ULTRASTRUCTURAL CHANGES IN THE FAT BODY CELLS OF MAMESTRA BRASSICAE (NOCTUIDAE, LEPIDOPTERA) DURING METAMORPHOSIS

by

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Received on June 19th, 1973

Introduction

In the larvae of the *Lepidoptera* the fat body is an organ which appears in the form of thin ribbons, lobes between the intestinal tube and the body wall. The lobes freely swim in the haemolymph, they are bound to the body wall and intestinal tube, respectively, only by a thin membrane of connective tissue. Parietal and visceral parts of the fat body are distinguished (Smith 1968; Chapman 1970). Its function is comparable to that of the liver, it synthetizes and/or stores lipids, glycogen and proteins (Kilby 1963; Price 1969; Chapman 1970).

In insects developing by holometamorphosis the fat body is a characteristic organ of the larval stage, in imagos it is fully absent, as a rule. In the course of postembryonal development well-defined changes can be observed in its cells (Gaudecker 1963; Ishizaki 1964; Walker 1966). In the young larval stages the cells are characterized by growth and a continuously increasing measure of storage of lipids (Locke and Collins 1965; Butterworth and Bodenstein 1967). During the last larval stage first the quantity of glycogen is increasing in an extreme measure. After that the cells of the fat body take up a great quantity of protein from the haemolymph by means of pinocytosis, as this was described regarding Calpodes (Locke and Collins 1965; 1968). The proteins taken up accumulate in the form of large granules (Gaudecker 1963; Ishizaki 1964; Walker 1966; Locke and Collins 1965; 1968). Later it is just the fat body which discharges a great quantity of proteins into the haemolymph; the electrophoretic mobility of these agrees with the one of the yolk proteins and also their entrance into the oocytes has been demonstrated in several insect species (Pan and coll. 1969; Engelmann and coll. 1971; De Loof and coll. 1972). At the end of the last larval stage large autophagic vacuoles appear in the cytoplasm of the fat body cells of the *Lepidoptera* and *Diptera* larvae, then the whole organ histolyzes in the first days of the pupal period (Butterworth and Bodenstein 1967; Gaudecker 1963; Locke and Collins 1965; 1968).

The circumstance that the said changes take place in the cells of the fat body at a definite time makes probable that, just as other steps of metamorphosis they also are consequences of the activation of particular genes at definite times, naturally through several intermediaries. These processes are controlled and influenced by the hormones of the insects (Highnam and Hill 1969; Engelmann and coll. 1971; Collins 1969; Thomasson and Mitchell 1972).

The examinations of the authors aimed at exactly describing with electron microscopic methods and determining as to time the steps of metamorphosis, especially the autophagic and heterophagic processes in the fat body cells of *Mamestra brassicae*. Since the changes of metamorphosis are well separated from one another in time, the cells of the fat body may serve with and excellent model system for a subsequent clearing of the control mechanism of the said steps.

Material und Method

Mamestra brassicae was kept and bred according to Szentessy's method (verbal communication). The larvae were kept in laboratory environment at 27°C and 75–80% relative vapour content and illuminated for daily 18 hours. The insect were fed on an artificial diet. Under such circumstances the duration of larval life was 34 days and the last (VIth) larval stage lasted 7 days. The single instars were determined according to their different colours.

For examination by the light microscope, pieces of the body of the larvae – in some instances the fat body separately – were fixed in Helly's or Bouin's mixture and embedded in paraffin. The sections were stained with haematoxylin-eosin, toluidine blue (pH = 3.5) and PAS reagent.

The material for electron microscopic examination was fixed in 4% glutaric aldehyde solved in 0.1 M phosphate buffer, then postfixed in a 2% OsO_4 solution and embedded in Araldit. The sections were contrasted by means of uranyl acetate and lead citrate. The examination was conducted by means of a UEMV – 100B electron microscope.

Results

Light-microscopic structure

The whole of the fat body as well as the single cells gradually increase in the course of the I–Vth larval stages. The lamellae of the fat body consist of a single layer of cells. The cytoplasm of the cells is highly basophilic, it contains numerous lesser or greater lipid vacuoles the number of which increases with time. The cell nucleus has processes and is relatively large, in it also the nucleolus is well recognizable.

In the first two days of the VIth stage a most rapid and considerable increase in the quantity of glycogen is conspicuous (Figure 1). Also the

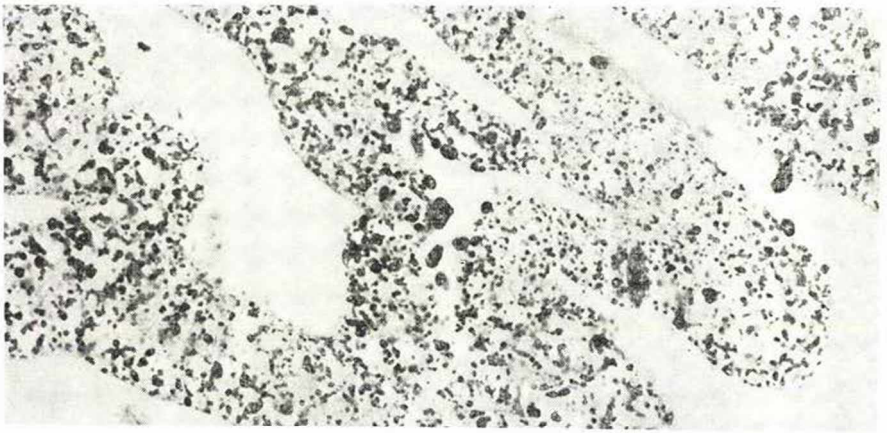

Fig. 1. 1st day of the VIth stage; the fat body cells are characterized by a marked accumulation of glycogen; PAS staining

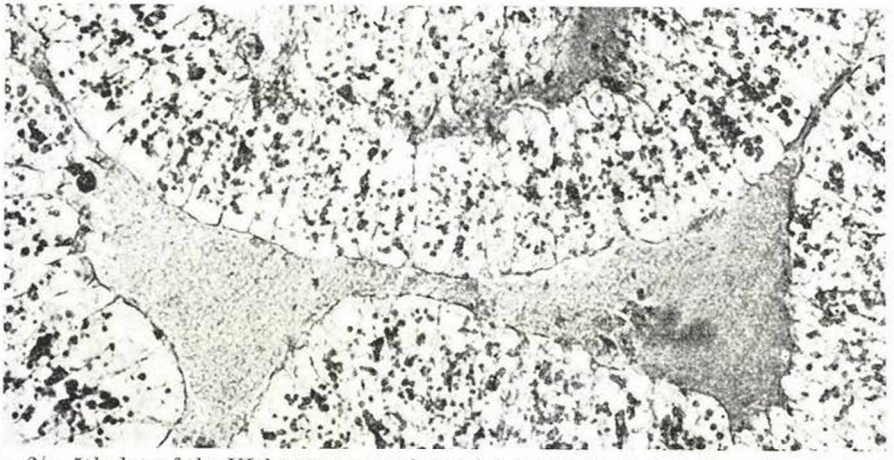

Fig. 2/a. 5th day of the VIth stage; granules staining with haematoxylin and eosin in the cytoplasm of the cells of the visceral lobe. Among the lamellae of the fat body there is a substance staining with eosin; haematoxylin-eosin staining

amount of the accumulated lipids is growing. The cells are swelling and become isodiametrical. The cytoplasm contracts to a thin network among the large lipid vacuoles.

The remarkable change of the third and fourth days is a concentration of the proteins of the haemolymph. In the beginning it appears only in the form of an eosinophilic mass among the cells and later also among the lobes of the fat body (Figure 2a). On the fourth day tiny basophilic granules are to be observed in the cytoplasm, mainly around the nuclei. These granules stain with haematoxylin and/or toluidine blue.

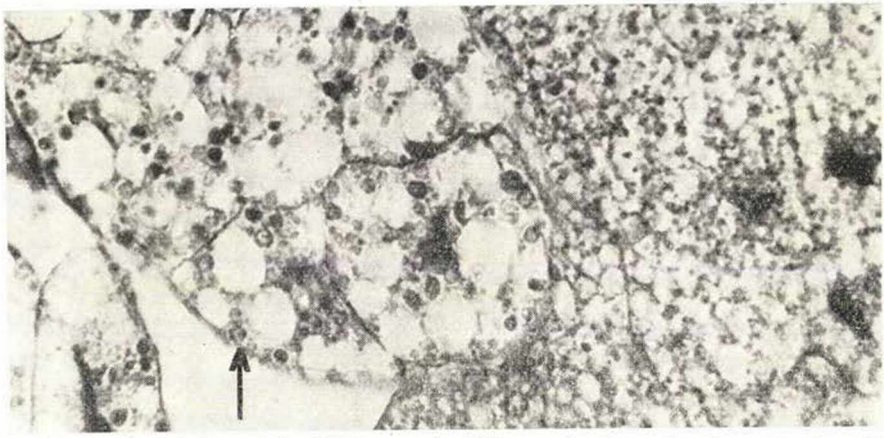

Fig. 2/b. On the 6th day of the VIth stage the difference between the visceral and parietal lobes of the fat body is well recognizable; haematoxylin – eosin staining

On the fifth day of the VIth stage numerous protein granules staining with eosin appear in the light microscopic picture, in the first place around the cell nuclei. Also the number of the granules staining with haematoxylin is increasing (Figures 2a and 2b).

On the sixth and seventh days the formation of granules intensifies to a degree that before pupation they completely fill up the cytoplasm.

In the pupal stage the cells of the fat body are swelling, in them there are several vast basophilic and a great quantity of eosinophilic granules. Types staining with both pigments are also to be found. The cell nuclei degenerate and then, on the 3rd day of the pupal stage, the fat body practically vanishes: only tiny connectival membranes are left from it. The interior of the abdomen is already filled up with the developing ovarioles at that time.

Electron microscopic structure

During the I–Vth larval stages a well-developed endoplasmatic reticulum of rough surface is to be observed in the cytoplasm of the cells which fills up the space between the lipid vacuoles and the cell nucleus. In many places also polyribosomes occur. Among the cisterns of the endoplasmatic reticulum there are numerous mitochondria, in many places active Golgi areas can be discerned. In the cells there are many microtubules, especially in their peripheral parts.

On the first two days of the VIth stage glycogen is accumulating in the cells. From that time on glycogen fills in continually growing fields among the cisterns of the endoplasmatic reticulum (Figures 4, 6 and 14). It is remarkable that the number of the Golgi apparatus is increasing and the Golgi vacuoles are filled with dense material. A qualitative change ensuing on the second day: numerous microbodies appear in the immedi-

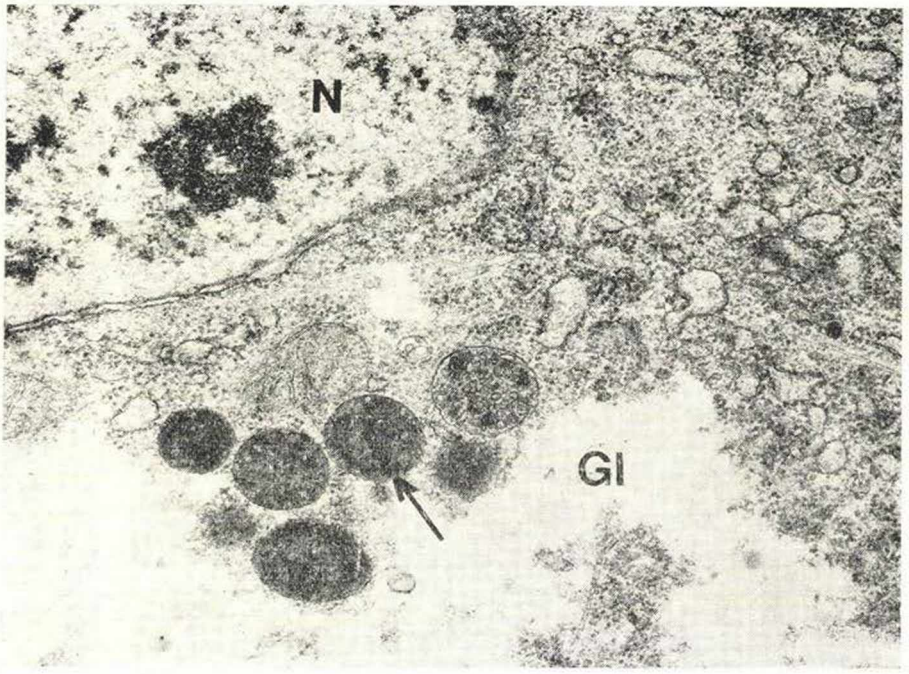

Fig. 3. Microbodies in the fat-body cells around the cell nuclei on the 2nd day of the VIth stage; N = nucleus, Gl = glycogen

ate environment of the cell nucleus (Figure 3). In the ground substance of these in numerous instances crystalline protein structure can be observed.

On the third day deep invaginations are to be noticed on the plasma membrane; they are filled with dense substance which probably corresponds to the proteins of the haemolymph (Figure 4). Similarly an event of the third day is the appearance of the multivesicular bodies (MVB). Morphologically these belong to two types. The ground substance of the first type is permeable by electrons, relatively few vesicles, arranged in groups can be observed in it; these are sorrounded by a dark areole (Figures 5 and 6). In cases it is to be seen that these bodies pinch off the direct environment of the Golgi areas (Figure 5). Within the second type of MVB there are vesicles to be discerned as well as thin tubules later arranged in glomerular form (Figures 6, 7 and 8). It is this second type which occurs in greater numbers in the cells of the fat body and is preserved up to the stage of pupation, yet inside it the structures degenerate (Figure 9). Simultaneously with the appearance the said bodies the number of Golgi areas increases in the cytoplasm. Equally at this time the first autophagic vacuoles appear in the course of development.

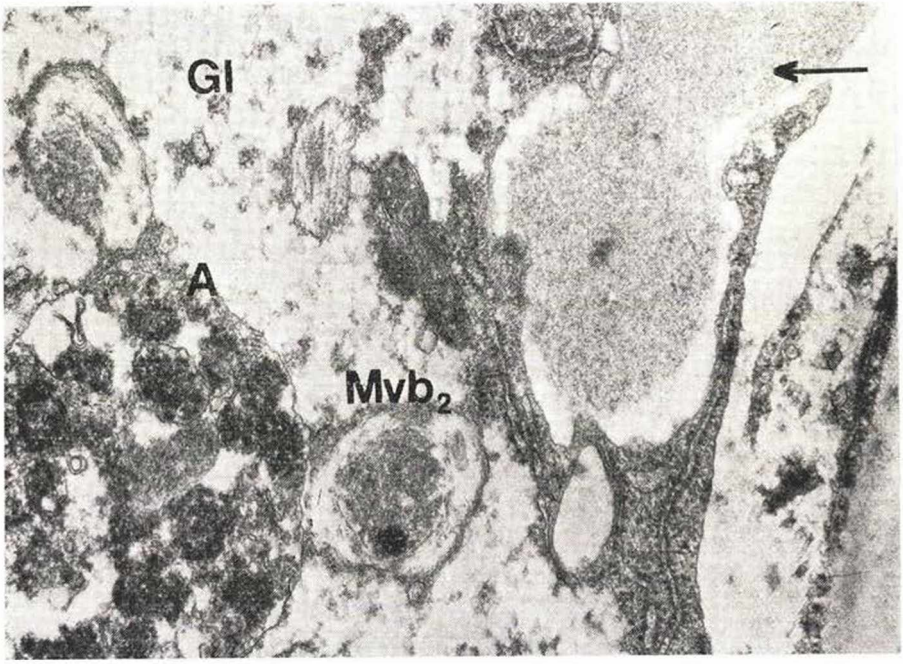

Fig. 4. Invagination containing dense substance on the plasma membrane on the 4th day of the VIth stage; Gl = glycogen, Mvb_2 = multivesicular body (of the IInd type), A = autophagic vacoule

On the fourth day the electron microscopic picture is characterized by the appearance of the autophagic vacuoles in great quantities. In them granulated parts of endoplasmatic reticulum, mitochondria, MVB-s can be recognized (Figures 4, 11 and 12). Characteristically, the autophagic processes start in most cases near the Golgi apparatus and the appearance of isolating cisterns can be observed beside them (Figure 10). Founded on a comparison of the light- and electron microscopic pictures it can be stated that the autophagic vacuoles are identical with the granules staining with haematoxylin which appear similarly on the fourth day. Their basophilia might be caused by the RNA content of the degrading ribosomes. In that stage there are also a great number of MVB's (especially of the second type) in the cells.

The eosinophilic protein granules appearing on the fifth day can be observed also at ultrastructural level. Probably they are formed of a third-type MVB, the ground substance of which is highly dense; in their marginal zone there are lighter vesicles, in their central part an initially small and later continually growing crystalline protein structure can be observed (Figures 13, 14 and 15). On the fifth day also the number of the autophagic vacuoles is increasing, parallelly with this the endoplasma-

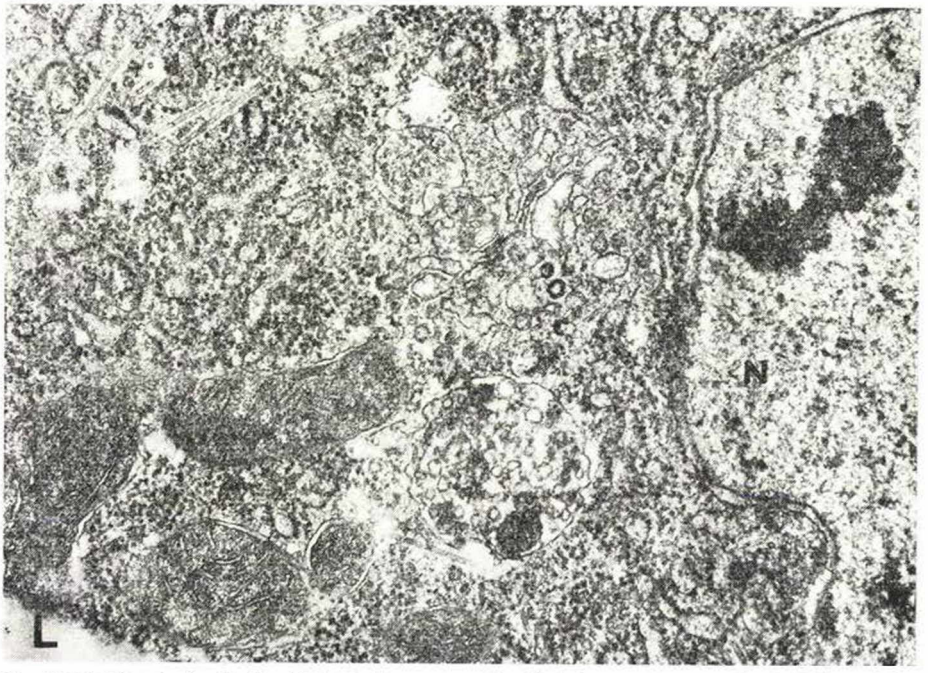

Fig. 5. Multivesicular body of the Ist type near the Golgi apparatus on the 3rd day of the VIth stage; N = nucleus, L = lipid vacuole

tic reticulum is forced back to a smaller and smaller area though everywhere the remaining parts seem to be capable of functioning .

The processes mentioned above become more and more expressed in the last two days of larval development. In the inside of the autophagic vacuoles the single cell organelles are already fully decomposed by that time or can be recognized but in traces (Figures 12 and 14). Interestingly, the autophagic vacuoles often also fuse with protein granules.

On the first day of the pupal stage, endoplasmatic reticulum and mitochondrium occur but sporadically in the cells which are filled up with a thin solution permeable by electrons. The cell nucleus is pycnotic, around it a great number of proteins and autophagic vacuoles accumulate (Figure 15). On the second and third days of the pupal stage the ground substance of the autophagic vacuoles is lightening and dense material occurs in them only in spots. Their size is growing, in some instances they are larger than the already degrading cell nuclei. On the third and fourth days of the pupal period the fat body histolyzes.

In the parietal lobe of the fat body the authors observed changes resembling those taking place in the visceral lobe. A substantial difference is, however, that here the protein granules appear onyl on the 7 th day of the last larval stage and their number is disproportionally smaller than in the visceral lobe.

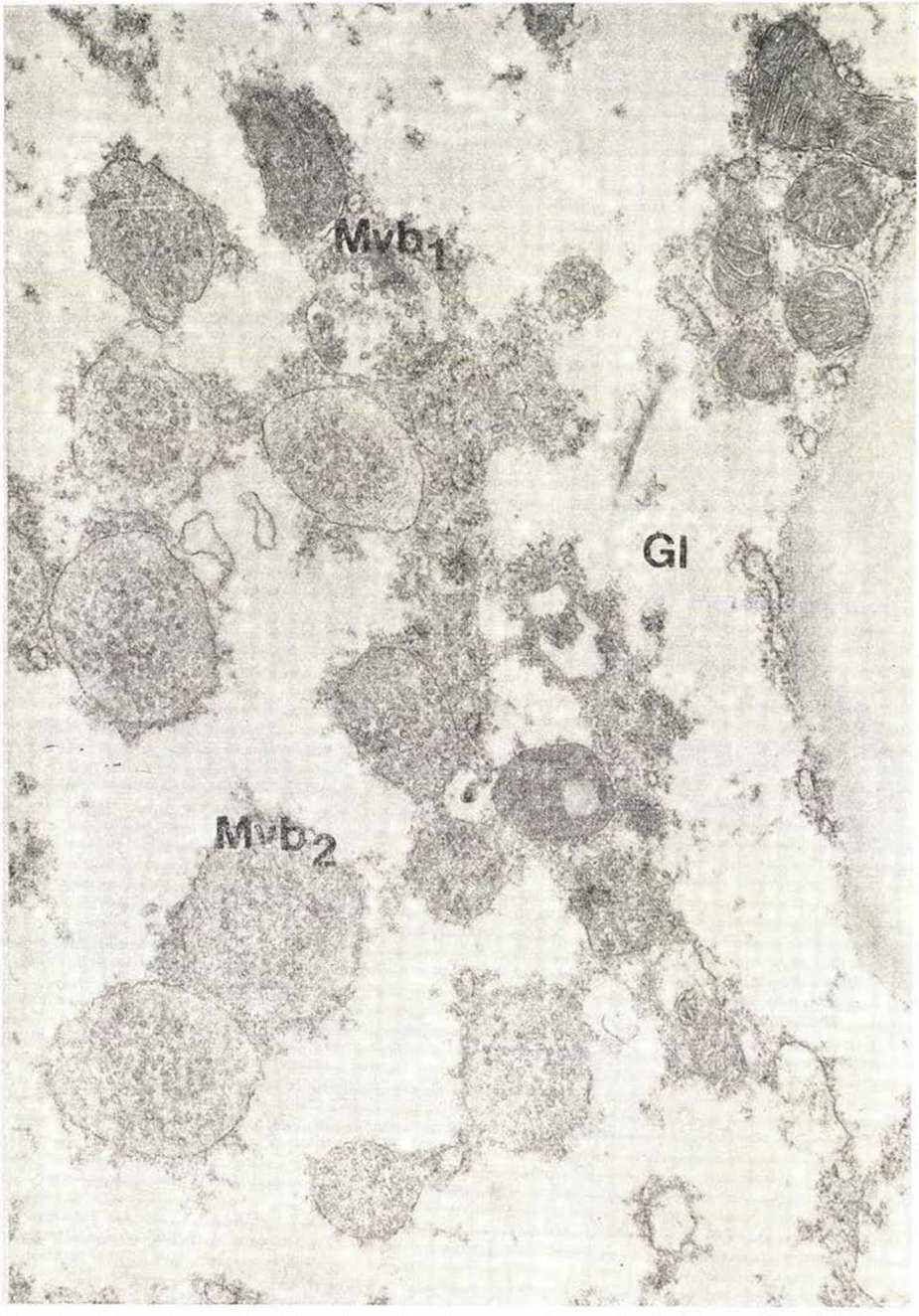

Fig. 6. The two types of multivesicular bodies appearing on the 3rd day of the VIth stage. Mvb_1 = multivesicular body (Ist type), Mvb_2 = multivesicular body (IInd type), Gl = = glycogen

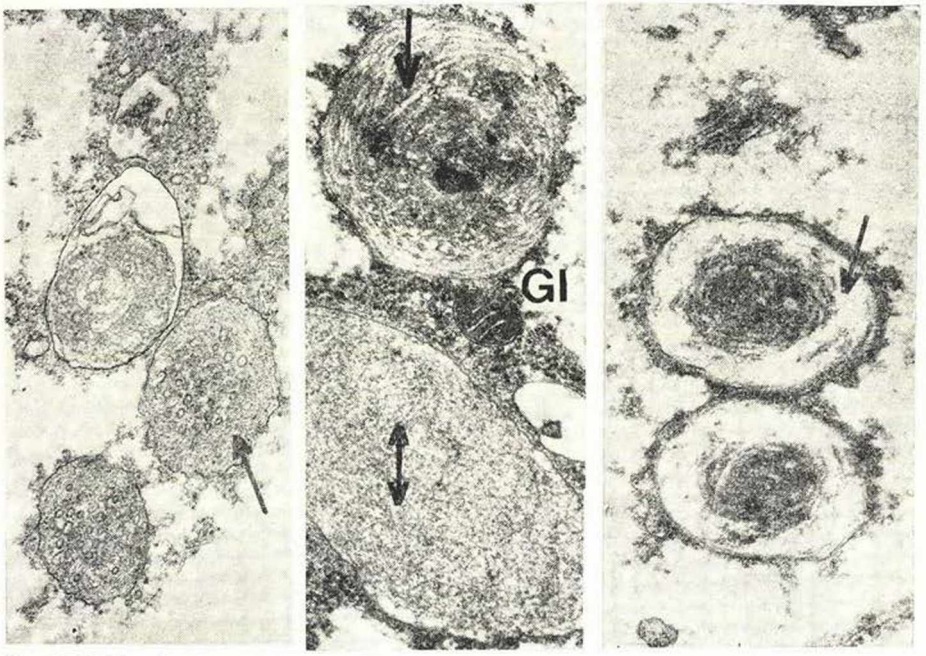

Fig. 7. Multivesicular bodies on the 4th day of the VIth stage. The coil-like structure in the inside of the Mvb_2 type is well observable

Fig. 8. On the 5th day of the VIth stage the coiling up of the tubules is well observable in the IInd type of the multivesicular bodies; Gl = glycogen, I = invagination filled with dense substance

Fig. 9. IInd type multivesicular bodies on the 2nd day of the pupal period

Discussion

According to several concordant results of investigation in various insect species *(Drosophila, Leucopheae, Schistocerca, Phylosamia, Leptinotarsa)*, in the first stages of larval development the fat body continually grows and the quantity of the lipids and glycogen accumulated in it is increasing (Butterworth and Bodenstein 1967; Walker 1966; Odhiambo 1968; De Loof 1969). In their examinations the authors also could make the same experience in *Mamestra brassicae*. The number and size of the lipid vacuoles further also the quantity of the glycogen granules intensely grows in the I–Vth stages.

Many microbodies appear round the cell nuclei on the 2nd day of the last larval stage in the visceral lobe of the fat body of the *Mamestra*. This has been mentioned only by Locke and Collins (1968) who conducted research work similarly on a *Lepidoptera* species *(Calpodes)*.

The appearance of the MVB-s is a general phenomenon in the course of the metamorphosis of the fat body (Ishizaki 1964; Gaudec-

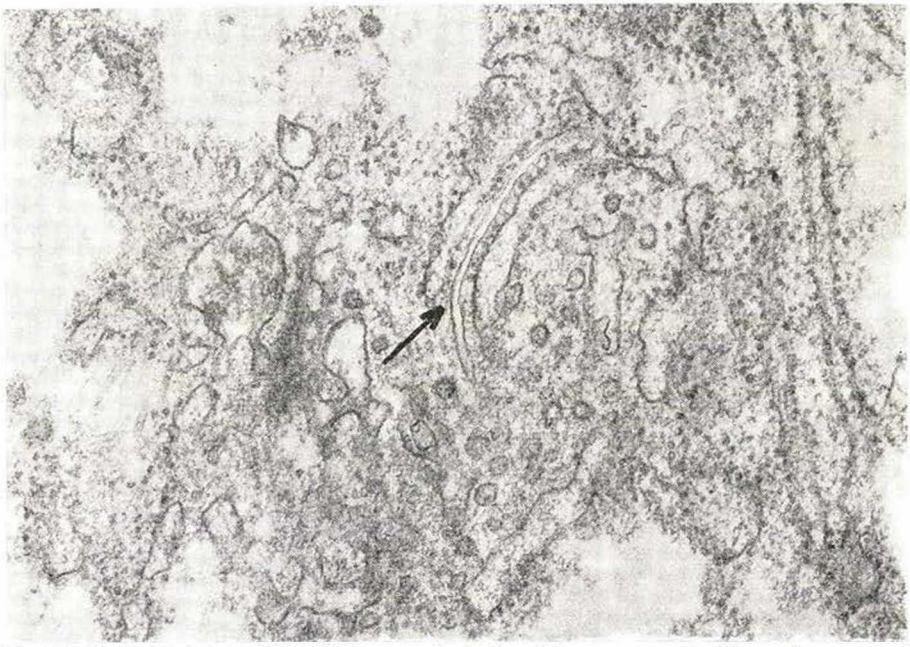

Fig. 10. On the 3rd day of the VIth stage isolating cisterns appear in the environment of the Golgi apparatus

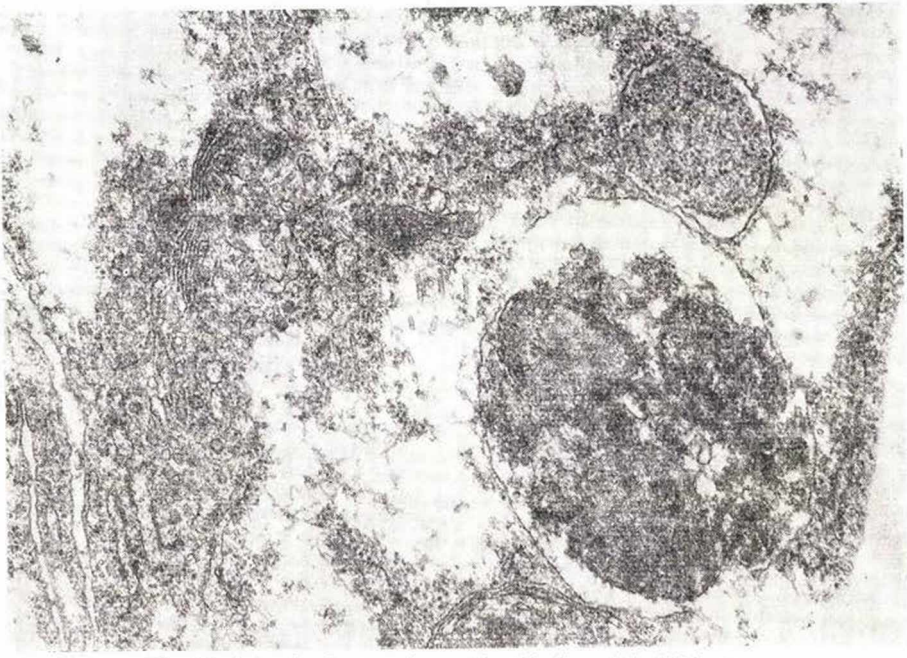

Fig. 11. Autophagic vacuoles on the 4th day of the VIth stage

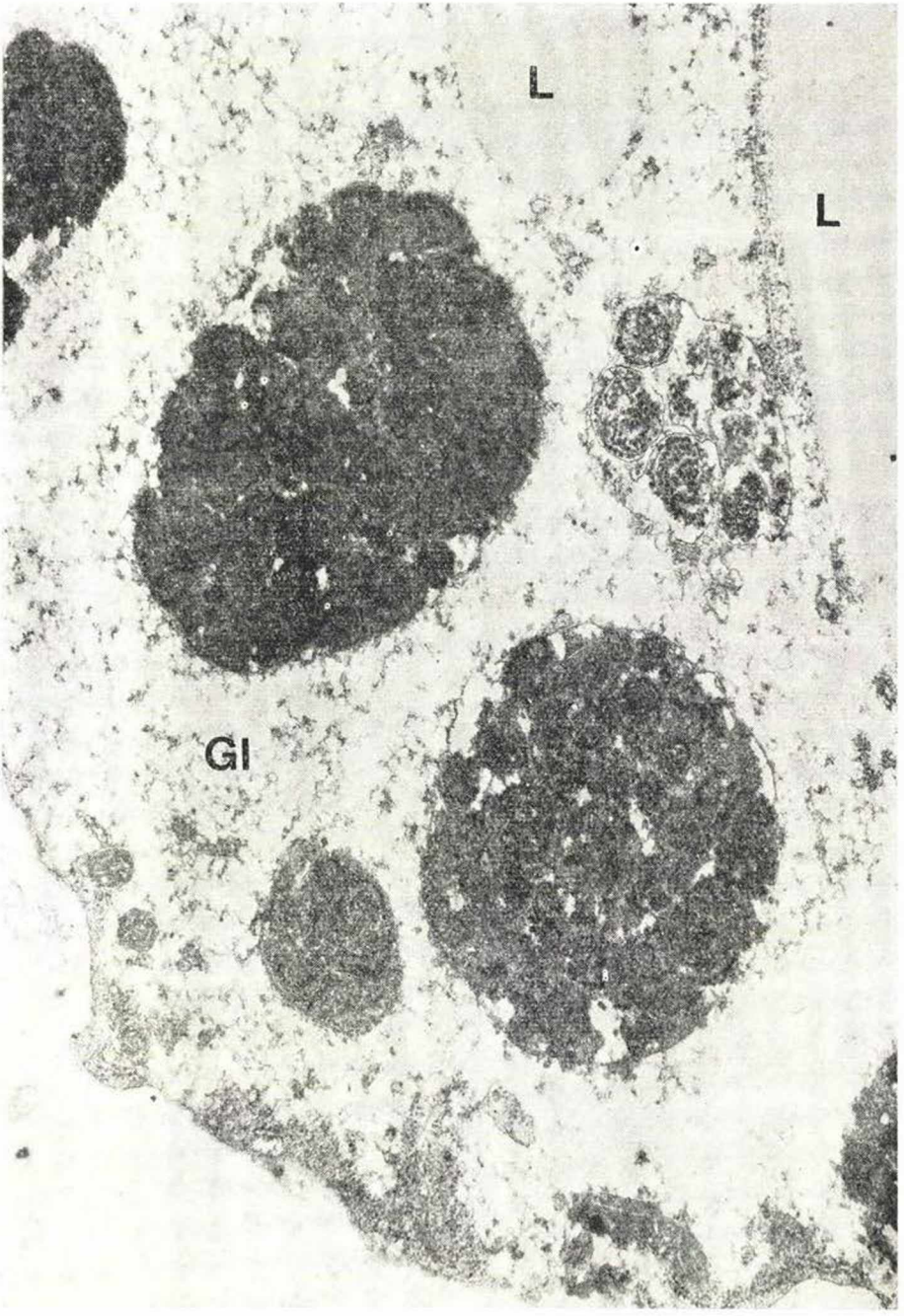

Fig. 12. Autophagic vacuoles near one another in the cytoplasm on the 6th day of the VIth stage; they contain early and degenerated substance; Gl = glycogen, L = lipid vacuole

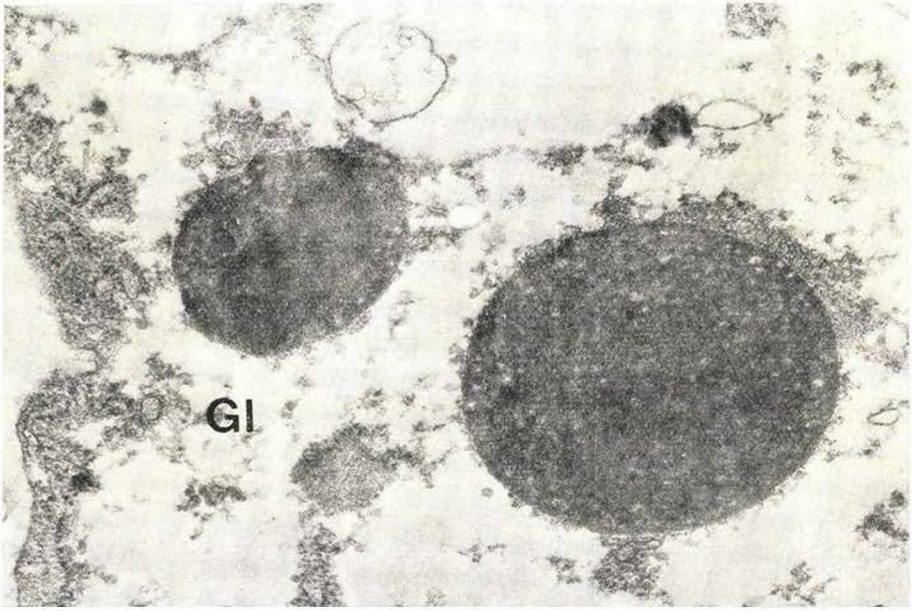

Fig. 13. IIIrd type multivesicular bodies with a protein crystal in their central zone on the 5th day of the VIth stage; Gl = glycogen

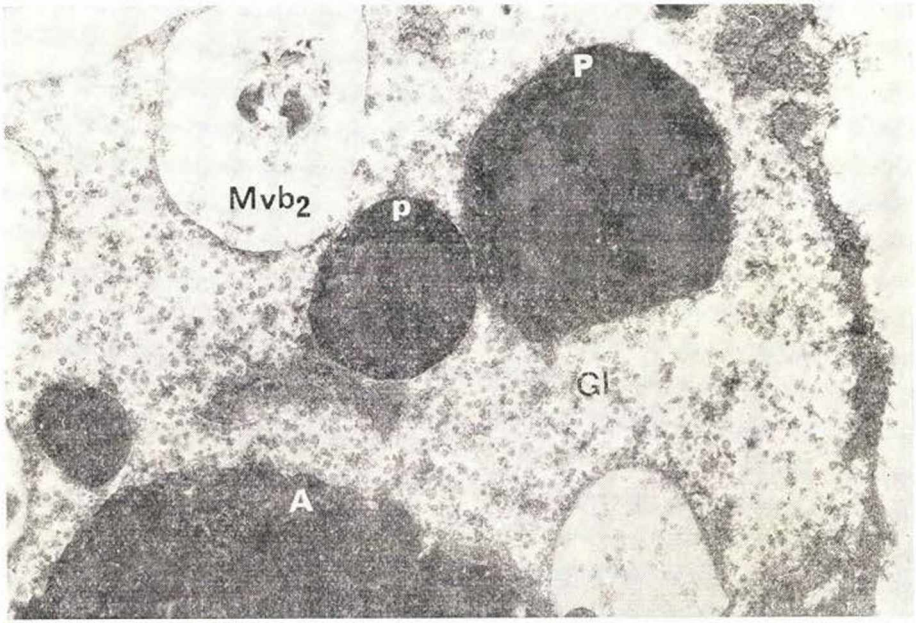

Fig. 14. Protein granules with vesicular structure in their marginal zone, degenerated autophagic vacuoles and IInd type multivesicular body on the 6th day of the VIth stage; P = protein granule, A = autophagic vacuole, Mvb_2 = multivesicular body (IInd type), Gl = glycogen

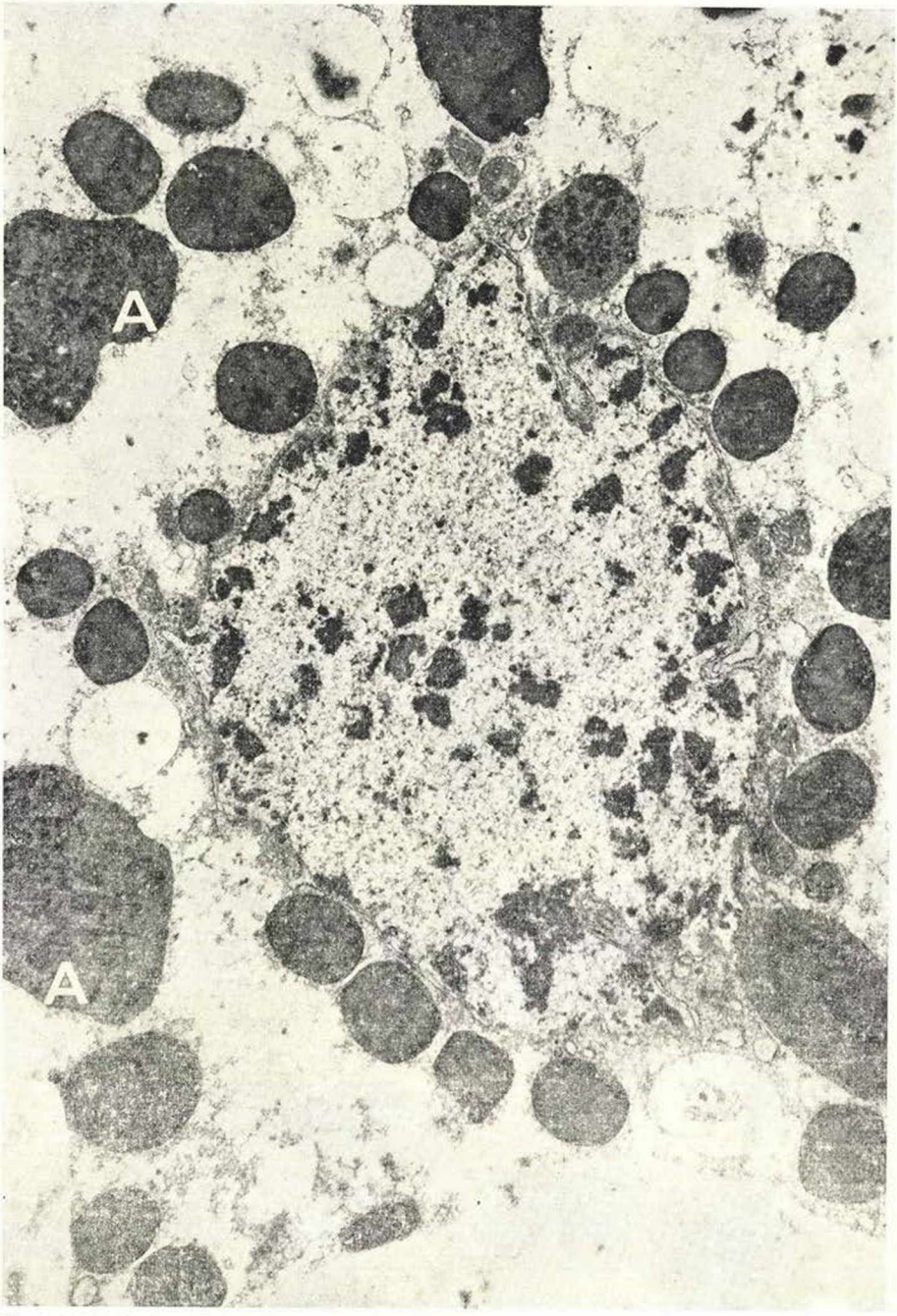

Fig. 15. Fat-body cell on the 2nd day of the pupal period; vast autophagic vacuoles (A) and numerous protein granules are to be seen round the cell nucleus

ker 1963; Walker 1966; Locke and Collins 1965; 1968); with the *Mamestra* it falls on the third day of the last stage. According to the findings of the authors and to the data of the pertinent literature the appearance of the MVB-s coincides with that of the deep invaginations coming in sight on the plasma membranes of the cells. In *Calpodes ethlius* pinocytotic vesicles were observed along the membrane of the invaginations (Locke and Collins 1968; Collins 1969). It is these pinocytotic vesicles – which contain proteins having their origin in the haemolymph – and the vacuoles of the Golgi apparatus that fuse into MVB-s (Locke and Collins 1965; 1968; Collins 1969; Collins and Downe 1972).

In the case of *Mamestra brassicae* the authors could distinguish three types by means of morphological methods. The type which appears first in the course of development is of very clear ground substance, in it few but dark vesicles can be seen. Similar MVB-s frequently also occur in the cells of insects and other animals. In the beginning the other type rather contains thin, coiled tubules than vesicles. The former are present in the cells up to the pupal stage although in the course of development the tubules decompose inside them. While in *Mamestra* this type of MVB is dominant, no similar ones have been described in other species. The third type agrees with the one described by the authors mentioned above (Gaudecker 1963; Locke and Collins 1965; 1968); from it the protein granules can be unambiguously deduced also in the case of the *Mamestra*. On the fifth and sixth day very often bodies can be seen on the marginal part of which the vesicular structure is still clearly discernible and there is already a protein crystal in their inside. The elucidation of the three kinds of MVB and of their interconnection calls for further investigation.

The protein granules are easy to recognize also with light-microscopic methods and it seems that they appear in the fat body cells of all insect species during development (Butterworth and Bodenstein 1967; Walker 1966; Odhiambo 1968; Locke and Collins 1965; 1968; De Loof 1971). Numerous authors are of the opinion that the protein content of the granules has its origin in the haemolymph and later, when released, it is embodied in the yolk substance of the ova (Pan and coll. 1969; Engellmann and coll. 1971; Patel and coll. 1972). As to their derivation and function, they can be considered heterophagic vacuoles since they contain proteins taken up from the outside and lytic enzymes which have their origin in the Golgi vesicles.

The formation of the protein granules is probably under hormonal control. According to experiments conducted in *Leptinotarsa*, their appearance is induced by the lack of juvenile hormone (De Loof and Lagasse 1970) and, in Calpodes, by an increased synthesis of ecdyson (Collins 1969).

As observed by the authors, there is a fundamental difference between the visceral and parietal lobes of the fat body as regards the appearance of the protein granules themselves. During development protein granul-

es are to be found much later and in much smaller quantity in the parietal lobe, while other phenomena of metamorphosis equally identifically affect also this lobe.

In the course of the development of the insects *Endopterygotes* the organs of the larval stage decompose. Regarding the salivary glands, intestinal canal and prothoracal glands the decomposition is effected by autophagic vacuoles which appear at a definite time in metamorphosis (Thomsen 1966; Beaulaton 1967; Schin and Clever 1968; Lockshin 1969; Aidelis and coll. 1971). The fat body is similarly a characteristic organ of the larval stage, numerous authors have described the origin of the autophagic vacuoles also in this respect (Ishizaki 1964; Walker 1966; Butterworth and Bodenstein 1967; Locke and Collins 1965; 1968). From among their examinations it is the development of *Calpodes ethlius* (Locke and Collins 1965; 1968) which most resembles the material of the authors discussed in the present paper. The difference is, however, that in *Mamestra brassicae* the formation of the autophagic vacuoles (4 days) takes place before that of the protein granules (5 days), while in the fat body cells of *Calpodes* they only appear on the day preceding pupation. In the course of the examinations discussed here the authors could not discover a difference between the way of pinching off of the mitochondria and of the endoplasmic reticulum in the way it was described in the case of *Calpodes* (Locke and Collins 1965).

Since the number of the autophagic vacuoles gradually grows from the 3rd day of the sixth larval stage, in the authors' opinion this process can be considered a specific secretory activity during which the cell is forming granules of RNP content by way of segregating its own rough-surfaced endoplasmic, reticulum. Then the material of the autophagic vacuoles is degraded into small molecules which become to be used up in the synthetic processes necessary for the development of the imago.

The exact timing of autophagy refers to the circumstance that the process is under strict control. The essential character of the latter needs to be cleared up by further investigation.

Summary

The changes ensuing in the cells of the fat body in the course of the development of the butterfly *Mamestra brassicae* were examined by the authors on light- and electron microscopic level.

The fat body consists of visceral and parietal lobes. In the larval stages the whole of the fat body and also the cells are growing, in them lipids and glycogen are accumulating. On the 2nd day of the last larval stage microbodies appear in the environment of the cell nuclei. On the 3rd day deep invaginations are being formed on the plasma membrane, which are filled with dense substance, presumably with protein originating in the haemolymph. Simultaneously with this, two types of multivesicular bodies were observed in the cytoplasm by the authors. In its light ground substance,

the first type contains few vesicles arranged in groups and having a dark areole. Besides numerous vesicles, tubules in coil-like arrangement can be recognized in the inside of the second type, its ground substance is dense. On the 4th day autophagic vacuoles appear in masses in all parts of the cytoplasm. On the 5th day protein granules begin to accumulate in the visceral lobe. These issue from a multivesicular body of a third type which is characterized by the feature that the lighter vesicles completely fill up the dark ground stance. First smaller and then continually greater protein crystals are to be recognized in their central parts. They probably contain proteins taken up from the haemolymph. In the cells of the parietal lobe the protein granules appear only later and in quite small numbers. On the 6th and 7th days the number of the protein- and autophagic vacuoles is increasing and the quantity of endoplasmatic reticulum decreases. The insect pupates on the 7th day. On the first two days only the degenerating nuclei and the said two types of granules can be observed in great number during the pupal stage, then on the 3rd day, the fat body histolyzes.

Since in the course of metamorphosis the auto- and heterophagic processes described above are strictly fixed as regards time and also well separable from each other, the authors are of the opinion that the fat body can serve with a suitable model system for clearing up the control mechanism of these processes.

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