

PHENOBARBITAL-INDUCED, MEMBRANE-LIKE SCROLLS IN THE OENOCYTES OF *MUSCA DOMESTICA* LINNAEUS

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INTRODUCTION

The administration of phenobarbital to mammals increases the metabolism of xenobiotic compounds by increasing the levels of RNA, phospholipid, protein, and compounds of the microsomal electron transport chain (5, 14, 15, 20). These biochemical changes are viewed ultrastructurally by a proliferation of smooth endoplasmic reticulum (SER) in hepatic parenchymal cells (15). Mammalian liver has been used for most biochemical and ultrastructural work with phenobarbital and other enzyme inducers. It was not shown until the last decade that microsomal detoxifying enzymes were present in insects (24), and only recently has it been shown that these enzymes are inducible in insects (1, 19, 25, 27, 29, 30). Perry et al. (18) have shown that phenobarbital increases levels of cytochrome P-450 and microsomal oxidase activity in susceptible and resistant houseflies, *Musca domestica*. Biochemical data on enzyme induction in insects are accumulating rapidly, but little complementary ultrastructural work has been done. The insect fat body often has been compared to the mammalian liver in its metabolic functions (8). The present results were obtained from examination of oenocytes, cells located in the

housefly fat body, after exposure of houseflies to phenobarbital and thioacetamide.

EXPERIMENTAL PROCEDURE

Treatment of Houseflies

Standardized fly rearing procedures were followed (2), employing a mixture of fly larval medium, an aqueous yeast suspension, nondiastatic malt solution, and water. From 100 to 120 newly-emerged, adult, insecticide-susceptible male and female flies¹ were allowed to feed ad lib. on a stock solution of 100 ml milk (condensed milk diluted 1:1 with distilled water) and 2 g sucrose, which was combined with either 0.25 g sodium phenobarbital or a mixture of 0.25 g sodium phenobarbital and 0.25 g thioacetamide. Controls were maintained on an equal amount of the stock solution. The flies were killed after feeding for 24, 48, or 72 h on their respective diets. The phenobarbital-treated flies were sluggish and had a mortality rate of about 13% as compared with 3% in the control group and 23% in the flies treated with phenobarbital and thio-

¹ The houseflies were a World Health Organization standard, insecticide-susceptible strain obtained from the Zoological Institute, University of Pavia, Italy.

FIGURES 1-4 Oenocytes of 48 h control flies.

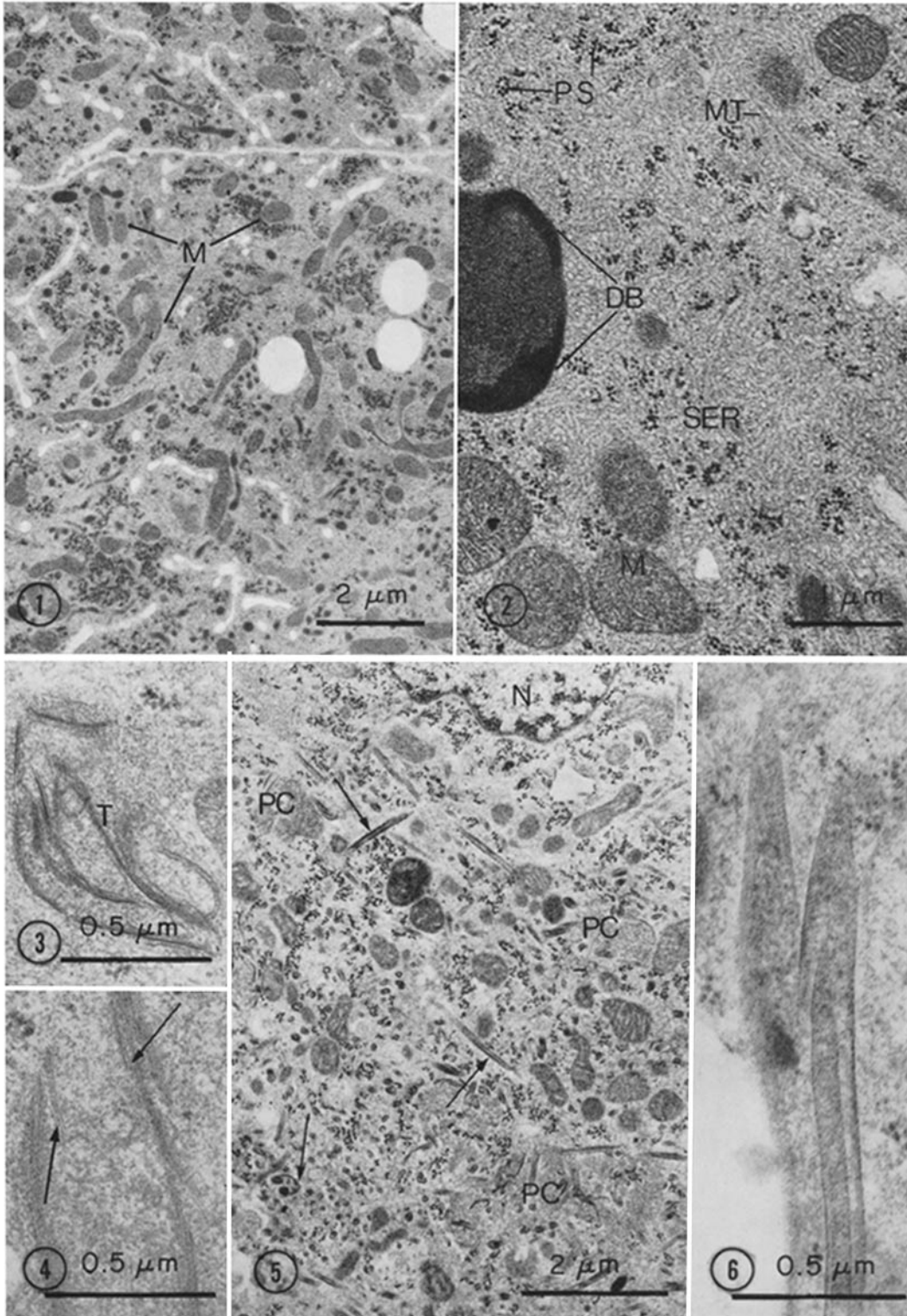
FIGURES 1 and 2 Portions of generalized views of oenocytes. *M*, mitochondria; *SER*, smooth endoplasmic reticulum; *DB*, dense body; *PS*, polysomes; *MT*, microtubule. Fig. 1, $\times 8,300$. Fig. 2, $\times 17,000$.

FIGURES 3 and 4 One of the "cytoplasmic inclusions", tubular elements (*T*), which appear to be membrane-like (arrows). Fig. 3, $\times 46,000$. Fig. 4, $\times 52,000$.

FIGURES 5 and 6 Oenocytes of flies fed phenobarbital for 48 h.

FIGURE 5 Generalized view of oenocyte. Note the production centers (*PC*) and abundance of scrolled structures (arrows). *N*, nucleus. $\times 11,000$.

FIGURE 6 Longitudinal view of scrolls. $\times 52,900$.



acetamide. Each experimental procedure was repeated with five different rearings of flies.

Tissue Preparation for Electron Microscopy

The flies were immobilized by chilling, and their abdomens were opened and immediately flooded with cold 4% glutaraldehyde in a 0.1 M sodium cacodylate and 0.008 M CaCl₂ buffer (pH 7.3). The fat body and associated oenocytes were excised, diced into approximately 1-mm³ pieces, placed into vials of the fixative, and stored for 3.5 h at 4°C. The tissue was rinsed with the buffer containing 0.25 M sucrose and postfixed for 1 h at 4°C with 1% OsO₄ dissolved in the same buffer. The tissue was again rinsed in the buffer containing 0.25 M sucrose, dehydrated in an ethanol series, embedded in Spurr resin (23), and polymerized for 8 h at 70°C. Thin sections were stained for 25 min in a saturated solution of uranyl acetate in 50% ethanol and for 25 min in lead citrate.

The fat body and associated oenocytes were taken from three male and three female flies and combined according to sex for each treatment and time period. Two fat body tissue samples from each of the respective daily treatments were examined ultrastructurally. This sampling procedure was repeated for all five rearings of flies; a total of 90 tissue samples were examined (30 samples/treatment).

RESULTS

Oenocytes are large cells of epidermal origin that occur almost universally in insects (4, 28). In the adult housefly, the oenocytes are intimately associated with the trophocytes of the fat body (Fig. 12). The normal oenocyte of adult flies is characterized by a cytoplasm densely packed with SER (Fig. 2). One or two large nuclei (Fig. 12), many clusters of polysomes, mitochondria, dense bodies, microtubules (Figs. 1

and 2), "cytoplasmic inclusions" (12) such as the tubular elements (Figs. 3 and 4), and a lack of rough endoplasmic reticulum further characterize the housefly oenocyte. No difference in the appearance of oenocytes from male or female flies was noted in any of the tissue examined; therefore, all figures are from female houseflies.

Treatments of flies with phenobarbital alone or in combination with thioacetamide for 24 h caused no consistent change in the ultrastructure. A striking alteration, however, occurred after 48 and 72 h of exposure to phenobarbital (Fig. 5). Many membrane-like scrolls (Fig. 6), rarely found in the oenocytes from control flies, were seen. These structures were found throughout the cytoplasm (Figs. 7–9) and occasionally in the nucleoplasm (Figs. 10 and 11) of the oenocytes. They appeared to be the same structures observed by Locke (12) in the oenocytes of *Calpodes* at the fourth and fifth molt and again at pupation. He stated that these scrolls were curved configurations of long, "membrane-like inclusions" (tubular elements) and were manufactured in "production centers", aggregations of a few microvesicles and dense granular material (Figs. 7 and 8). According to Locke, these membranous structures were lipid or lipoprotein, probably related to the agranular, tubular endoplasmic reticulum, and manufactured in the oenocyte for export. A definite continuity between these tubular elements and the tubular endoplasmic reticulum has been demonstrated in the oenocytes of *Culex*, where they increased in number shortly after pupal ecdysis (6).

Thioacetamide is thought to inhibit the migration of RNA from the nucleus to the cytoplasm (7, 9–11), which is evidently essential for the

FIGURES 7–11 Oenocytes of flies fed phenobarbital for 48 h.

FIGURES 7–9 Production centers (PC), longitudinal and cross sections of scrolls in cytoplasm. Fig. 7, × 38,000. Fig. 8, × 27,500. Fig. 9, × 55,100.

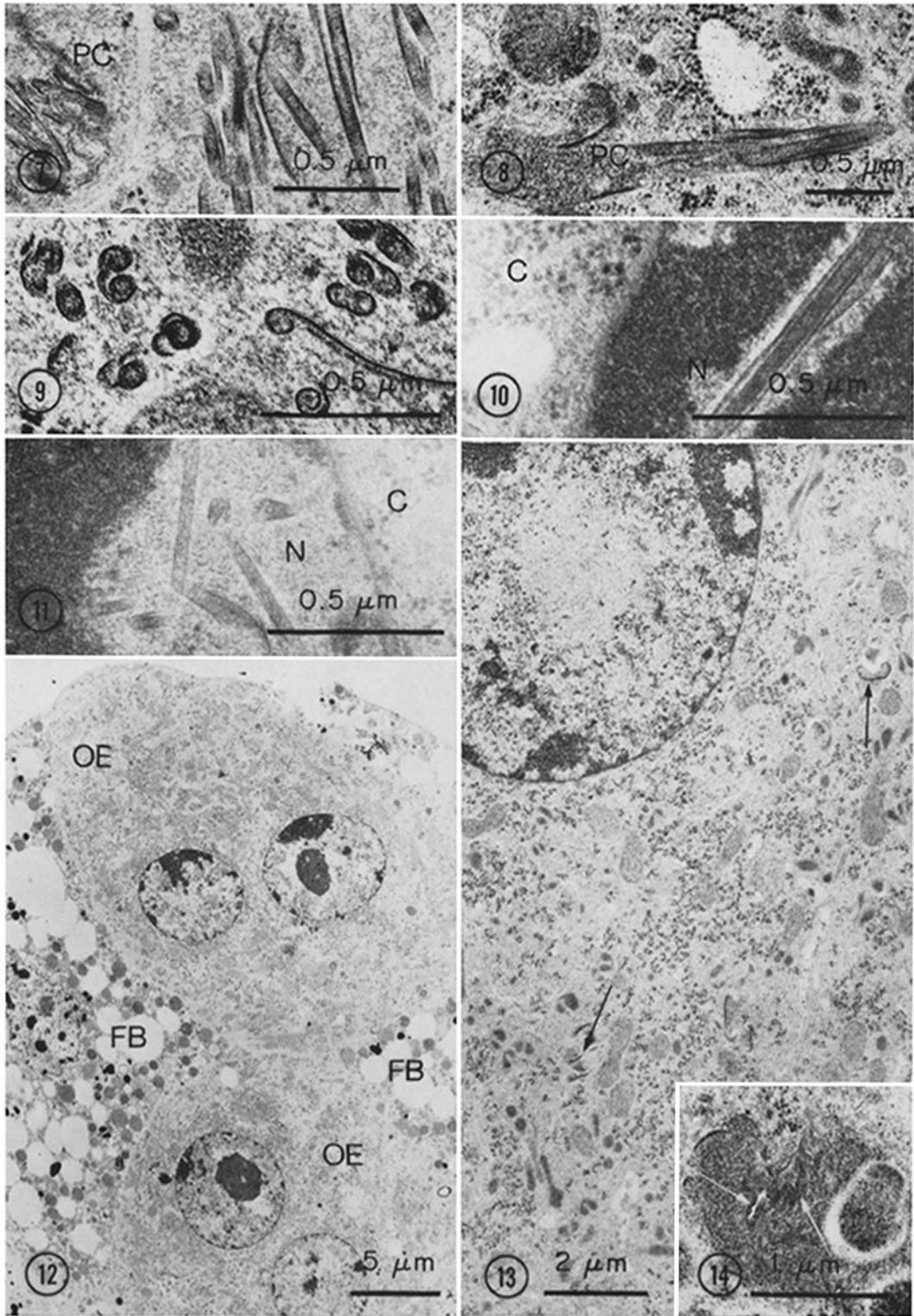
FIGURES 10 and 11 Scrolls present in the nucleus (N). C, cytoplasm. Fig. 10, × 65,000. Fig. 11, × 54,000.

FIGURES 12–14 Oenocytes of flies fed phenobarbital and thioacetamide for 48 h.

FIGURE 12 Association of oenocytes (OE) with trophocytes of fat body (FB). × 2,800.

FIGURE 13 Cytoplasm exhibiting a few scrolled structures (arrows). × 7,700.

FIGURE 14 Production center with some rather distorted scrolled structures (arrows). × 21,000.



formation of microsomes and their biosynthetic activity (16, 26). When thioacetamide is administered with phenobarbital, this combination is able to prevent the increase in *N*-demethylase activity and cytochrome P-450 content usually associated with phenobarbital administration (22). The ultrastructure of the oenocytes 48 and 72 h after treatment of flies with both phenobarbital and thioacetamide (Figs. 12 and 13) was noticeably different from that of the oenocytes of flies exposed to phenobarbital alone. The number of free scrolls in the cytoplasm was greatly reduced, and occasional production centers containing somewhat distorted scrolls (Fig. 14) were seen.

DISCUSSION

The functional significance of oenocytes is not known, but most investigators believe that they are sites of intermediary metabolism (4, 28). Oenocytes also have been implicated in steroid metabolism and detoxication (12). Our results may relate to the latter functions. Oenocytes, like cells engaged in steroid metabolism and detoxication, have a cytoplasm packed with SER. Structures thought to be intimately related to the SER have been seen in oenocytes at molting, pupal ecdysis (6, 12), and, now, after phenobarbital treatment. Like phenobarbital, the molting hormone, ecdysone, stimulates increases in RNA, protein, endoplasmic reticulum membranes (3, 13, 17, 21), and microsomal oxidases (30). Thus, it may be that the appearance of scrolled structures in the oenocytes accommodates the type of biochemical changes observed with both of these compounds.

Ultrastructural and biochemical phenomena associated with mammalian detoxication after phenobarbital administration have been described (5, 14, 15, 20). The same level of phenobarbital that previously had been shown (18) to increase the cytochrome P-450 content and mixed-function oxidase activity of houseflies has resulted, in our study, in the induction of scrolled structures thought to be closely related to the SER (6, 12). Thioacetamide, an inhibitor of phenobarbital-induced activities (22), seemed to interfere with the production of these scrolled structures. The fat body is often implicated as a possible organ of detoxication (24). The intimate spatial relationship of oenocytes and the fat body does

allow for the possibility that oenocytes may also be associated with detoxication. As Kilby (8) has pointed out, "The close association of oenocytes with the fat body means that much of the [biochemical] work with the fat body has involved the use of tissue 'contaminated' with oenocytes, and the possibility must be kept in mind that their presence may have contributed to the results observed."

To resolve some of these possibilities, a more detailed study of the ultrastructural effect of phenobarbital on fat body trophocytes, as well as studies involving the effects of other inducers and inhibitors on both oenocytes and trophocytes, is being conducted.

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