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The Effect of Age on the Ultrastructure of the Fat Body Cells of the Granary Weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae)

Mohammed S. Al-Khalifa

Zoology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

ABSTRACT. The changes with age in the ultrastructure of the fat body in female *Sitophilus granarius* are described. The fat body is much reduced in the aged animal. Ageing causes extensive ultrastructural changes in the cell organelles of the fat body cells including an increase in adielectronic lysosome-like bodies, autophagic vacuoles and lipid droplets, together with a decrease in glycogen particles. Many of the mitochondria have electron-translucent matrices. The changes observed are discussed in relation to previous observations on other insect species.

Most of the studies on the ultrastructure of the insect fat body have been concerned with the changes in the fat body during metamorphosis (Ishizaki 1965, Locke and Collins 1965, Walker 1966). Some studies deal with the ultrastructure of the adult fat body (Walker 1965, Odhiambo 1967, Deloofe and Lagasse 1970, Liu and Davies 1972, Thomsen and Thomsen 1974, Al-Khalifa 1981). Most previous work has been concerned with the changes which occur in the structure of the fat body during egg maturation, but little work has been done regarding changes with age. Sohal (1973) described fine structural alterations with age in the fat body of the adult male housefly, *Musca domestica* L.

The present study investigates the ultrastructural changes of *Sitophilus granarius* fat body with age.

Material and Methods

Stocks of *S. granarius* were maintained in our laboratory at 25°C in a 12 hr photoperiod. Newly emerged insects were placed into 1 lb honey jars and allowed to feed and oviposit in wheat grains. Females were taken at the age of 0, 30 and 90 days (maximum egg laying age).

Electron Microscopy and Cytochemistry

Females were dissected in ice-cold 5% gluteraldehyde (TAAB) fixative, the fat-body removed, placed in fresh fixative in 0.1 M sodium cacodylate and 0.17 M sucrose at pH 7.4 for 30 min, and then washed for 24 hr in cacodylate buffer with 0.34 M sucrose at 0-4°C. For cytochemical identification of acid phosphatase, the method of Muller and Palade as modified by Akai (1969) was used. Tissues were post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate and 0.7 M sucrose at 0-4°C for 90 min dehydrated in graded cold acetones and transferred to an acetone/TAAB resin mixture. Details of the electron microscopical and cytochemical identification are reported by King and Al-Khalifa (1980).

Results

At emergence, the abdominal haemocoele of the adult *S. granarius* is filled with fat body. The fat body cells resemble those described in other insects at a similar stage of development, each having an oval or spherical nucleus. Each cell may have more than one nucleus, which may be a result of nuclear budding, the cell thus becoming a syncytium (Fig. 1, 2).

The cytoplasm contains mitochondria, free ribosomes and small fragments of endoplasmic reticulum. Most of the mitochondria are oval or spherical with rather dense matrices (Fig. 2). A few small Golgi complexes are present and are associated with vesicles (Fig. 3).

The lipid droplets occlude a considerable part of the cytoplasm and show great variation in size (2-8 μm in diameter). There are adielectronic inclusions which give a positive reaction to histochemical tests for protein and these protein droplets are fewer in number than the lipid droplets (Fig. 2). They usually occur in the cytoplasm around the nucleus. Many discrete glycogen particles are present throughout the cytoplasm (Fig. 3).

At day 30 after emergence, the fat body still fills a considerable part of the abdominal haemocoele. The fat body cells at this stage are similar to those just described for the newly emerged females, but there is an increase in the number of protein droplets (Fig. 4) and there are many different sizes of lipid droplets filling a large part of the cell cytoplasm (Fig. 5, 6). Many glycogen particles and mitochondria are distributed throughout the cytoplasm and the rough endoplasmic reticulum (RER) and Golgi complexes are well developed (Fig. 5, 7), compared with those in the fat body cells of newly-emerged insects. There are several adielectronic bodies distributed throughout the cytoplasm, mainly in the cytoplasm proximal to the nucleus, which give a positive reaction to the test for acid phosphatase (Akai 1969) (Fig. 8).

After 90 days, the changes are more marked, the fat body is much reduced and often forms only a thin layer in the peripheral region of the abdominal haemocoele and a thin layer around the ovaries and other organs. Many alterations occur in the fine structure of the fat body cells with age. There is an increase in the number of adielectronic lysosome-like bodies (Fig. 10, 14). In addition, small electron dense vesicles, which are similar in size to those given off by the Golgi complex occur throughout the cytoplasm (Fig. 12) and in some profiles they lie in proximity to the dense bodies, indicating a possible mode of origin for the dense bodies. Many autophagic vacuoles occur throughout the cytoplasm and a few of these vacuoles contain mitochondria with clearly-defined cristae (Fig. 10, 11, 14). The site of origin of these autophagic vacuoles, however, has not been observed. The rough endoplasmic reticulum occurs as whorls of cisternae (Fig. 14, 15). Many of the mitochondria have electrontranslucent matrices (Fig. 11-14). The degeneration of cell organelles, in particular the mitochondria, may occur either by the direct combination of mitochondria with the adielectronic lysosome-like bodies (Fig. 12) or by shrinkage, so that they become dense with a few cristae (Fig. 10, 13, 15) or by the segregation and digestion in the autophagic vacuoles (Fig. 10), which occasionally occur in the fat body cells. In some profiles, the mitochondria become cup-shaped and progressively surround portions of the cytoplasm (Fig. 12).

Most of the protein droplets show some degree of peripheral degeneration (Fig. 13, 15), though occasionally the process begins deeper in the inclusion. The glycogen particles, which previoulsy were present in large numbers in the fat body cells of newly-emerged and 30 days after emergence animals. are now reduced in number in the fat body cells of old individuals (90 days after emergence). The sizes of the lipid droplets increase (4-11 μm in diameter) and they fill a large part of the cell volume (Fig. 9).

Discussion

The progressive development of cisternae of the RER and the Golgi complexes and the associated dense vesicles implement the increase in protein droplets during the period of egg production. A similar distribution of organelles was described by Locke and Collins (1965) in the fat body of larvae of the lepidopteran *Calpodes ethlius* Stoll.

The present investigation has shown that up to 90 days after emergence, the fat body of *S. granarius* undergoes a sequence of changes correlated with age. In 90 days old females, there is a high degree of disorganisation in the fat body cells compared with similar cells in newly-emerged females. The degradation of cytoplasmic organelles (*e.g.* RER and mitochondria) in aged fat body cells has often been related to the increase in the number of autophagic vacuoles and their activity. Similar observations were reported in insect fat body cells (Ishizaki 1965, Dean 1978), oenocytes (Locke 1969) and salivary glands (Schin and Clever 1975).

Hochschild (1971) reported an increase in the number of adielectronic lysosome-like bodies with age, in his review of lysosomes and ageing. He suggested that the leakage of hydrolytic enzymes from lysosomes may damage DNA and RNA as well as the cellular machinery for transcription and translation. The point is supported by histochemical evidence of greater fragility of the lysosomal membranes in neurons of old rats compared with those of young rats (Brunk and Brun 1972). de Priester and van der Molen (1979) reported that the autophagic activity will eventually convert the isolated cytoplasmic organelles of *Calliphora* fat cells into reserve substances during metamorphosis.

The decrease in number of cristae and their rearrangement in the mitochondria of the fat body cells of old *S. granarius* is probably an effect of ageing. The shrinkage of mitochondria has previously been recorded in the oenocytes of old houseflies (Sohal 1973) and the decrease and rearrangement of cristae has been observed in the flight muscles of old *Nasonia vitripennis* (Walker) (Davies 1974). Karnovsky (1963) reported similar changes in the mitochondria of the nephron from starved frogs and suggested that they were correlated with a reduction in cytochrome oxidase activity. Keeley (1970) reported a reduction in cytochrome oxidase activity with age in his study on *Blaberus discoidalis* Serville fat body mitochondria.

Similar alterations in the rough endoplasmic reticulum of the fat body cell of old females were described by Hopkins and King (1966) in the follicular epithelium cells of *Bombus terrestris*, which undergo degeneration. The disruption of rough endoplasmic reticulum is probably the result of the reduction and alterations in protein droplets, as many workers including Caro and Palade (1964) suggest that protein is synthesised within the rough endoplasmic reticulum.

The reduction of glycogen content in the fat body cells with age has been reported in other cell types. Sohal (1973) in his study of the fat body of male houseflies observed that the glycogen content is reduced with age and Takahashi *et al.* (1970) observed this in the flight muslces of old *Drosophila melanogaster.*

The increase in size of lipid droplets in the fat body cells of old females is mostly a result of the coalescence of small droplets to form bigger droplets. A similar observation was reported in *Calliphora* fat body cells by de Priester and van der Molen (1979).

The evidence suggests that during the early days of adult life and up to 30 days after emergence, the fat body cells show synthetic activity with an accumulation of protein and lipid droplets. When the female is 90 days old, the stored material of the fat body cells breaks down, together with other organelles such as RER, Golgi and mitochondria, which were previously involved in synthesis.

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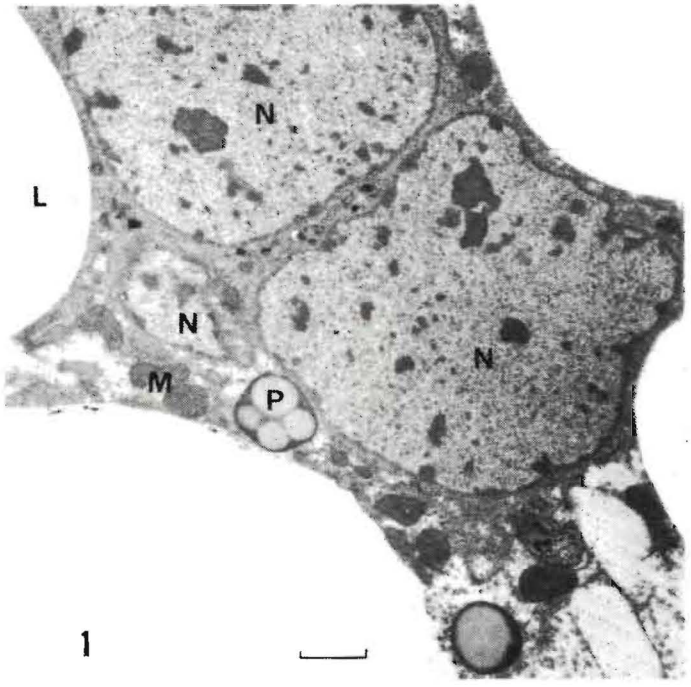
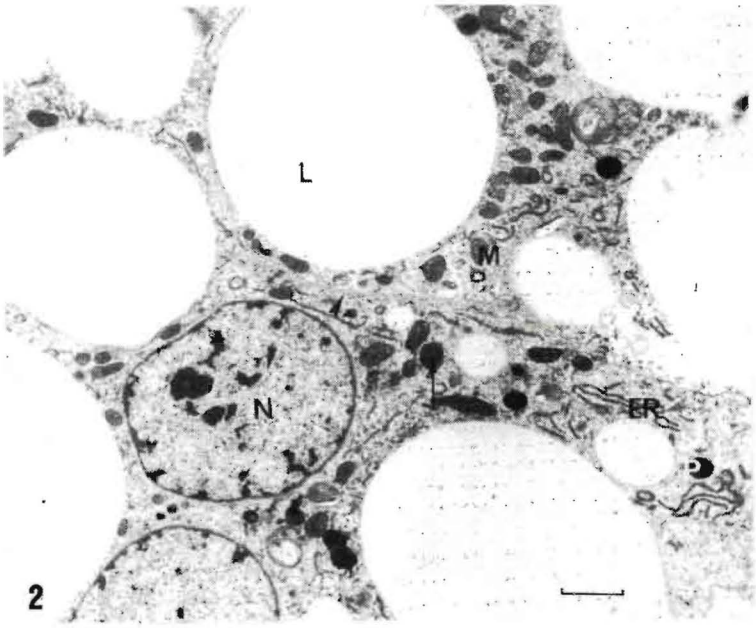


Fig. 1. An electron micrograph of fat body cell of newly emerged female. Note there is no plasma membrane between the three nuclei (N). Mitochondrion (M), Lipid droplet (L) and Protein droplet (P). Scale bar = 1 μm.

Fig. 2. Low power micrograph of fat body cells of newly emerged female. Note there is no plasma membrane between the two nuclei (N). The arrow indicates a mitochondrion with an electron dense matrix, endoplasmic reticulum (ER), lipid droplet (L), mitochondrion (M), plasma membrane (arrow head), and protein droplet (P). Scale bar = 2 μm.

Fig. 3. An electron micrograph from fat body cells of newly emerged female. Note small golgi complex (G) and glycogen particles (GY). Lipid droplet (L) and mitochondrion (M). Scale bar = 1 μm.

Fig. 4. An electron micrograph from fat body cells of a 30-day old female. Note there are many large protein droplets (P). Basement membrane (BM), intercellular space (ICS) and lipid droplet (L). Scale bar = 1 μm.



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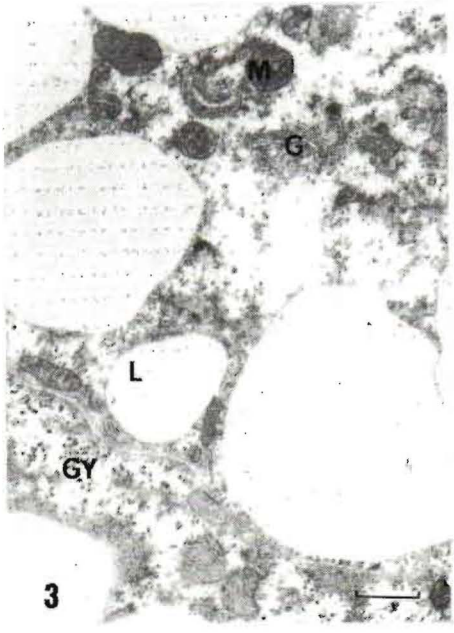

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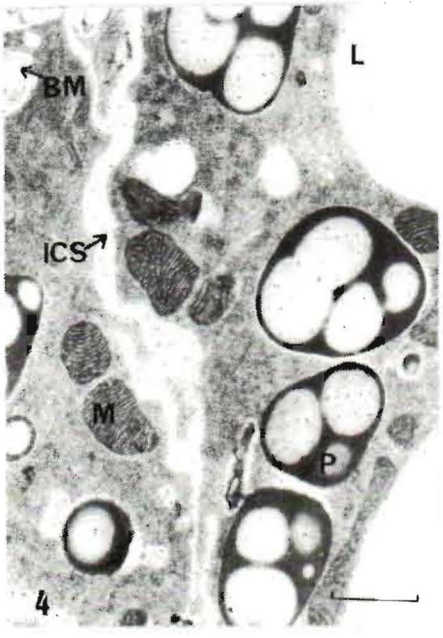

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Fig. 5-8. Fat body cells of 30 days old female.

Fig. 5. An electron micrograph showing the occurrence of many glycogen particles (GY). Lipid droplet (L) and mitochondrion (M). Scale bar = 1 μm.

Fig. 6. Low power micrograph showing a general view of a fat body cell with nucleus (N), many lipid droplets (L). Arrow indicates an adielectronic lysosome-like body and protein droplet (P). Scale bar = 2 μm.

Fig. 7. A high power micrograph showing well-developed rough endoplasmic reticulum (ER) and golgi (G) and associated vesicles (arrow-heads). Lipid droplet (L). Scale bar = 0.2 μm.

Fig. 8. Acid phosphatase localisation in the autophagic vacuoles (arrows). Mitochondrion (M) and nucleus (N). Scale bar = 1 μm.

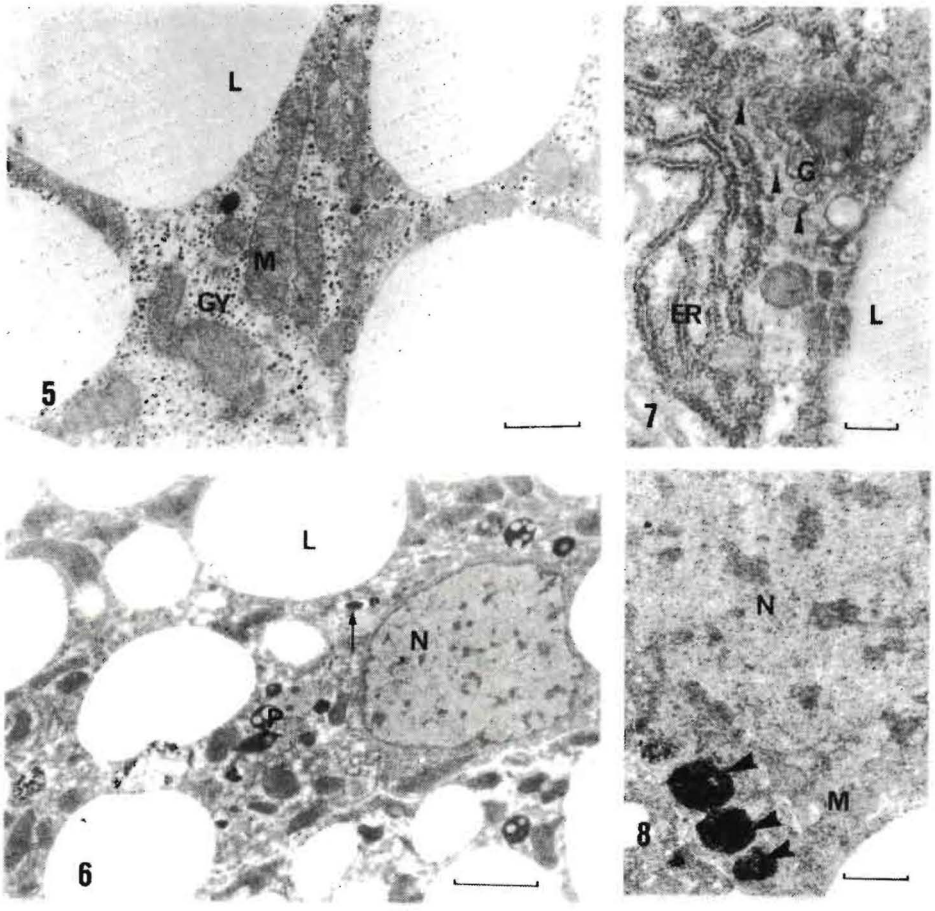
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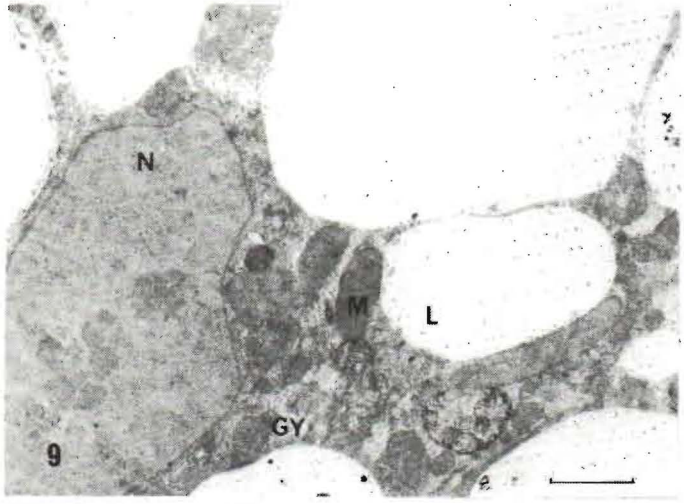
Fig. 9-11. Fat body cells of a female 90 days after emergence.

Fig. 9. An electron micrograph showing a general view of fat body cells with nucleus (N) and large lipid droplets (L). Glycogen (GY). Scale bar = 1 μm.

Fig. 10. An electron micrograph showing an autophagic vacuole (AV) containing a mitochondrion with well-defined cristae and an adielectronic lysosome-like body (arrow). Lipid droplet (L) and mitochondrion (M). Scale bar = 1 μm.

Fig. 11. High power micrograph showing autophagic vacuoles (AV) and mitochondrion (M) with less electron dense matrices. Note there is a degenerate organelle within the autophagic vacuole, which probably represents a mitochondrion (arrow). Scale bar = 0.5 μm.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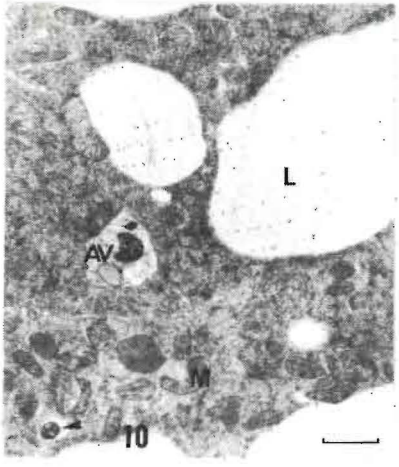
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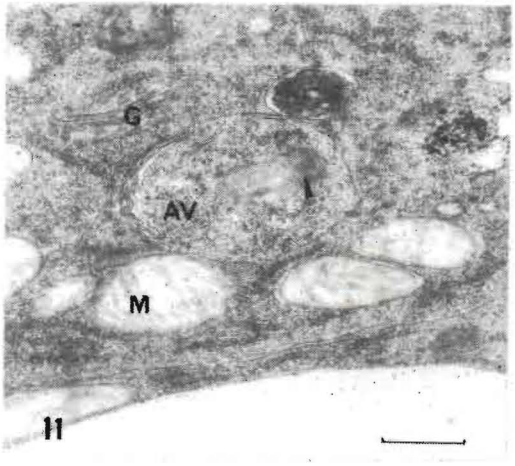
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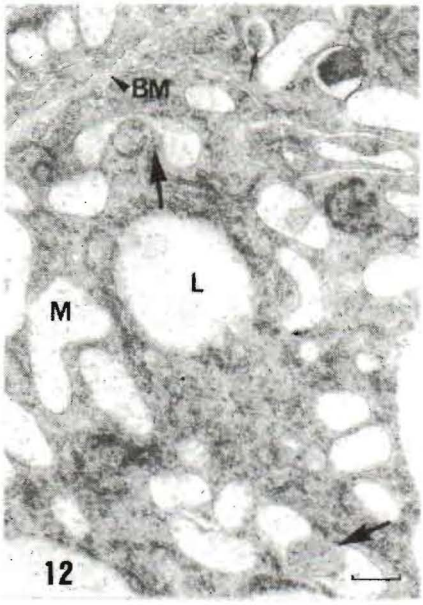
Fig. 12-15. Electron micrographs of fat body cells of a female 90 days old after emergence, showing the effect of age.

Fig. 12. Note most of the mitochondria (M) have less electron dense matrices, and several mitochondria engulf part of cytoplasm (large arrows). Note an adielectronic lysosome-like body in close proximity to a mitochondrion (small arrow). Basement membrane (BM) and lipid droplet (L). Scale bar = 0.5 μm.

Fig. 13. The degradation of mitochondria (M) and protein droplet (P). Lipid droplet (L) and nucleus (N). Scale bar = 0.5 μm.

Fig. 14. Note the whorl-like configuration of endoplasmic reticulum (ER) and an adielectronic lysosome-like body (arrow) in close proximity to autophagic vacuoles (AV). Lipid droplet (L) and mitochondrion (M). Scale bar = 0.5 μm.

Fig. 15. Note the whorl-like configuration of the endoplasmic reticulum (ER). Note the shrinkage of mitochondria (M), loss of their cristae and an increase in the electron density of their matrices. Note the degradation of protein droplets (P), lipid droplets (L) and trachea (T). Scale bar = 1 μm.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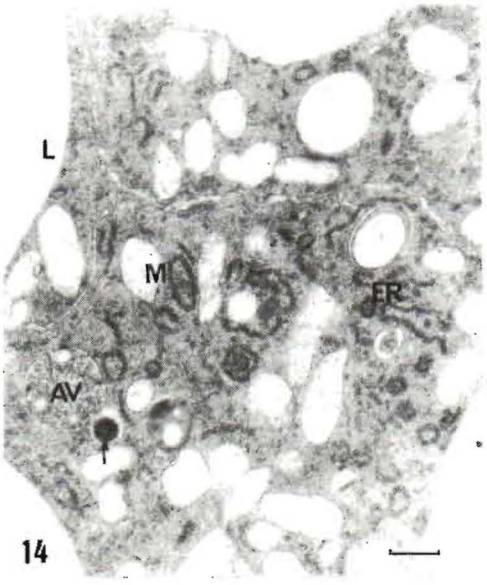
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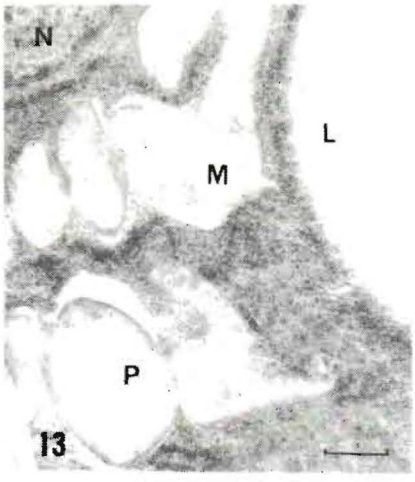
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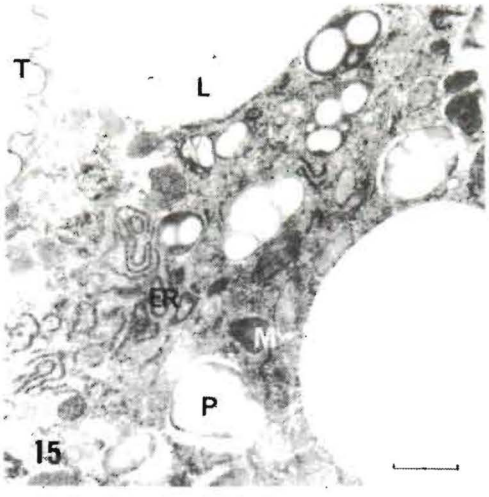
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تأثير العمر على التركيب الدقيق لخلايا الأجسام الدهنية في حشرة سوسة الحبوب

محمد صالح الخليفة

قسم علم الحيوان، كلية العلوم، جامعة الملك سعود ص.ب. ٢٤٥٥، الرياض ١١٤٥١، المملكة العربية السعودية

درست التغيرات في خلايا الأجسام الدهنية لأنثى حشرة سوسة الحبوب عندما تقدم بها العمر، ووجد أن كمية الأجسام الدهنية في أجسام الإناث قد نقصت أو قلّت كثيراً عندما بلغ عمرها تسعين يوما. ولقد لوحظ أن تقدم العمر يسبب تغيرات كبيرة في التراكيب الدقيقة لخلايا الأجسام الدهنية، بما في ذلك زيادة في الأجسام المحللة (Lysosomes) والفجوات ذاتية التغذية (Autophagic vacuoles) والقطرات الدهنية وانخفاض في كمية حبيبات النشاء الحيواني (الجليكوجين). أما بالنسبة للأجسام السبحية (الميتوكوندريا) فتصبح عديمة الرفوف (Cristae). التغيرات التي حدثت ولوحظت في هذه الخلايا المتقدمة السن نوقشت بالمقارنة بما وصف من قبل في خلايا الأجسام الدهنية وغيرها لأنواع أخرى من الحشرات.