*, as much as the two types of larvae of the latter fly differ from each other, this difference is nullified.

In the photomicrographs neurosecretory cells of groups 1, 3, and of both the hemispheres of the brain are distinct; all cells of group are not evident as their compact arrangement in tiers prevents their reproduction at one focus.

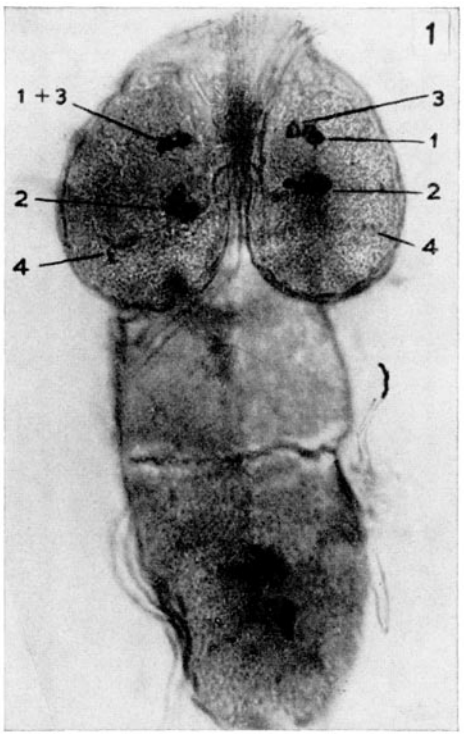

Fig. 1. Brain and ventral ganglion of first-instar larva showing all four groups of neurosecretory cells. × 170.

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