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Effects of a mammalian cholesterol biosynthesis inhibitor on adipocyte ultrastructure and metamorphosis in *Rhodnius prolixus* (Hemiptera)[1]

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Summary. A potent inhibitor of cholesterol biosynthesis in rodents [BMVA; 5-(4-biphenylyl)-3-methylvaleric acid] inhibits metamorphosis of 4th stage larvae of the blood-sucking bug *Rhodnius prolixus.*

It is well documented that the adipocytes of the haemophagus insect undergo a sequence of alterations in subcellular organization following a single nutrient meal[2]. The nutrient meal provides the necessary stimulus which initiates protein synthesis in the adipocyte[3]. Larval development and metamorphosis are dependent not only upon nutritional factors, but also upon the availability of the molting hormone (ecdysone) which restores the capacity of protein synthesis in the epidermal cells and ventral abdominal muscles. It is also well documented that dietary sources of sterols in the insect serve not only as structural components of cells and tissues, but also as precursors of essential metabolites and regulators (e.g. hormones)[4–6].

The biphenylyl methylated derivative of valeric acid (BMVA) appears to be a potent inhibitor of cholesterol biosynthesis in rodents[7,8]. The administration of BMVA to 4th instar larvae of *Rhodnius prolixus* interferes with molting and metamorphosis.

Materials and methods. 4th instar larvae of *Rhodnius prolixus* Stål (Hemiptera, Reduviidae) used in these studies had been starved for 2 months previously to reduce the large quantities of fat normally found in the insect adipocyte, allowing more critical electron microscopic observation. Employing an artificial feeding technique and heparinized human whole blood[9], 3 groups of insects (each group consisting of 55–60 larvae) received BMVA at concentrations of 10.5, 21.0, and 42.0 mg per 100 ml blood. A 4th group of insects was fed blood alone and served as a vehicle control. A minimum increase of $2\frac{1}{2}$ times the original body weight was the criterion used to ensure sufficient abdominal distention to provoke hormonal activation, initiating the molting phase. The onset of molting was recorded.

For ultrastructural investigations, 4 insects from each of the 4 experimental groups were sacrificed at 12 h and at daily intervals after feeding until day 6. The body cavity was cut open along the dorsal-ventral midline and Dalton's fixative (1% osmium tetroxide in dichromate buffer at pH 7.4) injected within. The upper cuticle was subsequently detached together with the net-like array of fat body. After 1h of fixation, the tissue was dehydrated in graded ethanol concentrations and 2 changes of epoxy propane and infiltrated with an epon-araldite mixture. Ultra-thin sections were cut and stained with uranyl acetate (30% w/v in ethanol) and Reynolds lead citrate[10]. Electron micrographs were taken at various levels of magnification for critical observation of fat body ultrastructure. For light microscopic investigations, epon-araldite embedded tissues were sectioned at 1 µm and stained with alkaline toluidine blue O[11]. The periodic acid Schiff reagent was utilized to determine visually the glycogen content in the adipocytes[12].

Results. 4th instar larvae of *Rhodnius prolixus* in all groups ingested approximately 2.7–3.1 times their body weight of human blood. The insects from all groups lost weight at a similar rate during the initial 5 days following feeding. In subsequent days, the weight loss in controls reached a maximum of 38% of the original weight recorded immediately after feeding. The rates of decrease in the mid- and low-dose groups were similar, although on day 6 insects in the mid-dose group lost significantly more weight. Similarly, an accelerated weight loss was observed in the high-dose insects from the 6th day on, reaching almost half of the original weight noted after the blood meal.

No significant differences among groups were observed regarding the normal onset and duration of molting. The 1st molt occurred between the 13th and 15th day after feeding in all groups. The remaining insects molted between the 13th and 23rd day after feeding. In this series of experiments, the most significant drug-related morphological effect was the inhibition of growth and development. All but one insect in the control group, and all the low-dose insects molted normally. In the mid-dose group 76% molted, while only 11% underwent ecdysis after receiving 42.0 mg BMVA/100 ml blood.

The electron microscopic investigations revealed that the general architecture of the fat body tissue and cells was well preserved in all groups, at all time intervals studied. In the 4th instar insects starved for 2 months, the adipocyte nuclei were generally ovoid in shape with well defined nucleoli and chromatin material. Chromatin was peripherally associated with the nuclear membrane and was scattered in clumps about the nucleoplasm and around the nucleolus (figure 1). The cytoplasm contained rough-surfaced endoplasmic reticulum consisting of single strands of cisternae concentrated mostly in the proximity of the plasma and

nuclear membranes. Small mitochondria with dense matrices, numerous lipid droplets, glycogen granules, dense lysosomes and free ribosomes also were seen within the cytoplasm. Smooth surfaced endoplasmic reticulum was observed rarely in the fat body of starved insects while Golgi complexes were noted in small numbers.

Following the nutrient meal, the fat body cells had a considerably different appearance. They underwent a sequence of significant alterations in subcellular organization which was apparent as early as 12 h after feeding (figure 2) and became fully manifested at 96 h. Increases in nucleolar, nuclear and cellular sizes were evident. Lipid droplets, protein material and glycogen rosettes accumulated significantly during this stage of development. The rough surfaced endoplasmic reticulum appeared as configurations of multi-layered stacked lamellae and distended vesicles. Golgi complexes were increased and associated with small vesicles containing electron dense inclusions. The electron microscopic investigations also demonstrated several varieties of partitioned mitochondria[13]. Autophagic vacuoles displaying recognizable portions of glycogen, mitochondria and cisternae of endoplasmic reticulum were evident following feeding and increased in number and size with time. Between 96 and 144 h after feeding the fat body cytoplasm appeared unchanged, with the exception that glycogen rosettes were not as abundant.

In *Rhodnius* treated with BMVA the fat body cells also underwent a sequence of alterations similar to those observed in control insects. The nucleolar, nuclear and cellular sizes increased, fat droplets, glycogen rosettes and protein material accumulated in the cytoplasm, endoplasmic reticulum increased in quantity, and dense lysosomes and autophagic vacuoles increased in number and size. Enlarged elongated mitochondria were increased in number when compared with starved insects and partitioned mitochondria were common. There were, however, morphological variations which were related to drug administration. Ultrastructural changes in mitochondria were consistently found in all treated groups, at all time intervals

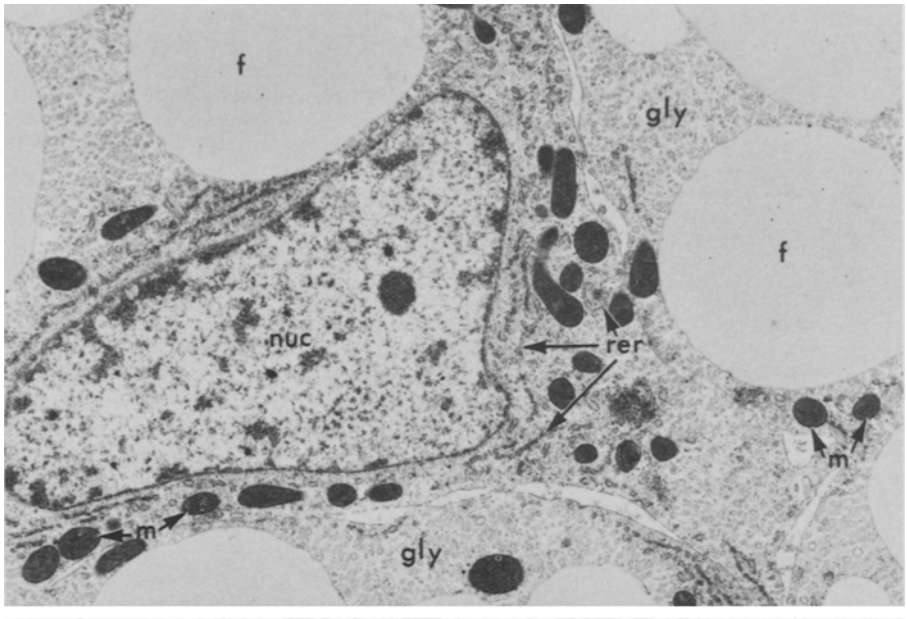

Fig. 1. Electron micrograph of a region of insect fat body in *Rhodnius* sacrificed after 2 months of food deprivation. Cells contain normal complement of organelles: nucleus (nuc), rough-surfaced endoplasmic reticulum (rer), mitochondria (m), fat droplets (f) and glycogen areas (gly). ×8400.

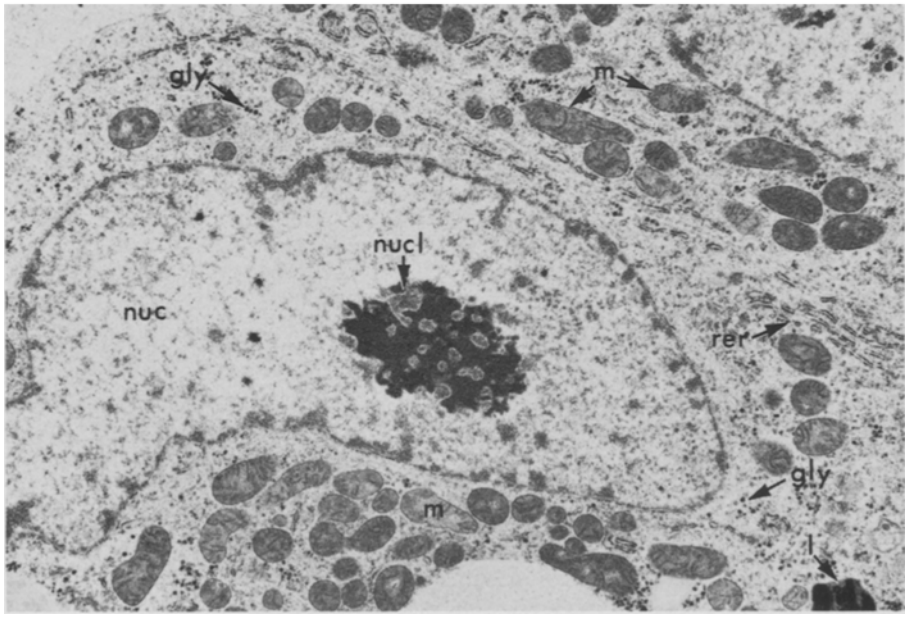

Fig. 2. Fat body cells 12 h after nutrient meal, showing prominent nucleolus (nucl), nucleus (nuc), mitochondria (m), rough endoplasmic reticulum (rer), glycogen rosettes (gly) and cytolysomes (l). ×8400.

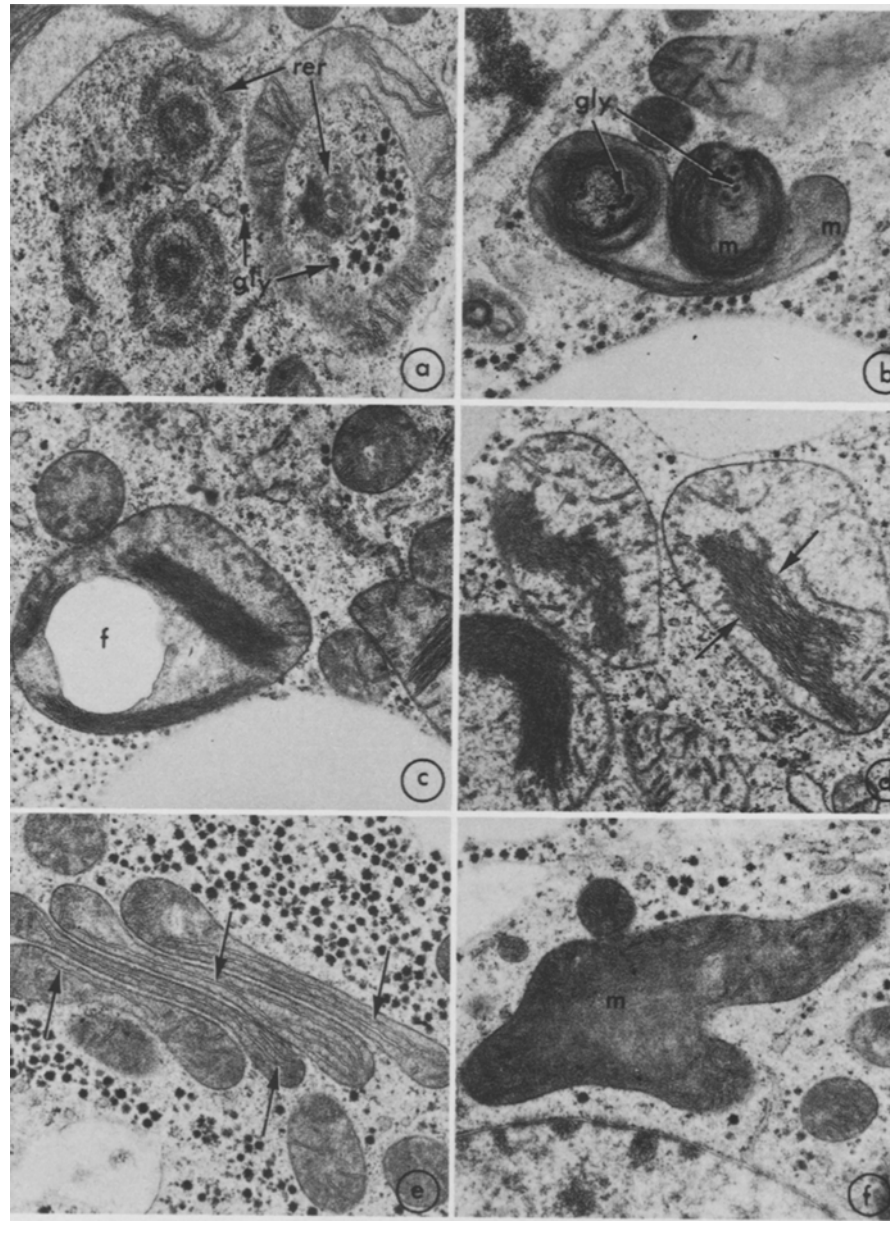

Fig. 3. Mitochondrial changes present in the fat body of *Rhodnius* receiving nutrient containing BMVA. Ring shaped mitochondria encompassing portions of cytoplasm, including endoplasmic reticulum (rer) and glycogen rosettes (a), other mitochondria (b), and small droplets of fat (c), were observed at all dose levels and time intervals investigated. Mitochondrial swelling was evident (d), together with parallel arrangement of cristae (arrows). Club-shaped alterations (e) with parallel stacks of cristae (arrows), and megamitochondria (f) were common. × 19,000.

studied, but were more evident in the insects given the high dose of BMVA. Fat body cells showed mis-shapen mitochondria diffused throughout the cell (figure 3). Among the variety of mitochondrial changes, there were ring-shaped varieties usually found encircling portions of endoplasmic reticulum, glycogen rosettes, other mitochondria, or sometimes droplets of fat. Some mitochondria were swollen with their cristae accumulated in parallel stacks within the matrix. Club-shaped alterations and megamitochondria were observed regularly.

Discussion. The administration of a potent inhibitor of cholesterol biosynthesis in rodents to 4th instar larvae of *Rhodnius prolixus* inhibited molting and metamorphosis, possibly by interfering with the sterol precursors used in the biosynthesis and/or metabolism of the molting hormone. Following compound administration, ultrastructural changes of adipocytes confirmed the availability of nutritional factors necessary for normal insect development. Drug-related ultrastructural changes were confined to conformational alterations of mitochondria.

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