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DIFFERENTIATION IN THE HONEYBEE LARVA

A HISTOLOGICAL, ELECTRON-MICROSCOPICAL AND PHYSIOLOGICAL STUDY OF CASTE INDUCTION IN *APIS MELLIFERA MELLIFERA* L.

(with a summary in Dutch)

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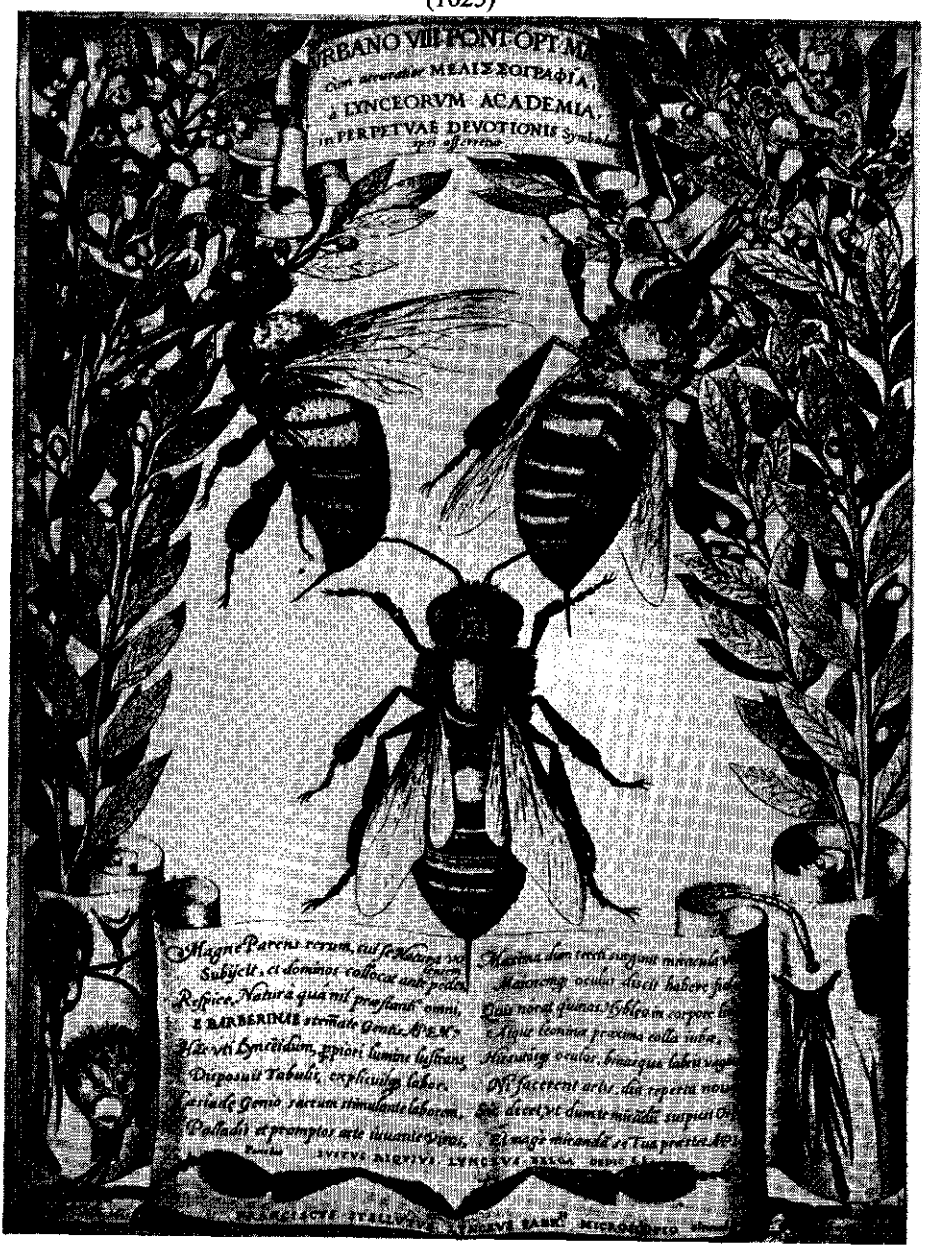

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1. INTRODUCTION

1.1. General introduction

In many insect species individuals from one sex, or both sexes, occur in more than one form. This phenomenon is called polymorphism when variation is discontinuous and so distinct as to be recognizable without morphometric analysis (Richards, 1961). In social insects the castes, although sometimes structurally nearly indistinguishable, may show specific behavioural and physiological differences (Michener, 1961).

Two different systems exist on which polymorphism can be based. When in a population the heterozygote is fitter than the corresponding homozygotes, in a number of generations an equilibrium will be attained resulting in the coexistence of different forms. This situation is called balanced polymorphism. The frequency of the different forms is a result of selective forces during a number of generations and, as stated, based on differences in fitness. In the other system the development of different forms in a generation is induced by the environment.

Differences between larvae or adults of the same species may then appear as phase polymorphism in locusts, pteromorphism in aphids, seasonal polymorphism in butterflies and leafhoppers, and caste polymorphism in social insects.

Metamorphosis, differentiation and regeneration are processes which, on the subcellular level, are brought about by activation – or derepression – of groups of genes that are involved in the development of larval, pupal and imaginal structures and functions. There are indications that during certain sensitive periods these nuclear processes are directly or indirectly regulated by the action of messengers from the cellular environment such as hormones (Wigglesworth, 1970).

Caste polymorphism has developed in close relation with the division of labour in a social insect colony. Michener (1961) submits that the division of labour antedated the morphological features of polymorphism.

Queen and worker dimorphism in the honeybee is not due to genetical differences between individuals. Differentiation is regulated via the food the young female larvae receive from the nurse bees (Perez, 1889). Therefore, queen and worker bee are to be considered as different phenotypes. The queen is the only sexually developed female in the colony. She is specialized in reproduction. Another important function of the queen is the secretion of pheromones which control processes such as building queen cells and the development of eggs in the ovaries of worker bees. The worker bee is morphologically unfit to copulate or store sperm and therefore cannot produce female offspring. Sometimes eggs may develop in the worker ovary, but as these eggs remain unfertilized only drones result. The worker bee is adapted to fulfill social duties such as nursing the queen, the drones and the brood, maintaining a constant high temperature

in the brood nest, comb building (wax secretion) and collecting nectar and pollen. As RICHARDS (1961) remarked, the development of the worker caste allowed the queen to evolve much further from the ancestral type than might have been possible in the absence of the worker.

Caste dimorphism in the honeybee has attracted the attention of scientists for a long time. The following topics related to the problem have been studied:

a. origin and composition of the food of queen and worker larva
b. postembryonic development of queen and worker bee
c. differences in structure and behaviour of adult queen and worker bee.

Morphogenetical and other physiological processes are governed by endocrine and nervous mechanisms. In the present study the role of hormones in caste differentiation of the honeybee was investigated. With histological, electron microscopical and physiological methods queen and worker bee were compared during larval development. The influence of the endocrine system, particularly that of the corpus allatum, on differentiation was given special attention. With histochemical methods an attempt was made to estimate the relative amount of glycogen and lipids in the fat cells of queen and worker larva during development.

1.2. LARVAL FOOD AND DIFFERENTIATION

Larval food plays a crucial role in inducing caste polymorphism. Quantitative as well as qualitative differences seem to be involved in regulating larval development. In this Section literature on origin and composition of larval food is reviewed. For the chemical composition the reader is referred to HAYDAK (1968).

1.2.1. *Brood care and larval food.*

Larval food is a secretion from the head glands of nurse bees: young worker bees that during the first three weeks of their life have well-developed food glands. Little is known about the interactions between the nurse bees and the larvae of both castes (TOWNSEND and SHUEL, 1962).

Honeybee larvae are regularly fed in their cells. LINDAUER (1952) calculated that a worker larva receives about 150 feeding visits during its development, taking a total time of about two hours. One nurse bee might raise 2 to 3 worker larvae. JUNG-HOFFMANN (1966) found that for raising a queen larva 1600 feeding visits were necessary with a total duration of 17 hours, the total amount of food offered to one queen larva being bout 1.5 gram.

For the larval food several terms are in use:

Brood food: a general term for the food fed to bee larvae.

Bee milk: a general term for the secretion product of the head glands of nurse bees.

Royal jelly (RJ): the food supplied to queen larvae.

Worker jelly (WJ): the food supplied to young worker larvae up to the moment of food change (during the first 3½ days of larval life).

Modified worker jelly (mixed food; MWJ): the food of worker larvae more than 3½ days old.

This terminology, mainly based on the source of larval food used for feeding experiments, will be used in this thesis.

In the food, VON RHEIN (1933) described two secretions which nurse bees separately offered to worker larvae. SMITH (1959) ascertained the same fact for queen larvae. JUNG-HOFFMANN (1966) and REMBOLD (1969) gave some data on the sources of bee milk. According to REMBOLD the secretion of the hypopharyngeal glands forms the basis of the food. The presence or absence of mandibular gland secretion should qualify it as RJ or WJ, respectively. There are indications (TOWNSEND and SHUEL, 1962) that sugars in brood food originate from the honey stomach. JUNG-HOFFMANN, following VON RHEIN, analysed the food of queen and worker larvae. The author also distinguished two secretions: a clear and a milky one. The clear one presumably is composed of secretion of the hypopharyngeal glands and honey, the white one may contain secretion of the mandibular glands in addition to secretion of the hypopharyngeal glands. Queen larvae not only receive more food, but the ratio white component to clear component is about 1:1 as against 1:3 to 1:4 for worker larvae. Worker larvae of more than 3½ days old receive in addition to the clear secretion a yellowish component about every third feeding. The colour of this component can be attributed to the presence of pollen, so that besides the clear secretion, the content of the honey stomach is probably involved. Older worker larvae are rarely fed white component. VON RHEIN (1956) assumed that addition of pollen is a way to 'economize glandular food' in the colony.

1.2.2. *Influence of larval food on postembryonic development.*

Several investigators have been engaged in rearing honeybee larvae in vitro mainly because to control circumstances inside the hive is very difficult, especially where feeding the larvae is concerned. VON RHEIN (1933) investigated the influence of larval food on development. He concluded from his experiments in vitro that three kinds of bee milk can be distinguished:

a. Worker jelly: with this food the larva becomes a worker. When fed to older larvae it prevents them from passing through metamorphosis.
b. 'Royal jelly for young larvae': with this food the young larva is induced to become a queen. Older larvae receiving this food die prematurely.
c. 'Royal jelly for older larvae': this food enables the older larvae to develop into adult queens, provided they have been fed 'royal jelly for young larvae' earlier in larval life. It stimulates growth and makes metamorphosis possible.

WEAVER (1955, 1958) found that larvae reared in vitro on stored RJ develop into queens, workers and intercastes. It was concluded that RJ contains a very labile substance which induces caste differentiation. According to WEAVER the amount of food did not play a role as his experimental larvae had an excess of food at their disposal. SMITH (1959) made a detailed study of the effect of storage on the differentiating capacities of RJ. He concluded that the activity indeed decreased on storage, but not to the extent WEAVER supposed. He succeeded,

unlike VON RHEIN, in raising young larvae on 'RJ for older larvae'. SMITH suggested that a certain amount of RJ is needed for the development of a worker larva into a queen. PETIT (1963) observed that larvae grew better and faster when she added honey (up to 20%) to RJ. SHUEL and DIXON (1968) added sugar to WJ and found that this mixture, unlike pure WJ, allowed four-day-old worker larvae to reach the adult stage. MITSUI et al. (1964) collected RJ from queen cells of different ages and compared ovary development of larvae reared on these samples. The authors found that feeding RJ from three-day-old queen cells resulted in adults with about 150 ovarioles per ovary, as compared with 45 to 70 ovarioles per ovary in the other treatments. They gave no explanation for this finding.

Since 1957 research workers from the Max Planck Institute of Biochemistry in Munich have been trying to identify the 'determining Principle' in RJ. Biopterin, neopterin and pantothenic acid, larger amounts of which are found in RJ than in WJ, appeared to have no direct influence on caste differentiation (BUTENANDT and REMBOLD, 1957; 1958). REMBOLD (1969) claimed that he found a 'factor' in the dialysate of RJ. Added to larval food this very labile substance – the structure of which is still unknown – may promote the differentiation of worker larvae into queens. This finding is very interesting, but until now more detailed data are lacking.

1.3. GROWTH OF QUEEN AND WORKER LARVA

In the colony, honeybee larvae are reared under very favourable circumstances. Temperature in the brood nest is maintained at about 35 °C; food of high quality is supplied frequently and at regular intervals. Therefore, it is not surprising that the larvae of queens and workers – with a weight of 0.1 mg only just after hatching – attain a body weight of about 300 mg and 150 mg respectively, in only six days.

STABE (1930) and WANG (1965) collected data on the weight of queen and worker bees during larval development. The data of WANG have been used in Fig. 1. Worker larvae are heavier than queen larvae during the first days. Differences are most pronounced during the third day. Only after the fourth day do queen larvae become heavier than worker larvae. The authors gave no satisfactory explanation for the difference in weight during the first three days.

Histological observations made during this study showed that in worker larvae a considerable fraction of the fat body is occupied by liquid containing vacuoles. In queen larvae these large vacuoles are normally missing. The difference in weight may be caused by a higher moisture content of worker larvae. Biochemical analysis (STRAUS, 1911) indeed showed that young worker larvae contain more water than older ones. MELAMPY et al. (1940) found a higher moisture content in young worker larvae than in queen larvae of the same age.

The duration of the developmental stages was studied by several authors at the beginning of this century. In 1963 JAY reviewed literature concerned. There is

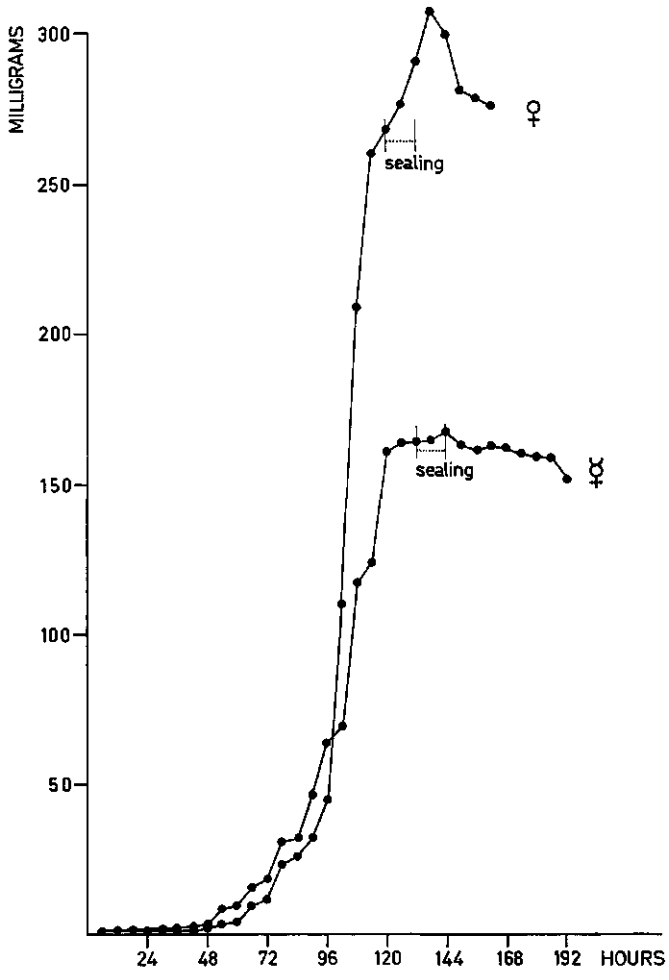


FIG. 1. Growth rates of honeybee larvae (after WANG, 1965).

considerable variation in the data. Part of this variation could be attributed to differences in temperature in the brood nest as MILUM (1930) showed this factor to have a pronounced influence on the duration of development.

BERTHOLF (1925) counted the number of larval moults. His data are presented in Fig. 2. During the first four larval instars no differences in developmental rate can be observed between queen and worker. The duration of the fifth larval instar of the queen is one day shorter than that of the worker, and the duration of the pupal stage is even four days less. Consequently, the total difference in time

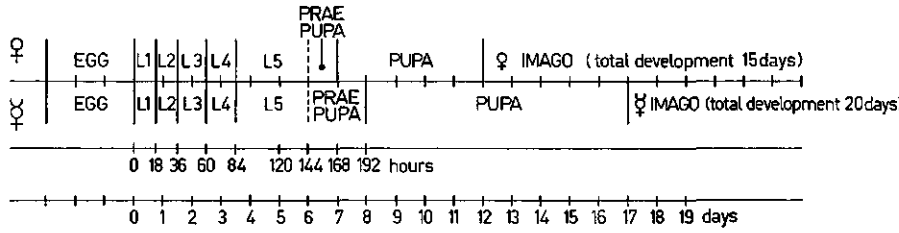


FIG. 2. Postembryonic development of queen and worker larva (after BERTHOLF, 1925).

needed for adult development of queen and worker amounts to five days. The praepupal phase, as the name indicates, is not a clearly defined stage. It is characterized by the pupal structures becoming visible within the larval skin after spinning of the cocoon. BERTHOLF found that ecdysis only takes 30 minutes.

In the present study data were collected on the duration of moulting by comparing histological preparations of larvae that were fixed at regular intervals of 3 hours. About 10 hours before the next exdysis the deposition of a new cuticle by the epidermal cells became visible. In the following hours the cuticle increased in thickness. The process – easily followed near the outlet of the spinning glands – can be used to correct the age of the larvae in experiments. Observations on the duration of the larval instars were in agreement with those of BERTHOLF.

2. MATERIALS AND METHODS

2.1. Experimental animals

Colonies of the Dutch brown bee – an indigenous race of *Apis mellifera mellifera* L. – kept in 6 or 10 frame hives were used for all experiments, unless otherwise stated. To obtain larvae of known age a queen was restricted to one brood frame by fitting queen excluder screens to both sides of the empty frame. The queen was allowed to lay eggs for a certain period (normally 6 hours). Subsequently the batch of eggs was marked with pins. After three days only eggs that hatched within the course of 6 hours were maintained. Other eggs and larvae were removed. Queen larvae were obtained by grafting worker larvae of appropriate age in natural queen-cups, which were fitted under a horizontal bar halfway up the brood frame. The larvae were dry grafted, unless otherwise stated. Immediately after grafting frames with larvae were placed in colonies which had been queenless for about 24 hours. Variations in growth rate were more pronounced in queen larvae than in worker larvae. In the experiments larvae that were too retarded in development were not used. In experiments involving mutual comparison of queen and worker, only larvae from the same batch of eggs were used. Some of these larvae remained in the queenright colony and were therefore raised as workers. Others were grafted in queen cups and reared as queen larvae in queenless colonies. Preliminary trials showed that worker larvae when 48 hours old were large enough to process histologically. They are well suited to study changes in development upon grafting. Also, functional queens still can be produced by raising two-day-old worker larvae in queen cells. Queen and worker larvae were taken from the hives at regular intervals and fixed at once.

2.2. Techniques

In Table 1 ages of larvae in the experimental series are listed as are their numbers and the period they remained in queen cells. The difference in age of larvae at the start of an experiment can be 6 hours. When the larvae grow up physiological age differences may become more pronounced. As the first four larval instars last only about 24 hours each it is important to determine the age of the larvae as accurately as possible. As stated in Section 1.3 this can be done within certain limits by observation of the moulting process. This method was used to correct the ages of larvae in some of the experiments (see Fig. 2), but the ages of the individual larvae were not corrected. In experiments in which older worker larvae were used for transfer into queen cups ecdysis took place some hours later in queen larvae than in corresponding worker larvae. This is probably due to grafting. No corrections in age were made in that case.

In Table 2 histological methods have been summarized. Other data on materials and methods will be given at the appropriate place elsewhere in this thesis.

TABLE 1. List of experiments: A: age of larvae (in hours). B: time fed as queen larva (in hours). C: number of larvae per age group.

♀ larvae		Fixations:																			
		1		2		3		4		5		6		7		8		9		10	
Exp. series		A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C
1		15	4	29	2	35	4	41	3	58	4	64	3	70	4	82	4	88	2	94	2
2		34	3	37	1	40	3	43	1	46	3	49	1	52	3	55	1	58	3	61	1
3		48	2	60	1	72	2	75	2	78	3										
4		24	2	36	4	48	4	60	4	74	4	84	2								
5		35	4	47	3	59	2	71	3	83	2										
6		18	2	35	3	47	2	59	3	71	2	83	2	95	2	106	2				
7		48	3	54	2	60	2	66	2	72	2										
8		48	4	60	2	66	2	72	2	78	2	84	3								
9		70	3	82	4	88	4	94	4	106	2										
10		50	2	62	3	74	4	88	3	100	4										

♀ larvae	Grafting age (in hours)	Fixations:																					
		1			2			3			4			5			6			7			8			9			10		
Exp. series		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	5–10	15	10	3	29	24	3	35	30	3	41	36	3	58	48	3	64	54	3	70	60	3	82	72	3	88	78	2	94	84	2
2	34	37	3	4	40	6	4	43	9	3	46	12	4	48	15	5	52	18	5	55	20	4	58	24	3	61	27	3			
3	48	60	12	3	72	24	3	75	27	2	78	30	2																		
4a	12	24	12	4	36	24	4	48	36	5																					
b	24	36	12	3	48	24	4	60	36	3																					
c	36	60	24	3	74	38	2																								
d	48	74	26	4	84	36	5																								
5	23	35	12	4	47	24	4	59	36	4	71	48	4	90	60	3															
6a	18	35	17	1	47	29	3	59	41	3	71	53	3	83	65	3	95	77	3												
b	68	95	27	2	107	39	4																								
7	48	54	6	2	60	12	3	66	18	2	72	24	1																		
8	48	60	12	2	66	18	2	72	24	2	78	30	2	84	36	3															
9	70	82	12	4	88	18	3	94	24	3	106	36	2																		
10	50	62	12	2	74	24	2	88	38	2	100	50	2																		

Table 2. Histological methods

Object	Fixative	time	Rinsing	time	Fixative	time	Rinsing, staining	time	Dehydration	time
1. Larvae (skin ruptured)	Bouin	24–48 h	alc. 70%	overnight					alc. 80, 90, 96%, M.B.: subsequently M.B.C.:	15–30 min each > 48 h
2. Larvae (intact)	Carnoy 70%	1–12 h*	alc. 70%	overnight					alc. 80, 90, 96%, M.B.: subsequently M.B.C.:	15–30 min each > 48 h
3. Larvae (intact)	Carnoy 100%	1–6 h*	alc. 100%	30 min					alc. 100%, M.B.: subsequently M.B.C.:	15–30 min each > 48 h
4. Fat tissue dissected	Formol (10% in H_2O)	5 min	H_2O bidest	5 min			Sudan III			
5. Larval heads	2% Glut. ald. phosph. b.0.1M.: 0.17 M sucrose pH 7 4°C	2 h	Phosphate b. 4°C: 0.34 M sucrose idem 4°C:	10 min (2×) overnight	1% OsO_4: (in Phosph. b.)	2 h	H_2O bidest: subsequently uranyl acetate 1% in H_2O:	15 min (2×) 30 min	alc. 70, 80, 90, 96, 100 (2×) %: subsequently prop. oxyde (2×):	15 min each 15 min each
6. Larval heads	3% Glut. ald. cacodyl. b.: 0.1 M pH 7.3 4°C	1½ h	Na-cacodylate b.: 4°C 0.34 M sucrose idem 4°C:	10 min (2×) overnight	1% OsO_4: (in veronal-acetate b.)	2 h	H_2O bidest: subsequently uranyl acetate 1% in H_2O:	15 min (2×) 30 min	hexyleen glycol 10, 20, 40, 60, 80 100%:	30 min each

Continuation

No. Embedding	time	sectioning	staining	time	microscope
1. Benzol (3×) paraplast (melt. point 58–60°C) (3×): blocks of paraplast	15 min each	5, 7, 10 μ* Leitz microtome	Mallory		Olympus E.H. Wild M. 20
2. Benzol (3×) paraplast (melt. point 58–60°C) (3×): blocks of paraplast	15 min each	5, 7, 10 μ* Leitz microtome	Mallory, Y.H.		
3. Benzol (3×) paraplast (melt. point 58–60°C) (3×): blocks of paraplast	15 min each	5, 7, 10 μ* Leitz microtome	P.A.S., Diastase-P.A.S.		
4.		gentle squash			
5. Prop. oxyde: E.A. = 3:1; 1:1; 1:3 : E.A. 4°C: Embedding in gelatin capsules Hardening: 20°C 40°C 70°C :	30 min each over night 24 h each	L.M.: 1–5 μ E.M.: ± 500A L.K.B. ultratome III	Uranyl-acet.: subseq. Lead citrate:	30 min 30 min	Siemens Elmiskop 1
6. Hex. glycol.:E.R.L. = 1:1; 1:2 : E.R.L. 4°C : Embedding in gelatin capsules Hardening: 70°C. :	30 min each over night 24–48 h	L.M.: 1–5 μ E.M.: ± 500 A L.K.B. ultratome III	Uranyl-acet.: subseq. Lead citrate:	30 min 30 min	

* Depending on larval age.

Explanations: Bouin; satured picric acid (H_2O): formalin: acetic acid = 15:5:1
b: buffer
Carnoy 70%; alc. 70%: chloroform: acetic acid = 6:3:1
Carnoy 100%; alc. 100%: chloroform: acetic acid = 6:3:1
E.A.: Epon (812)-Araldite (6005; Ladd's)
EM.: Electron microscopy
E.R.L.: E.R.L.-4206 (Spurr, 1969)
Glutar aldehyde: previously treated with active charcoal (FAHIMI and DROCHMANS, 1965)
L.M.: light microscopy
Mallory: according to MALLORY (1900), adapted for insect tissue.
M.B.: methyl benzoate
M.B.C.: methyl benzoate + 2% celloidin
P.A.S.: periodic acid Schiff's reaction (PEARSE, 1960)
Prop. oxyde: propyleen oxyde
Sudan III: according to DADDI (1896)
Uranyl acetate, leadcitrate: according to REYNOLDS (1963), adapted for the honeybee,
Y.H.: Iron-haematoxylin; according to HEIDENHAIN (1892), adapted for insect-tissue

3. FOOD PERCEPTION

It is well known that endocrine processes in the insect may be controlled by environmental factors (DE WILDE, 1971). According to DETHIER (1963): 'the accomplishment of many sexual, reproductive, social and feeding activities of insects depends to a great extent upon the detection and assessment of specific chemical aspects of the environment'.

No information is available on the sensory information which the larva of the honeybee receives from the food. NELSON (1924) described two small spines on the labium, located on each side of the common opening of the silk glands, slightly ventrally to it. Also on each maxilla the author mentioned a spine similar to that on the labium. However no further information was given.

Histological and electron microscopical examination of these spines in 4th instar larvae revealed that each of them is composed of a coupled pair of sensilla (Fig. 12). The top of each of them is formed by an infolded conical papilla (Fig. 13a). Five dendrites can be seen running to the tip of the sensillum (Fig. 13d). However, not all of the dendrites reach the extreme tip of the papilla (Figs 13b, c). Possibly one of the neurons has a mechanoreceptive function. The nerves of the sensillum appear to end in the suboesophageal ganglion. Sheath cells surround the dendrites of the sensillum. It is difficult to distinghuish between tormogen and trichogen cells (Fig. 14).

Electrophysiological recording showed that the sensilla contain sugar and salt receptors. WANG and SHUEL (1965) and BER-LIN CHAI and SHUEL (1970) found that addition of sugar to the food stimulated larval development, whereas acids often hampered this development. It is attractive to think in terms of growth regulation by feeding stimulant c.q. deterrent action.

The 'determining principle' in RJ might have a pheromone function and act on differentiation via chemoreceptors.

4. FAT BODY

4.1. INTRODUCTION

The function of the fat body of an insect is not merely restricted to storage of fat, as the name would suggest. The fat body plays a more complicated role in insect metabolism, in many respects comparable with that of the liver in vertebrates. It regulates haemolymph composition by processes like uptake, storage, synthesis and release of proteïns, carbohydrates and lipids.

In the larval honeybee, organs that are involved in intake, digestion and storage of food are about the only well developed structures. The adipose tissue of the mature larva comprises 60% of the body weight (BISHOP, 1961). It is stuffed with food reserves that enable the development of imaginal structures during the late larval and pupal stage when no food is consumed. STRAUS (1911) found that more than 50% of the dry weight of full grown larvae consists of nutrients. In Chapter 1 the role of food in inducing caste differences was discussed. If one considers the importance of nutrition it is surprising how little attention has been paid to the adipose tissue. SCHNELLE (1923, 1924) gave a morphological description of the development of this tissue in worker larvae. But as he only mentioned lipids as store, his findings are of little value. BISHOP (1922, 1958) was mainly interested in the formation of proteïn granules in the fat cells of mature larvae and pupae. His observations on earlier developmental stages of the fat cells – mainly restricted to queen larvae – were brief and incomplete. In his biochemical research (RONZONI and BISHOP, 1929) some attention was given to the importance of glycogen as a food reserve, but no statement was made on the site of storage. Most of the other authors (NELSON, 1924; SNODGRASS, 1956; BOEHM, 1965; HOFFMANN, 1968) referred to the work of SCHNELLE and BISHOP when reviewing the larval fat body. Therefore it was decided to include in this study a histological and histochemical examination of the adipose tissue.

4.2. FAT BODY OF THE WORKER LARVA

In the development of larval fat-cells several phases can be distinguished which are more or less corresponding with the larval instars. The fat body can be subdivided into a peripheral and a central part, the latter being situated mainly ventrally against the midgut. Both parts pass through the same phases, but the peripheral part is earlier in development. As the ventral part is more extensive than the peripheral part the description will be based on the former, unless otherwise stated. The growth of the fat cells (Fig. 3) parallels larval growth (Fig. 1). In the larva that has just hatched the cell-diameter is about 5 to 8 μm, in the full-grown larva 60 to 80 μm (SCHNELLE, 1924). In addition to light microscopic photographs some electron microscopic photographs have been included.

The reader is referred to the accompanying text (Figs 15 to 24).

First phase: multiplication. In the first larval instar (0–20 h) the fat cells lie more or less separated or in small clusters. Mitoses can be seen which indicates that the number of cells is still increasing. In the nuclei of non-dividing cells the heterochromatin is found as a few granules. The amount of glycogen (PAS) and fat (Sudan III) is very small.

Second phase: (18–36 h): Vacuolization. In this larval instar some glycogen may be stored, though individual differences can be observed. In many cells small vacuoles appear, one or two of which increase in size. According to SCHNELLE (1924) and BISHOP (1922) these vacuoles should contain fat. The authors mentioned Sudan III as fat stain, but in their papers no concrete indication can be found that they actually applied this staining method to young worker larvae. It is therefore not clear how the authors came to their conclusion. NELSON (1924) referred to the work of Bishop but distinguished between small fat globules and large vacuoles that he assumed to contain 'food reserve'. No further indication about the nature of the content was given.

In this study living larval fat tissue was dissected, fixed in formol (see Table 2) and stained with Sudan III. For the large vacuoles the result was always negative. Unlike the few small fat gluboles present in these cells they did not take up the stain. In this stage the large vacuoles were PAS negative as well. Electron microscopic photographs show membranes extending into the lumen of the vacuoles (Fig. 19). The cell nucleus contained many evenly spread granules of heterochromatin. No mitoses were found in larvae of more than 24 hours old. Increase in volume of the fat body therefore is reflected in the increase in size of single fat cells (Fig. 3).

Third phase: increasing vacuolization. In the third instar (30–60 h) the large vacuoles increase in size so that in many cells the nucleus seems to be pushed aside. In histological sections these cells have the typical appearance of a signet-ring. During the first period some glycogen can be stored in the peripheral part of the cell near the nucleus. Besides these 'signet-ring' cells another cell type can be observed in which the large vacuole is missing. In these cells glycogen in varying amounts can be found at the periphery. The number of small fat globules also increases. In most larvae the second period – during which a new cuticle is being formed (between 50 and 60 h) – is characterized by a remarkable decrease in glycogen and fat stores. The plasm is spread evenly within the cells except for the large vacuoles which give the tissue a spongy appearance. The chromatin in most nuclei is so clustered that space is formed between the chromatin and the cell plasm.*

* This phenomenon can often be observed in tissues after Bouin or Carnoy fixation. In tissues fixed with glutaraldehyde – OsO_4, the nuclei remain unshrunk. Not all tissues show clustering of nuclei; sometimes only a few cells in a tissue give this reaction to fixation. These facts could indicate that shrivelling of nuclei is not only a fixation-artefact but also an indication of physiological activity within the nucleus.

Fourth phase (60–85 h): cell growth, increase in amount of cytoplasm, is more pronounced in this phase than during earlier ones. During the first period (60–75 h) glycogen and some fat are accumulated. During the second period (75–85 h) these reserves seem to be released again to a certain extent. Individual differences, however, make it difficult to give a general description of this phenomenon. Chromatin in the nuclei is clustered as in the previous stage.

Fifth (gorging) phase: During the first period of the fifth instar (85–144 h) the larvae increase so much in weight that BERTHOLF (1925) called it the 'gorging' stage: After $3\frac{1}{2}$ days the larvae receive MWJ. In the fat body this is reflected in an enormous increase in the amount of glycogen. Fat accumulation also increases. The nucleus is surrounded by a homogeneous mass of cytoplasm which contains cell organelles. Many of the large vacuoles contain glycogen before they are taken up into the cytoplasm. In larvae of about 100 to 110 hours old, the band of cytoplasm around the nucleus has disappeared. The plasm is spread as a finely branched network throughout the cell. Fat vacuoles are mainly located near the, distorted, nucleus.

Development was followed up to 110 hours. Older larvae were not included in present experiments. For a description of protein formation and, in a later stage of histolysis of fat cells, the reader is referred to the work of BISHOP (1922, 1958).

4.3. FAT BODY OF THE QUEEN LARVA

Worker larvae of different ages were grafted in queen cells (Table 1). Histological changes in the structure of the fat cells were recorded. (For light microscopic and electron microscopic description the reader is also referred to the Figs 15 to 24). Within certain limits the developmental pattern appeared to be independent of the age of the larva at the moment of grafting. With the understanding that the response upon grafting as expressed in the uptake of nutrients in fat cells was faster in older larvae than in younger ones, the following description based on experiments with larvae transferred at 48 hours of age is applicable to other ages as well. In Fig. 4 the development of fat cells in queen larvae is visualized.

In Section 4.2 fat cells of 48-hour-old worker larvae were described. At that stage the cells generally contain little reserve material. Within 12 hours after larvae have received RJ, glycogen (PAS +) is accumulating in all fat cells. The large, watery, vacuoles appear to contain PAS-stainable matter before they disappear in the surrounding cytoplasm. In about 16 hours the cells become loaded with glycogen, leaving only a tiny band of cytoplasm around the nucleus. Areas containing glycogen are irregular in form which gives the fat body an untidy appearance. It is possible that in this stage vacuoles in the fat cells contain glycogen as well as lipids, or glycolipids. Electron microscopy does not reveal many clearly separated fat vacuoles besides the glycogen containing areas, whereas in Sudan III preparations irregular fat vacuoles occur. In the

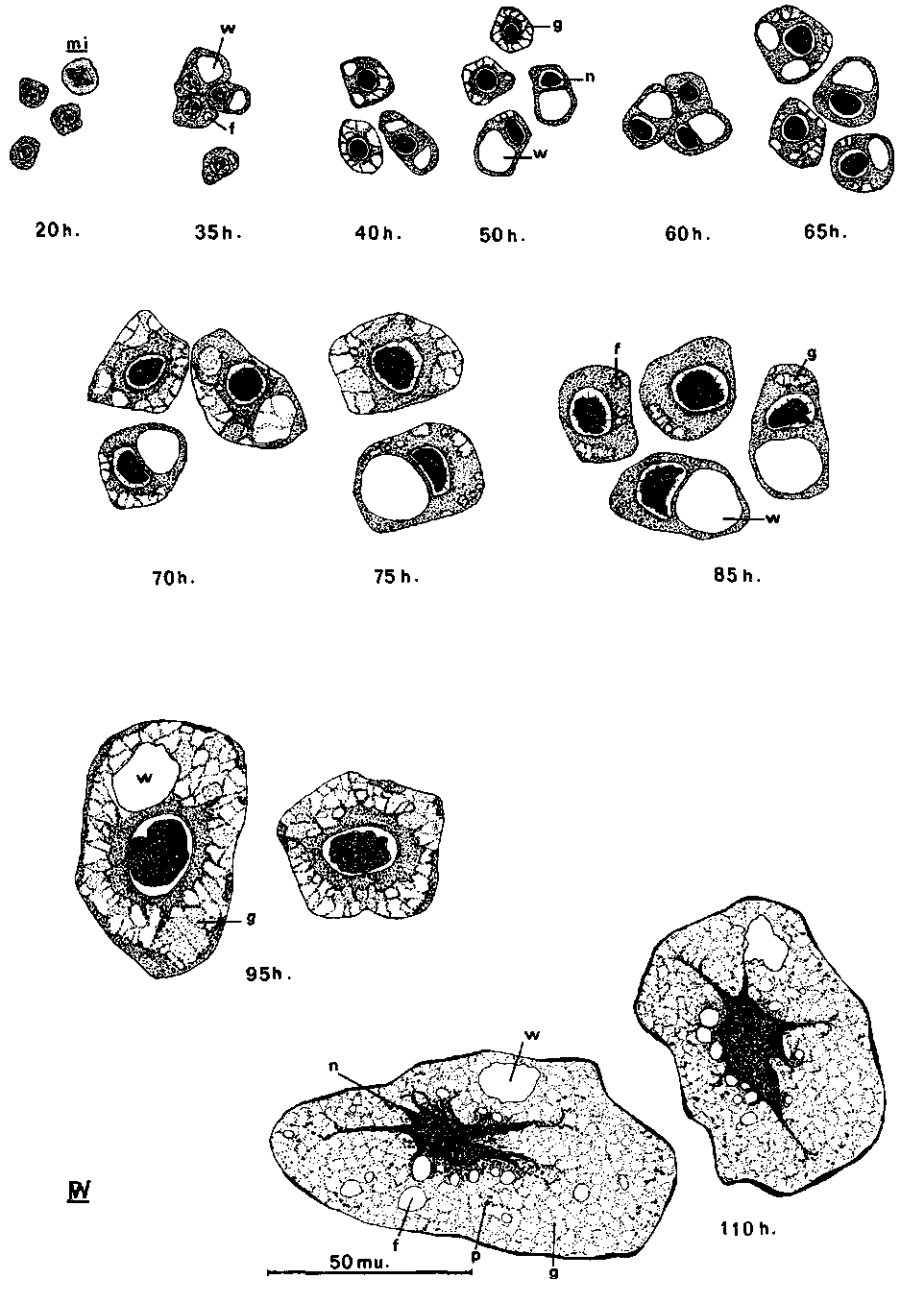


FIG. 3. Development of fat cells in the worker larva (time in hours; explanation of abbreviations: see pages: 60, 61).

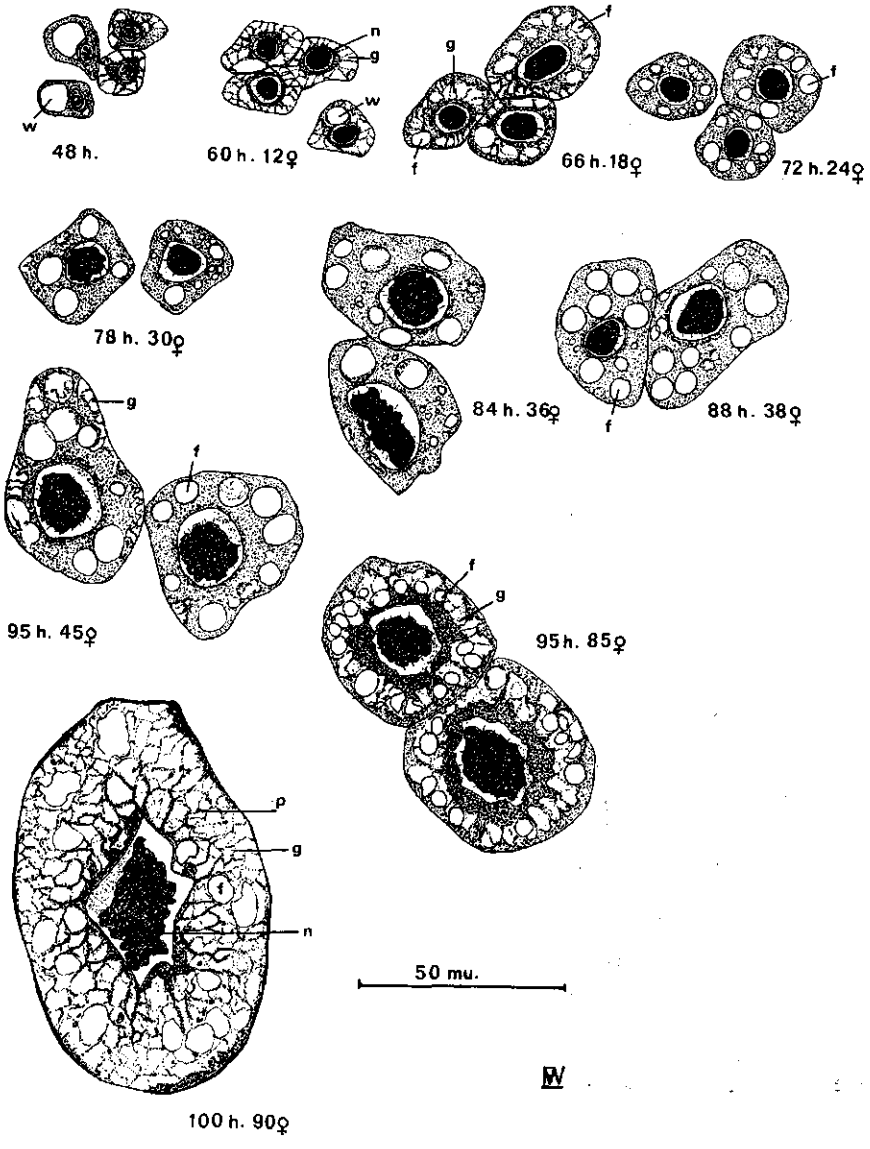


FIG. 4. Changes in fat cells of the queen larva, after grafting (time in hours,...♀: time during which the larvae were nursed as queen larvae).

period between 18 and 36 hours after transfer to queen cells glycogen moves inwards and forms a ring in the cytoplasm halfway between nucleus and cell border. The amount of glycogen decreases, so that at the end of this period hardly any trace can be found. The irregular spaces in the cytoplasm become more and more rounded, forming numerous spherical fat vacuoles (Figs 21 to 24). These observations give the impression that glycogen is converted into fat. The peripheral fat-cells react in the same way as the ventral cells but their response is slower and not all large vacuoles may disappear. In many of the larvae described above within 24 to 36 h after transfer into queen cells (age 72–84 h) a new cuticle is formed. It is possible that part of the glycogen is used for this cuticle formation. As in the worker larvae, the next period (85–100 h) is characterized by a rapid uptake of glycogen. Also the amount of fat increases.

Later stages of development of the fat body have been described by BISHOP (1922, 1958). Biochemical analysis of the fat body of queen and worker larva is needed to reveal qualitative and quantitative differences. Histological investigation can only give an impression of quantities that are stored.

4.4. DISCUSSION

Contrary to general opinion the fat body of worker larvae contains relatively little reserves during the first three and a half days of development. Fat is present only in small amounts. Glycogen storage presumably depends on the moulting stage, which is not abnormal in insects (LABOUR, 1970; WIGGLESWORTH, 1947). STABE (1930) found that some hours before and after ecdysis honeybee larvae consume little food. Watery vacuoles have been found in the fat body of some insects (LABOUR, 1970; WIGGLESWORTH, 1967). WIGGLESWORTH (1942) described two types of vacuoles in fat cells of the mosquito larva, *Aedes aegypti*, the first type containing fat, the other having an aqueous content. Some of the watery vacuoles contained glycogen. Uric acid crystals were often found after fixation. The author considered that uric acid was being formed as a result of proteïn consumption for energy purposes during starvation. In larvae that were not starved, acid accumulation was greatly reduced or did not occur at all. In starved larvae uric acid presumably was discharged into the haemolymph after feeding was resumed. In *Rhodnius prolixus* (WIGGLESWORTH, 1967) watery vacuoles first appeared when starved larvae were fed again; they subsequently disappeared. BUTTERWORTH et al. (1965) described spherical vacuoles in fat cells of *Drosophila*, which failed to stain with any of the procedures they used. The authors considered these vacuoles to be empty because the saturated lipids were extracted during fixation. It would not be surprising if these vacuoles had a watery content. Further investigation is needed to determine the function of watery vacuoles in the insect fat body.

There is no evidence in support of the statement that the large vacuoles in the fat body of worker larvae contain fat. Sudan III which dissolves in, and therefore stains, saturated lipids, gave negative results. Fixation with glutaraldehyde-OsO_4 which normally preserves unsaturated lipids did not preserve the

content of the large vacuoles. The percentage of water in young worker larvae is higher than in queen larvae (section 1.3). In queen larvae large vacuoles are missing.

Biochemical analyses of whole worker larvae (STRAUS, 1911; MELAMPY et al., 1940) are in good agreement with present histological findings on the reserves in fat cells during development. STRAUS showed that during the first three days of development little glycogen and fat was present in worker larvae. Between the third and fourth day there was a considerable increase in glycogen and, to a smaller extent, in fat (calculated as percentage of fresh weight). MELAMPY et al. (1940) showed that 3 to 4 day-old queen larvae contained more lipids than worker larvae of the same age. In the next period there was a substantial increase in reducing substances in queen larvae. In worker larvae more than 4 days old a moderate increase in lipids and a large increase in reducing substances was observed. According to STRAUS (1911), MELAMPY et al. (1940) and RONZONI and BISHOP (1929) part of the glycogen of honeybee larvae is converted into fat. In the one-day-old worker pupa (STRAUS, 1911) food reserve consists for 27% of the dry weight of glycogen and for 18% of lipids. Nearly all food-stores in the pupa of the worker as well as of the queen are used for adult development (MELAMPY et al., 1940; MELAMPY and OLSAN, 1940). As a consequence, in just emerged adults these stores are depleted.

Considering present observations on the fat body of worker larvae the conclusion seems justified that worker larvae are kept short of food during the first three days of larval life. Indeed LINEBURG (1924) and LINDAUER (1952) found that worker larvae receive little attention during the second day. However, the situation is not as simple as that. In all experiments in the present study, the midgut of larvae was found to contain food. It is well known that an excess of WJ alone does not give rise to adult workers with the same weight as queens. On the contrary, VON RHEIN (1933) found that rearing larvae on WJ prevents adult development. This is not surprising in view of our present knowledge of the poor food situation in the fat body of worker larvae during the time they receive WJ. Fed on WJ they simply cannot store enough reserves to make metamorphosis possible. Only after the larvae receive MWJ much glycogen is being stored. SHUEL and DIXON (1968) added sugar to WJ and obtained some adult worker bees from four-day-old worker larvae. DIXON and SHUEL (1963) reared just hatched larvae in vitro on modified WJ and RJ for three days. From their data it can be concluded that addition of sugars to WJ did not increase the amount of glycogen markedly, but rather that sugar was converted into lipids, which therefore were found in rather large amounts. However, these results obtained from experiments in vitro should be considered with caution as larval growth was greatly reduced.

The experiments at least suggest that sugar is an important factor in larval development. HAYDAK (1968) reviewed the literature on the composition of larval food. A comparison of the nutritive value of WJ and RJ does not explain the low storage capacity of WJ although one has to bear in mind that data of several authors do not agree at all. DIXON and SHUEL (1963) found 9.9%

(of wet weight) sugar in RJ as compared to 3.4% in WJ. HAYDAK (1943) and VON PLANTA (1888) could not demonstrate large differences. Even if the data of DIXON and SHUEL are correct, WJ cannot be considered as deficient in the main nutrients. In addition one wonders why worker larvae do not consume more food to compensate for a possible sugar deficiency.

Histological comparison of larvae fed in a colony (in which only one brood frame was left) on an excess of WJ with normally fed larvae surprisingly showed that the latter stored more glycogen whereas in the former watery vacuoles were larger. JUNG-HOFFMANN (1966) found that worker larvae are fed variable amounts of white (mandibular) component, depending on the situation in the colony. After $3\frac{1}{2}$ to 4 days worker larvae store large amounts of food. After $3\frac{1}{2}$ days larvae seldom receive the white component. REMBOLD (1969) stated that WJ merely consists of hypopharyngeal-gland secretion and that addition of mandibular-gland secretion alters the physiological properties to those of RJ. However, this theory seems too simple.

Accepting that mandibular-gland secretion of nurse bees in a queenright colony has an inhibitory effect on feeding one can only speculate about how this inhibition is achieved. DIXON and SHUEL (1963) postulated that: 'concentration of water-soluble acids in the natural diets might be a mechanism of nutritional regulation of early larval growth'. In this respect the possibility of chemosensory regulation has obviously been overlooked. After transfer of larvae to queen cells the above supposed inhibition is removed immediately. Larvae in all experiments (this thesis) stored large amounts of nutrients in the fat body soon after they received RJ. An explanation for the observations of HOFFMANN (1966) that RJ contains large quantities of white component may be that there is a rapid change in mandibular-gland secretion after the queen has been removed from the colony (nurse bees are capable of feeding RJ to queen larvae within a few hours after the removal of the queen). Another possibility is that in the colony nurse bees capable of rearing queen larvae are always present; they might be prevented from offering RJ-secretion to larvae by the influence of the queen. REMBOLD (1969) found evidence that in a queenless colony mandibular-gland secretion of some nurse bees was altered. However, up to the present insufficient attention has been paid to the role of nurse bees to give a reliable statement on this point.

5. ENDOCRINE SYSTEM OF THE LARVA

5.1. INTRODUCTION

It is generally assumed that hormones are involved in the regulation of caste differentiation in the honeybee. Several hypotheses have been put forward, but until now no conclusive evidence has been reported. In this chapter a description will be given of the structure of the neurosecretory cells (NCS), the corpus cardiacum (CC), the prothoracic gland (PtG) and the corpus allatum (CA). Except for the CA, the description has been taken mainly from literature. As far as is known there has been no research on the ultrastructure of the endocrine system of the honeybee larva. In the present study some attention was paid to the fine structure of various endocrine centres. These observations on ultrastructure will be reported at the appropriate places in the description to follow. As the corpus allatum was studied in more detail it will be described in a separate section. In Fig. 5 the endocrine system and some related structures are drawn. In Fig. 5a the organs have been somewhat spaced out. In Figs 5b-f different cross-sections of the head are presented, showing the orientation of the endocrine organs.

5.2. NEUROSECRETORY CELLS

L'HÉLIAS (1950) briefly described the endocrine system: from each CC a short nerve runs up to the brain where it is found to bifurcate, one branch running to the pars intercerebralis where a group of large neurons are situated, the other extending more laterally ending in a group of large neurons which have the same appearance as those in the pars intercerebralis. However, the author did not suggest that these neurons were neurosecretory. FORMIGONI (1956) described in the pars intercerebralis of third instar larvae two groups of 60–65 cells which fuse during the pupal stage. No neurosecretory (NS) material could be demonstrated in these cells during larval and pupal development. During the praepupal phase the author observed a decrease in their size. From each group of cells a nerve runs to the opposite CC, forming a chiasm in the central neuropile of the brain. Dorsally to the pars intercerebralis 5 to 6 neurons differentiated during the second larval instar. Before the pupal moult these cells showed a marked swelling. Half-way the pupal stage they became very chromophile and vacuolated. It is not clear from this description whether these cells correspond with the lateral cells described by L'HÉLIAS.

NELSON (1915) described neuroblasts in the brain of the bee larva: 'In the peripheral region of the brain and ganglia of the ventral chord of mature larvae are to be seen here and there cells of large size, which in their mode of division and other characters are plainly to be identified as neuroblasts'. CANETTI, SHUEL and DIXON (1964) published a remarkable paper on the neuroblasts.

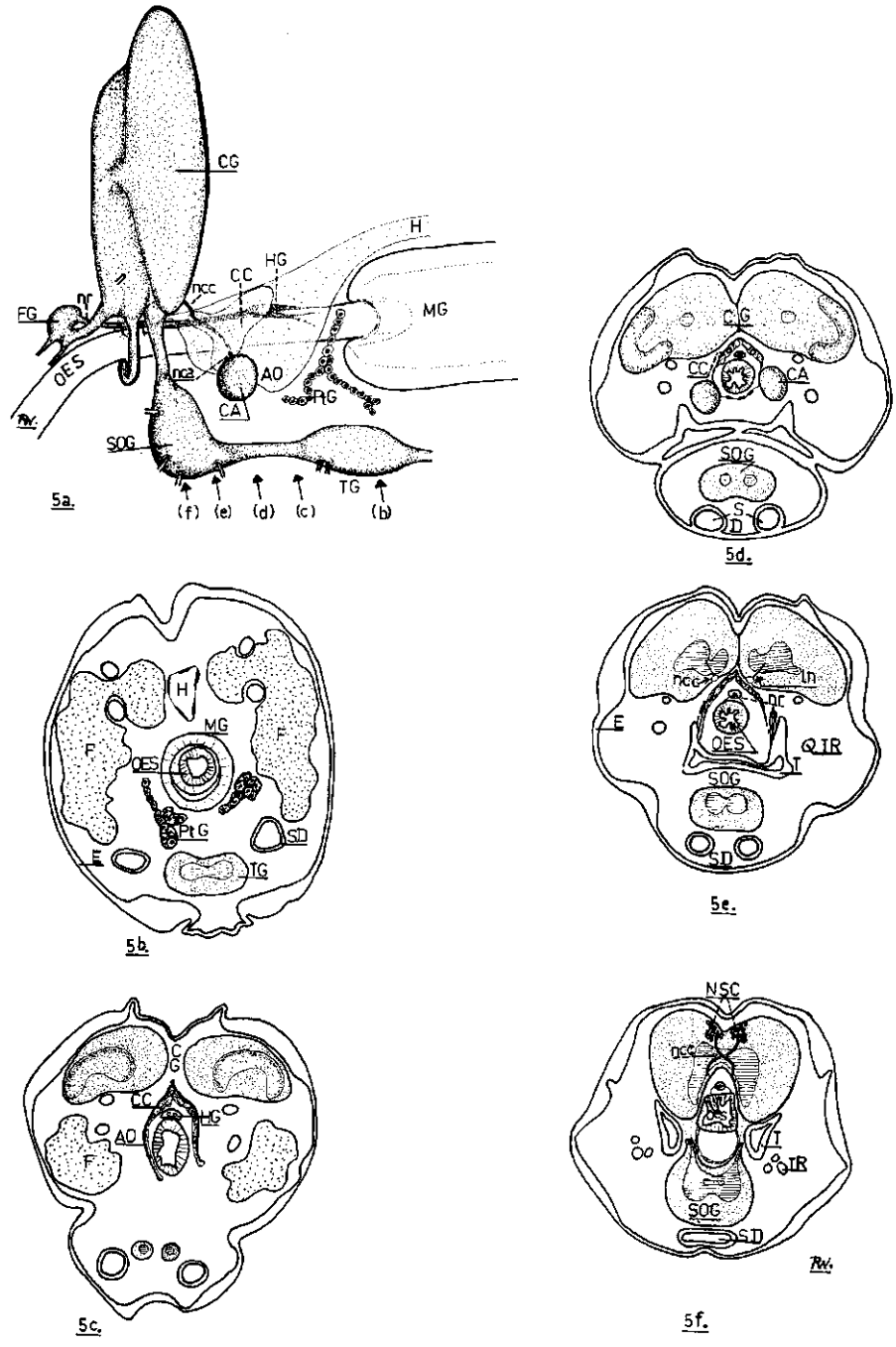


FIG. 5. Endrocrine system of the honeybee larva (semi schematic).
5a. Model, after Nelson (1915), Schaller (1950), Lukoschus (1952) and own observations.
5b–f. transverse sections through the endocrine system (abbreviations see p. 60, 61).

Using the aldehyde fuchsin stain (GABE, 1953) the authors were able to demonstrate stainable matter within these cells. In queen larvae between 40 and 80 hours the number of granules was significantly larger than in worker larvae. The authors suggested that the neuroblasts have a NS function.

RITCEY and DIXON (1969) investigated the development of the endocrine system of the two female castes. The authors found an increase in diameter of the NSC in queen larvae of 84 to 96 hours (it is interesting to note that they considered them to be fourth instar larvae). Subsequently a decrease was observed followed by an increase again during the pupal period. In the worker the diameter of the NSC remained constant at the age of 84–96 hours and then increased during the second day of the praepupal phase. The maximum size for both castes was attained in late praepupae and pupae. With the PAF stain (GABE, modified by EWEN, 1962) NSC were observed in all parts of the brain and other ganglia. In the protocerebrum a medial, a lateral and a ventral group of NSC were described. In the deutocerebrum and tritocerebrum NSC were mentioned, but from the paper it is not clear where these NSC were located. Axons from these cells runned to the CC via the nervus corporis cardiaci. Until now in no insect investigated have such large numbers of NSC been observed. In the discussion the authors stated that in as early as the third larval instar NSC were active. This conclusion was based on the fact that NS granules should be present. A closer examination of the paper, however, reveals that in the description no information is given on NSC in the third larval instar. The only detailed pictures of the brain are of medial NSC and the youngest larvae in which these are shown were about 84–96 hours old. Thus, the reported observations and statements are rather incomplete so that it is difficult to interpret the results. The conclusions the authors derive from their observations are rather speculative.

In the present study it was not intended to examine the NSC extensively, but merely to obtain some information on the presence of NS granules in the brain of young larvae. For this purpose a number of brains were sectioned. NS granules could not be detected in any of the neuroblasts examined (Fig. 25a). Except for mitochondria and a few golgi areas few organelles were present. In some cells large lysosome-like bodies could be seen (Fig. 25b). Perhaps these are the structures CANETTI et al. (1964) took for NS material. In three larvae examined (54, 66 hours of age) cells were found to contain some NS granules (Figs 26a, b, c). As in this stage the amount was very small it is not surprising that staining did not make NS material visible by light microscope.

Further evidence that NSC are functional can be found in the ventral region of the brain, near the aorta wall. Here, many axons contain NS granules (Fig. 26d). A detailed investigation of synthesis, storage and release of NS material during larval development is necessary to define the function of neurosecretion in caste differentiation.

DÖHMER (1958), BIEDERMANN (1964) and VAN LAERE (1970) described the NSC in the pars intercerebralis of adult honeybees. The descriptions will not be reviewed here as they do not contribute essential information to caste formation.

5.3. CORPUS CARDIACUM

The CC in the honeybee larva appear ventrally of the brain as thickenings of the wall of the aorta which forms a roof over the oesophagus. SCHALLER (1950) described the CC as isles of glandular cells intergrown with connective tissue. FORMIGONI (1956) found two types of cells in the larval CC, viz: large polyhedric cells situated dorsally and small cells more laterally. The latter passed through several mitoses at the end of the larval stage and a close contact was made with the axons of the nervus corporis cardiaci (NCC). The findings of LUKOSCHUS (1956a) are in good agreement with those of FORMIGONI. The volume of the CC increased in the late praepupal phase, during cuticle formation. Shortly before the pupal moult the volume decreased again. RITCEY and DIXON (1969) also described the CC of older larvae. They distinguished between two parts: 1.a glandular part which occupies a position dorsal and lateral to 2.a ventral nervous part from where axons extend backwards to the NSC of the brain.

Several authors gave different descriptions of the innervation of the CC. SCHALLER (1950) described a nerve (NCC) near the place where the aorta comes close to the cerebral ganglion. This nerve finds its origin in the brain and enters into the CC. L'HÉLIAS (1950) stated that the NCC fuse with the N. recurrens after the latter has bifurcated. Also RITCEY and DIXON (1969) demonstrated a nervous connection between the CC and the hypocerebral ganglion. HANAN (1955) stated that a distinct ring of neural and aortal tissue surrounds the oesophagus at the level of the CA. Besides these connections, the author found the CC to be dorsally connected by nervous tissue.

Electron microscopic examination (this study) revealed that the aorta wall is composed of connective-tissue like cells. The CC cells are as it were embedded in this aortal tissue. Many CC cells contained NS granules, but only in small amounts in the stages examined. Figs 27 to 31 give an impression of the aorta-CC complex. In agreement with the findings of RITCEY and DIXON, the CC cells were mainly observed in the dorsal part of the aorta. Besides the glandular cells which resemble the NSC in the brain many axons could be found, mainly located at the inner periphery of the aorta wall. In the lower part of the aorta many axons were seen to end in axon terminals. In all larvae examined many axons contained NS granules. Figs 30b, c show the release into the haemolymph of NS granules from an axon located in the aorta wall. Thus, the aorta of the honeybee larva serves as a place of release of NS substance. The ventral part of the aorta (Fig. 31b), near the place where the CA are situated, does not contain axons except for the nervus corporis allati (NCA). At present, it can only be presumed that the NS granules originate from NSC in the brain as well as from CC cells. Although it is probable that the former contain the 'brain hormone' (in JOLY, 1968) there is not sufficient information at present for any definite statement in this connection. Nervous connections between the N. recurrens and the CC as described by L'HÉLIAS and RITCEY and DIXON were not observed (which does not mean that they do not exist). Electron microscopic pictures of the aorta complex show many single axons running in undefi-

nable directions. Innervation is far more complex than suspected from light microscopic research, with which only relatively large nerves can be traced.

5.4. Prothoracic glands

L'Hélias (1952) first described the PtG of the honeybee. Laterally of the oesophagus, near the midgut, two groups of cells are situated (Fig. 32c). Lukoschus (1952) found that each group consists of three main chains, the first running along tracheae in the direction of the CA, the second running more dorsally along the midgut near the tracheae of the wing discs and the third chain ending more laterally near the prothoracic spiracle. Formigoni (1956) stated that the cells showed cyclic activity. Before ecdysis they increased in volume and the cytoplasm contained vacuoles. In full-grown queen larvae the cells were larger than in worker larvae. During the pupal stage the glands disappeared. Lukoschus (1956) measured the nuclear volume and found in both castes an increase from the end of the 4th larval instar onward. At the beginning of the 5th instar the nuclei were larger in queen larvae than in corresponding worker larvae. Just in the beginning of the pupal moulting during the mitotic period the difference was at a maximum, the nuclei of the PtG in queen larvae being about ten times as large as those of worker larvae. In the cells of the PtG of queen larvae vacuolization occurred and an excretory duct was formed, opening into the haemocoel. Subsequently degeneration of the gland took place. In the worker larva these phenomena were less pronounced. Ritcey and Dixon (1969) confirmed the data obtained by Lukoschus. O'Brien and Shuel (1972) estimated the development of PtG in larvae reared on different diets in vitro. Measuring the nuclear diameters in larvae of 4 days old they obtained data on the activity of the gland. One of the conclusions they reached was that ecdysone might be involved in the 'exploitation of caste development', a term the authors did not define. As stated earlier experiments in vitro should be considered with caution. However, there are also several other reasons why their results are not conclusive. First of all the differences in nuclear diameter between queen and worker used as a criterion for activity were not pronounced in larvae of that age (90–100 h). One wonders why the authors did not use older fifth instar larvae (see Lukoschus 1956) where differences in volume amount to a factor 10. Secondly, there is a striking difference between the values given for control workers in different experimental groups. This difference is statistically significant (as are the values given for different treatments).

It is interesting to note that in larvae that received WJ with added sugar for the whole period of feeding (up to 100 h), nuclei of the PtG almost reached diameters also found in queen larvae. This links up with observations on the storage situation in the fat body after feeding with WJ (see Section 4.2). It seems likely that addition of sugar to WJ stimulated feeding behaviour.

5.5.1. *Introduction*

NELSON (1915) first described the embryological development of the CA: 'These bodies arise as ectodermal ingrowth located in front of the bases of the first maxillae'. At a later stage: 'the corpora allata appear as irregular cellular outgrowths springing from the mesial side of the mandibular apodemes'. They subsequently lose their attachment and by that time are located near the tentorium, where they remain during the larval stage. The author, not knowing their function, suggested that it might be glandular. As in other insects the CA is innervated by a prolongation of the NCC (SCHALLER 1950).

PFLUGFELDER (1948) measured the volume of the CA during development of the worker larva. Whereas the body gained 550 times in weight, the volume of the CA increased 20 to 30 fold. In the praepupal phase the volume of the CA was reduced to half that of the last larval instar. The author found the same relations in queen larvae. No differences in volume between the CA of queen and worker larvae were mentioned. PFLUGFELDER also measured nuclear volumes and found an increase from about 40 μm^3 in the first instar to about 900 μm^3 during the 5th instar. In the praepupal phase the nuclear volume was reduced to about 400 μm^3. (From the number of measured nuclei, being about 160, and the fact that each CA contains about 80 nuclei (PFLUGFELDER 1948) it can be concluded that the author only measured one animal to represent each figure). LUKOSCHUS (1956a) determined the volume of the CA in worker and queen larvae, in the latter from the third day on. Unlike PFLUGFELDER, the author observed a marked increase in the volume of the CA in queen larvae older than $3\frac{1}{2}$ days compared with worker larvae. LUKOSCHUS related this difference in volume to the fact that worker larvae receive MWJ after $3\frac{1}{2}$ days. According to the author this food restricted the activity of the endocrine system. In the praepupal phase a decrease in CA volume occurred which was less pronounced in the queen. The author compared body weight and CA volume and concluded that during larval development the ratio volume to weight decreased in both castes. Only during the 5th instar the increase in volume parallelled the increase in weight. From LUKOSCHUS' paper it can be concluded that a difference in larval weight occurred after $2\frac{1}{2}$ days, the queen larva weighing more than the worker larva. It is interesting to note that difference in CA volume occurred about 24 hours later (see Section 5.5.2). CANETTI et al. (1963) gave some pictures which they claimed to be representative for the size of the CA in both castes. In queen larvae, 64–72 h old, the increase in volume was more pronounced than in worker larvae. As no data were presented it is not possible to ascertain the moment of divergence in development. According to the authors nuclear degeneration took place in the third and fourth instar of queen larvae. During the fifth instar intercellular spaces were visible which they interpreted as a probable cessation of activity. By then the nuclei were 'reorganized'. In the worker larva these processes were less pronounced. The authors came to the conclusion that CA activity is arrested earlier in the queen larva than in the worker larva. WANG and SHUEL (1965) and

TABLE 3. Size of the CA during larval development according to:

WANG and SHUEL (1965) volume (in $mm^3 \times 10^{-3}$)			RITCEY and DIXON (1969) diameter (in μm)		
age (h)	♀	☿	age (h)	♀	☿
48	0.8	0.8	54–84	71–100	68–78
60	2.0	1.7	84–96	96–101	75–79
72	2.4	1.7	96–	98–111	78–83
90	4.7	2.8			
102	6.2	3.3			

RITCEY and DIXON (1969) reported differences in diameter of the CA, as shown in Table 3. RITCEY and DIXON added that the CA of queen larvae attain about maximum size early in the third instar.

5.5.2. *Caste related differences in corpus allatum volume*

5.5.2.1. Methods

To determine the earliest moment after grafting at which duality in CA development can be observed, measurements were carried out on histological preparations. With the aid of a drawing tube (camera lucida) the largest perimeter of each gland was drawn and the surfaces were measured with a planimeter. No efforts were made to calculate 'real' volumes, as the only aim was to compare queen and worker mutually. In figs 6 to 8e, arbitrary planimeter values (nonius units) are plotted against the age of the larvae. As these values correspond with the *surface*, differences in *volume* are greater.

5.5.2.2. Results

During larval development of the worker the CA increased markedly in size, as is shown in Fig. 6. Considerable variation was observed, partly due to relatively large differences in age between individuals reared from the same batch of eggs. Data were collected in age groups of 6 hours. In each group the mean surface of the CA was determined. In Table 4 and Fig. 7 these mean values are presented.

The figures give the impression that increase in size of CA is not continuous: there seems to be a brief delay in growth of the gland during the hours after ecdysis. Because of the variation in the data (vertical bars in Fig. 7 indicate highest and lowest value per age group) further detailed measurements are needed to ascertain cyclic activity of the CA. Worker larvae of different ages were grafted in queen cells. Queen larvae from different experiments were collected into 5 groups, according to the moment of grafting. In Table 4 the mean values per age group are given. In Figs 8a–e the data are expressed graphically.

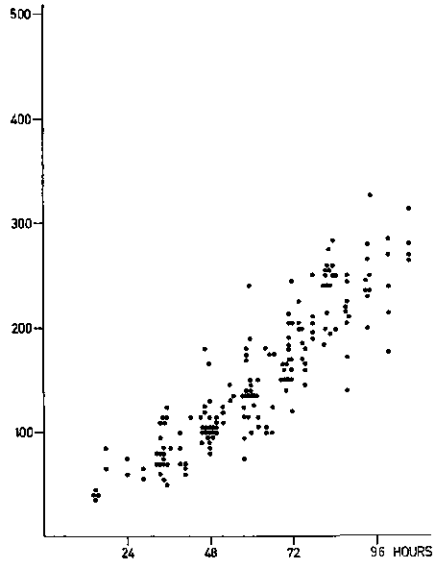


FIG. 6. Surfaces of CA of worker larvae (in arbitrary units). Each dot represents the mean of both CA of one larva.

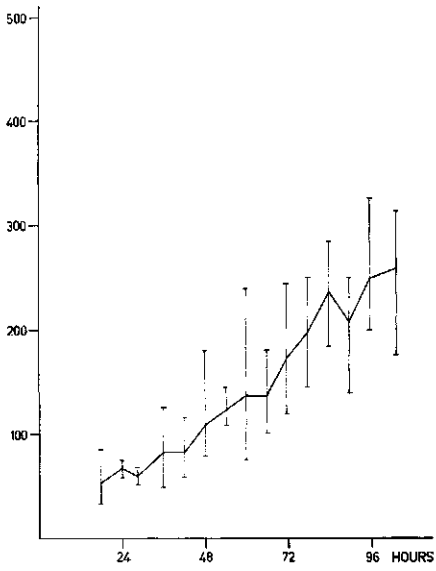


FIG. 7. Surfaces of CA of worker larvae. Means per age group. Vertical bars indicate variation within age groups.

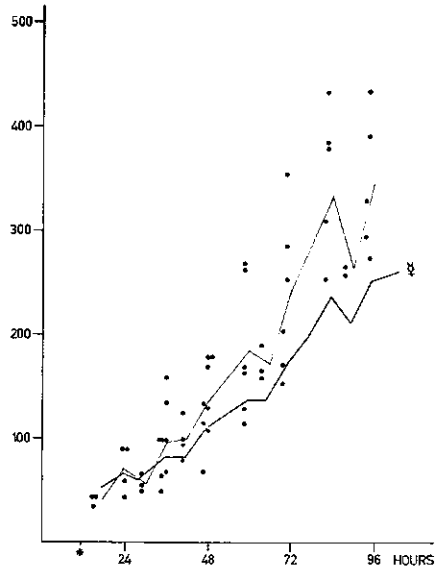


FIG. 8a. Surfaces of CA of queen larvae, grafted when 5 to 18 hours old (first instar), compared with worker larvae.
* moment of grafting

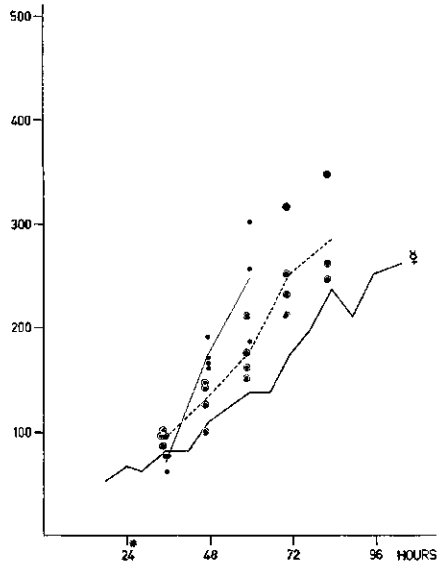


FIG. 8b. Surfaces of CA of queen larvae, grafted when 24 hours old (second instar), compared with worker larvae.
* moment of grafting ●: b_1; ⊙: b_2 (Table 4)

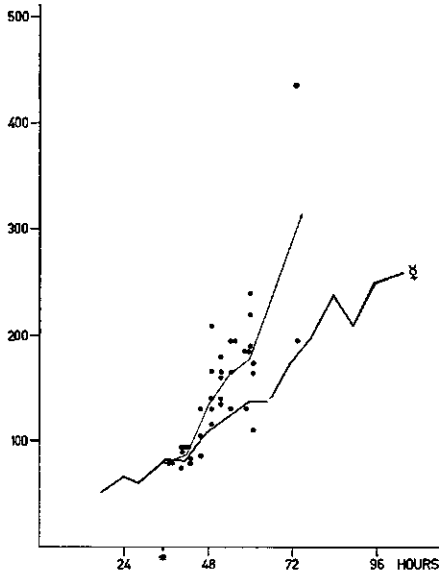


FIG. 8c. Surfaces of CA of queen larvae, grafted when 34 to 36 hours old (second-third instar), compared with worker larvae.
* moment of grafting.

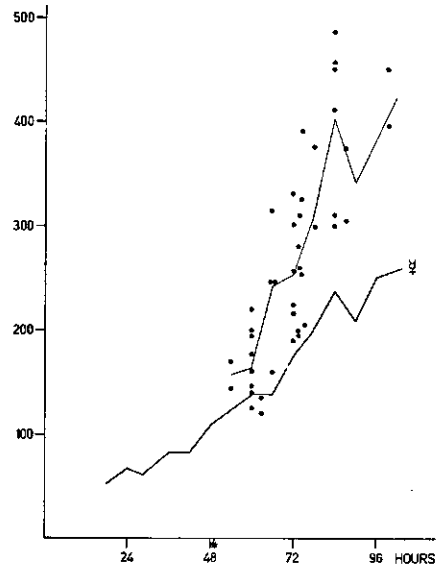


FIG. 8d. Surfaces of CA of queen larvae, grafted when 48 to 50 hours old (third instar), compared with worker larvae.
* moment of grafting.

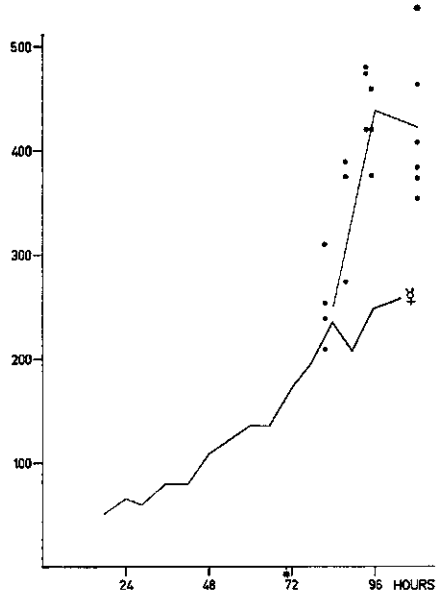


FIG. 8e. Surfaces of CA of queen larvae, grafted when 70 hours old (fourth instar), compared with worker larvae.
* moment of grafting.

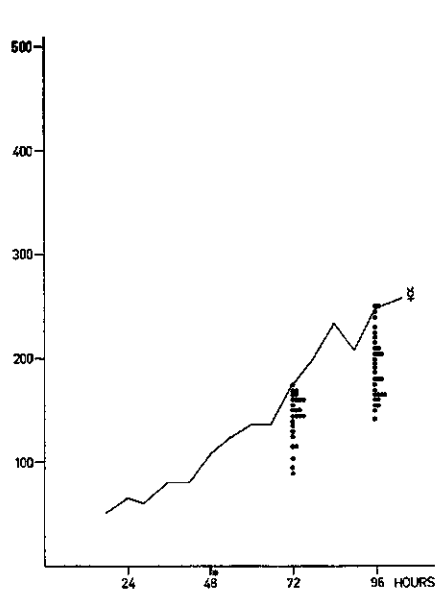


FIG. 8f. Surfaces of CA of queen larvae, grafted when 48 hours old in artificial queen-cups, compared with worker larvae.
* moment of grafting.

TABLE 4. Means (per age group) of surfaces of corpora allata (in arbitrary units). A: mean surface. B: number of larvae per age group.

caste: / age group (hours)	☿		♀a*		♀b_1		♀b_2		♀c		♀d		♀e	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
15– 21	51	6	42	3										
21– 27	67	2	71	4										
27– 33	60	2	57	3										
33– 39	84	19	98	8	70	3	94	4	80	2				
39– 45	81	7	100	3					88	7				
45– 51	109	25	136	8	171	4	128	4	131	9				
51– 57	128	6							163	9	158	2		
57– 63	138	25	186	6	247	3	174	4	178	9	162	10		
63– 69	137	7	172	3							241	4		
69– 75	178	22	238	6			251	4			250	11		
75– 81	188	12							315	2	308	6		
81– 87	239	17	333	6			283	3			402	6	254	4
87– 93	209	9	263	2							340	2	347	3
93– 99	252	8	345	5									438	6
99–108	259	8									422	2	425	6

*a: grafted at the age of 5–18 hours (first instar)
b_1: grafted at the age of 24 hours (second instar)
b_2: grafted at the age of 24 hours, in queen cells with RJ of 3-day-old larvae
c: grafted at the age of 34–36 hours (second-third instar)
d: grafted at the age of 48 hours (third instar)
e: grafted at the age of 70 hours (fourth instar)

The increase in mean CA surface of queen larvae, upon receiving RJ, appeared to be more pronounced than that of corresponding worker larvae, that were fed WJ and thereafter MWJ. Within 12 to 24 hours after grafting a difference in mean CA size could be observed, independent of the age of the larvae at the moment of grafting (figs 8a–e). However, the rate of increase appeared to be age dependent. The older the larvae at the moment of grafting, the faster and the more pronounced the reaction. The gland, as it were, seemed to compensate for the relatively late change in the developmental pattern.

In the following section ultrastructural changes accompanying this dichotomy in CA development will be discussed.

5.5.3. *Ultrastructure of the corpus allatum*

5.5.3.1. Materials

Larvae were transferred to queen cells at the age of 48 hours and subsequently placed in a queenless colony. Table 5 gives the ages at which the larvae were fixed for electron-microscopical investigation, as well as the period queen larvae were nursed in queen cells. Worker larvae from the same batch of eggs were nursed in a queenright colony (see also Chapter 2. For methods see Table 2). Per larva normally one CA was investigated, as preliminar examination revealed that both CA of a larva had the same ultrastructure.

5.5.3.2. Results

First the ultrastructure of the larval CA will be described. Next a brief characterization of changes in the fine structure of the CA during development of queen and worker larva will be given.

TABLE 5. Larvae of different ages and castes used for electron-microscopical investigation. A: age of the larvae (h) B: age at the moment of grafting (h) C: number of larvae investigated.

	♀		☿
A	B	C	C
48	–	–	2
54	6	2	3
60	12	4	3
66	18	2	2
72	24	6	5
78	30	3	3
84	36	6	6
96	48	4	4
108	60	3	3
123	72	1	–
147	96	2	2
171	120	3	2
	total	36	35

5.5.3.2.1. General description

The spherical or ovoid CA is composed of about 80 cells. As the number of cells remains constant, changes in size of the gland can be attributed to changes in nuclear and cytoplasmic volume. Electron microscopy reveals that the gland is surrounded by a thin layer of fine granular stroma (glycocalyx; Fig. 36a).

Nucleus: the spherical nuclei are located more or less centrally in the cells*. Chromatin is spread as a diffuse meshwork throughout the nucleus. In most sections of nuclei some large masses of electron dense material are present. With the staining used it is difficult to distinguish between chromosomal heterochromatin and nucleoli. VAN LAERE (1970) suggested that similar structures in nuclei of CA cells in the adult honeybee are nucleoli. The nuclear membrane is smooth in outline. Nuclear pores are numerous.

Cytoplasm: the amount of cytoplasm per cell may vary greatly, depending on age and caste of the larva (Section 5.5.2). Electron density of the cytoplasm in different cells in a CA may show considerable variation.

Free ribosomes are situated in small clusters. In young CA these groups of ribosomes lie close together, whereas in later stages they may be more separated.

Rough endoplasmic reticulum (RER) is present in limited amounts. Small cisternae are scattered in the cytoplasm. Surrounding the golgi complexes and SER structures, long RER cisternae may be found, sometimes interconnected with the SER (Figs 35, 45, 46). In some stages groups of long profiles of RER may be seen close to the nucleus. Ribosomes are not attached to the RER in abundance.

Golgi complexes are found mainly near the nucleus. In cross-section they may appear as stacks of cisternae or as irregular masses of small tubules. The complex is composed of a number of parallel membrane-lamellae. The membranes of such a golgi saccule may be found lying close together or more dilated, presumably representing different stages of activity. At the dorsal side of the complex there are small golgi vesicles which may have an electron dense appearance (Fig. 35c). At the ventral side of the active golgi complex translucent vacuoles seem to be released into the cytoplasm.

Small vacuoles: similar small, membrane bound vacuoles are present in considerable amount throughout the cytoplasm of active cells (figs 35d, e). At the periphery of the CA these vacuoles can be seen in all stages of exocytosis, presumably discharging their content into the haemolymph (figs 36, 37). (In electron microscopic sections no content is present which might indicate a lipoid nature). However, it is sometimes difficult to distinguish between exocytosis and pinocytosis (fig. 36c). In some stages membrane-bound vacuoles are also present in the intercellular spaces (fig. 37d).

Smooth endoplasmic reticulum (SER) is the most variable structure in the CA. It may take several forms. In the most common form present in every CA it appears as small isolated vesicles, scattered through the cytoplasm (Fig. 34b).

* Nuclear degeneration as described by CANETTI et al. (1964) was not observed in any of the CA examined.

In some stages it may be orientated into parallel strains, forming concentric layers (whorls). These whorls often enclose a golgi complex (Fig. 46b). In the third form to be described the SER occupies large areas in the cell, the small vesicles laying close together, excluding most organelles (Figs 49, 50). In older CA the vesicles in the SER areas may be lying so close to each other that the structures formed look like a jigsaw (Fig. 51b).

Mitochondria seem to be present in a rather constant number. The density distribution therefore largely depends on the amount of cytoplasm in the cell. Often they lie in groups in the cytoplasm. The form varies with age and caste. Ovoid mitochondria are most numerous, but in several stages long, slender, mitochondria of elaborate forms are present. The mitochondrial matrix may be electron opaque or rather dense. Cristae are not very numerous (Figs 34a, b).

Large vacuoles occur in variable numbers in different stages, sometimes forming groups at the periphery of the CA (Fig. 38). These vacuoles have an electron opaque content and always lack a surrounding membrane. THOMSEN and THOMSEN (1970) described similar vacuoles in the blow fly (p. 37) suggesting that the content, being of lipid nature, might contain JH. It is not clear how these vacuoles arise as they appear to be rather uniform in size. Nor is there any indication as to how they disappear. No exocytosis was observed.

Lysosome-like bodies are present here and there in the cell. The most common structure is a very electron dense granule (primary lysosome: TONER and CARR, 1971). In older CA they may be seen in variable sizes and forms (Figs 44a, 52, 53). In Fig. 40a another type of lysosome-like body is shown. In SER areas often one or two spherical *electron dense bodies* are found (Figs 47c, d). These dense bodies are always closely related to the SER. As no membrane seems to surround the bodies their nature is rather obscure.

Axons: The CA is innervated by a bundle of axons (Fig. 39a) extending from the aorta wall. This nerve is supposed to be a prolongation of the NCC. Single axons may be seen in the intercellular spaces of the CA cells. Some of these axons contain NS granules (Figs 39b, c) but this NS material does not occur in the considerable amounts sometimes found in the CA of other insect species. However, there seems to be an increase in NS material corresponding with increasing age of the larva.

5.5.3.2.2. Changes in ultrastructure

Worker larva: 48 hours old (fig. 40). *RER:* scarce. Some cisternae in close relationship to the golgi area. *Golgi complex:* in most cells the lamellae lie close together, in some cells somewhat dilated and surrounded by a few golgi vacuoles. *SER:* scarce, a few vesicles scattered in the cytoplasm. *Small vacuoles:* scarce. *Mitochondria:* ovoid, some rodlike; matrix not very dense. *Large vacuoles:* rather numerous.

Worker larva: 54 hours old. *Nucleus:* some scattered heterochromatin granules. *Large vacuoles:* not numerous. No further obvious changes if compared with the 48-hour-old larva.

Queen larva: 54 hours old, 6 hours in queen cell. *Nucleus:* some scattered

heterochromatin granules. *RER:* long cisternae surrounding some golgi areas. *Golgi complex:* lamellae somewhat dilated. *Small vacuoles:* in some cells rather numerous. *Mitochondria:* most ovoid, some rodlike. *Large vacuoles:* not numerous.

Worker larva: 60 hours old (fig. 41). *RER:* scarce. *Golgi complex:* lamellae somewhat dilated. *SER:* a small whorl surrounding some golgi complexes. Scattered SER scarce. *Small vacuoles:* rather numerous in some cells. *Mitochondria:* most ovoid, some rodlike. *Large vacuoles:* rather numerous.

Queen larva: 60 hours old, 12 hours in queen cell (figs 45, 46, 47). *RER:* cisternae well developed, often surrounding golgi complexes, giving rise to spider-like structures (Fig. 45). *Golgi complex:* lamellae dilated, small vacuoles surrounding the golgi complex. *SER:* in many cells whorls surrounding the golgi complex. *Small vacuoles:* rather numerous in some cells. *Mitochondria:* rather multiform. *Large vacuoles:* not numerous.

Worker larva: 66 hours old. *RER:* scarce. *Golgi complex:* lamellae lying rather close together. *SER:* scarce. *Small vacuoles:* scarce. *Large vacuoles:* rather numerous in some cells.

Queen larva: 66 hours old, 18 hours in queen cell. *RER:* scarce. *Golgi complex:* lamellae somewhat dilated, a few golgi vacuoles. *SER:* whorls in many cells in one larva, 'none' in the other larva. *Small vacuoles:* not very numerous. *Large vacuoles:* in one larva scarce, in the other larva rather numerous.

Worker larva: 72 hours old (Fig. 42a). *RER:* in some cells a small group of rather long cisternae. *Golgi complex:* lamellae somewhat dilated. *SER:* scarce. *Small vacuoles:* scarce. *Mitochondria:* ovoid and rodlike. *Large vacuoles:* rather numerous.

Queen larva: 72 hours old, 24 hours in queen cell (figs 47b, 48). *RER:* long cisternae, often surrounding SER whorls. *Golgi complex:* lamellae dilated, some golgi vacuoles. *SER:* whorls in many cells. In this stage also areas of closely packed SER vesicles may be seen, excluding most organelles (mitochondria). *Small vacuoles:* numerous in some cells. *Mitochondria:* multiform. *Large vacuoles:* rather scarce.

Worker larva: 78 hours (Fig. 43a). *RER:* scarce. *Golgi complex:* lamellae dilated. *SER:* in a few cells, a small whorl. *Small vacuoles:* in some cells rather numerous. *Mitochondria:* most ovoid, matrix rather dense. *Large vacuoles:* numerous.

Queen larva: 78 hours old, 30 hours in queen cell. *RER:* scarce. *Golgi complex:* lamellae dilated. *SER:* abundant, scattered in the cytoplasm. Some chains. In many cells whorls and areas of closely packed vesicles. *Small vacuoles:* rather numerous. *Mitochondria:* multiform; matrix dense. *Large vacuoles:* rather numerous.

Worker larva: 84 hours old. *Intercellular spaces:* near the periphery of the gland somewhat dilated. *RER:* in some cells groups of long cisternae. *Golgi complex:* lamellae more or less flattened. *SER:* scarce. *Small vacuoles:* rather scarce. *Mitochondria:* most ovoid, also multiform; matrix dense. *Large vacuoles:* rather numerous.

Queen larva: 84 hours old, 36 hours in queen cell. (Figs 49, 50). *Intercellular spaces:* somewhat dilated at CA periphery. *RER:* long cisternae surrounding SER formations. *Golgi complex:* lamellae dilated. *SER:* abundant, scattered in the cytoplasm. In many cells areas of closely packed vesicles, some whorls. *Small vacuoles:* not numerous. *Mitochondria:* multiform, dense matrix. *Large vacuoles:* scarce.

Worker larva: 96 hours old. *Intercellular spaces:* somewhat dilated at CA periphery. *RER:* scarce. *Golgi complex:* flattened lamellae. *SER:* scarce, small whorls in a few cells. *Small vacuoles:* scarce. *Mitochondria:* most ovoid, dense matrix. *Large vacuoles:* not numerous.

Queen larva: 96 hours old, 48 hours in queen cell (Fig. 51). *Intercellular spaces:* somewhat dilated at CA periphery. *RER:* in some cells a small group of cisternae. *Golgi complex:* lamellae dilated, numerous golgi vacuoles. *SER:* abundant. In some areas the SER vesicles lie so close together that jigsaw-like structures are formed. *Small vacuoles:* numerous. *Mitochondria:* multiform, dense matrix. *Large vacuoles:* not numerous.

Worker larva: 108 hours old. *Intercellular spaces:* dilated to some extent, near the periphery of the CA. *RER:* scarce. *Golgi complex:* most lamellae flattened, sometimes dilated. *SER:* scarce. *Small vacuoles:* rather scarce. *Mitochondria:* most ovoid, matrix rather dense. *Large vacuoles:* not numerous.

Queen larva: 108 hours old, 60 hours in queen cell. *RER:* scarce. *Golgi complex:* lamellae dilated in many cases. *SER:* abundant, jigsaw structures in some cells. *Small vacuoles:* numerous. *Mitochondria:* multiform, dense matrix. *Large vacuoles:* scarce.

Worker larva, 123 hours old; no material available because of poor fixation.

Queen larva, 123 hours old: 72 hours in queen cell (one larva). *RER:* scarce. *Golgi complex:* lamellae somewhat dilated. *SER:* not abundant; some jigsaw structures. *Small vacuoles:* rather numerous. *Mitochondria:* most ovoid, some long and slender. Matrix rather dense. *Large vacuoles:* rather numerous. *Lysosome-like bodies:* rather numerous.

Worker larva: 147 hours old (Fig. 44b). *Nuclear membrane:* somewhat wavy in outline. *RER:* small groups of cisternae. *Golgi complex:* lamellae rather close together. *SER:* scarce. *Small vacuoles:* scarce. *Mitochondria:* most ovoid, matrix rather dense. *Large vacuoles:* not numerous.

Queen larva: 147 hours old, 96 hours in queen cell (Fig. 52). *RER:* scarce. *Golgi complex:* lamellae rather close together. *SER:* rather scarce. *Small vacuoles:* rather scarce. *Large vacuoles:* numerous. *Lysosomes:* numerous.

Worker larva: 171 hours old (Fig. 44a, c). *Nuclear membrane:* somewhat wavy in outline. *RER:* groups of long cisternae in some cells. *Golgi complex:* lamellae somewhat dilated. *SER:* scarce. *Small vacuoles:* scarce. *Mitochondria:* multiform, matrix not dense. *Large vacuoles:* rather numerous. *Lysosomes:* rather numerous.

Queen larva: 171 hours old, 120 hours in queen cell (Fig. 53) (just before pupation). *Cell membrane:* dilated near the CA surface. *RER:* scarce. *Golgi complex:* lamellae lying close together. *SER:* scarce. *Small vacuoles:* scarce.

Mitochondria: mostly ovoid. *Large vacuoles:* not numerous. *Lysosomes:* rather abundant.

5.5.3.3. Discussion

5.5.3.3.1. Ultrastructural criteria of CA activity

Since 1960 a number of articles have been published on ultrastructure of the CA. A brief review will be presented here. It is generally agreed that cells are larger in active than in inactive CA. This is mainly due to a more developed cytoplasm. In active CA ribosomes tend to be more numerous. Mitochondria are mostly larger, elongated and polymorphic; the mitochondrial matrix often is electron dense, cristae are more numerous, whereas in inactive glands the mitochondria are smaller and ovoid. The golgi complex does not show large differences in activity, but often small vacuoles are observed in the surroundings of the golgi complex in active CA. About the activity of the endoplasmic reticulum (ER) there is less agreement. In some insect species, active glands contain rough endoplasmic reticulum (RER), in others the smooth endoplasmic reticulum (SER) is abundant if compared with the inactive gland. The functional role of the ER in JH synthesis is largely unknown. From vertebrate histology it is known that SER often is involved in synthesis of lipoids (FAWCETT et al., 1969). Several authors observed a different rate of development of the ER during the period the gland was supposed to exert its maximum activity. As also JOLY et al. (1968) remarked one can have reservations as to the validity of the choice of active and inactive stages in several studies.

SCHARRER (1964) compared CA of the Cockroach, *Leucophaea maderae*, in different stages of activity. The length of the terminal oocytes was used as a criterion for the degree of activity of the CA. Active CA were taken from females just before ovulation whereas inactive CA were obtained from females that were pregnant or at the start of a reproductive cycle (just emerged adults). In active CA the author observed rough ergastoplasmic units, which showed a tendency to form whorls, located mainly near the nucleus. In inactive CA only short RER chains existed. No signs of JH synthesis were found. SCHARRER (1971), however, found that in the golgi complex a product was synthesized, which in the form of electron dense bodies was transported to the periphery of the cell. These 'C-bodies' eventually passed the cell membrane without undergoing structural tranformations. Little was said about a possible relationship between C-bodies and activity of the gland. Differences in the amount of this material between individuals occurred depending on the phase of the intermoult period. Much C-body material was observed in CA with a low level of activity, mainly in the intercellular compartment of the gland. According to the author the C-body material had little chance to be retained and accumulated in active CA.

ODHIAMBO (1966a) investigated the ultrastructure of active CA in the male desert locust, *Schistocerca gregaria*. The most conspicuous structure appeared to be an extensive network of SER. According to the author it was formed in the golgi region. The SER was found in three forms, viz: small isolated vesicles

close to the golgi apparatus; long, bead-like, rows of small vesicles stacked into lamellae (whorls), while in the third type the vesicles were swollen. The vesicles, possibly containing the JH, might be stages in a process of transport towards the periphery of the gland. However, no extrusion of vesicles was observed. According to the author the SER is the principal agent in the elaboration of the hormone. ODHIAMBO (1966b) compared active and inactive CA from mature and newly emerged male adults of *Schistocerca gregaria*, respectively. The SER was found to show marked differences, in the active gland being far more developed as compared with the inactive gland and excluding most of the cytoplasmic organelles. In newly emerged adults no SER vesicles were seen.

THOMSEN and THOMSEN (1970) found in the blow fly, *Calliphora erythrocephala*, that SER was highly developed in active CA, whereas little RER was present. In inactive glands the SER tended to form whorls and myelin-like structures. As a criterion for CA activity these authors introduced large lipid droplets, which were supposed to contain JH. These droplets developed in close spatial relationship to vacuoles that budded off from the SER. The authors suggested that JH, or a precursor, might be synthesized in the tubules of the SER and collected in membrane-bound vacuoles. Subsequently, the vacuoles might loose their membranes. The hormone was supposed to dissolve in lipid, forming the already described lipid droplets. These were more numerous in active than in inactive glands. No release of lipid into the haemolymph was observed.

JOLY et al. (1967, 1968) compared CA of the desert locust, *Locusta migratoria*. Inactive CA were obtained by cauterization of the neurosecretory 'C-cells' in the brain. Inactivation of the CA was characterized by an accumulation of SER, followed by the formation of whorls which tended to disappear as myelin-bodies. Maximum activity coincided with the beginning of SER formation, rather than with the presence of the maximum amount of SER.

FAIN-MAUREL and CASSIER (1969) described small vacuoles in active CA of *Locusta migratoria*, mainly in the form of SER. Formation and structure of the SER appeared to be related to the physiological state of the insect during sexual maturation and oviposition. In inactive glands the SER was seen to form whorls, as described by JOLY et al. (1968).

PANOV and BASSURMANOVA (1970) found no SER in CA of the bug, *Eurygaster integriceps*. RER was far more developed in active than in inactive CA.

AGGARWAL and KING (1969) demonstrated SER in the larval CA of *Drosophila melanogaster*. The authors found evidence that the SER during the first larval instar developed by budding off from the nuclear surface. During the larval stage an increase in the amount of SER was observed. According to the authors electron transparent areas presumably contained JH in living cells, the contents being extracted during cytological processing. 'Allatum bodies' (curved stacks of flattened, disc-shaped, membraneous sacs) were supposed to represent resting stages of the SER. According to the authors, JH presumably was released from the gland by diffusion as they did not find signs of release.

TOMBES and SMITH (1970) compared active and inactive (diapause) CA of the adult alfalfa weevil, *Hypera postica*, but did not find structural signs of secreto-

ry activity. Only the amount of cytoplasm in active CA was larger.

WAKU and GILBERT (1964) described electron dense bodies which they supposed to be related to secretory substance in active CA of the silkmoth, *Hyalophora cecropia*. Concentric circles of ER were demonstrated besides a more dispersed ER. At that time, however, cytological processing was not yet very reliable (which is demonstrated by the pictures presented). Consequently no exact interpretation of ER structures can be given. From this review of the literature it can be concluded that there is little agreement on ultrastructural signs of JH secretion.

In the present study differences in ultrastructure were observed in CA of honeybee larvae of different ages and castes. These structural differences could be interpreted in terms of activity as follows: In the inactive gland RER is scarce. Lamellae of the golgi complex lie close together. Golgi vacuoles are scarce as are small vacuoles in the cytoplasm. The first sign of resumed activity is the formation of spider-like RER cisternae (Fig. 35a) which may be interconnected with the golgi cisternae (Fig. 45b). The golgi lamellae may be dilated. Subsequently SER whorls are seen surrounding some golgi complexes. RER cisternae remain in close contact with the SER (Fig. 46). This arrangement gives the impression that SER whorls are formed by co-operation of the RER and the golgi complex. Such a mechanism of SER formation is not merely speculative as HIGGINS and BARNETT (1972) found evidence that RER is involved in the biogenesis of SER by delivering the proteinaceous part of SER membranes. The role of the SER seems to be an indirect one. It is likely that SER mainly determines the secretory potential of a cell. The most direct sign of secretory activity is the formation of small vacuoles in the golgi complex. Lysosomes are present in considerable numbers at the end of the larval stage. The large vacuoles are a puzzling feature. Usually more of these fat containing (?) vacuoles are found in the worker larva than in the queen larva. If Thomsen's (1970) assumption is correct that these vacuoles contain JH, they might serve as a place of storage of JH during the period that little hormone is being released. For the present moment, however, there is no evidence to support this theory.

Not all cells in the CA become activated during a secretory period. Cells lying at the periphery of the gland show a tendency to be more active than those in the centre.

5.5.3.2.3. Cyclic activity in the corpus allatum of the worker larva.

If one accepts the above criteria for activity it may be concluded that the CA of the worker larva seems to release moderate amounts of JH. SER is rather scarce. In some stages SER whorls may be found, but no areas of closely packed SER vesicles develop as in the queen larva. Mitochondria tend to be more ovoid than those of the queen larva. There are fewer small vacuoles in active cells in the CA of the worker larva than in those of the queen larva.

Cyclic activity may be described as follows: the CA of larvae 48 and 54 hours old may secrete JH in limited amounts. RER sometimes surrounds the golgi complex which may indicate that the cells are in preparation for the next se-

cretory period. At 60 hours JH secretion, and release, takes place. At 66 hours most cells are inactive again. In larvae of 72 hours old RER formations indicate resumed activity: initiation of the next secretory cycle. At 78 hours some cells are actively secreting. In the CA of larvae, 84 hours old, RER formations indicate structural activity; small vacuoles are present in limited numbers, indicating that the gland is secreting JH. From 96 hours on, up to 171 hours, secretory activity seems to be constant and at a rather low level. However, as the intervals between successive stages examined were considerable (96, 147, 171 hours), periods of activity may have occured in between. Dilations of the intercellular spaces are seen in some older CA. Membrane bound vacuoles are sometimes found to fill the space which might indicate that besides the border cells the more centrally situated cells are actively secreting.

5.5.3.2.4. Cyclic activity in the corpus allatum of the queen larva.

In the queen larva of 54 hours old, RER cisternae indicate the initiating of an activity period. The presence of rather numerous small vacuoles in some cells means that JH secretion is taking place. At 60 hours, RER cisternae may be quite conspicious; the golgi complex actively produces small vacuoles. Whorls of SER indicate that the cells are preparing for a high level of secretion. At 66 hours, the CA is secreting at a rather low rate. SER whorls are present, but RER surrounding these whorls is scarce. At 72 hours, RER is present again in long cisternae surrounding the SER which indicates that SER formation is resumed. Cells are actively secreting. The same holds for CA of larvae, 78 hours old. However, RER by then is scarce again. At 84 hours, SER formation occurs, but JH secretion is at a rather low level. At 96 and 108 hours the CA is actively secreting again. RER is not abundant, which means that SER formation does not occur at a high rate. In SER areas jigsaw-like structures might indicate that SER is inactivated to some extent. At 123 hours, SER areas are not as abundant as in the previous stages. Secretion still continues, but golgi complexes are not very active. At 147 hours and at 171 hours the cells seem to be rather inactive although intercellular spaces may contain membrane-bound vacuoles, indicating that JH is still being released. SER is rather scarce. Lysosome-like bodies are numerous.

From the above description it can be concluded that also the CA of the queen larva shows cyclic activity. Secretory activity seems to be at a higher level than in the CA of the worker larva. After grafting the amount of SER increases steadily up till 96 hours. From 123 hours on it decreases again. Mitochondria of elaborate shapes frequently occur. In older CA the intercellular spaces may be dilated. Membrane-bound vesicles may fill up this space.

The present author is aware that 'translation' of structural features in terms of activity is speculative. With a labelled precursor of JH combined with ultrathin cryotome sections more reliable information might be obtained on the way the hormone is synthesized and released. At the present moment, however, a labelled precursor is not available.

6. THE ROLE OF JUVENILE HORMONE IN CASTE DIFFERENTIATION

6.1. Introduction

JH plays an important role in morphogenesis in the prae-imaginal stages as well as in protein synthesis in the adult. In the previous chapter some histological criteria for the activity of the CA were described. As histology at best gives a static picture of the situation at the moment of fixation, it is difficult to obtain information of processes such as uptake or release of substances.

Piepho (in Wigglesworth, 1970) implanted fragments of insect integument in the body cavity of insects and found that these implants reacted in the same way as the host tissue in the type of cuticle that was formed. As no nervous or other connections existed between host and implant, information had to be transferred via the haemolymph. Determination of hormone concentrations in the haemolymph of insects in different physiological stages can supply information about the part the hormone plays in regulating processes. Changes in development after experimental variation of the hormone concentration may indicate that the hormone is involved in certain processes.

The hormone titre results from synthesis and release on the one hand, and from inactivation and excretion on the other (De Wilde c.s., 1971). By CA implantation and extirpation experiments the hormone level can be varied and the subordinated processes studied (in Wigglesworth, 1970). However, care must be taken when interpreting implantation experiments, as little is known about regulation of CA activity. Sometimes implanted CA were found to react on the physiological situation of the host (Joly, 1967). JH in the insect may be inactivated at a fast rate. De Kort (1970) found that injected JH had a half-life of 1 hour in the colorado beetle. Whitmore c.s. (1972) found that in *Hyalophora gloveri* certain esterases may be involved in de-activation of JH. During larval life a relatively high JH titre is maintained (Joly, 1968; Wigglesworth, 1970). The notation 'relatively' is important in this respect. Bartelink (de Wilde et al., 1971) found 50.000 Galleria units/ml haemolymph in 4th instar *Philosamia cynthia* larvae, whereas in haemolymph of larvae of the colorado beetle the JH titre was below the limit of detection (de Wilde, pers. comm.).

In the larva, JH is supposed to inhibit development of adult structures. Prior to pupation the JH titre would be lowered to enable processes involved in metamorphosis to take place. A rise in the ecdysone titre would induce the moulting process. Metamorphosis therefore would be the result of a relatively low JH titre, combined with a relatively high ecdysone titre.

6.2. JUVENILE HORMONE TITRES IN THE HAEMOLYMPH

6.2.1. *Methods*

To determine the concentration of JH in the blood, about 160 mg of clear haemolymph were collected. Larvae were punctured dorsally, just laterally of the heart vessel and the outpouring haemolymph was collected with a capillary (Pasteur's pipette), and immediately cooled in melting ice. Some glutathione was added to prevent tyrosinase activity. The samples were tested for the presence of JH activity with the *Galleria* wax-test of SCHNEIDERMANN, modified by DE WILDE et al. (1968).

6.2.2. *Results*

In Table 6 JH titres are given for larvae of different ages and castes. In interpreting these data it should be noted that the variation in this test remains within a factor-two limit.

The hormone titres in worker larvae less than 5 days old were on the boundary of detection, the amount of JH in the highest-concentration group (undiluted) being just detectable. In the praepupa an increase in concentration was observed. In queen larvae the titre obviously increased within about 30 hours after grafting and then remained high up to the last stage checked (6 days of post-embryonic development). In this study there was not enough time available for further experiments. It would be interesting to add data on the JH titres in

TABLE 6. JH concentrations in the haemolymph ($^1/_2B<X<2B$)

A: age of larvae (days)	B: galleria units/g haemolymph ♀		C: number of larvae per sample ☿	
A	B	C	B	C
3[1]	3000	20	< 200	30
3[1]	–	–	< 300	30
4[2]	2400	14	< 200	13
5[3]	1300	5	< 100	5
5[3]	2200	5	200	5
6[4]	600	7	600	8
6[5]	1400	3	1300	5

[1]: 72 h ♀: 28 hours after grafting
[2]: 99 h ♀: 51 hours after grafting
[3]: 123 h ♀: spinning, ☿: just prior to sealing
[4]: 130–140 h ♀: after spinning (beginning praepupa), ☿: spinning
[5]: 147 h ♀: praepupa ☿: beginning praepupa.

In extracts from fresh RJ no JH activity could be demonstrated.

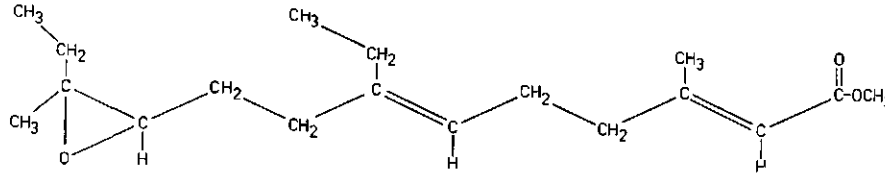
younger as well as in older stages of both castes and further investigate the relation between structure and function of the gland. Evidence on cyclic activity of the CA during the larval stage might be obtained with animals of precisely known age.

6.3. Topical application of juvenile hormone

To ascertain the possible role of JH in caste differentiation worker larvae of different ages were treated with the hormone and some caste characters of the adults obtained were examined (Wirtz and Beetsma, 1972).

6.3.1. *Methods*

Worker larvae of known age were treated with JH. The larvae normally lie on their side in the cell. A drop of 1 to 2 μl of the hormone (Biojine 100^{R}, Procida SA, see formula), in a conc. of 1 to 2.10^{-3} in acetone, was applied laterally on



Methyl, 10 epoxy, 7 ethyl 3,11 dimethyl 2,6-tridecadienoate (trans, trans, cis): Röller formula, *Cecropia* hormone.

each larva with a 10 μl syringe. The frames, containing the treated larvae were kept in an incubator (35 °C) for about 30 min to allow the acetone to evaporate, and then replaced in a queenright or queenless colony. It was necessary to take the frames out of the colony immediately after the cells were sealed to prevent the treated larvae from being removed by the worker bees. Development was completed in the incubator.

As the acetone solution spreads readily over the larva and the surrounding food as well as over the cell surface, it is not possible with this method to give an estimation of the amount of JH that penetrates into a larva. It is possible that the JH exerts its effect over a longer period because of release from the cell wall (see Section 6.3.2). Further improvement of the method of application is needed, particularly with regard to the optimum concentration and moment of application. The experiments were carried out late in the season (second half of July, August and September).

6.3.2. *Results*

After application of JH the larvae were again nursed by the worker bees in the colony. As the food in the midgut of 4½ day-old larva (after JH treatment at the age of 3 to 3½ days) contained much pollen it was concluded that the JH-

treated larvae were nursed as normal worker larvae. Also, no worker cell containing a JH-treated larva was drawn out like a queen cell. The cells were capped like normal worker cells, although some cappings were bulgy. It appeared that this was because the JH-treated larvae had moved towards the opening of their cell – presumably in search for more food – and were lying halfway in the cell. JH-treated larvae showed a tendency to become heavier than their normal sister-worker larvae. During the larval stage mortality in most experiments was very low but increased markedly after sealing of the cell. Nursing of JH-treated larvae in queenless colonies did not differ from that in queenright colonies. However, experiments are needed to investigate whether worker bees prefer cells containing JH-treated larvae for the construction of queen cells, after the colony has become queenless. Capping of the cells containing JH-treated larvae took place some hours earlier than normal, depending on the effectiveness of the application. Cocoons of JH-treated larvae that completed pupation showed the tendency to be heavier than those constructed by control workers. JH-treated larvae completed their development some days earlier than controls (WIRTZ and BEETSMA, 1972). The expression of queenlike phenomena was dependent on the moment of application as well as on the dose of JH applied. The best results were obtained by treating 3 to $3\frac{1}{2}$ day-old worker larvae with one dose of 1 to 2 μg Biojine-100. The adults concerned were about the size of normal worker bees but their appearance was queenlike (Figs 54, 55). In most experiments the sealings were artificially removed before the adults were expected to emerge because it was observed that they had difficulties in breaking their cocoon.

When JH was applied to the cell wall in a way that it did not come into direct contact with the larvae, the creatures died during the pupal stage (Table 7, 47–58). The pupae were abnormal in several ways. Mandibles were neither queenlike nor worker-like; they were sickle-shaped. Browning of the epidermis, normally occurring late in the pupal stage, took place before the eyes were dark brown giving the animals a patchy appearence. JH must have reached the larval epidermis via the comb from which small quantities were apparently released during development.

The important question arises as to what extent adults from JH-treated larvae may approach natural queens. For this purpose the most queenlike looking adults from different topical application experiments were investigated on several caste characters (Table 7, 1–35). Some more worker-like adults have been included (Table 7, 36–46). It was observed that adults from untreated larvae which were reared in cells bordered by cells with JH-treated larvae, were sometimes slightly queenlike in some characters. Most likely JH had passed through the cell wall. There is little variation in morphological characters in natural worker-bees. In natural queens caste characters may show more variation.

TABLE 7. Comparison of some caste characters of adults – obtained from JH-treatment of about 3-day-old worker larvae – with those of natural queens and worker bees.

adult	1	2	3	4	5	6	7a	7b	8
♀ (S)	–	A	A	4*	2.3	1.6	2.6	3.8	160
♀ (S)	A	A	A	3*	2.1	1.4	2.6	3.6	180
♀ (S)	A	A	A	4*	2.3	2.0	2.5	3.7	–
♀ (S)	A	A	A	4*	2.5	2.0	2.6	3.8	150
♀ (S)	A	A	A	4*	1.7	2.0	2.6	3.7	150
♀ (S)	A	A	A	4*	1.6	1.8	2.6	3.8	175
♀ (L2–3)	A	A	A	2*	2.5	1.8	–	3.7	180
♀ (L2–3)	A	A	A	3*	1.6	2.0	–	3.6	175
♀ (± L3)	A	A	A	3*	1.3	1.6	2.5	3.6	155; 160
♀ (± L3)	B	B	A	6*	1.9	2.3	–	4.0	160; 170
♀ (± L3)	A	A	A	2*	2.0	1.6	–	3.7	145; 145
☿	E	E	E	10	2.6	–	2.9	3.9	6
☿	E	E	E	10	2.4	2.5	2.6	3.8	4
☿	E	E	E	10	2.5	2.7	2.8	3.8	4
☿	E	E	E	10	2.5	2.6	2.8	3.7	1; 3
1	C	B	B	6	1.9	2.3	2.6	3.7	120
2	B	A	A	5	1.9	1.4	2.5	3.5	120
3	C	B	B	5	1.8	2.0	2.6	3.7	115
4	B	B	B	4	1.6	1.3	2.2	3.5	140
5	B	B	B	3	1.6	1.3	2.3	3.5	150
6	B	A–B	A–B	5	2.3	1.7	2.3	3.7	–
7	C	C	C	5	1.3	1.3	2.3	3.5	160
8	C	D	C	5	2.3	1.8	2.7	3.7	50
9	C	B	C	6	1.9	1.6	2.1	3.5	–
10	A–B	A	A	4	1.1	1.8	2.5	3.5	145
11	A–B	A+	A+	2	1.3	1.3	2.3	3.5	170
12	B	A	A	4	1.2	1.1	2.3	3.7	110
13	B–C	C	B	6	0.9+	2.0	2.4	3.7	125
14	C	D	D–E	7	1.4+	2.3	2.6	3.7	80
15	C	C–D	C–D	6	1.4	1.8	2.8	3.8	140
16	B	C	D	6	2.0	1.6	2.6	3.6	130
17	B	B	A–B	6	1.6	2.1	2.6	3.8	65
18	B	B	B	6	1.5	2.0	2.6	3.7	120; 110
19	B–C	C	B	5	1.6	2.1	2.6	3.7	85
20	C	B	B	5	2.1	2.1	2.7	3.7	120
21	B	A	A	4	2.0	1.7	2.5	3.7	165
22	B	B	B	5	2.3	1.8	2.6	3.7	125
23	B	D	D	5	1.9	1.6	2.5	3.5	150
24	C	B	B	5	2.1	2.5	2.7	3.6	85
25	B–C	D	E	5	1.3	1.7	2.6	3.6	145
26	B	B	B	5	1.9	1.6	2.5	3.5	155
27	C	B	B	6	2.5	2.6	2.6	3.5	65
28	C–D	C	C	6	2.0	2.4	2.4	3.5	85
29	C	B	B	6	1.8	2.3	2.6	3.5	120
30	C	A–B	D	5	2.0	2.5	2.5	3.5	120
31	B	A–B	A	4	1.9	1.6	2.4	3.5	170
32	D	D	C	6	2.0	2.2	2.6	3.4	165
33	E	E	D	7	2.0	2.3	2.8	3.7	86
34	C	C	C	5	2.1	2.4	2.7	3.7	50
35	C	A–B	B–C	5	2.2	1.6	2.4	3.5	100

TABLE 7. (continued)

adult	1	2	3	4	5	6	7a	7b	8
36	E	E	E	6	2.8	2.2	2.7	3.7	40; 50
37	C	E	D	6	1.9	2.1	2.6	3.6	85
38	E	D	E	5	2.0	2.1	2.7	3.7	100
39	C	E	E	6	2.1	2.3	2.6	3.7	15
40	D	E	D	7	2.2	1.7	2.5	3.4	–
41	D	E	E	8	2.1	2.3	2.5	3.6	–
42	C–D	E	E	6	1.8	2.1	2.5	3.6	115
43	D	E	E	4	1.9	2.2	2.6	3.6	25
44	D	E	E	6	2.5	2.8	2.6	3.7	85
45	C	D	D	6	2.0	2.3	2.6	3.6	60
46	D	D	D	7	2.1	2.4	2.8	3.7	30
47	D△	A	A	6	0.9	1.2	2.4	3.7	75; 120
48	D△	A–B	A–B	6	1.8	1.4	2.4	3.9	65; 60
49	D△	D	C	–	2.3	1.8	2.6	4.0	95
50	B	A–B	A–B	6	1.9	1.6	2.6	3.7	78; 85
51	D△	B	C	6	2.2	1.8	2.6	3.7	120
52	D△	B	C	6	2.1	1.6	2.2	3.7	165
53	D△	B	B	5	2.6	2.0	2.1	3.8	105
54	C△	A–B	B	6	0.5	0.6	2.0	3.5	150
55	C△	A–B	A	7	1.9	1.5	2.5	–	–
56	C△	A	A	5	2.0	1.7	2.1	3.7	140
57	C△	A	A	5	1.6	1.4	2.1	3.8	90
58	D△	E	E	7	2.5	1.9	2.3	3.7	150

Explanation of Table 7

	1: mandibles	2: hairs on surface of tibia of hindleg	3: basitarsal hairs (pollencomb)
A:	strongly notched	short hairs on whole surface	no rows of hairs
B:	notched	short hairs on 75% of surface	25% of hairs in rows
C:	slightly notched	short hairs on 50% of surface	50% of hairs in rows
D:	nearly smooth	short hairs on 25% of surface	75% of hairs in rows
E:	smooth	no short hairs on surface, (pollen basket)	100% of hairs in rows, (pollen comb)

4: sting: number of barbs. The sting of the worker bee has 8 to 10 pronounced barbs (fig. 54f). In queens (*) sometimes a few hummocks are the only signs (Fig. 54d). Mostly, the sting of the queen bears 1 to 2 small barbs and 1 to 2 rudimentary barbs; the latter have been included in the countings.

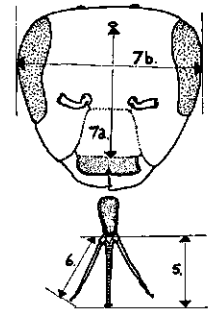
5: length of the glossa in mm

6: length of the labial palpus in mm

7a: length of the head in mm

7b: width of the head in mm

8: number of ovarioles per ovary. The ovary was dissected and ovarioles were carefully tore apart and counted under a stereo microscope (accuracy: ± 5 ovarioles, in the large ovaries)



♀ (S): swarm queens. ♀ (Lx): queens from larvae, grafted at the age of × days)

\+ malformed

△: mandibles atypical (sickle-shaped).

6.4. Discussion

Adult queen and worker differ in many respects. Lukoschus (1956b) listed a number of morphological, anatomical and physiological features of adult queen and worker. The reader is referred to this publication. For a long time the dichotomy in development between adult queen and worker has been regarded as not being strict, since intermediate forms can be produced (Klein, 1904). Gradual series of intercastes ranging from queenlike to worker-like can be obtained in three ways: *a.* by transferring worker larvae of more than 3 days old into queen cells in queenless colonies, *b.* by rearing worker larvae on RJ in the laboratory, *c.* by altering the JH titre.

a. Effects of transferring worker larvae above the critical age: A number of articles have been published on the influence of larval age (at the moment of grafting) on the expression of caste characters (Alpatov, 1928; Komarov, 1934, 1935; Gontarski, 1941; Vagt, 1955; Weaver, 1957 a, b; Naulleau, 1960; Weiss, 1971; Woyke, 1971). There is general agreement that successive grafting of larvae more than 3 days old results in an increase of worker-like morphological characters. The number of ovarioles decreases markedly (Table 9). Less unanimity exists on the influence of the moment of grafting in the first 2 to 3 days, on ovary development. Jordan (1960) and Woyke (1971) reported evidence for an increase in ovarial development, the number of ovarioles in adults being larger the earlier the larvae were placed in queen cells. Other authors could not demonstrate this effect (Becker, 1925, Eckert, 1934, Weiss, 1971). In Table 9 some data on this subject are presented. According to Vagt (1955) and Woyke (1971) the size of the spermatheca decreases progressively when larvae more than ½ day old are used for queen rearing.

In the queen larva the number of ovarioles per ovary increases to about 160 to 180, whereas in the full-grown worker a maximum of about 130 ovarioles per ovary is attained (Zander et al., 1916). Subsequently in the worker, during the praepupal and pupal period, a regression in the number of ovarioles occurs, resulting in adults with about 1 to 20 ovarioles per ovary. Table 8 shows the development of the ovary during the larval stage according to Zander et al (1916) and Wang and Shuel (1965).

There are indications that worker bees in the colony can distinguish between queen and worker larvae. Larvae more than 3 days old are often not accepted when placed in queen cells (Vagt, 1955; Eckert, 1934; Woyke, 1971). The reverse is also true: older queen larvae placed in worker cells are usually removed by the worker bees. No information is available on the cause of this distinction between larvae of both castes. Woyke (1967) found that diploid drones secrete a 'cannibalism' substance during a certain period of larval development and are subsequently eaten by the worker bees. An identical mechanism might be involved in the recognition of larval caste.

b. Effects of rearing worker larvae on RJ in vitro: Weaver (1957a, 1958) showed that adults obtained from larvae reared in vitro on RJ may exhibit worker-like characters. Smith (1959) also reported intercastes developing from

TABLE 8. Ovary development during the larval stage.

larval age (hours)	number of ovarioles per ovary in larvae.			
	ZANDER et al. (1916)		WANG and SHUEL (1965)	
	♀	☿	♀	☿
36	70	–	–	–
48	100	70	95	90
60	–	–	100	100
72	130	80	110	100
90	–	–	115	110
102	–	–	120	110
120	155	130	–	–

TABLE 9. Classified numbers of ovarioles per ovary, in adults grafted at different larval ages. (figures above 25 rounded to the nearest 5).

age at the moment of grafting (days)	authors: BECKER	ALPATOV	ECKERT	KOMAROV	WEAVER	WEISS	WOYKE
egg	–	155	–		–	175	160
½	–	–	160		–	180	–
1	160	–	160		165, 170	180	155
1½	–	–	155	145–190	–	180	–
2	160	–	150		170	175	145
2½	–	–	160		–	–	–
3	165	160	160		150	–	135
3–4	150, 45, 35, 7	50, 35, 7	–	115–180	110, 50, 15	–	–
4	3	4	–	–	–	–	110
'normal' queen			130–185	135–190	115–215	165–200	150–170
normal worker			–	2–24	1–9	3–11	–

experiments in vitro with different samples of RJ. The author classified the intercastes into 4 groups based on several morphological and anatomical characters. Queens, intercastes and workers were produced in about equal numbers. Workers raised in vitro usually were larger and had better developed ovaries than normal worker bees reared in the colony, whereas queens reared in the laboratory had less developed ovaries than colony-reared queens.

In the colony, queen and worker type are strictly separated in the sense that normally no intercastes develop. As mentioned before this is mainly achieved via the selective behaviour of worker bees towards the larvae. However, there are indications that larval food also helps prevent development of intermediate forms. On the one hand RJ is supposed to contain a substance which promotes

the development of queenlike characters. On the other hand present findings indicate restriction of larval feeding on WJ during the first 3½ days. After this period 'normal' feeding seems to be restored (MWJ) enabling the larva to store enough reserves to pass metamorphosis. WANG and SHUEL (1965) altered the sugar and 'water soluble acid' (extracted from bee milk) concentration of WJ and RJ. Addition of acids to WJ and RJ resulted in a decrease of body weight as well as in a reduction in the number of ovarioles as compared with controls. Addition of sugar to WJ-plus-acids reduced the hampering effect of this food. Although the authors did not draw this conclusion it is likely that the acceptability of the food offered to the larvae was altered, and consequently the amount of food ingested, this in its turn influencing body weight and ovary development.

c. Altering the JH titre. BER-LIN CHAI and SHUEL (1970) implanted CA of 3 and 4 day-old queen larvae into 4-day-old worker larvae. They observed an increase in the number of ovarioles in the adults compared with control workers, both reared in vitro. CA of worker larvae did not exhibit this effect. DIXON and MOSER (1971) reported that CA of 48-hour-old queen larvae acted repressively on glucoside secretion in accessory sex-glands of female cockroaches, *Periplaneta sp.* (see SHAAYA and BODENSTEIN, 1969), whereas, on the contrary CA of 72-hour-old queen larvae stimulated glucoside secretion. According to the authors CA of 48-hour-old worker larvae were actively secreting. CA of 72-hour-old worker larvae were seen to be far less active than corresponding queen larval CA.

From topical application experiments (this study) it can be concluded that indeed the JH acts as a mediator between the food and the differentiation of caste characters. It remains to be investigated in what way feeding regulates the functioning of the CA. Possible mechanisms will be discussed in chapter 8.

7. EXPERIMENTS IN VITRO

In chapters 4 and 5 a description was given of the dichotomy in the development of the fat body and the CA in colony-reared queen and worker larvae. In this chapter some data will be presented on the development of these organs in larvae reared in vitro.

7.1. Materials and methods

Larvae were reared in plastic queen-cups in the incubator at 35 °C and 96% RH. Food was refreshed every 24 hours by regrafting the larvae in queen-cups that contained new food. RJ was collected from queen cells with larvae of about 3 days old. RJ was supplied fresh (unstored) or after storage at –20 °C for several months. Before use the refractive index of the RJ was ajusted to 27% with a refractometer (Smith, 1959) and stirred thoroughly. Young larvae were provided with about 0.1 ml of food, older larvae received about 0.2 ml. Worker larvae, 48 hours old, were grafted in queen-cups containing the following diets:

1. Fresh (unstored) RJ
2. Stored RJ
3. Stored RJ plus mandibular glands of 13-day-old worker bees from a queen-right colony.
4. Stored RJ plus mandibular glands of 13-day-old worker bees from a queen-less colony.
5. Sweetened condensed milk.

Mandibular glands were taken out, taking care that the content was not spoiled, and immediately added to the stored RJ. The food was mixed thoroughly before the larvae were placed in the queen cells. 48-hour-old larvae (one larva per queen-cup) received 2 pairs of glands in 0.1 ml RJ. 72-hour-old larvae received 4 pairs of glands in 0.2 ml RJ. The refractive index of sweetened condensed milk – in undiluted form containing 8% milk fat and 20% fat free and water free milk substances – was adjusted to 86%. Addition of more water made this food too liquid to support the larvae.

7.2. Results

Larvae reared in the laboratory developed markedly slower than controls in the colony. The fourth larval instar lasted up to about 100 hours (controls about 84 hours). Therefore it is difficult to relate the results to natural development. In Table 10 some data are presented.

It is clear that increase in size of the CA of larvae in vitro is little compared with normal worker larvae (Fig. 8f, Table 10) and disproportional compared

TABLE 10. CA and fat body development of larvae reared in vitro for 24 and 48 hours (worker larvae, grafted when 48 hours old).

	Larval age: 72 hours					Larval age: 96 hours				
Diet	A	B	fat body* type	glycogen	fat	A	B	fat body* type	glycogen	fat
1	4	160	♀	+	++	9	200	♀	+	+++
2	5	150	☿–♀	++	+	7	210	(☿)–♀	++	++
3	5	150	☿–(♀)	+	+	6	195	☿	++	+
4	7	155	☿	+	+	7	170	☿	++	+
5	5	105		+++	++	2	160		—	+++

A: number of larvae
B: mean CA surface (methods: section 5.5.2.1)
*: explanation see text.
— : almost absent
+ : little amount
++ : moderate amount
+++: considerable amount

with queen larvae of the same chronological age. With Diet 1, one might expect a queenlike development of the CA as fresh RJ was provided. However, natural diets offered did not induce marked differences in CA size although the CA of larva reared on diets 3 and 4 (Table 10) tended to be smaller. In larvae reared on the artificial diet (5) CA growth was still less. Mortality on this diet was high.

The influence of the diets was clearly reflected in the fat body. The fat cells of larvae reared on fresh RJ (Diet 1) showed the same developmental pattern as seen in queen larvae (Fig. 4). Large watery vacuoles disappeared; fat containing vacuoles were formed. In larvae reared on stored RJ (Diet 2) for 24 hours an intermediate type of development was seen. The central part often developed queenlike, the peripheral fat-cells remaining worker-like. Individual differences were pronounced, some larvae being more queenlike than others. Fat cells in most larvae reared on stored RJ for 48 hours were quite queenlike, some were worker-like to some extent. The majority of larvae of 72 hours old reared on Diet 3 remained worker-like. In two larvae some fat cells were queenlike. Larvae of 96 hours old (Diet 3) were worker-like. All larvae reared on Diet 4 for 24 and 48 hours were worker-like. The reaction of the fat body of larvae reared on Diet 5 was queenlike in some respects. The cells were loaded with glycogen after 24 hours. After 48 hours fat cells in the surviving larvae contained numerous small fat vacuoles; glycogen was practically missing at this stage (Fig. 16j).

7.3. DISCUSSION

Results of laboratory-rearing differ from those of colony-rearing in two respects. Firstly, survival rate is low mainly because of mortality during the pupal moult. Secondly, larvae reared on RJ in vitro do not necessarily develop

into queens; also workers and intercastes may be formed. Little is known about the factors that cause these differences. Temperature and humidity have been studied and there is general agreement that 35 °C and 96% RH are optimum conditions for larval development in vitro. During the pupal stage the larvae may be kept at 60% RH (SMITH, 1959). Therefore the most logical presumption seems to be that food is different in vitro compared with colony circumstances.

In the experiments in vitro (Section 7.2) some histological data were obtained on the fat body and the CA. As the experiments were not repeated the following considerations may only serve as a working hypothesis. Fig. 8f shows that *fresh* RJ did not stimulate CA growth as was expected. It is questionable whether the fresh RJ supplied remained fresh during the 24 hours that larvae were fed on it at a temperature of 35 °C. Much 'labile factor' might be lost during this time. On the other hand the reaction of the fat body was identical to that of larvae reared on RJ in the colony. *Stored* RJ obviously had lost more of its differentiating capacities. The fat body remained partly worker-like. Addition of mandibular glands altered fat-cell development into the direction of that found in lavae fed WJ. Little food was stored. On the milk diet (5) the larvae made large stores in the fat body, but nevertheless most of them died within a few days.

These observations might be explained by: 1. a very labile substance in RJ that activates the endocrine system; 2. substances in larval food that regulate feeding. From other insects it is known that the endocrine system may be controlled via sensory information (chapter 3).

8. DISCUSSION

In this chapter, an attempt will be made to evaluate and interrelate some factors that are, or presumably are, involved in caste differentiation in the honeybee larva. The scheme presented (Fig. 10) does not pretend completeness, but is merely a framework to support the discussion.

In the role of larval food, two aspects have to be distinguished: 1. The food as nourishment, ensuring the presence of sufficient reserves for development into the adult. 2. The possible role of factors in the food, inducing processes that are more directly involved in caste differentiation. Literature on the subject proves that in practice distinction of these aspects is very difficult. HAYDAK (1943) submitted that the nutritional state of the larva is the only factor inducing caste differentiation. The author postulated that the full-grown ovary of the queen larva secretes a hormone which in sufficient quantity causes structural and physiological changes characteristic for queen development. In the worker larva which is, according to HAYDAK, undernourished from the third day of the larval stage on the rudimentary ovary does not have this potential. However, REMBOLD (1969) found evidence for a 'determining principle' in RJ.

In Chapter 4 was shown that during the first $3\frac{1}{2}$ days of development worker larvae are undernourished compared with queen larvae. The observation that worker larvae may be heavier during this period is misleading in this respect, since they apparently have a higher moisture content. Stores in the fat cells are very limited. Large watery vacuoles might indicate that protein is being used for energy production. Abundant supply of WJ does not seem to alter this situation. According to HOUSE (1969) feeding activity of insects may depend on the quantitative composition of food. In chapter 3, evidence was presented that honeybee larvae can perceive elements in their food via chemoreceptors situated on the mouth parts. Mandibular gland secretion (Chapter 7) and sugars from the honey stomach, therefore, might regulate feeding. In the fourth instar, when the larvae receive MWJ, normal feeding seems to be restored. From this time on the development of fat cells resembles that in queen larvae after grafting. The presence of pollen in MWJ has no direct effect on caste formation. In 4th and 5th instar larvae changes in food are no longer of influence on the future caste of the adult.

A few hours after a worker larva has been placed in a queen cell – in the colony – accumulation of food in the fat body takes place. Glycogen is being stored and large vacuoles disappear. Then, increasing amounts of fat can be demonstrated. Feeding, therefore, seems to occur at a higher rate than in the corresponding worker larva on WJ. The mechanism by which this might be controlled has been described above.

In addition, there are indications that RJ contains a very labile factor that induces differentiation. The NES presumably is activated resulting in a high JH titre. At present there is no information how this activation is achieved. Three

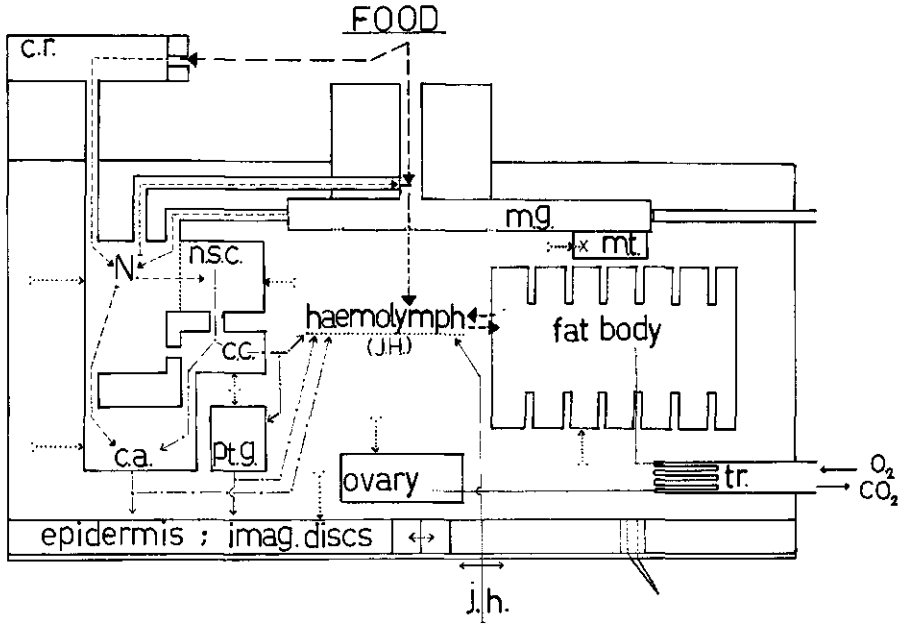


FIG. 10. Scheme of some organs (and interrelations) possibly involved in caste differentiation in the honeybee (jh: topical applied juvenile hormone). Abbreviations p. 60, 61.

possible levels of action will be discussed. 1. In the first model the endocrine system is activated by the food surrounding the larva. Impulses from chemoreceptors activate nervous centres which, in turn, control the NS cells (p.12). These cells release NS material and bring other endocrine centres into action. 2. MWJ and RJ, unlike WJ, seem to permit normal feeding. When the intestine is extended stretch receptors might send signals to the nervous system which in its turn activates the NSC. (The events that follow may be similar to those described sub 1). Here the amount of food in the intestine, indirectly controlled by information from the chemoreceptors, activates the endocrine system. 3. Substances from the food are taken up in the blood which surrounds the organs in the body cavity. It is possible that this way endocrine centres are activated.

The first model seems the most attractive. In the social insect system, pheromones are a basic feature. Although the way of perception is still a matter of discussion, chemoreception seems to be the most likely (VELTHUIS, 1971). The second model is found to function in some insects in controlling moulting (WIGGLESWORTH, 1970). From experiments in vitro (this study, chapter 7) it is less likely that this model functions in the honeybee. In larvae, reared on fresh RJ, fat body development was queenlike whereas the CA did not increase in size markedly. RJ apparently was ingested in sufficient amount but failed

to activate the endocrine system. However, the experiments should be repeated (including a check on the amount of food ingested) to ascertain these findings. At present there is no information to support the third model. LÜSCHER (1972) reared larvae of the termite, *Kalotermes flavicollis*, on diets containing synthetic JH. In his experiments, LÜSCHER observed that pre-soldiers developed as a result of this treatment. He concluded that JH might be a pheromone transmitted via trophallaxis.

Several authors studied differences in the metabolism of queen and worker larvae during the first days of development. As to the haemolymph constitution these studies include: dehydrogenase isozymes (TRIPATHI and DIXON, 1969), esterases (TRIPATHI and DIXON, 1968), proteins (LIU and DIXON, 1965) and free amino-acids (LUE and DIXON, 1967). As to the respiratory metabolism they include the work of MELAMPY and WILLIS (1939), SHUEL and DIXON (1959) and SHUEL and DIXON (1968). These studies show that the metabolic rate is at a higher level in the queen larva than in the worker larva. REMBOLD (1969a, b) found that cytochrome-C and cytochrome-oxydase were present in larger amounts in mitochondria of queen larvae than in worker larvae. However, there is no evidence that these early differences are directly connected with caste differentiation.

The number of ovarioles (WOYKE, 1971) and the size of the spermatheca of adults (VAGT, 1955; WOYKE, 1971) are reduced with increasing age of the larvae (at the moment of grafting) from egg hatching on. It is likely that the nutritional state of the larva influences these characters (VON RHEIN, 1933). Morphological caste characters appear to be determined at a later stage (Section 6.3). There is general agreement that the fifth-instar larva (> 84 h) has lost the potential to develop into a queen. When JH was applied during the fourth instar the adults that developed were queenlike in all morphological characters that were checked. This result implicates that in the natural situation the endocrine organs have to be activated some hours earlier. Indeed there are indications (Chapter 5) that dichotomy in development of the CA increases markedly during the fourth larval instar (60–84 h).

At the present moment no information is available on the way the NS system is involved in caste differentiation. Electron microscopy (chapter 5) revealed that in 54-hour-old larvae NS material is present. As there are no indications that the principles of endocrine regulation in the honeybee larva differ from those in other insects, it may be assumed that subordinated centres (CA, PtG) are governed by the NS system (see NOVÀK, 1966; KNOWLES and BERN, 1966).

The role of the PtG in caste development is not known. Observations of LUKOSCHUS (1955) suggest that ecdysone may be involved in development of epidermal caste structures during the praepupal and pupal period. Differences in activity in the PtG of queen and worker are found during the late fourth and fifth instar.

Histology and electron-microscopy revealed cyclic activity of the CA. In the hours following ecdysis the activity is low (66 h). Ultrastructural signs of resumed secretory activity are found at the start of the formation of the new

cuticle. In the period before and during ecdysis the gland is active. These findings are in good agreement with those of WILLIAMS (1961) in the silkworm, *Hyalophora cecropia*, in so far as the experiments are comparable. It is interesting that the periods of low activity closely follow the periods when little food is consumed. Data on the control of activity of the CA are scarce and contradictory. Literature on this subject will not be discussed here (see JOLY, 1968). We have shown that in the honeybee JH is involved in the development of caste characters (Chapters 5 and 6). An interesting feature is that in the honeybee larva two levels of JH exist. A high titre gives rise to queens whereas a low titre results in worker bees. However, larval moults in both castes coincide: this interesting problem needs to be investigated. Ovary development, as expressed by the number of ovarioles, appears to be very plastic and environment dependent (Section 6.4). In the ovaries of adults – obtained after topical application of JH to worker larvae – ovariole numbers were often as high as in normal queens. It seems likely that regression in the ovary of the worker is caused by a low JH titre. However, the possibility that JH-treated larvae ingested more MWJ and thus had large food stores at their disposal than normal workers cannot yet be excluded. On the other hand, cocoons were rather heavy and therefore made a considerable demand on reserves.

LUKOSCHUS (1955a, b, 1962) made a detailed histological study of the development of epidermal structures. He summarized the findings on caste characters stating that in the worker the number of nuclear divisions – including normal mitoses and endomitoses – during the praepupal and pupal period is larger than in the queen. It would be interesting to investigate the influence of JH and ecdysone on mitotic activity of epidermal cells during that period.

SUMMARY

In the first chapter, the constitution of larval food and the part this food plays in inducing caste differentiation are discussed. Postembryonic development as expressed by larval growth and moulting are vizualized in figs 1 and 2.

In Chapter 2, data on experimental animals (Table 1) and histological methods (Table 2) are presented.

In Chapter 3, contact chemoreceptors located on the labium and the maxillae are described. These sensilla appear to contain salt and sugar receptors. The possible role of the sensilla in discriminating larval food is mentioned.

In Chapter 4, fat body development in queen and worker larvae is described. Contrary to general opinion fat cells of the worker larva during the first three days appear to contain little stores. During this period worker larvae receive WJ. In the next period – when larvae are fed MWJ – accumulation of glycogen and fat occurs. Thus, there are indications that WJ restricts feeding. Large vacuoles in the fat cells of the (young) worker larva appear to have a watery content. Many of these vacuoles disappear during the 5th instar. Larvae in queen cells – in a queenless colony – normally are fed RJ. Within 18 hours after transfer of 48-hour-old worker larvae into queen cells (grafting), the fat body stores considerable amounts of glycogen. Watery vacuoles disappear and the amount of fat increases markedly: numerous spherical fat vacuoles appear in the cytoplasm. By then hardly any glycogen is present. In the fifth-instar queen larva, glycogen is being accumulated again. These observations suggest that worker larvae, unlike queen larvae, are undernourished during the first three days of postembryonic development.

In Chapter 5, the endocrine system of the larva is described. Little information is available on the part the different endocrine centres play in caste differentiation. Histology and electron-microscopy reveal cyclic activity in the CA of larvae of both castes. The CA in the queen larva seems to reach a high level of secretion within 24 to 36 hours after grafting.

In Chapter 6, the relation of JH to caste differentiation is discussed. JH titres in the haemolymph of queen larvae appear to rise within 28 hours after grafting, if compared with worker larvae of the same age. RJ seems to activate the endocrine system in some way. Topical application of JH on 3-day-old worker larvae results in the development of queenlike adults in worker cells. Thus, convincing evidence is present that JH is involved in caste differentiation.

In Chapter 7, some data are presented on the development of the fat body and the CA in larvae reared in vitro. The results suggest: 1. that mandibular gland secretion induces a worker-like type of fat body; 2. that RJ upon storage quickly loses its differentiation-promoting capacities. The CA does not increase in size markedly. Accumulation of food in the fat cells of larvae reared in vitro on fresh RJ, however, may be similar to that in colony-reared queen larvae.

In Chapter 8, present knowledge on caste differentiation in the honeybee is discussed.

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SAMENVATTING

In het eerste hoofdstuk wordt de samenstelling van larvevoedsel besproken, alsmede de rol van het voedsel bij het induceren van kastendifferentiatie. Fig. 1 en 2 geven een beeld van de groei en de vervellingen van de larve.

In hoofdstuk 2 wordt ingegaan op het materiaal (Tabel 1) dat voor de experimenten werd gebruikt en op de toegepaste histologische technieken (Tabel 2).

In hoofdstuk 3 wordt de bouw van chemoreceptoren, gelegen op het labium en de maxillen, besproken. Deze zintuigen blijken zout en suiker receptoren te bevatten. In het kort wordt ingegaan op de mogelijkheid dat ze een rol spelen bij het herkennen van het larvevoedsel.

In hoofdstuk 4 wordt de ontwikkeling van het vetweefsel in koninginnelarven en werksterlarven besproken. In tegenstelling tot wat algemeen wordt aangenomen blijken de vetcellen van werksterlarven gedurende de eerste drie dagen, dat wil zeggen in de periode dat de larven werkstergelei gevoerd krijgen, weinig reserves te bevatten. In de daaropvolgende periode, tijdens welke de larven mengvoedsel krijgen, vindt opslag van glycogeen en vet plaats. Deze waarnemingen wijzen erop dat de larven zich slechts in beperkte mate voeden met werkstergelei. De inhoud van de grote vacuolen in het vetweefsel van de (jonge) werksterlarve blijkt waterig te zijn. Veel van deze vacuolen verdwijnen gedurende het 5e larvale stadium. Larven in koninginnecellen – in een moerloos volk – worden normaal gevoerd met koninginnegelei. Binnen 18 uur na het overbrengen van 48 u. oude werksterlarven in koninginnecellen (overlarven) zijn in het vetweefsel grote hoeveelheden glycogeen aanwezig. De waterige vacuolen verdwijnen en de hoeveelheid vet neemt aanmerkelijk toe: in het cytoplasma ontstaan vele bolvormige vet-vacuolen. In dit stadium is glycogeen nauwelijks meer aanwezig. In koninginnelarven vindt gedurende het 5e larvale stadium weer opslag van glycogeen plaats. De waarnemingen wijzen erop dat werksterlarven, in tegenstelling tot koninginnelarven, gedurende de eerste drie dagen van het larvestadium in een toestand van ondervoeding verkeren.

In het vijfde hoofdstuk is het endocriene systeem van de larve beschreven. Omtrent de rol die diverse endocriene centra vervullen met betrekking tot de kastendifferentiatie is nog weinig bekend. Uit histologisch en electronen-mikroskopisch onderzoek blijkt dat het CA, in dieren van beide kasten, cyclische activiteit vertoont. Het CA van koninginnelarven lijkt, binnen 28 uur na het overlarven, een hoge graad van activiteit te bereiken.

In hoofdstuk 6 wordt ingegaan op het verband tussen de JH titer in de haemolymphe en de kastendifferentiatie. De JH titer in koninginnelarven blijkt binnen 28 uur na het overlarven omhoog te gaan, vergeleken met even oude werksterlarven. Er zijn aanwijzingen dat koninginnegelei het endocriene systeem activeert. Na het bedruppelen van 3 dagen oude werksterlarven met JH blijken koningin-achtige imagines te ontstaan in gewone werkstercellen. Dit houdt in dat JH betrokken is bij het ontstaan van het kastendimorphisme.

In hoofdstuk 7 zijn enige gegevens vermeld met betrekking tot de ontwikkeling van het vetweefsel en de CA van dieren, die in vitro waren opgekweekt. De proeven doen vermoeden: 1. dat het secreet uit de mandibulaire klieren de ontwikkeling van een werksterachtig vetweefsel induceert; 2. dat bewaren van koninginnegelei een snelle afname van de differentiatie-inducerende eigenschappen tot gevolg heeft. Het CA blijkt weinig in grootte toe te nemen. Hiertegenover staat dat de opslag van reservevoedsel in dieren welke in vitro op koninginnegelei zijn opgekweekt, sterk gelijkt op die in koninginnelarven welke in het volk worden opgekweekt.

In hoofdstuk 8 wordt de huidige stand van zaken met betrekking tot de kennis van de kastendifferentiatie bij de honingbij besproken.

LIST OF ABBREVIATIONS (singular or plural)

a	axon(s)
AO, ao	aorta
c	cuticle
CA, ca	corpus allatum
CC, cc	corpus cardiacum
CG, cg	cerebral ganglion
Cr	chemoreceptor
CW, cw	cell wall
d	dendrite(s)
e	enveloping cell
E	epidermis
f	fat vacuole
F	fat cell
FG	frontal ganglion
g	glycogen
gl	glycocalyx
go	golgi complex
gv	golgi vacuole
H	heart
HG	hypocerebral ganglion
imag. disc	imaginal disc
is	intercellular space
JH, jh	juvenile hormone
L, l	lysosome-like body
ln	lateral branch of NCC
LV	large (fat) vacuole
m	mitochondrion
MG, mg	midgut
mi	mitosis
mt	malpighian tubule
mu	muscle tissue
MWJ	modified worker jelly
n	nucleus
N	nervous system
NCA, nca	nervus corporis allati
NCC, ncc	nervus corporis cardiaci
NES, nes	neuroendocrine system
np	nuclear pore
nr	nervus recurrens
NS	neurosecretory (granule, substance)
NSC, nsc	neurosecretory cells

oe	oenocyte
OES, oes	oesophagus
ov	ovary
p	protein granule
PAS	periodic-acid Shiff
PtG, ptg	prothoracic gland
r	ribosome(s)
RJ	royal jelly
s	scolopale
sc	sensory cell
SD, sd	silk duct
SOG, sog	suboesophageal ganglion
sv	small vacuole
T	tentorium
TG	thoracic ganglion
TR, tr	trachae (tracheole)
W	watery vacuole
WJ	worker jelly

N.B. grafting: transferring a worker larva into a queen cell.

REFERENCES

AGGARWAL, S. K. and KING, R.C. (1969). A comparative study of the ring glands from wild type and 1(2)-gl mutant *Drosophila melanogaster*. J. Morph. **129**: 171–200.

ALPATOV, W. W. (1928). The influence of the conditions of development on the organism of the worker bee and the queen. Am. Bee J. **68**: 115–117.

BECKER, F. (1925). Die Ausbildung des Geschlechtes bei der Honigbiene. Erlanger Jb. Bienenk. **3**: 163–223.

BER-LIN CHAI and SHUEL, R. W. (1970). Effects of supernumerary corpora allata and farnesol compounds on ovary development in the worker honeybee. J. apicult. Res. **9**: 19–27

BERTHOLF, L. M. (1925). The moults of the honeybee. J. econ. Ent. **18**: 380–384.

BIEDERMANN, M. (1964). Neurosekretion bei Arbeiterinnen und Königinnen von *Apis mellifica* L., unter natürlichen und experimentellen Bedingungen. Z. wiss. Zool. **170**: 256–308.

BISHOP, G. H. (1922). Cell metabolism in the insect fat body. J. Morph. **36**: 567–601.

BISHOP, G. H. (1958). Nuclear and cytoplasmic changes in fat body cells of the queen bee during metamorphosis. J. exp. Zool. **137**: 501–525.

BISHOP, G. H. (1961). Growth rates of honey bee larva. J. exp. Zool. **146**: 11–20.

BOEHM, B. (1965). Beziehungen zwischen Fettkörper, Oenocyten und Wachsdrüsen Entwicklung bei *Apis mellifica* L. Z. Zellforsch. mikrosk. Anat. **65**: 74–115.

O'BRIEN, D. M. and SHUEL, R. W. (1972). Influence of diet upon the development of prothoracic glands and oenocytes in female honeybee larvae. J. apicult. Res. **11**: 13–21.

BUTENANDT, A., REMBOLD, H. (1957). Über den Weiselzellenfuttersaft der Honigbiene I. Isolierung, Konstitutionsermittelung u. Vorkommen der 10-hydroxy-Δ^2-decensäure. Hoppe-Seyler's Z. physiol. Chem. **308**: 284–289.

BUTENANDT, A., REMBOLD, H. (1958). Über den Weiselzellenfuttersaft der Honigbiene. II. Isolierung von 2-amino-2-hydroxy-6-(-1,2-dihydroxy-propyl-)-pteridin. Hoppe-Seyler's Z. physiol. Chem. **311**: 79–83.

BUTTERWORTH, F. M., BODENSTEIN, D. and KING, R. C. (1965). Adipose tissue of *Drosophila melanogaster*. An experimental study of larval fat body. J. exp. Zool. **158**: 141–154.

CANETTI, S. J., SHUEL, R. W. and DIXON, S. E. (1964). Studies in the mode of action of roya jelly in honeybee development. IV Development within the brain and retrocerebral complex of female honeybee larvae. Can. J. Zool. **42**: 229–233.

CARLETON, H. M. (1957). Histological technique. Oxford Univ. Press. London.

CHAUVIN, R. (1968). Traité de biologie de l'Abeille. Masson et Cie. Paris.

DADDI (1896): in PEARSE (1960), blz. 303.

DETHIER, V. G. (1963). The Physiology of Insect Senses. Methuen and co. Ltd, London. Chapter V.

DIXON, S. E. and MOSER, E. (1972). Duality in function in corpora allata of honeybee larvae. Can. J. Zool. **50**: 593–595.

DIXON, S. E. and SHUEL, R. W. (1963). Studies in the mode of action of royal jelly in honeybee development. III The effect of experimental variation in diet on growth and metabolism of honeybee larvae. Can. J. Zool. **41**: 733–739.

DÖHMER, I. (1958). Das Neurosekretorische System der Honigbiene, insbesondere der Arbeiterinnen verschiedenen Alters. Diss. Univ. Berlin: 1–39.

ECKERT, J. E. (1934). Studies in the number of ovarioles in queen honeybees in relation to body size. J. econ. Ent. **27**: 629–635.

EWEN, A. B. (1962): An improved aldehyde fuchsin staining technique for neurosecretory products in insects. Trans. Amer. microsc. Soc. **81**: 94–96.

FAHIMI, N. D. and DROCHMANS, P. (1965). Essais de standardisation de la fixation au glutaraldehyde. II. Influence des concentrations en aldehyde et de l'osmolarité. J. Microsc. **4**: 737–748.

FAIN-MAUREL, M. A. and CASSIER, P. (1969). Etude infrastructurale des corpora allata de

Locusta migratoria migratorioides, phase solitaire, au cours de la maturation sexuelle et des cycles ovariens. C. r. Acad. Sci., Paris. t. **268**: 2721–2723.
FAWCETT, D. W., LONG, J. A. and JONES, A. L. (1969). The ultrastructure of endocrine glands. Recent Progr. Horm. Res. **25**: 315–380. Chapter 3.
FORMIGONI, A. (1956). Neurosécrétion et organes endocrines chez *Apis mellifica* L. Annls. Sci. Nat., Zool. **11**: 283–291.
GABE, M. (1953): Sur quelques applications de la coloration par la fuchsine-paraldéhyde. Bull. Microsc. appl. **3**: 153–162.
GONTARSKI, H. (1941). Ueber Zwischenformen von Königin und Arbeiterin im Staat der Honigbiene (*Apis mellifica* L.). Z. wiss. Zool. **154**: 345–356.
HANAN, B. B. (1955). Studies of the retrocerebral complex in the honey bee. Part 1: Anatomy and histology. Ann. ent. Soc. Am. **48**: 315–319.
HAYDAK, M. H. (1943). Larval food and development of castes in the honeybee. J. econ. Ent. **36**: 778–792.
HAYDAK, M. H. (1958). Do the nurse honey bees recognize the sex of the larvae? Science **127**: 1113.
HAYDAK, M. H. (1968): in CHAUVIN (1968), pag. 302–333.
HEIDENHAIN, M. (1892): in CARLETON (1957), pag. 109.
L'HÉLIAS, C. (1950). Étude des glandes endocrines post-cérébrales de la larve d'*Apis mellifica* L. Bull. Soc. Zool. Fr. **75**: 70–74.
L'HÉLIAS, C. (1952). Étude de la glande prothoracique chez la larve d'*Apis mellifica* L. Bull. Soc. Zool. Fr. **77**: 191–195.
HIGGINS, J. A., BARRNETT, R. J. (1972). Studies on the biogenesis of smooth endoplasmic reticulum membranes in livers of phenobarbital-treated rats. J. cell. Biol. **55**: 282–298.
HOFFMANN, I. (1968): in CHAUVIN (1968), pag. 68–100.
HOUSE, H. L. (1969). Effects of different proportions of nutrients on insects. Ent. exp. et appl. **12**: 651–669.
JAY, S..C. (1963). The development of honeybees in their cells. J. apicult. Res. **2**: 117–134.
JOLY, P. (1967). Comparaison du volume et de l'activité physiologique des Corpora allata de *Locusta migratoria* L. Annls. Soc. ent. Fr. (n.s.) **3**: 601–608.
JOLY, P. (1968). Endrocrinologie des Insectes. Masson et Cie, Ed. Paris.
JOLY, L., PORTE, A., GIRARDIE, A. (1967). Caractères ultrastructuraux des corpora allata actifs et inactifs chez *Locusta migratoria*. C. r. Acad. Sci., Paris t. **265**: 1633–1635.
JOLY, L., JOLY, P., PORTE, A. et GIRARDIE, A. (1968). Étude physiologique et ultrastructurale des corpora allata de *Locusta migratoria* L. en phase grégaire. Archs. Zool. exp. gén. **109**: 703–728.
JORDAN, R. (1960). Die Zucht der Königin, ausgehend vom Ei. Bienenvater **81**: 3–7.
JUNG-HOFFMANN, I. (1966). Die Determination von Königin und Arbeiterin der Honigbiene. Z. Bienenforsch. **8**: 296–322.
KLEIN, J. (1904). Futterbrei und weibliche Bienenlarve. Die Bienenpflege **5**: 80–84.
KNOWLES, F. and BERN, H. A. (1966). Function of neurosecretion in endocrine regulation. Nature **210**: 271–272.
KOMAROV, P. M. (1934). Influence of the age of the larvae and of the number of generations upon the development of the queen's sex organs. The Bee World **15**: 81–83.
KOMAROV, P. M. (1935). Uebergangsformen bei weiblichen Honigbienen. Zum Polymorphismus sozialer Insekten I. Arch. Bienenk. **16**: 152–166.
KORT, C. A. D. DE (1970): in DE WILDE et al. (1971).
LABOUR, G. (1970). Étude histologique et histochimique du corps adipeuse au cours du développement post-embryonaire du doryphore. Archs. Anat. microsc. Morph. exp. **59**: 235–251.
LAERE, O. VAN (1970). Bijdrage tot de studie van de endocriene organen bij de honingbij (*Apis mellifera* L.). Thesis Univ. Gent.
LINDAUER, M. (1952). Ein Beitrag zur Frage der Arbeitsteilung im Bienenstaat. Z. vergl. Physiol. **34**: 299–345.
LINEBURG, B. (1924): The feeding of honeybee larvae. Bull. U.S. Dep. Agric. no. 1222: 25–37.

LIU, T. P. and DIXON, S. E. (1965). Studies on the mode of action of royal jelly in honeybee development. VI Haemolymph protein changes during caste development. Can. J. Zool. **43**: 873–879.

LUE, P. F. and DIXON, S. E. (1967). Studies in the mode of action of royal jelly in honeybee development. VII The free amino acids in the haemolymph of developing larvae. Can. J. Zool. **45**: 205–214.

LUKOSCHUS, F. (1952). Ueber die Prothoraxdrüse der Honigbiene (*Apis mellifica* L.) Naturwissenschaften **39**: 116.

LUKOSCHUS, F. (1955a). Die Bedeutung des innersekretorischen Systems für die Ausbildung epidermaler Kastenmerkmale bei der Honigbiene (*Apis mellifica* L.) Insectes soc. **2**: 221–236.

LUKOSCHUS, F. (1955b). Untersuchungen zur Metamorphose der Honigbiene (*Apis mellifica* L.) Insectes soc. **2**: 147–162.

LUKOSCHUS, F. (1956a). Untersuchungen zur Entwicklung der Kastenmerkmale bei der Honigbiene (*Apis mellifica* L.). Z. Morph. Ökol. Tiere **45**: 157–197.

LUKOSCHUS, F. (1956b). Zur Kastendetermination bei der Honigbiene. Z. Bienenforsch. **3**: 190–199.

LUKOSCHUS, F. (1956c). Die Kastenentwicklung bei der Honigbiene, Avitaminose oder endokrine Anaplasie? Verh. dt. Zool. Ges. Hamburg: 278–283.

LUKOSCHUS, F. (1962). Ausbildungsformen von Kutikula und kutikularen Kleinorganen bei der Honigbiene (*Apis mellifica* L.). Z. Morph. Ökol. Tiere **51**: 547–574.

LÜSCHER, M. (1972). Environmental control of juvenile hormone (JH) secretion and caste differentiation in termites. Gen. and Comp. Endocrinol. suppl. **3**: 509–514.

MALLORY, F. B. (1900): in CARLETON (1957), pag. 107.

MELAMPY, R. M. and WILLIS, E. R. (1939). Respiratory metabolism during larval and pupal development of the female honeybee (*Apis mellifica* L.). Physiol. Zool. **12**: 302–311.

MELAMPY, R. M. and OLSAN, R. D. (1940). Total carbohydrate and glycogen content of the developing honeybee. Proc. Soc. exp. Biol. Med. **45**: 754–758.

MELAMPY, R. M., WILLIS, E. R. and MCGREGOR, S. E. (1940). Biochemical aspects of the differentiation of the female honeybee (*Apis mellifica* L.). Physiol. Zool. **13**: 283–293.

MICHENER, C. D. (1961). Social polymorphism in Hymenoptera. In Insect Polymorphism: 43–56. Kennedy, J. S. Roy. Entomol. Soc. London.

MILUM, V. G. (1930). Variations in time of development of the honey bee. J. econ. Ent. **23**: 441–447.

MITSUI, T., SAGAWA, T., SANO, H. (1964). Studies on rearing honey bee larvae in the laboratory. I The effect of royal jelly taken from different ages of queen cells on queen differentiation. J. econ. Ent. **57**: 518–521.

NAULLEAU, G. (1960). Les transpositions de larves de différentes castes d'*Apis mellifica* dans les cellules d'autres castes. Étude des types intermédiaires entre reine et ouvrières. Annls. Abeille **3**: 65–83.

NELSON, J. A. (1915). The embryology of the honey bee. Princeton, New York: 161–164.

NELSON, J. A. (1924). Morphology of the honeybee larva. J. Agric. Res. **28**: 1167–1213.

NOVÀK, V. J. A. (1966). Insect Hormones. Methuen and Co Ltd. London. 478 pp.

ODHIAMBO, T. R. (1966a). The fine structure of the corpus allatum of the sexually mature male of the desert locust. J. Insect Physiol. **12**: 819–828.

ODHIAMBO, T. R. (1966b). Ultrastructure of the development of the corpus allatum in the adult male of the desert locust. J. Insect Physiol. **12**: 995–1002.

PANOV, A. A. and BASSURMANOVA, O. K. (1970). Fine structure of the gland cells in inactive and active corpus allatum of the bug, *Eurygaster integriceps*. J. Insect Physiol. **16**: 1265–1281.

PEARSE, A. G. E. (1960). Histochemistry. J. and A. Churchill Ltd. London.

PEREZ, J. (1889): In CHAUVIN (1968) part 1, pag. 47.

PETIT, I. (1963). Étude 'in vitro' de la croissance des larves d'abeilles (*Apis mellifica* L.). Annls Abeille **6**: 35–52.

PFLUGFELDER, O. (1948). Volumetrische Untersuchungen an den Corpora Allata der Honig-

biene, *Apis mellifica* L. Biol. Zbl. **67**: 223–241.
PLANTA, A. VON (1888): in CHAUVIN (1968) part 1, chapter 5 (III).
REMBOLD, H. (1969). Biochemie der Kastenbildung bei der Honigbiene. 22. Int. Bienenz. Kongr. München pp. 552–554.
REVEL, J. P. (1963). Electron microscopy of glycogen. Histochemical Soc. Symp. Washington: 104–114.
REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. cell Biol. **17**: 208–212.
RHEIN, W. VON (1933). Ueber die Entstehung des weiblichen Dimorphismus im Bienenstaate. Roux' Arch. Entw. Mech. **129**: 601–665.
RHEIN, W. VON (1956). Ueber die Ernährung der Arbeitermade von *Apis mellifica* L. insbesondere in der Altersperiode. Insectes soc. **3**: 203–212.
RIBBANDS, C. R. (1964). The behaviour and social life of honeybees. Dover Publ., inc., New York.
RICHARDS, O. W. (1961). An introduction to the study of polymorphism in insects. R. ent. London. Symp. insect Polymorphism. paper 1.
RITCEY, G. M. and DIXON, S. E. (1969). Post-embryonic development of the endocrine system in the female honeybee castes, *Apis mellifera* L. Proc. ent. Soc. Ont. **100**: 124–138.
RONZONI, E. and BISHOP, G. H. (1929). Carbohydrate metabolism in the honeybee larva. 4th Int. Congr. Ent. **2**: 361–365.
SCHALLER, F. (1950). Étude morphologique du complexe endocrine rétrocérébral de la larve d'abeille (*Apis mellifica* L.). C. r. Soc. Biol. Paris, **144**:1097–1100.
SCHARRER, B. (1964). Histophysiological studies on the corpus allatum of *Leucophaea maderae*. IV Ultrastructure during normal activity cycle. Z. Zellforsch. mikrosk. Anat. **62**: 125–148.
SCHARRER, B. (1971). Histophysiological studies on the corpus allatum of *Leucophaea maderae*. V Ultrastructure of sites of origin and release of a distinctive cellular product. Z. Zellforsch. mikrosk. Anat. **120**: 1–16.
SCHNELLE, H. (1923). Ueber den feineren Bau des Fettkörpers der Honigbiene. Zool. Anzeiger **57**: 172–179.
SCHNELLE, H. (1924). Der feinere Bau des Fettkörpers der Honigbiene (*Apis mellifica* L.). Arch. Bienenk. **6**: 83–117.
SHAAYA, E. and BODENSTEIN, D. (1969). The function of the accessory glands in *Periplaneta americana* L. The role of the juvenile hormone in the synthesis of protein and protocatechuic acid glucoside. J. exp. Zool. **170**: 281–292.
SHUEL, R. W. and DIXON, S. E. (1959). Studies in the mode of action of royal jelly in honeybee development. II Respiration of newly emerged larvae on various substrates. Can. J. Zool. **37**: 803–813.
SHUEL, R. W. and DIXON, S. E. (1968). The importance of sugar for the pupation of the worker honeybee. J. Apicult. Res. **7**: 109–112.
SHUEL, R. W. and DIXON, S. E. (1968). Respiration in developing honeybee larvae. J. Apicult. Res. **7**: 11–19.
SMITH, M. V. (1959). Queen differentiation and the biological testing of royal jelly. Thesis, Cornell Univ., Agr. Exp. Sta. Ithaca, New York, 1957.
SNODGRASS, R. E. (1956). Anatomy of the honey bee. Comstock Publ. Ass. Ithaca, New York.
SPURR, A. R. (1969). A low-viscosity Epoxy Resin Embedding Medium for electron microscopy. J. Ultrastruct. Res. **26**: 31–34.
STABE, H. A. (1930). The rate of growth of worker, drone and queen larvae of the honeybee, *Apis mellifera* L. J. econ. Ent. **23**: 447–453.
STADHOUDERS, A. M. (1965). Particulate glycogen. A correlated electron-microscopical and biochemical study. Thesis Univ. Nijmegen.
STRAUS, J. (1911). Die chemische Zusammensetzung der Arbeitsbienen und Drohnen während ihrer verschiedenen Entwicklungsstadien. Z. Biol. **56**: 347–397.
THOMSEN, E. and THOMSEN, M. (1970). Fine structure of the corpus allatum of female blowfly *Calliphora erythrocephala*. Z. Zellforsch. mikrosk. Anat. **110**: 40–60.
TOMBES, A. S. and SMITH, D.S. (1970). Ultrastructural studies on the corpora cardiaca-alata

complex of the adult alfalfa weevil, *Hypera postica*. J. Morph. **132**: 137–148.
TONER, P. G. and CARR, K. E. (1971). Cell structure. Churchill Livingstone, Edinburgh.
TOWNSEND, G. F. and SHUEL, R. W. (1962). Some recent advances in apicultural research. A. rev. Ent. **7**: 481–499.
TRIPATHI, R. K. and DIXON, S. E. (1968). Haemolymph esterases in the female larval honeybee, *Apis mellifera* L., during caste development. Can. J. Zool. **46**: 1013–1017.
TRIPATHI, R. K. and DIXON, S. E. (1969). Changes in some haemolymph dehydrogenase isozymes of the female honeybee, *Apis mellifera* L., during caste development. Can. J. Zool. **47**: 763–770.
VAGT, W. (1955). Morphologische Untersuchungen an Nachschaffungskönigинnen von *Apis mellifica*, die aus verschiedenen alten Larven gezüchtet wurden. Z. Bienenforsch. **3**: 73–80.
VELTHUIS, H. H. W. (1971). The mechanism of action of queen substances in the honeybee. Thesis Univ. Utrecht.
WAKU, Y. and GILBERT, L. I. (1964). The corpora allata of the silkmoth, *Hyalophora cecropia:* An ultrastructural study. J. Morph. **115**: 69–96.
WANG, D. I. (1965). Growth rates of young queen and worker honeybee larvae. J. apicult. Res. **4**: 3–5.
WANG, D. I. and SHUEL, R. W. (1965). Studies in the mode of action of royal jelly in honeybee development V. The influence of diet on ovary development. J. apicult. Res. **4**: 149–160.
WEAVER, N. (1955). Rearing of honeybee larvae on royal jelly in the laboratory. Bee World **36**: 157–159.
WEAVER, N. (1957a). Effects of larval age on dimorphic differentiation of the female honey bee. Annls ent. Soc. Am. **50**: 283–294.
WEAVER, N. (1957b). Experiments on dimorphism in the female honey bee. J. econ. Ent. **50**: 759–761.
WEAVER, N. (1958). Rearing honeybee larvae in the laboratory. Proc. X int. Congr. Ent. **4**: 1031–1036.
WEISS, K. (1971). Ueber Ausbildung und Leistung von Königinnen aus Eiern und jungen Arbeitermaden. Apidologie **2**: 3–47.
WHITMORE, D., WHITMORE, E. and GILBERT, L. I. (1972). Juvenile hormone induction of esterases: a mechanism for the regulation of juvenile hormone titer. Proc. natn. Acad. Sci. U.S.A. **69**: 1592–1595.
WIGGLESWORTH, V. B. (1942). The storage of protein, fat, glycogen and uric acid in the fat body and other tissues of mosquito larvae. J. exp. Biol. **19**:56–77.
WIGGLESWORTH, V. B. (1947). The epicuticle of an insect, *Rhodnius prolixus*. Proc. R. Soc. B. **134**: 163–181.
WIGGLESWORTH, V. B. (1967). Cytological changes in the fat body of *Rhodnius* during starvation, feeding and oxygen want. J. cell Sci. **2**: 243–256.
WIGGLESWORTH, V. B. (1970). Insect hormones. Oliver and Boyd. Edinburgh.
WILDE, J. DE (1971). Hormones and insect diapause. Mem. Soc. Endocr. **18**:487–514.
WILDE, J. DE, STAAL, G. B., KORT, C. A. D. DE, LOOF, A. DE and BAARD, G. (1968). Juvenile hormone titer in the haemolymph as a function of photoperiodic treatment in the adult colorado beetle (*Leptinotarsa decemlineata* SAY). Proc. K. ned. Akad. Wet. **71**: 321–326.
WILDE, J. DE, KORT, C. A. D. DE and LOOF, A. DE (1971). The significance of juvenile hormone titers. Mitt. schweiz. ent. Ges. **44**: 79–86.
WILLIAMS, C. M. (1961). The juvenile hormone. II Its role in the endocrine control of moulting, pupation, and adult development in the Cecropia silkworm. Biol. bull. **121**: 572–585.
WIRTZ, P. and BEETSMA, J. (1972). Induction of caste differentiation in the honeybee (*Apis mellifera*) by juvenile hormone. Ent. exp. and appl. **15**: 517–520.
WOYKE, J. (1967). Diploid drone substance – cannibalism substance. Apimondia. 21st Int. Apic. Congr. Maryland. p. 471.
WOYKE, J. (1971). Correlations between the age at which honeybee brood was grafted, characteristics of the resultant queens, and results of insemination. J. apicult. Res. **10**: 45–55.
ZANDER, E., LÖSCHEL, F., MEIER, K. (1916). Die Ausbildung des Geschlechtes bei der Honigbiene (*Apis mellifica*, L.). Z. angew. Ent. **3**:1–74.

FIG. 12. Labial sensilla-styloconica (contact chemoreceptors) of fourth-instar larvae.
a. Innervation of the sensillum (a) from the SOG. Note conical papilla on top of the sensillum (arrow).
b. Arrangement of enveloping cells (e) and sensory cells (sc) of the coupled sensilla. Larva halfway through the moulting stage (double cuticle).
c. Bipolar neuron (sc) and enveloping cell (e). Larva not far from ecdysis (double cuticle, c).

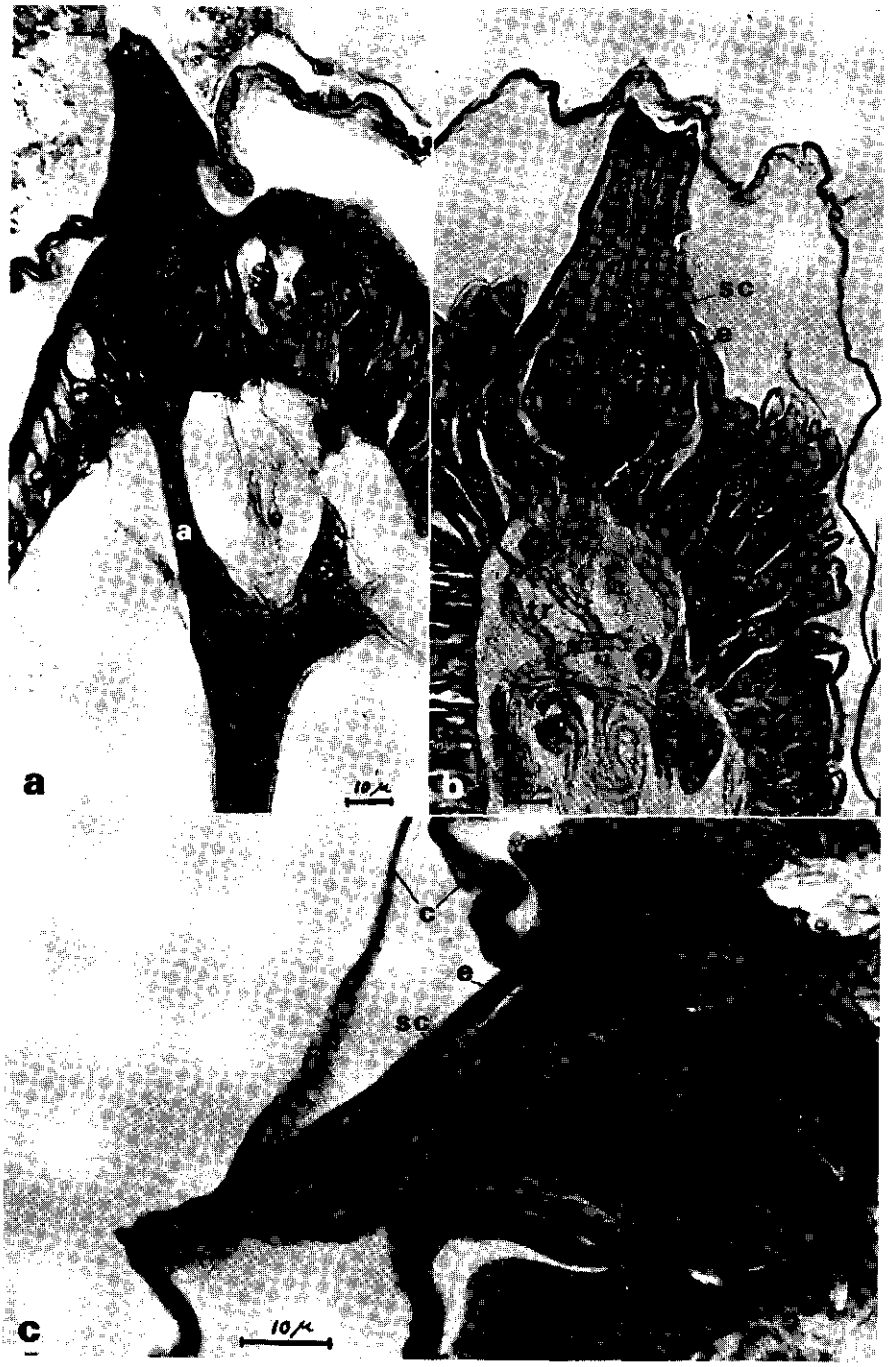

FIG. 12

FIG. 13. Electron-micrographs of the top of the sensilla (early fourth-instar larvae).
a. Conical papilla with terminal aperture (arrow). 37500 ×
b Cross section of the tip of the papilla. One dendrite in the receptor lymph cavity. 50000 ×
c Cross section of the tip of a papilla, more inward than 2b. Note four dendrites (arrow). 54000 ×
d. Cross section through the sensillum, just below the papilla. Five dendrites within the scolopale (s) running freely in the receptor lymph cavity. Note microtubules (arrow). 55000 ×

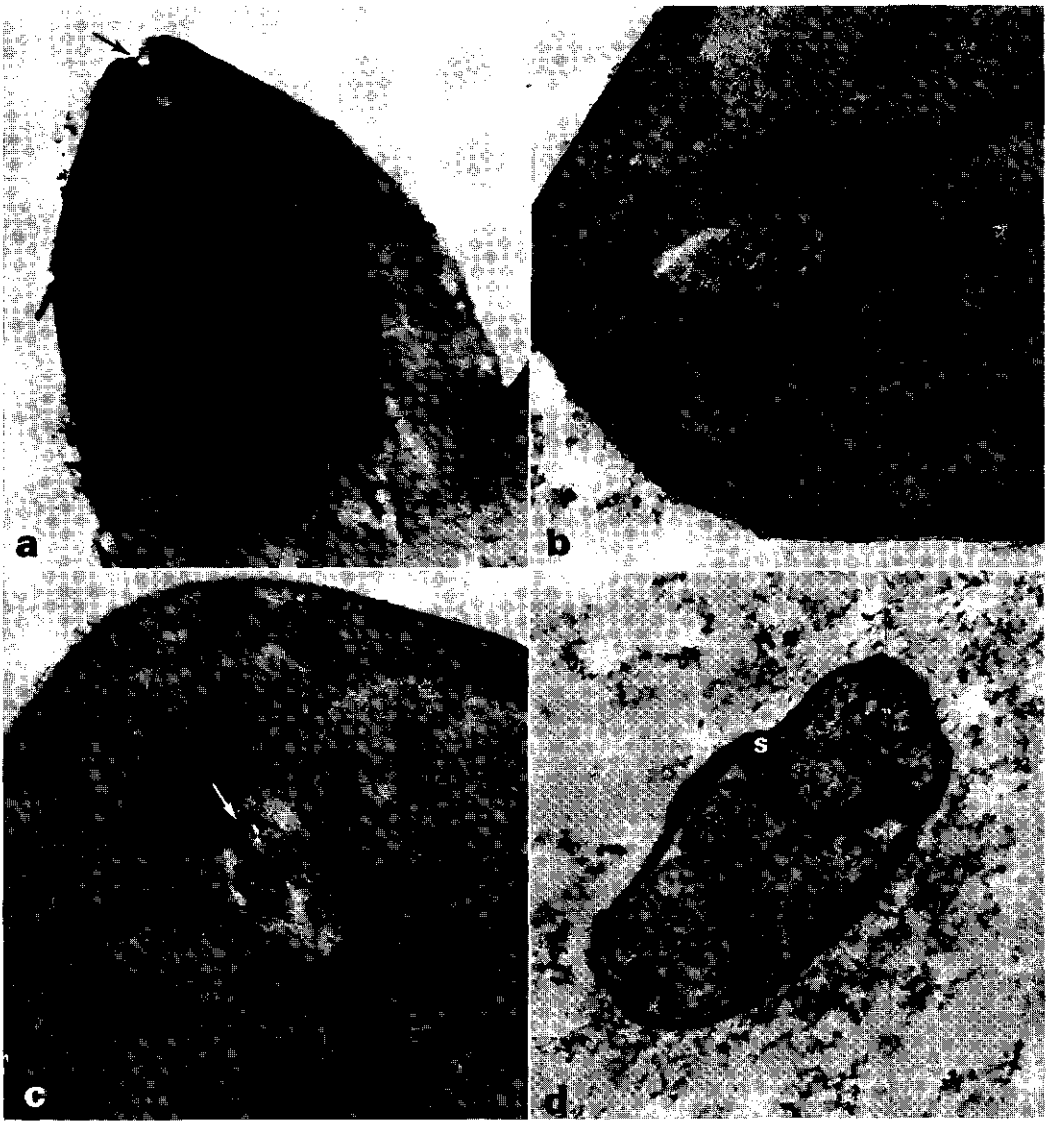

FIG. 13

FIG. 14. Cross sections of sensillum.

a. Enveloping cells (e) located in the epidermis and surrounding the dendrites (arrows). 4000 ×

b. Oblique section through the nuclear region of the enveloping cells. Note dendrites (d) in one of the enveloping cells. 10000 ×

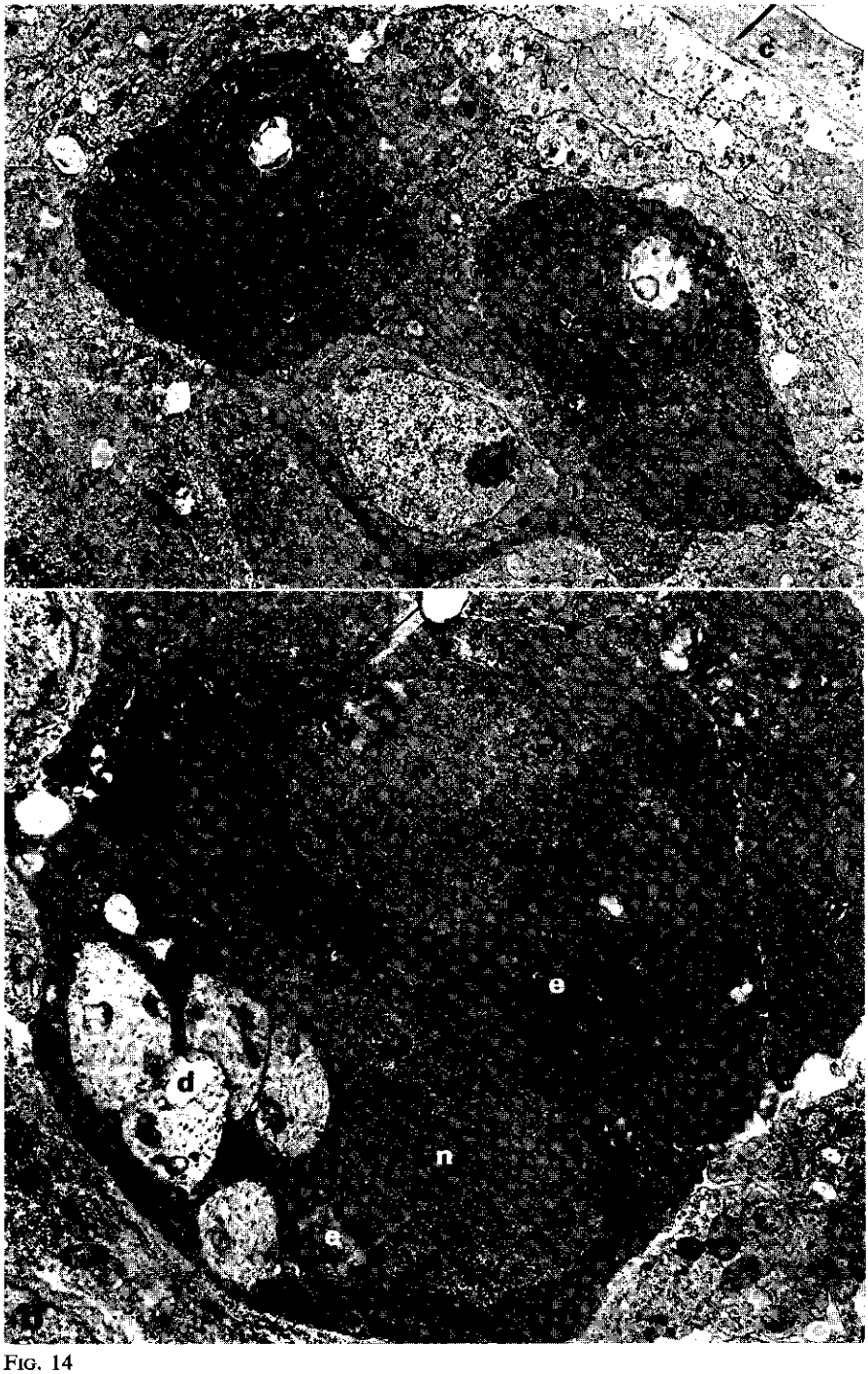

FIG. 14

FIG. 15. Development of fat cells.

a. 20-h-old worker larva. Dividing fat cells (arrow). Bouin fixation; Mallory

b. 20-h-old worker larva. Young fat cells. Formation of watery vacuoles in some cells (arrow). Bouin; Mallory

c. 58-h-old worker larva. Fat cells containing watery vacuoles and only little food stores. Bouin; Mallory

d. 70-h-old worker larva. 'Signet-ring' type cells with large watery vacuoles (w) and some glycogen (g) containing cells. Bouin; Mallory

e. 106-h-old worker larva Carnoy (70%) fixation. Mallory. Glycogen lost during histological processing.

f. 106-h-old worker larva. Carnoy (100%). PAS. Note large amounts of glycogen, also in watery vacuoles (arrow).

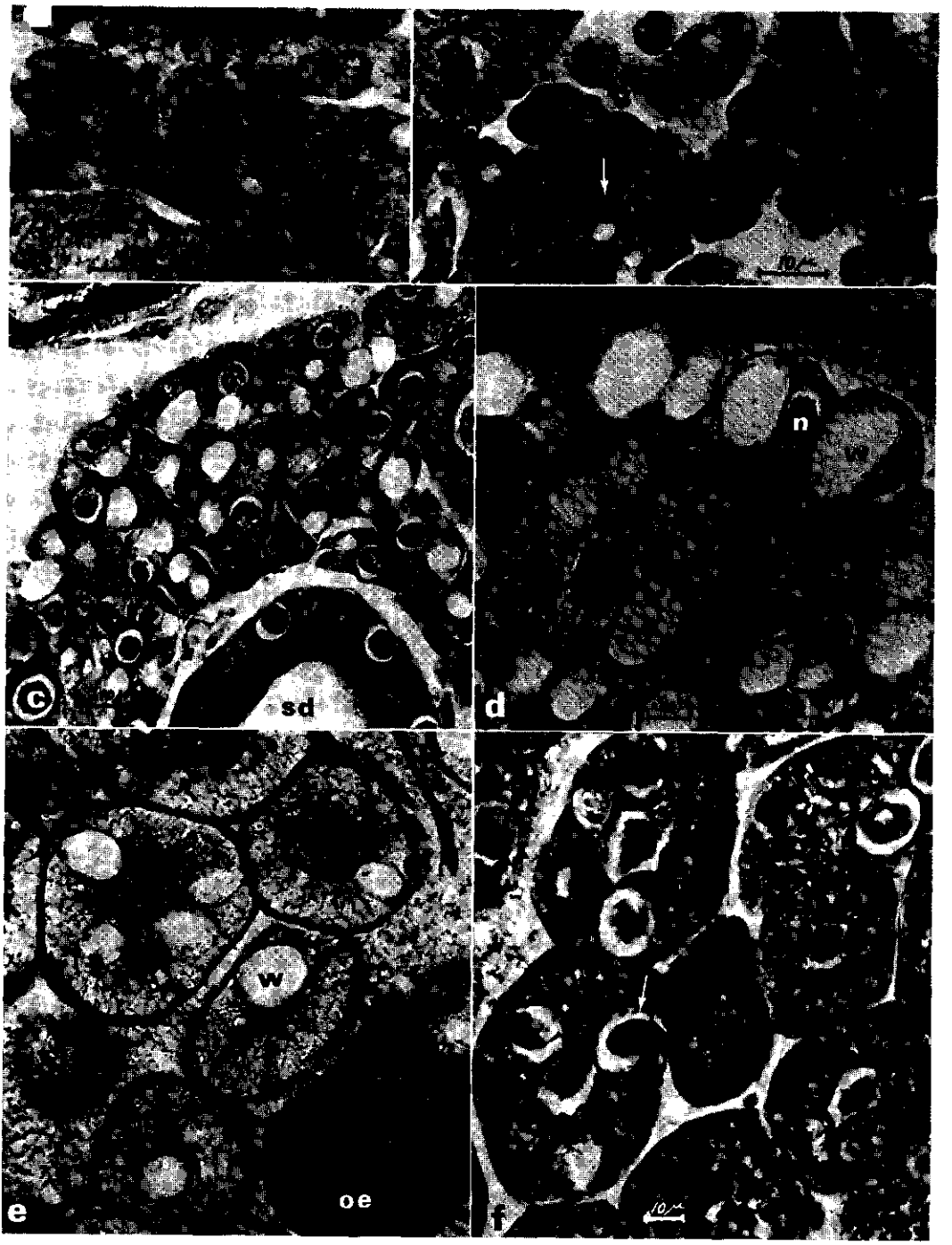

FIG. 15

FIG. 16. Development of fat cells: glycogen. (b-e, h-j: same magnification as a; g: same magnification as f).

a. 60-h-old queen larva (12 h in queen cell) Bouin; Mallory. Glycogen lost during histological processing.
b. 66-h-old queen larva (18 h in queen cell). Carnoy (100%); PAS. Note large amounts of glycogen.
c. d. 72-h-old queen larvae (24 h in queen cell). Mallory. c: Carnoy; d: Bouin. Numerous fat vacuoles, little glycogen.
e. 96-h-old queen larva (48 h in queen cell). Carnoy (100%); PAS. Much glycogen, large fat vacuoles (f).
f. 50-h-old worker larva. Carnoy (100%); PAS. Little glycogen in fat cells. Note glycogen in cells of the silk duct (arrow).
g. 66-h-old worker larva. Carnoy (100%); PAS. Glycogen as small granules in the cells. No glycogen in large vacuoles.
h. 84-h-old worker larva. Bouin; Mallory.
i. 84-h-old worker larva. Carnoy (100%); PAS. Glycogen as small granules in the cells. Some glycogen in large vacuoles.
j. 96-h-old larva fed for 48 hours with condensed milk. Carnoy; Mallory. Numerous fat vacuoles.

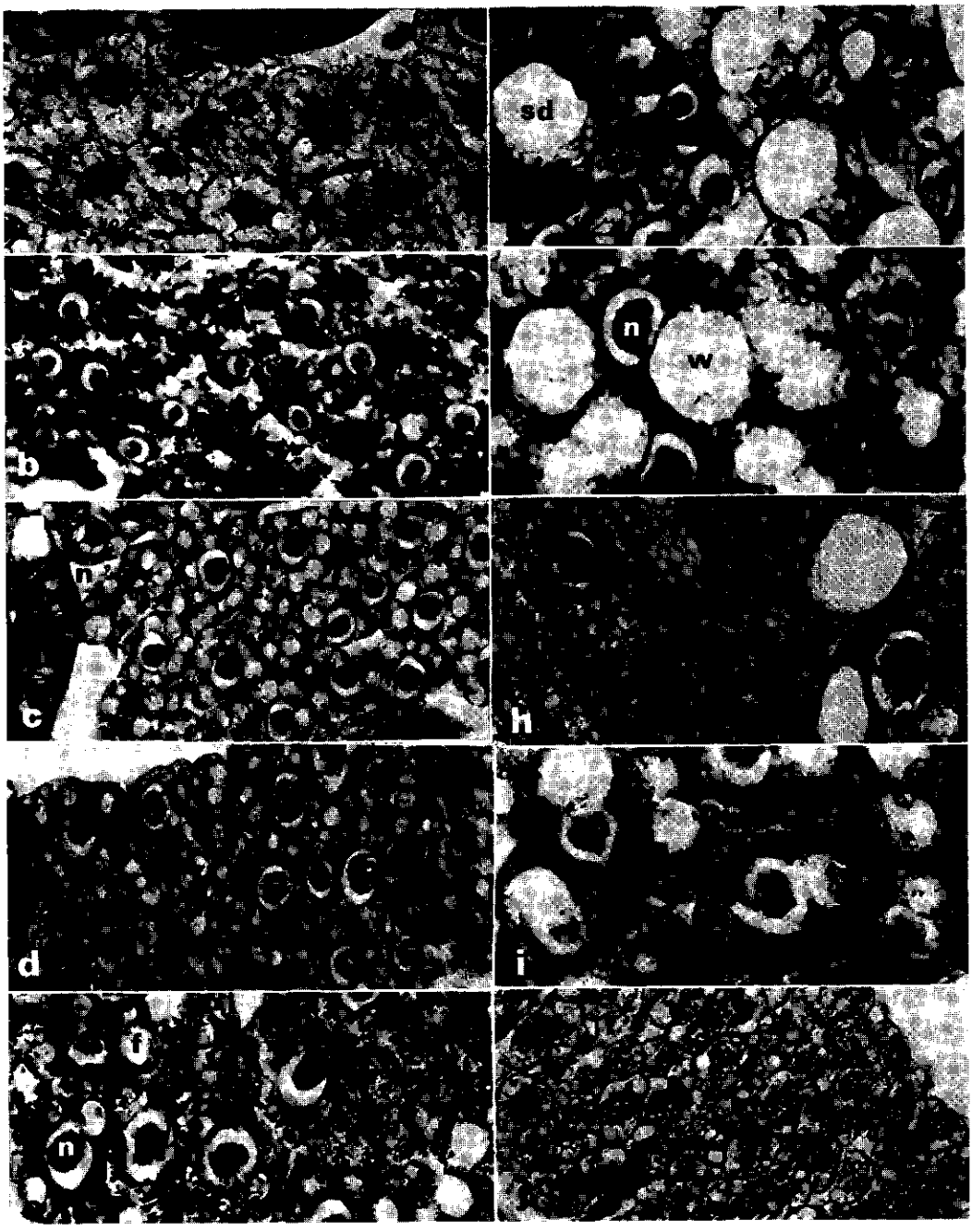

FIG. 16

FIG. 17. Development of fat cells: fat vacuoles (b–h: same magn. as a) Formol fixation; Sudan III.

a. 24-h-old worker larva. Only a few small fat vacuoles (arrow).
b. 66-h-old queen larva (18 h in queen cell). Many fat vacuoles.
c. 82-h-old queen larva (12 h in queen cell).
d. 94-h-old queen larva (24 h in queen cell).
e. 72-h-old worker larva. Small fat vacuoles (arrow), large watery vacuoles (W).
f. 78-h-old worker larva.
g. 82-h-old worker larva. Large fat cell containing fat vacuoles (f) and a large watery vacuole (W).
h. 107-h-old worker larva. Large fat cell containing numerous fat vacuoles.

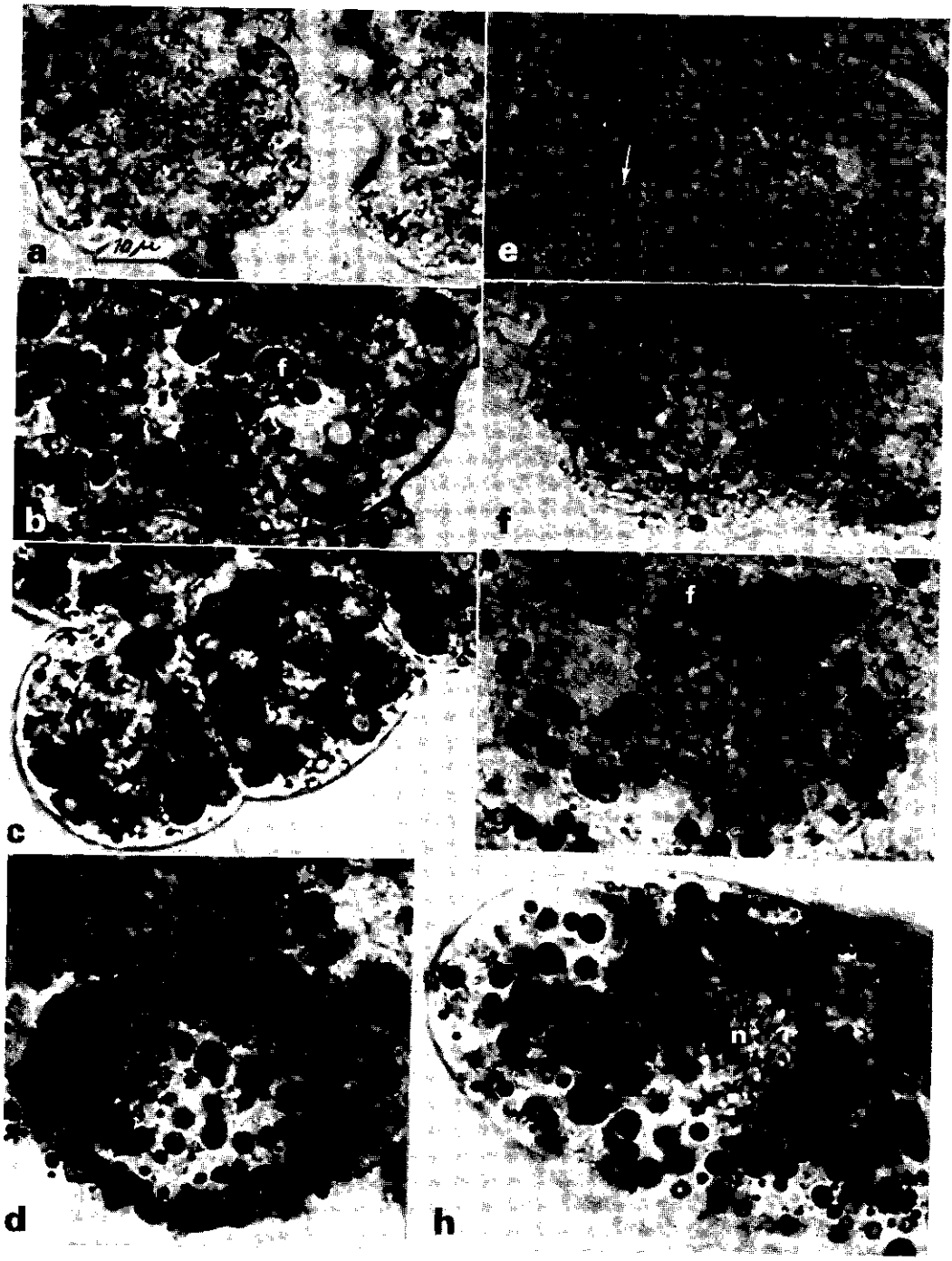

FIG. 17

FIG. 18. Glycogen containing fat cells of a 54-h-old worker larva. 5000 × (see STADHOUDERS, 1965 and REVEL, 1963 for electron microscopy of glycogen).

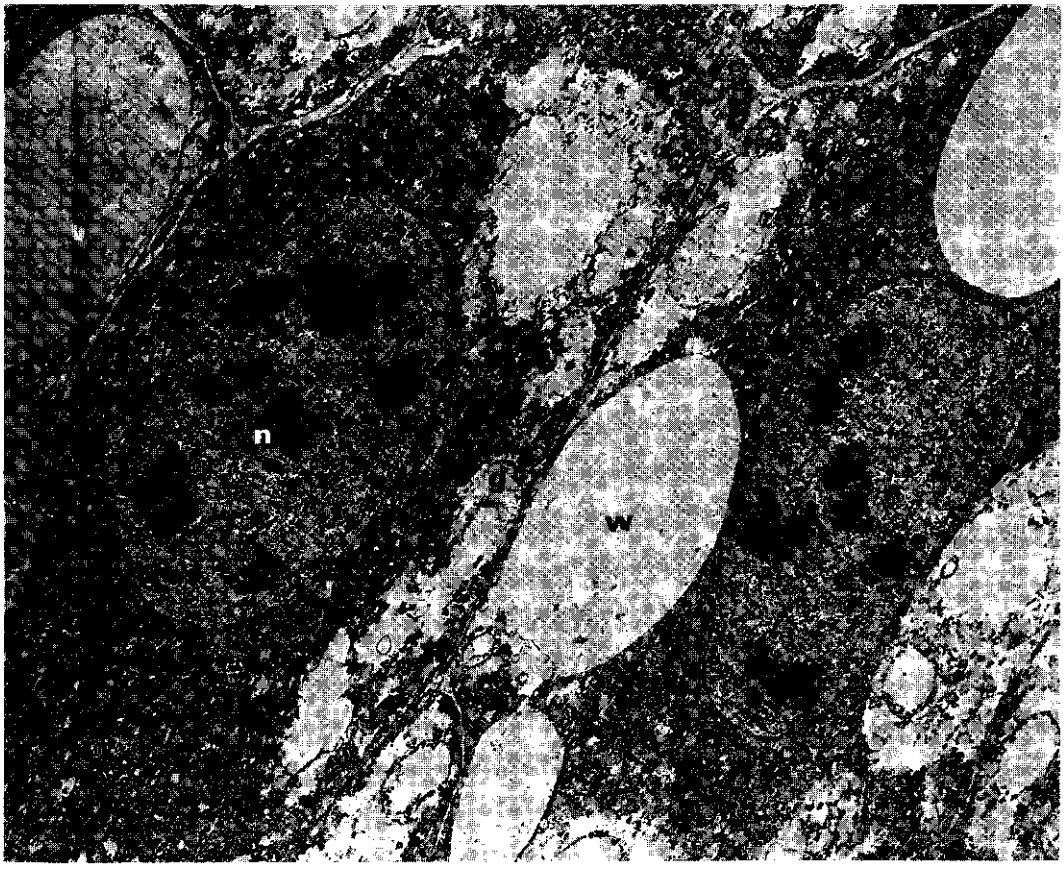

FIG. 18

FIG. 19. Watery vacuoles in fat cells.

a. 78-h-old worker larva. 'Signet-ring' type of fat cell. Large watery vacuole (W), a few small fat vacuoles (f). 4000 ×.

b. 78-h-old worker larva. Large watery vacuoles appear to be membrane bound. Membranes extending into the lumen of the vacuole (fixation-artefact?) 32500 ×

c. 48-h-old worker larva. Large watery vacuole containing membranes. Membranes still partly attached to the cytoplasm surrounding the vacuole (arrow). 17500 ×

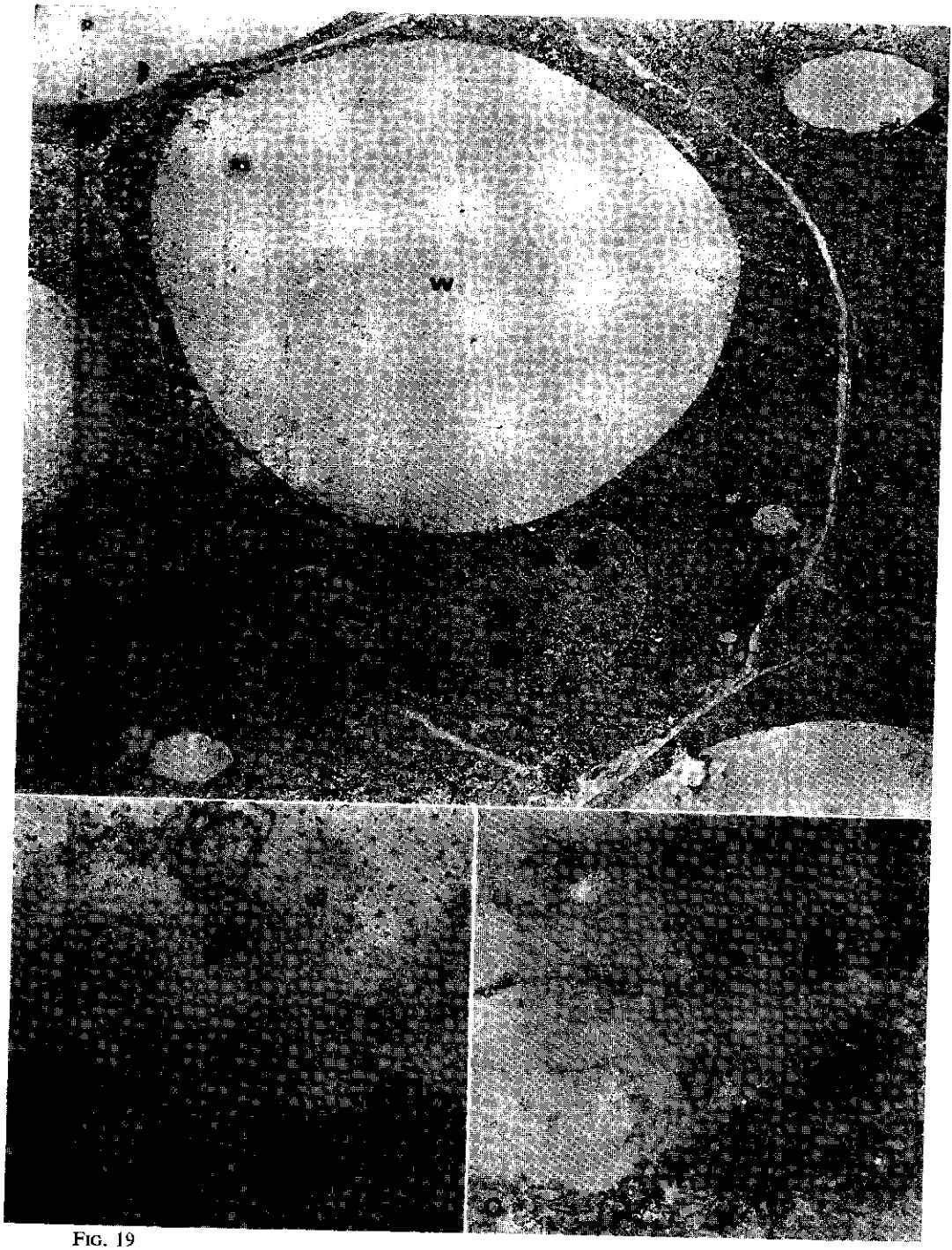

FIG. 19

FIG. 20. Development of fat cells.
a. 72-h-old worker larva. Little reserves in the 'signet-ring' type of fat cell. 8000 ×
b. 78-h-old worker larva. Fat cell in the head region. Close arrangement of RER and mitochondria (arrow). RER cisternae dilated. This orientation may indicate protein synthesis. 17500 ×
c. 84-h-old worker larva. RER cisternae dilated. Protein-like granules near the nucleus. 10000 ×

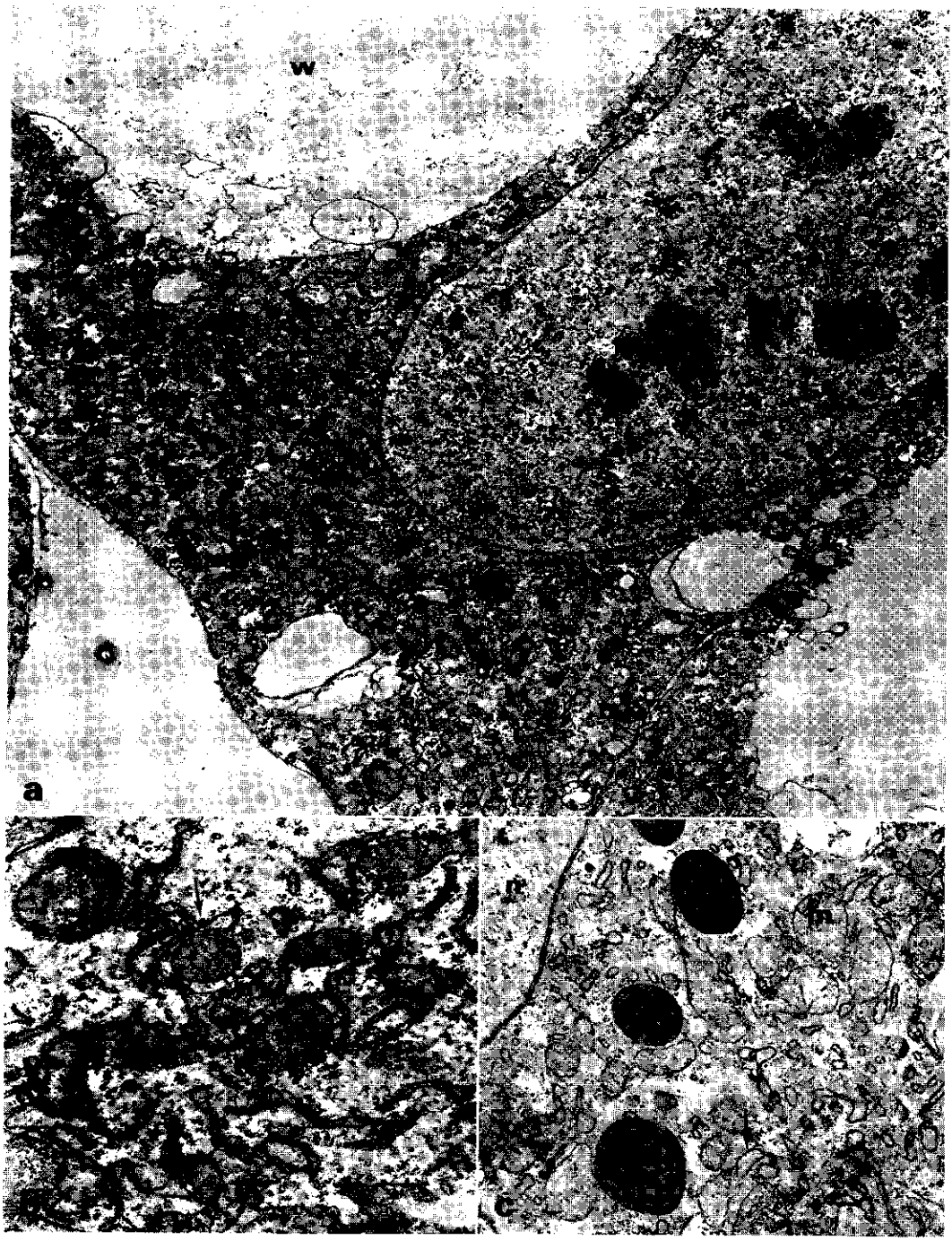

FIG. 20

FIG. 21. Development of fat cells.
60-h-old queen larva (12 h in queen cell). Accumulation of glycogen in cytoplasm and watery vacuole. Organelles in the plasm surrounding the nucleus.
Many ring-shaped mitochondria (arrow). 5000 ×

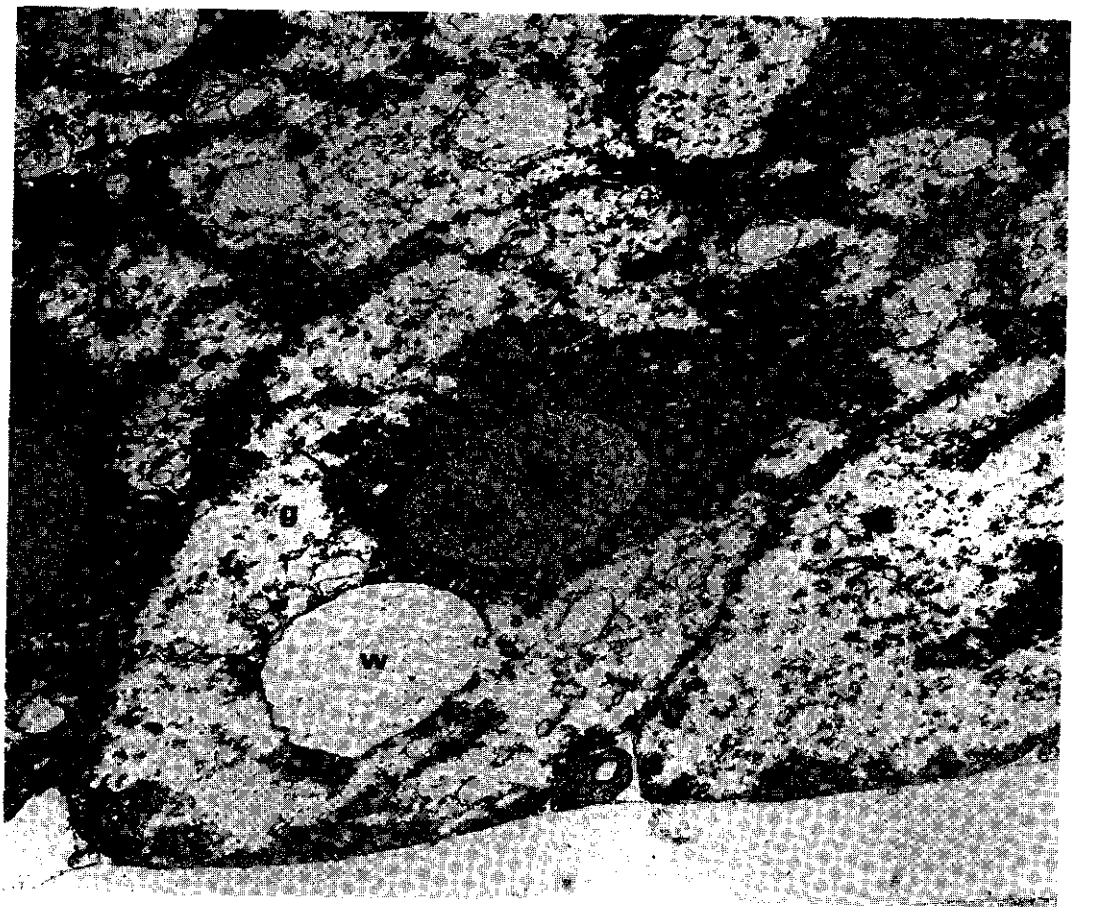

FIG. 21

FIG. 22. Development of fat cells.

a. 72-h-old queen larva (24 h in queen cell). Fat cells containing numerous fat vacuoles (f) and some transitional stages between glycogen areas and fat vacuoles (arrow). Outline of some fat vacuoles still irregular. 4000 ×

b. 72-h-old queen larva (24 h in queen cell). Detail of fat vacuole and glycogen area. 10000 ×

c. 72-h-old queen larva (24 h in queen cell). Outline of fat vacuoles rather smooth. Note invaginations of cell walls (arrow). 4000 ×

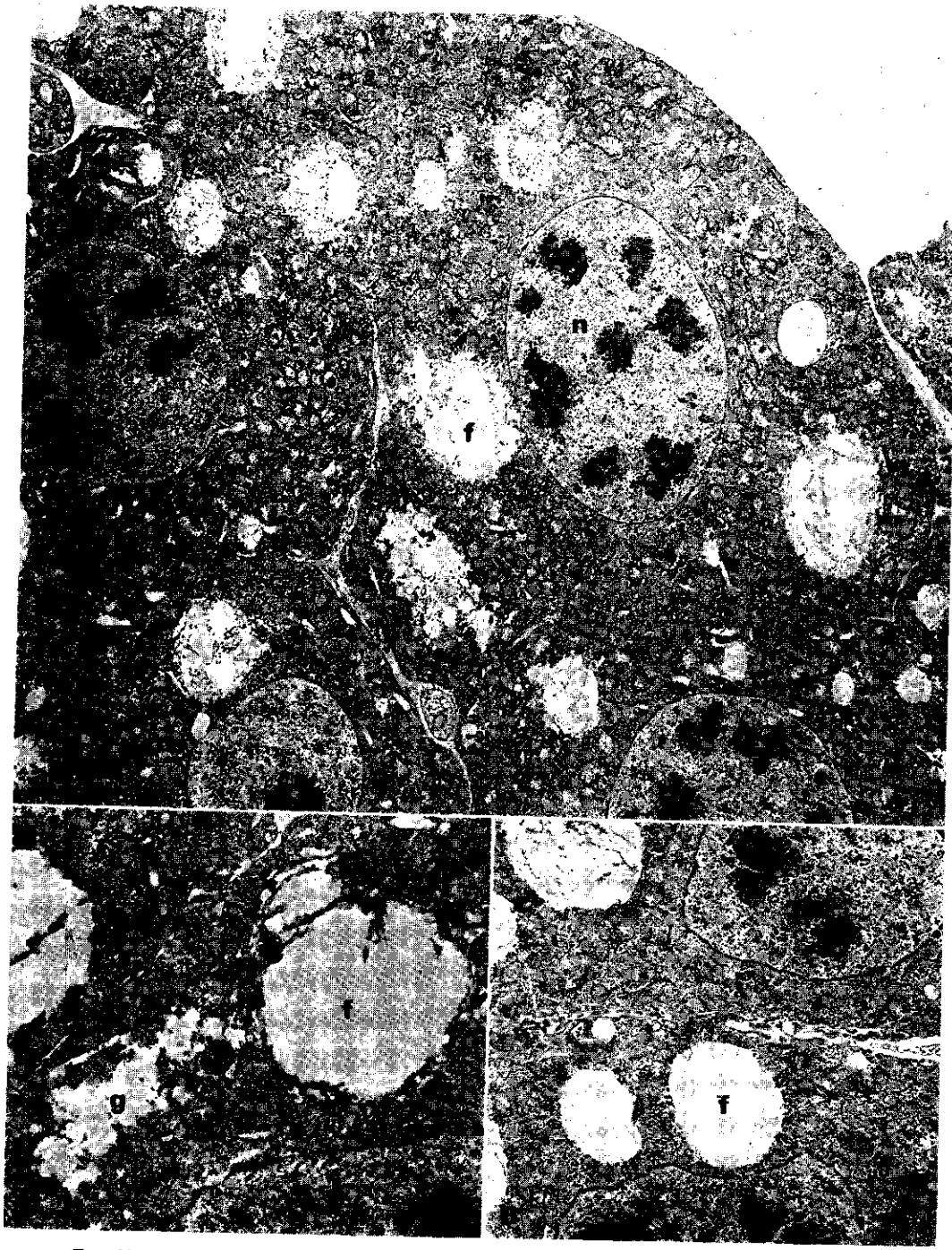

FIG. 22

FIG. 23. Development of fat cells.

a. 84-h-old queen larva (36 h in queen cell). Numerous large fat vacuoles containing some protein-like substance. Nucleus irregular in outline. Protein-like granules in cytoplasm near nucleus (arrow). 4000 ×

b. Detail of same cell showing protein-like, membrane-bound bodies. 16000 ×

c. 78-h-old queen larva. (30 h in queen cell). Detail of protein-like substance in fat vacuole. 22500 ×

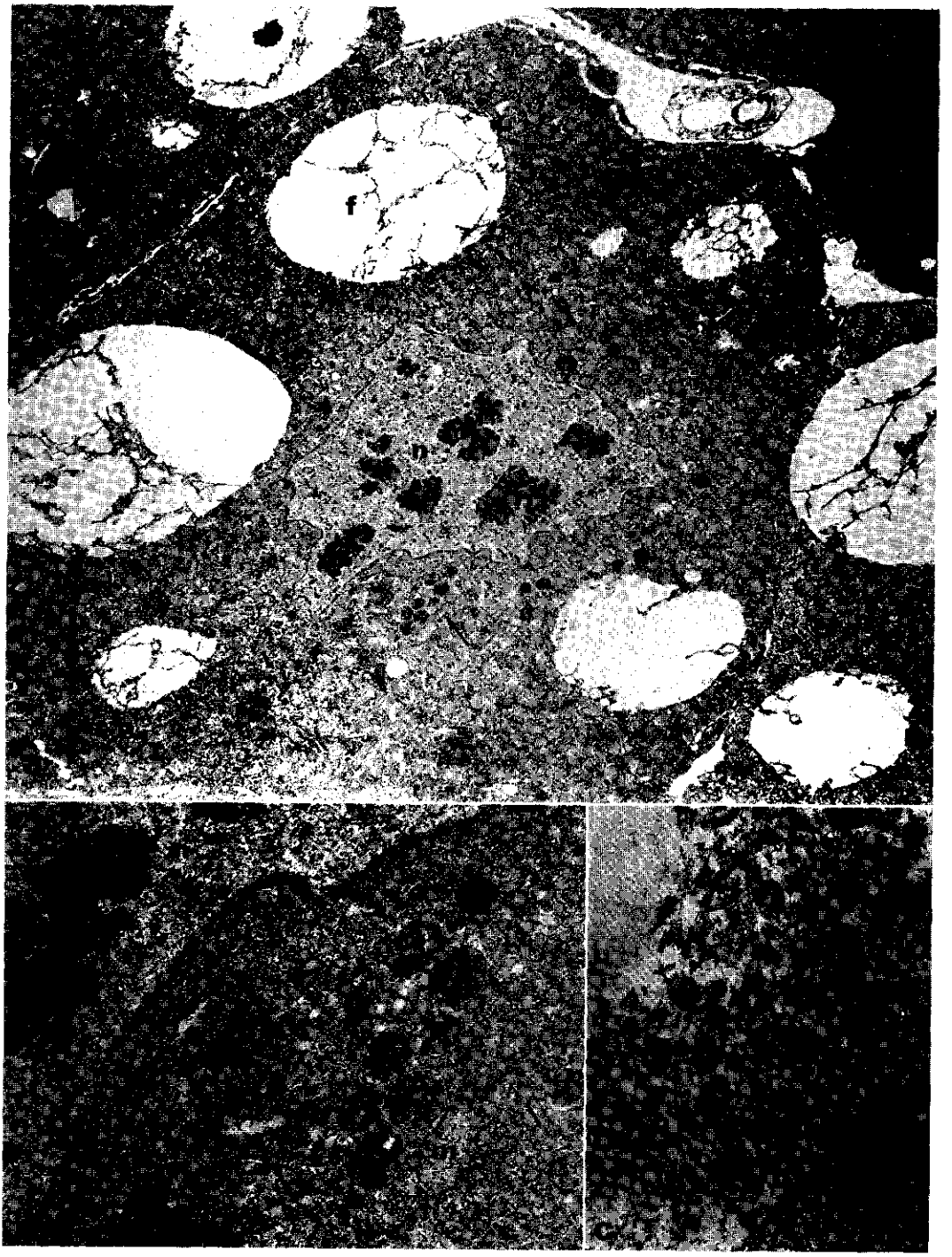

FIG. 23

FIG. 24. Development of fat cells 84-h-old queen larva. Fat cell containing fat vacuoles and glycogen areas. Protein-like substance in fat vacuoles (small arrows). Cell wall invaginated (arrow). 4000 ×

FIG. 24

FIG. 25. Cerebral ganglion.
a. 54-h-old queen larva (6 h in queen cell). Neuroblast. Daughter cell just tied off (arrow). 8000 ×
b. Lysosome like bodies in neuroblast. Note double cell wall (arrow). 30000 ×

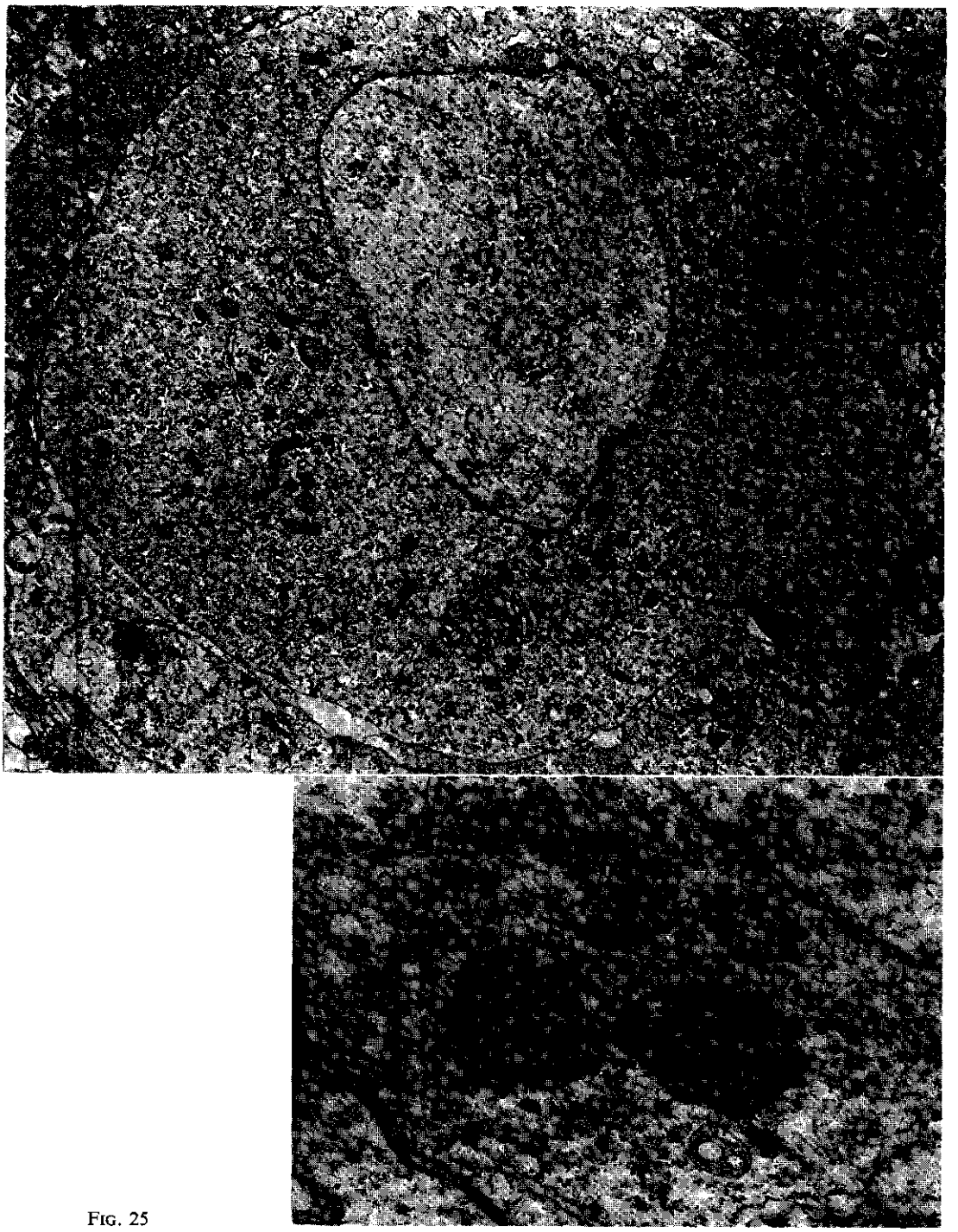

FIG. 25

FIG. 26. Neurosecretion in the brain.
a. 54-h-old worker larva. Neurosecretory product in the golgi complex (arrows). 45000 ×
b. Same larva. Neurosecretory granules in the cytoplasm (arrow) of a NSC. 30000 ×
c. 84-h-old queen larva. (36 h in queen cell). Neurosecretory product in the golgi complex of a NSC in the brain. 37500 ×
d. 60-h-old queen larva (12 h in queen cell). Neurosecretory granules (arrow) in axons in the region of the brain near the aorta. 12500 ×

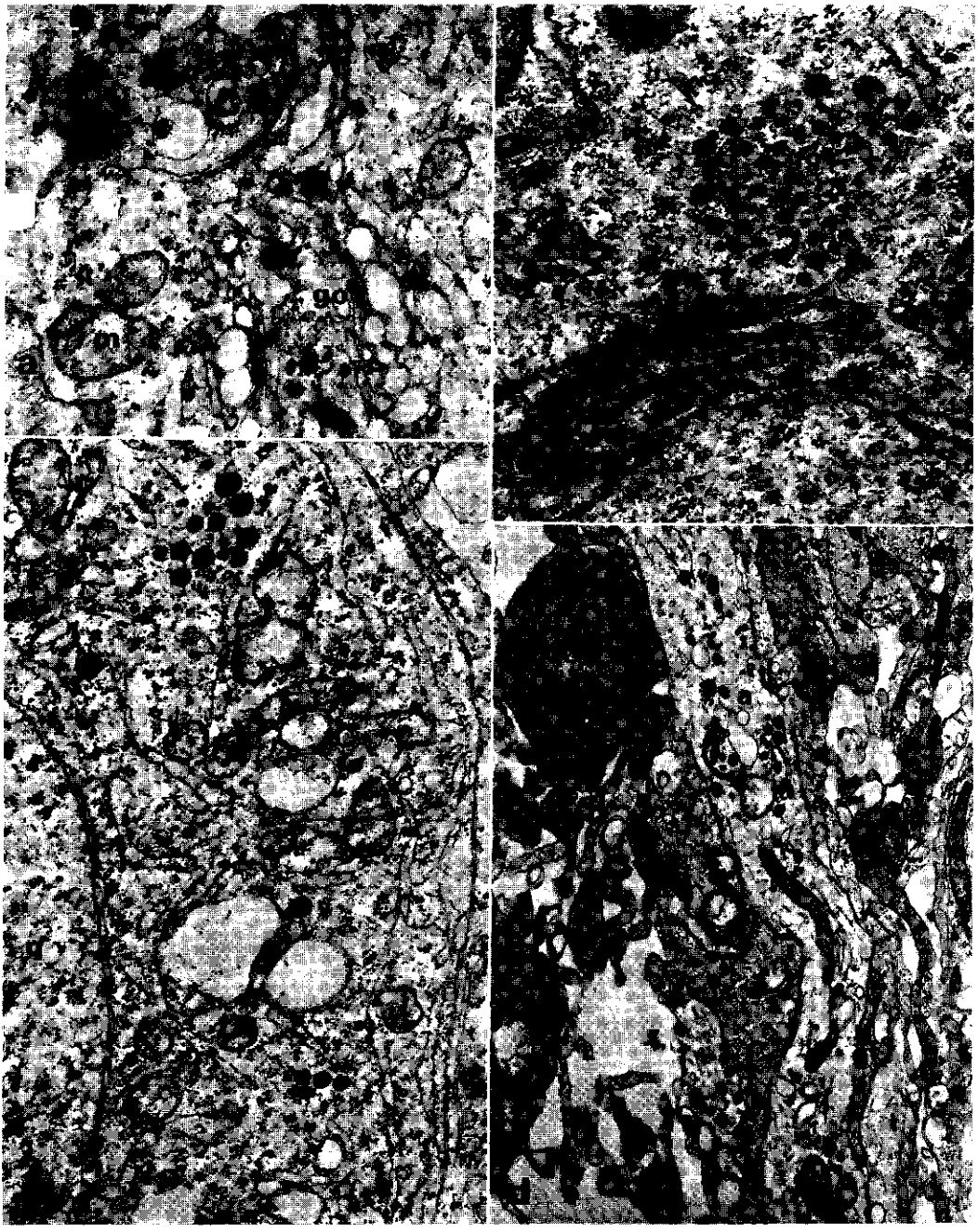

FIG. 26

FIG. 27. Corpus cardiacum.
78-h-old queen larva (30 h in queen cell). Transverse section through the aorta-CC complex. Neurosecretory CC cells (cc) and axons (a) both containing NS granules (arrows). mu: muscle tissue of the oesophagus. 4000 ×

FIG. 27

FIG. 28. Corpus cardiacum.

a. 78-h-old queen larva (30 h in queen cell). Transverse section through the aorta-CC complex and nervus corporis cardiaci (NCC). Axons contain neurosecretory granules (arrow). (figs 27 and 28a overlap) 4000 ×

b. Same larva as 28a. CC cells in the upper part of the CC. 4000 ×

c. 60-h-old queen larva (12 h in queen cell). Neurosecretory substance in the golgi complex of a CC cell (arrow).

FIG. 28

FIG. 29. Corpus cardiacum.

a. 60-h-old queen larva (12 h in queen cell). Medial part of the CC. Axon terminals (arrow) containing neurosecretory granules. Near the inner border of the aorta numerous axons contain NS granules. 4000 ×

b. 96-h-old queen larva (48 h in queen cell). CC cell with actively secreting golgi complex bordered by axons (a) containing a few NS granules. 20000 ×

c. 78-h-old worker larva. Axons with numerous NS granules. 22000 ×

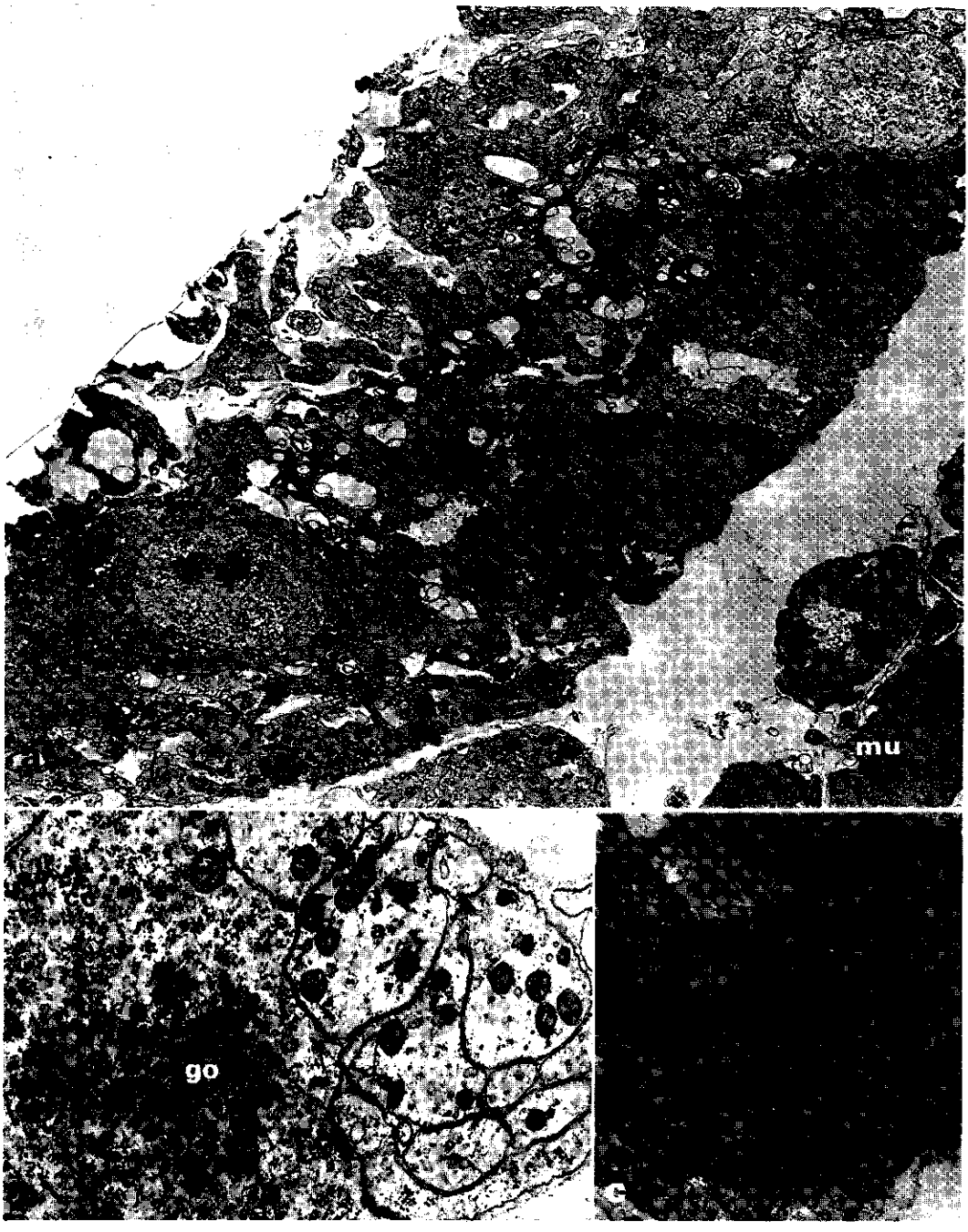

FIG. 29

FIG. 30. Corpus cardiacum.

a. 60-h-old queen larva (12 h in queen cell). Lower part of the aorta-CC complex with some axon terminals (arrow). In the lower left-hand corner the aorta wall is composed of connective-tissue like cells. In this area axons are scarce. 4000 ×

b. 54-h-old worker larva. Different stages of release of NS granules into the haemolymph. 20000 ×

c. Detail of 30b. 84000 ×

FIG. 30

FIG. 31. Corpus cardiacum, aorta.
a. 54-h-old worker larva. Overall picture of the CC (same area as 30 b and c). 6000 ×
b. 60-h-old worker larva. Lower part of the aorta wall, near the CA. Connective-tissue like cells; no axons. 16000 ×

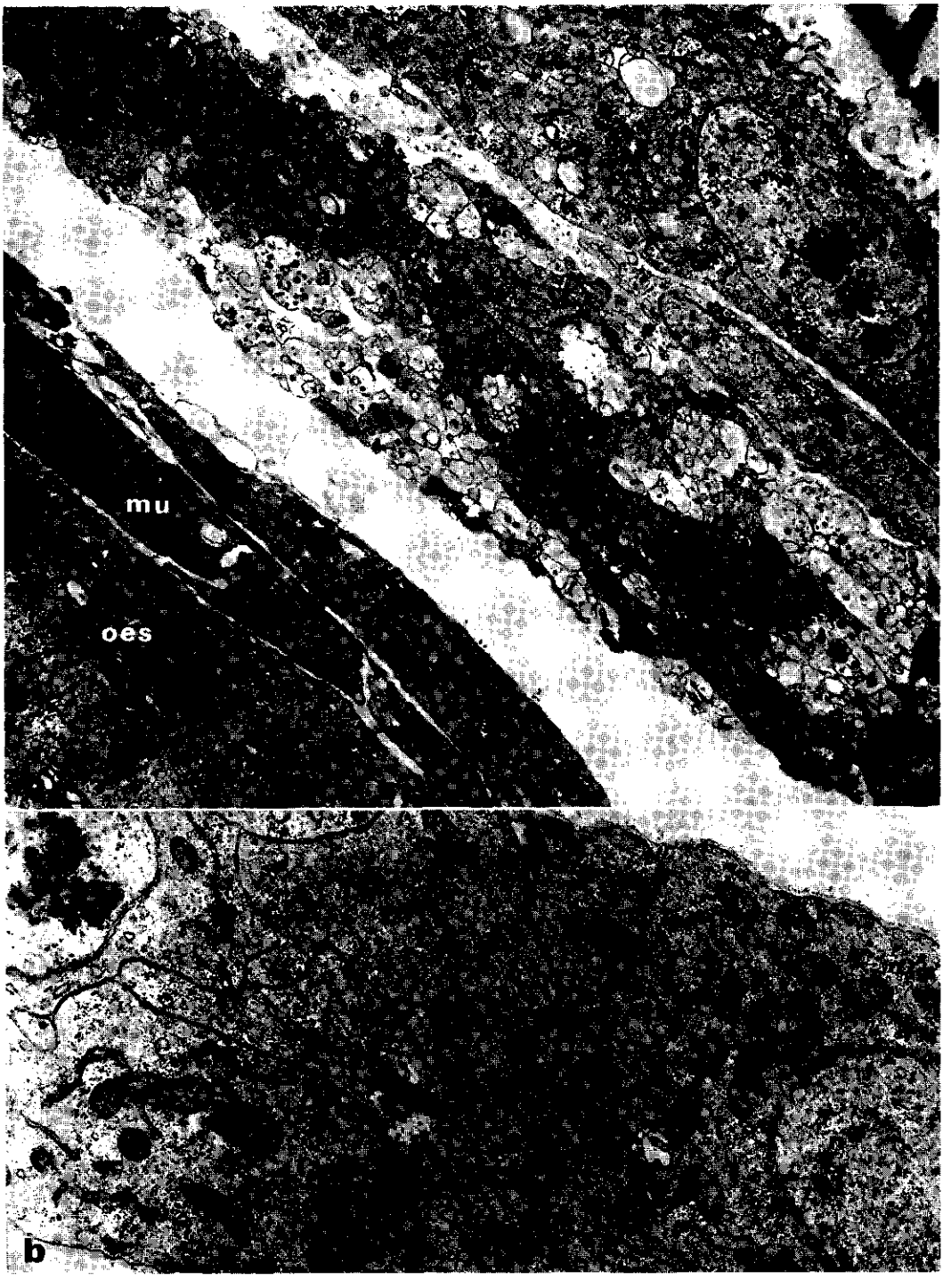

FIG. 31

FIG. 32.

a. Longitudinal section of a 52-h-old queen larva (18 h in queen cell). Note that a considerable part of the body cavity is occupied by the midgut. Carnoy (70%); Mallory

b. Corpus allatum of a 78-h-old queen larva (30 h in queen cell). Carnoy (70%); Mallory.

c. Longitudinal section through the sub-oesophageal region. Prothoracic-gland cells near the midgut (arrow). Carnoy (70%); Mallory.

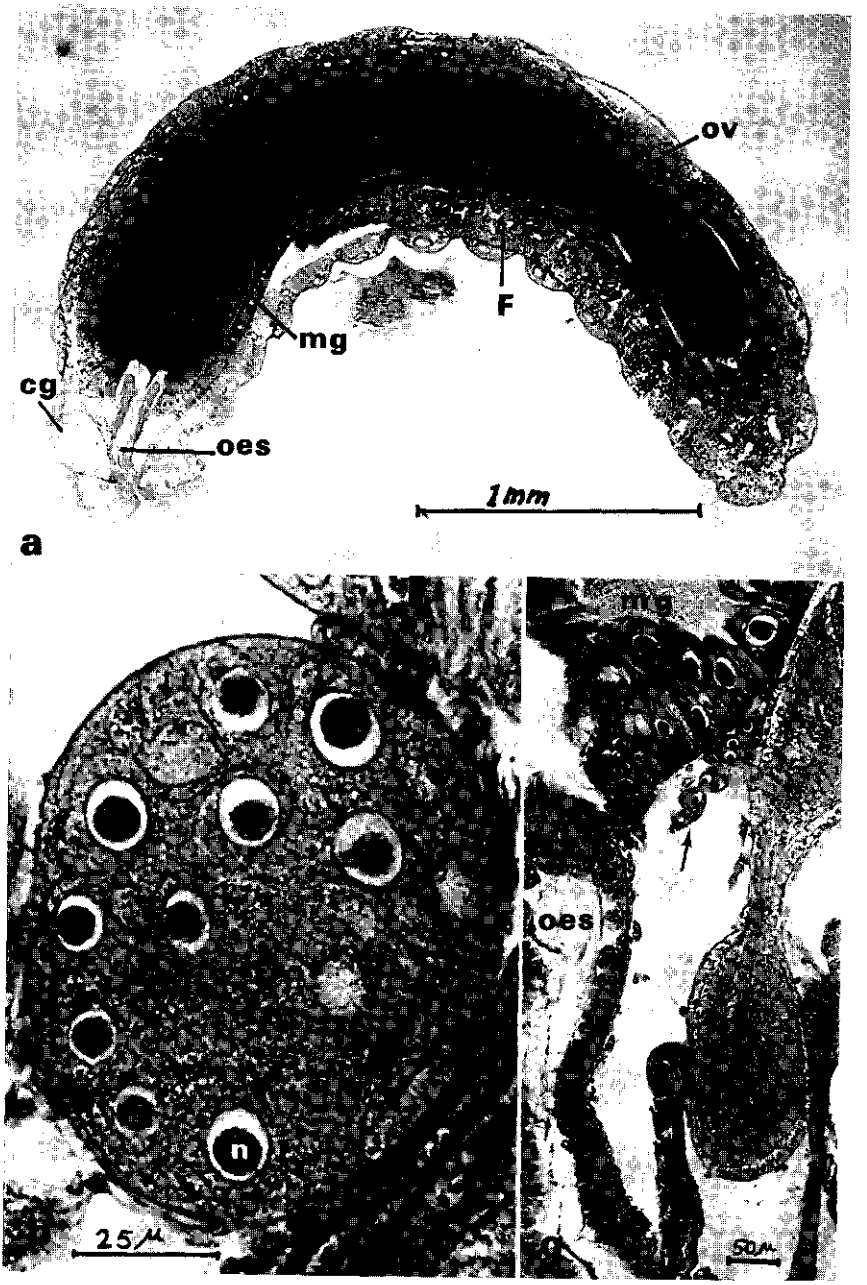

FIG. 32

FIG. 33. Corpus allatum.
60-h-old queen larva (12 h in queen cell). Overall picture of the CA (lateral section). Note SER whorls (arrows) 4000 ×

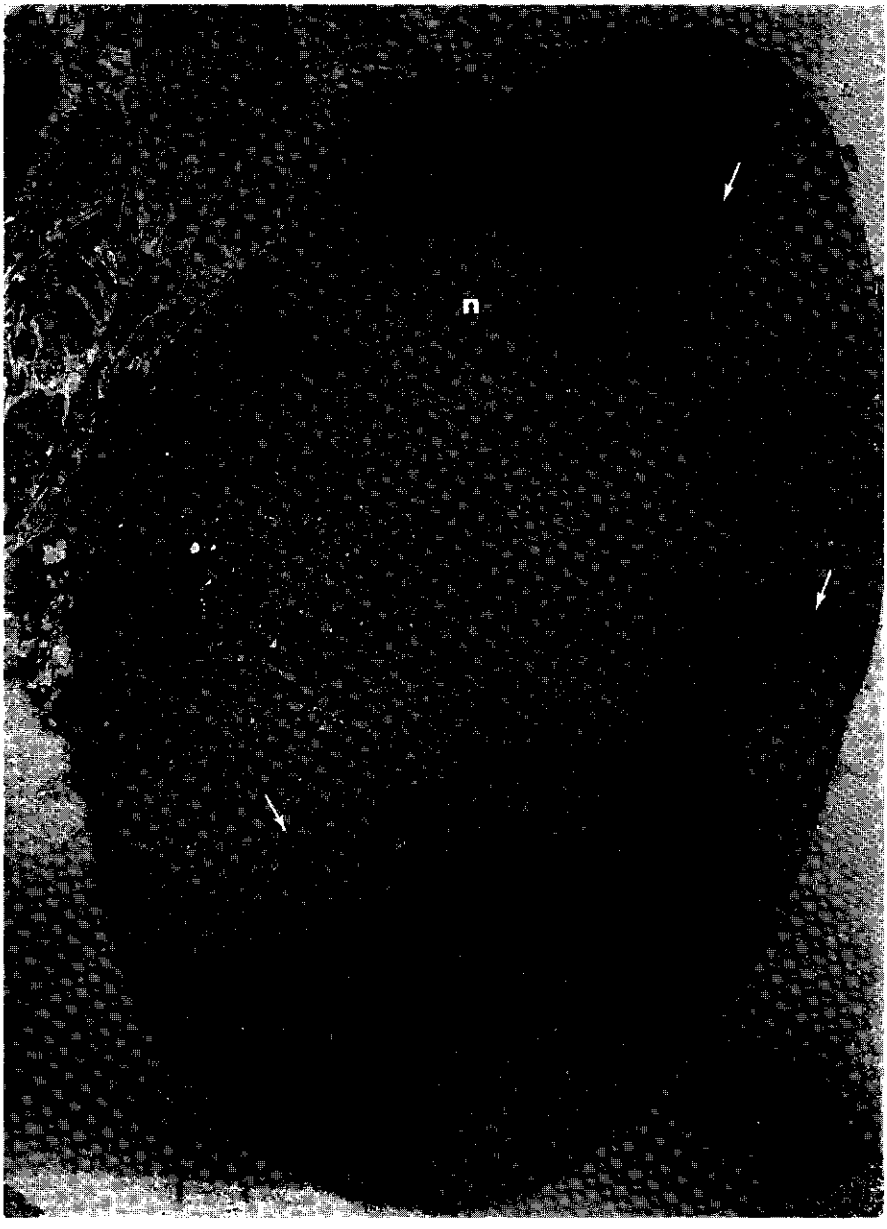

FIG. 33

FIG. 34. Corpus allatum.

a. 60-h-old queen larva (12 h in queen cell). Small RER cisternae, little SER. Membrane lamellae of the golgi complex dilated. Ovoid and rod-shaped mitochondria (m) with few cristae. A few small vacuoles (sv). Note nuclear pores (small arrow). 24000 ×

b. 78-h-old worker larva. Ovoid mitochondria and little SER. 42000 ×

c. 84-h-old queen larva (36 h in queen cell). Lysosome-like body (L.) 55000 ×

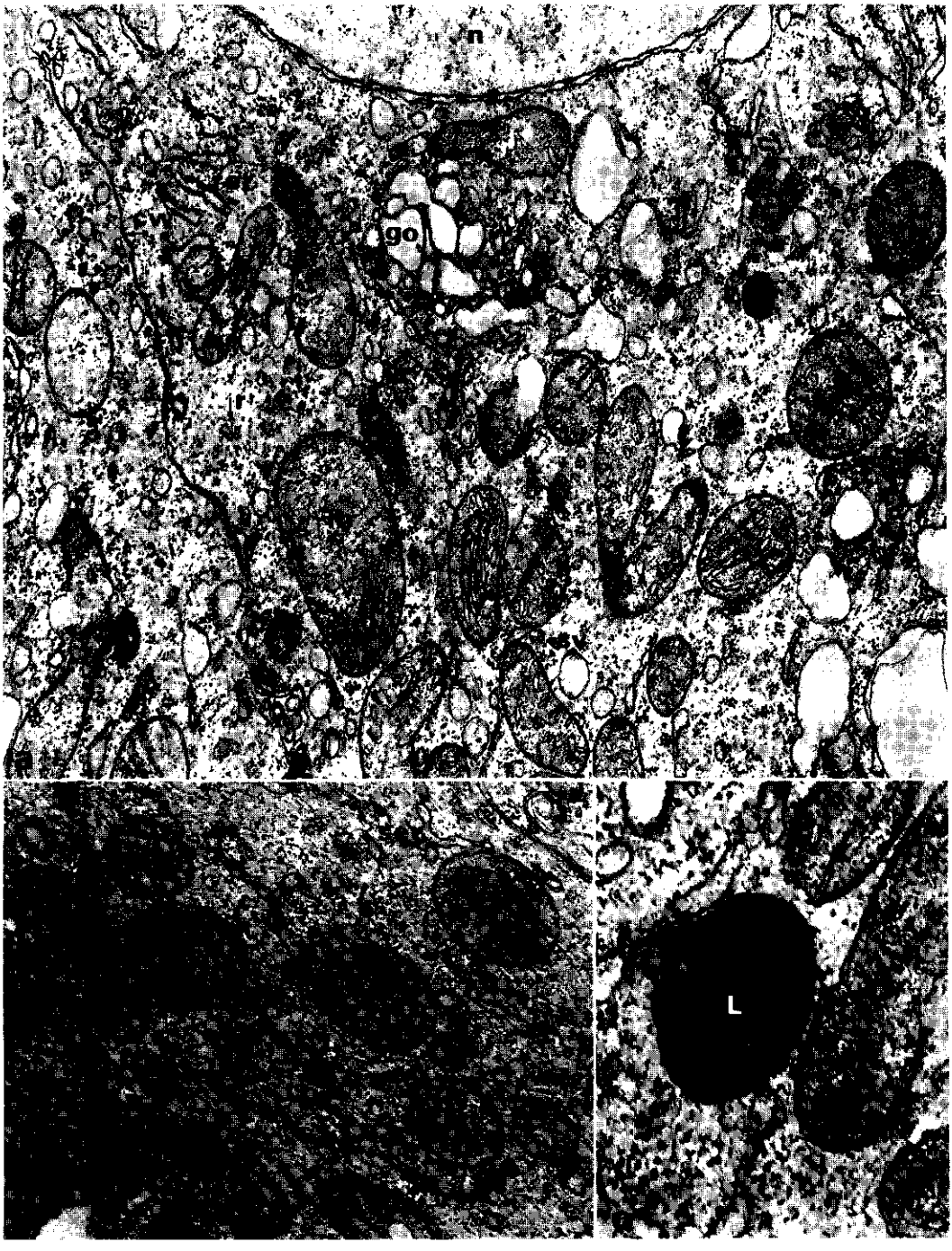

FIG. 34

FIG. 35. Corpus allatum.

a. 72-h-old queen larva (24 h in queen cell). Spider-like RER cisternae surrounding the golgi complex. Some golgi vacuoles budding off from the golgi complex. 18000 ×

b. 84-h-old queen larva (36 h in queen cell). Some golgi vacuoles (gv) near the active golgi complex. Much SER. 22000 ×

c. 72-h-old queen larva (24 h in queen cell). Many golgi vacuoles (gv). Much SER. Note small golgi vesicles (arrow). 40000 ×

d. 60-h-old queen larva (12 h in queen cell). Small – membrane bound – vacuoles (SV) in the cytoplsm. Arrow: microtubule. 40000 ×

e. 78-h-old queen larva (30 h in queen cell). Numerous small vacuoles in the cytoplasm. 20000 ×

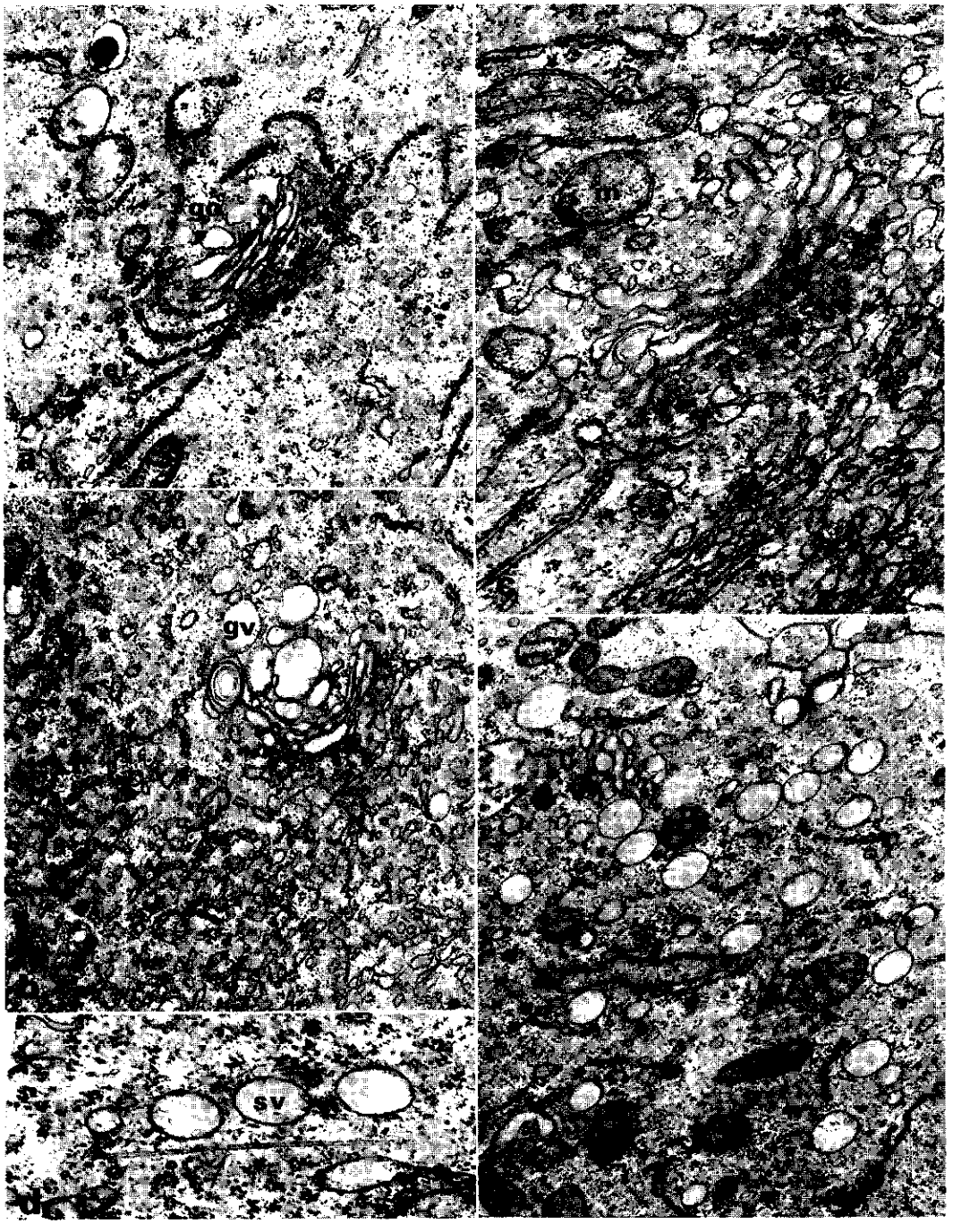

FIG. 35

FIG. 36. Corpus allatum.

a. 84-h-old queen larva (36 h in queen cell). At the border of the CA just inside the cell membrane (arrow) dense cytoplasmic areas are a common feature. The function of these electron dense spots is unknown. Note the surrounding glycocalyx (gl). 160000 ×

b. 96-h-old queen larva (48 h in queen cell). Small vacuoles in different stages of extrusion at the border of the CA. In the intercellular space also membrane bound vacuoles may be observed (arrow). 36000 ×

c. 60-h-old queen larva (12 h in queen cell). Formation of a pinocytotic vesicle (?) at the border of the CA. 68000 ×

d. 123-h-old queen larva (72 h in queen cell). Release of small vesicles at the border of the CA. 44000 ×

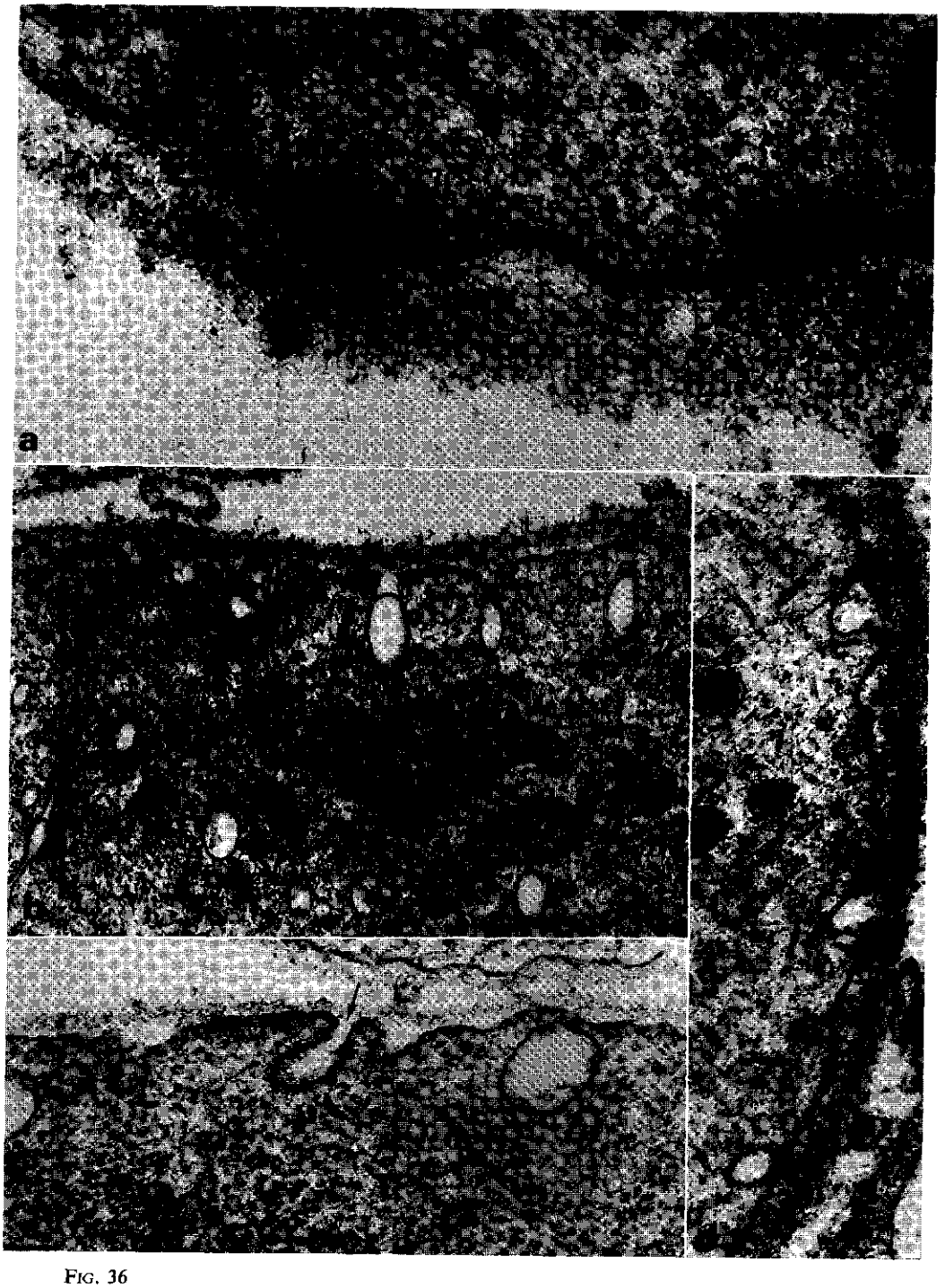

FIG. 36

FIG. 37. Corpus allatum.
a. 78-h-old worker larva. Release of small vacuoles at the CA border. 36000 ×
b. 78-h-old worker larva. Release of small vacuoles at the CA border and small vacuoles in the intercellular spaces (arrows). 90000 ×
c. 78-h-old worker larva. Release of small vacuoles at the CA border (arrows) 40000 ×
d. 84-h-old worker larva. Intercellular spaces containing membrane-bound small vacuoles (arrow). 62000 ×

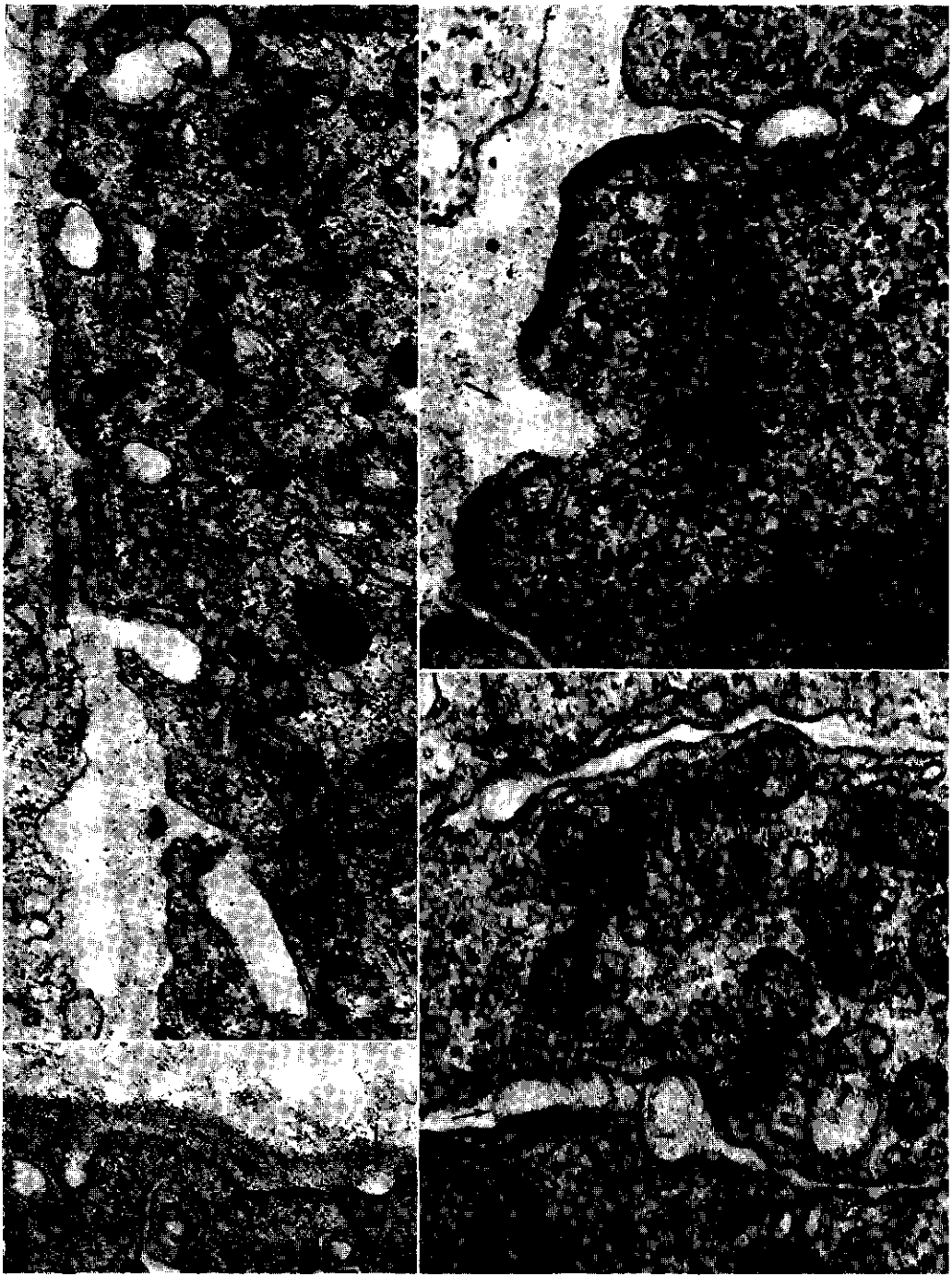

FIG. 37

FIG. 38. Corpus allatum.
84-h-old queen larva (36 h in queen cell). Numerous large vacuoles with an electron-opaque content near the CA border. 16000 ×

FIG. 38

FIG. 39. Innervation of the corpus allatum.

a. 84-h-old queen larva (36 h in queen cell). Nervus corporis allati entering the CA. Arrow: CA border. Note numerous small vacuoles in the cytoplasm of the CA cell. 8000 ×

b. 84-h-old queen larva (36 h in queen cell). Axon in the CA, containing a few NS granules. 40000 ×

c. 84-h-old queen larva (36 h in queen cell). Axon in the CA, containing numerous NS granules. 24000 ×

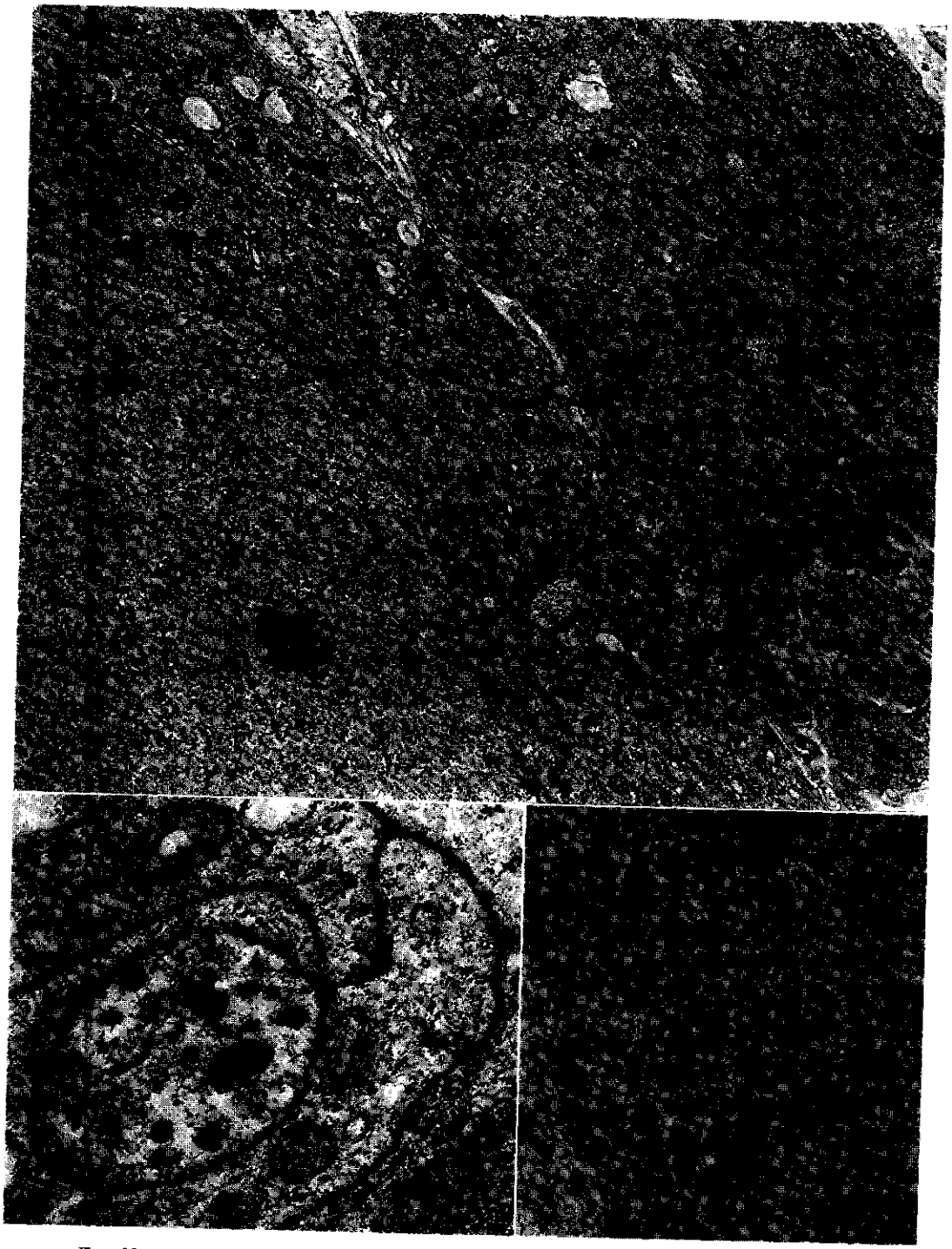

FIG. 39

FIG. 40. Corpus allatum.

a. 48-h-old worker larva. Overall picture of an inactive CA cell. Note lysosome-like bodies (L). 16000 ×

b. Same CA as a. More active cell at the CA border. RER cisternae near golgi complex. Some golgi vacuoles bud off from the golgi complex. 20000 ×

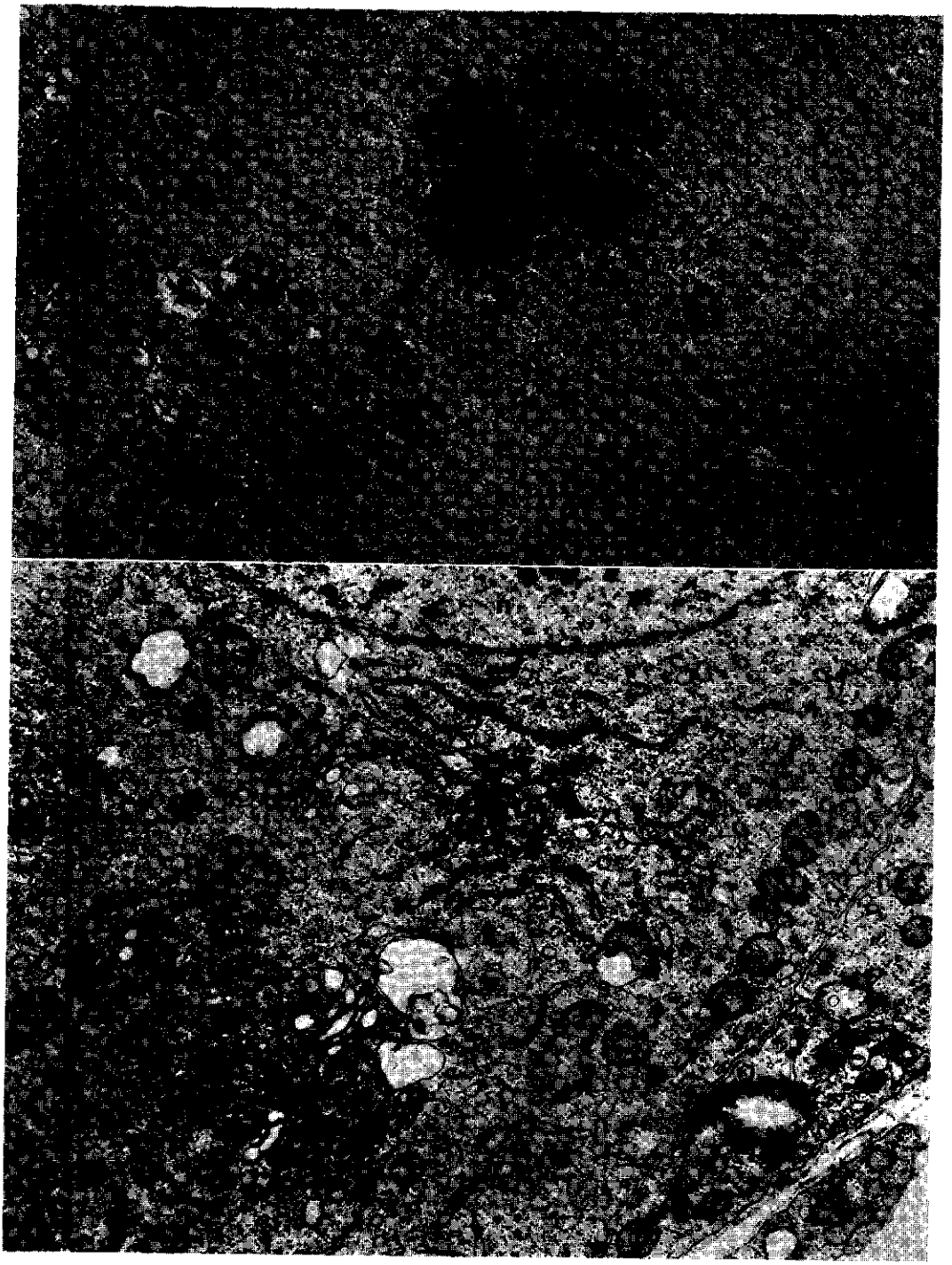

FIG. 40

FIG. 41. Corpus allatum.

a. 60-h-old worker larva. Overall picture of a CA cell. Some small vacuoles in the cytoplasm. A few large vacuoles. Golgi complexes rather extensive but not so active. 8000 ×

b. Same larva as a. Small SER whorl. Note the connection between SER and RER (arrow). 56000 ×

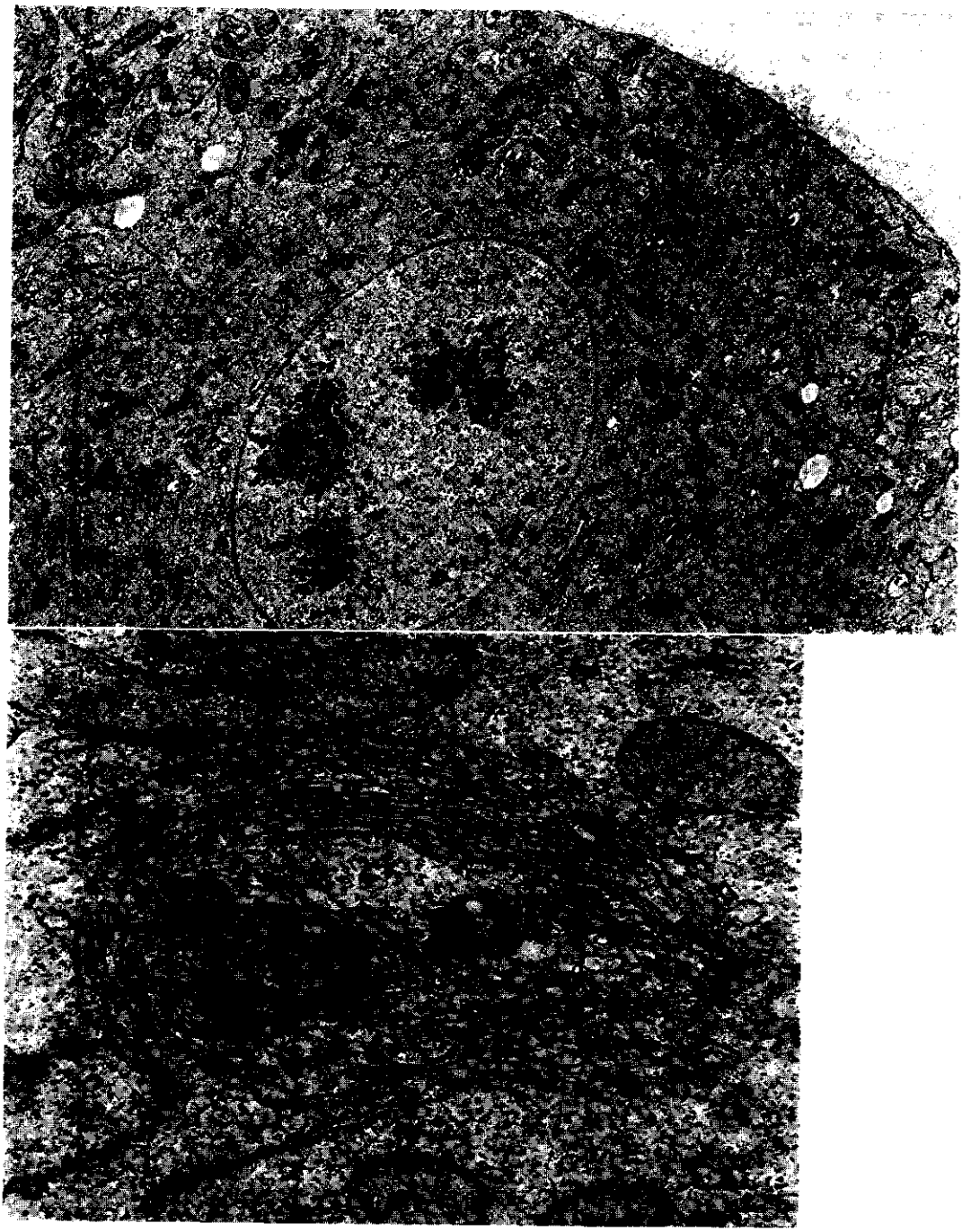

FIG. 41

FIG. 42. Corpus allatum.
a. 72-h-old worker larva. Golgi lamellae somewhat dilated. A few small vacuoles. Little SER. 20000 ×
b. 78-h-old worker larva. SER whorl surrounds a large vacuole. 48000 ×

FIG. 42

FIG. 43. Corpus allatum.
a. 78-h-old worker larva. Overall picture of active cells. Note numerous small vacuoles (arrow) in the cell at the CA border. 8000 ×
b. 84-h-old worker larva. Inactive cell. 8000 ×

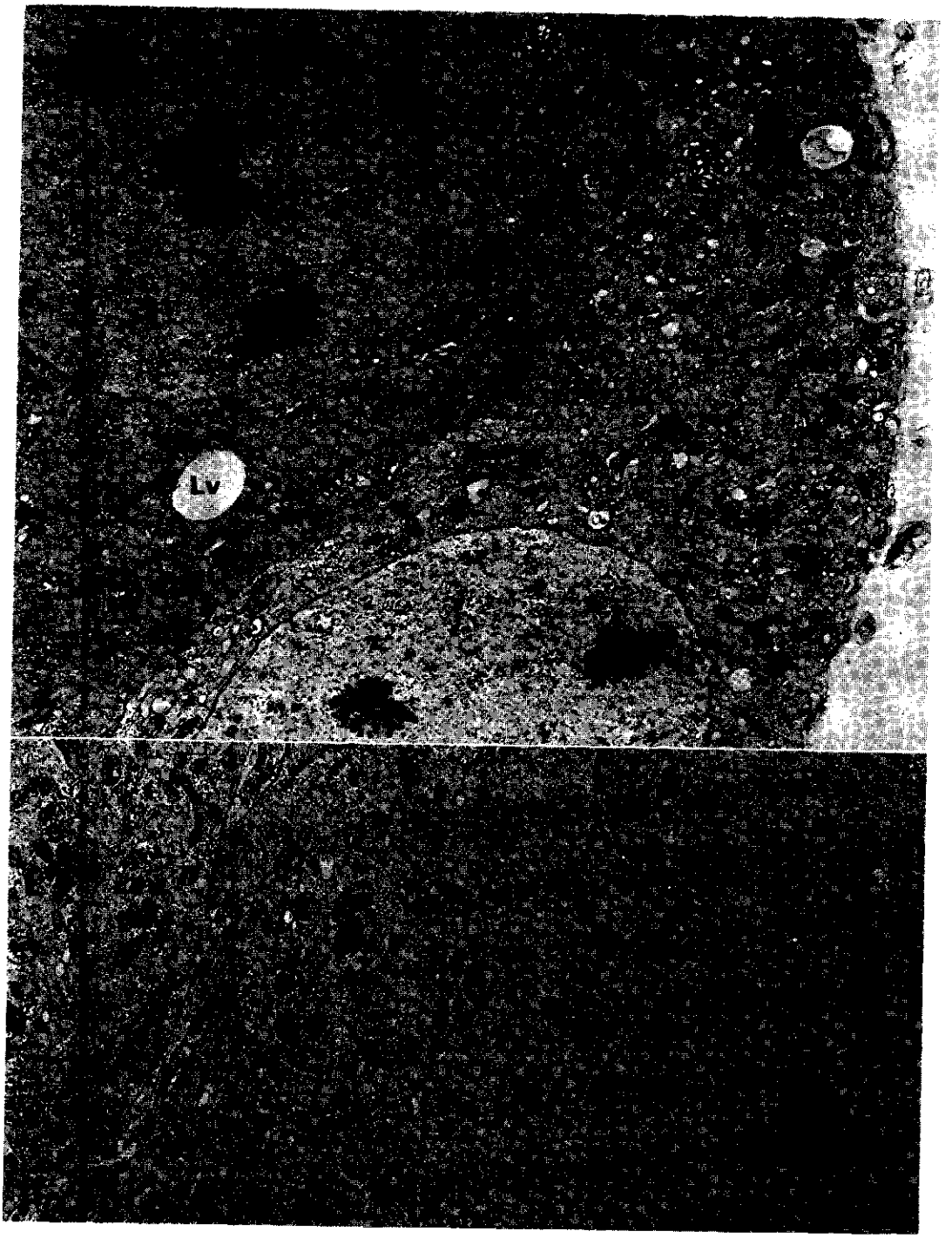

FIG. 43

FIG. 44. Corpus allatum.
a. 171-h-old worker larva. Numerous lysosome-like bodies. Little SER. Golgi complex inactive. 18000 ×
b. 147-h-old worker larva. Overall picture of a cell. Ovoid mitochondria. 4000 ×
c. 171-h-old worker larva. Detail of a golgi complex and some large RER cisternae. 30000 ×

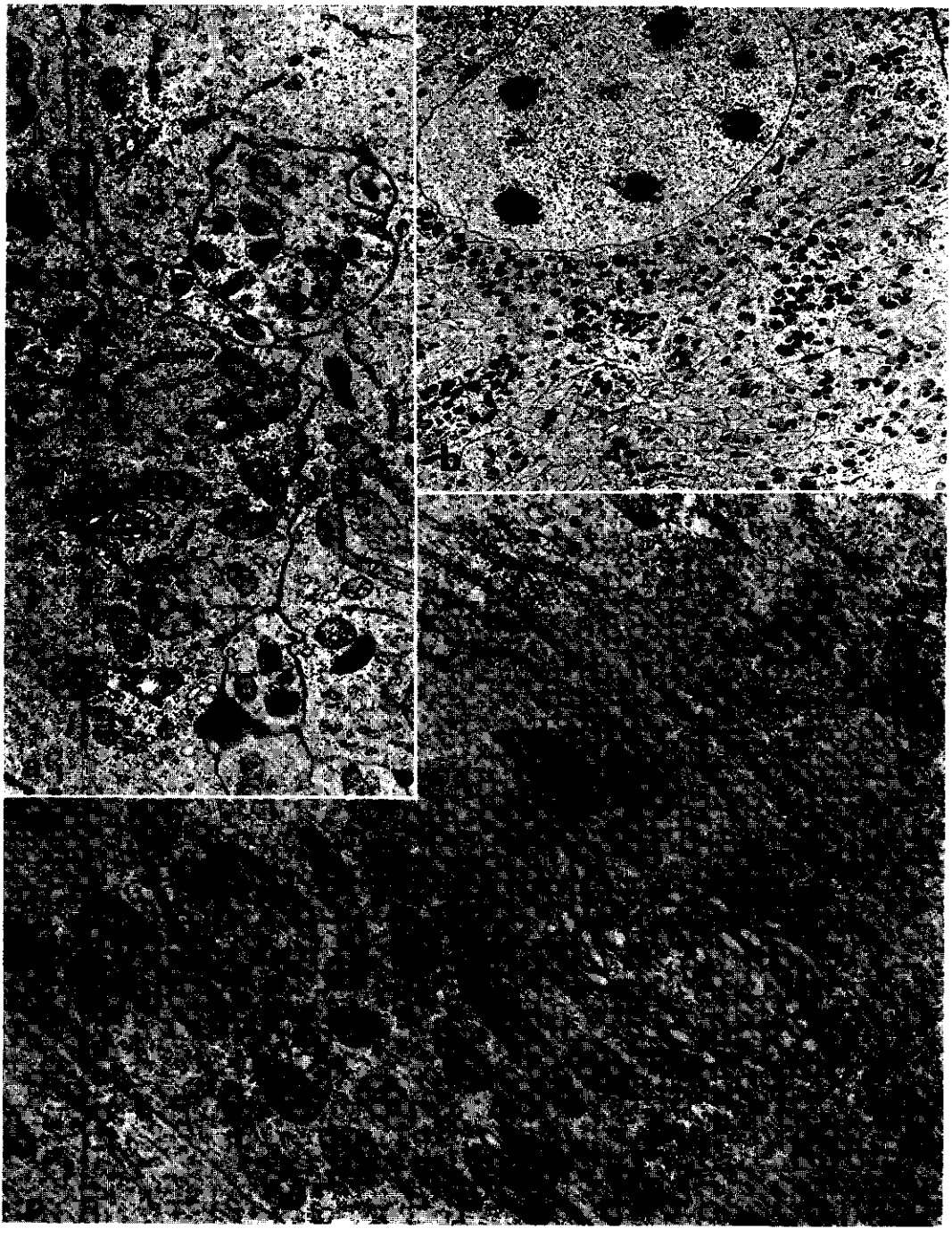

FIG. 44

FIG. 45. Corpus allatum.

a. 60-h-old queen larva. (12 h in queen cell). Numerous spider-like RER cisternae surrounding golgi complexes. Several small vacuoles (arrow). Note difference in plasm of the CA cells. Golgi lamellae dilated. 6000 ×

b. 60-h-old queen larva (12 h in queen cell). Detail of a golgi complex. RER cisternae (arrow) connected with it. 27500 ×

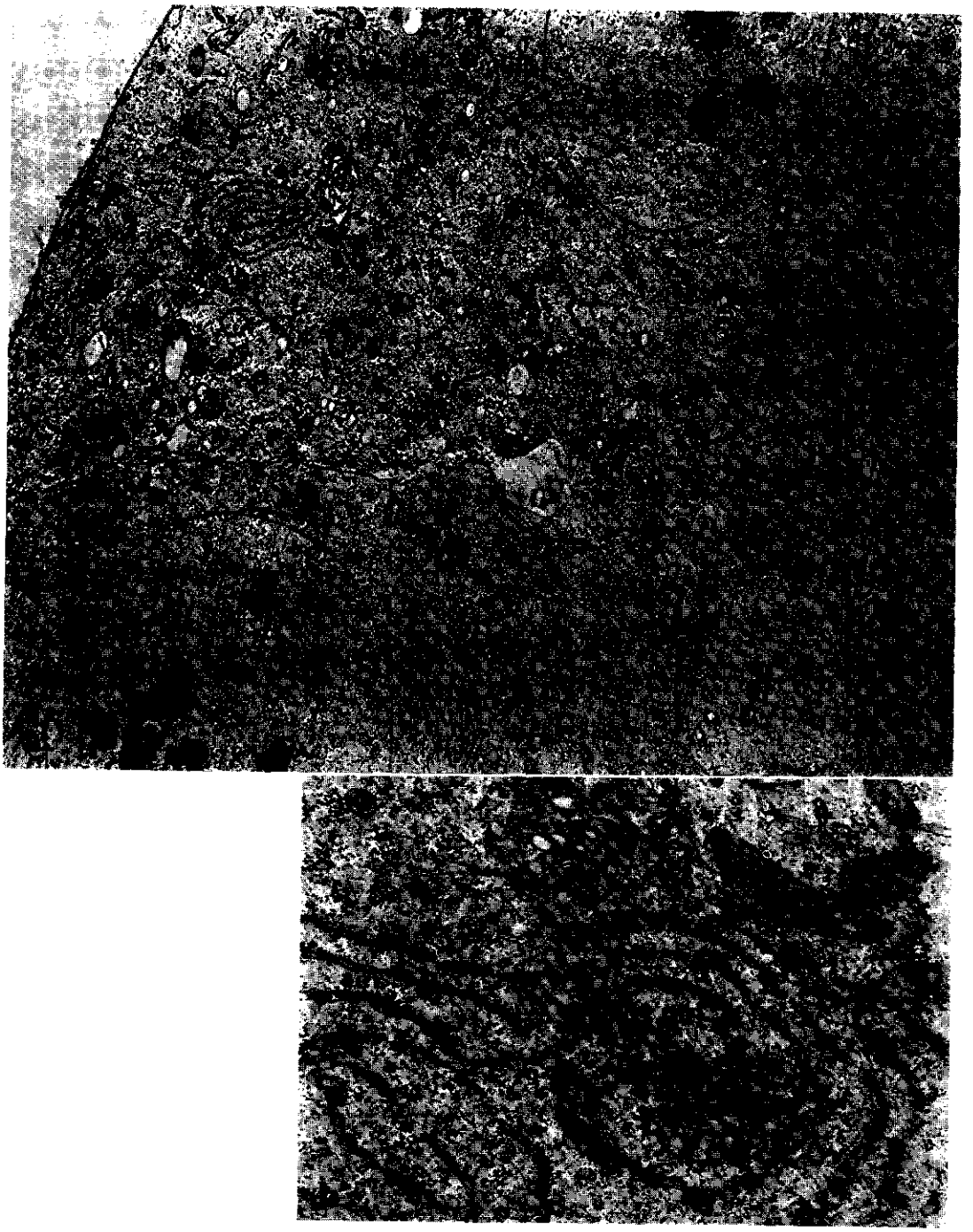

FIG. 45

FIG. 46. Corpus allatum.
a. 60-h-old queen larva (12 h in queen cell). SER and RER in close relationship. 28000 ×
b. Same larva as a. Golgi-SER-RER complex near the nucleus. 24000 ×

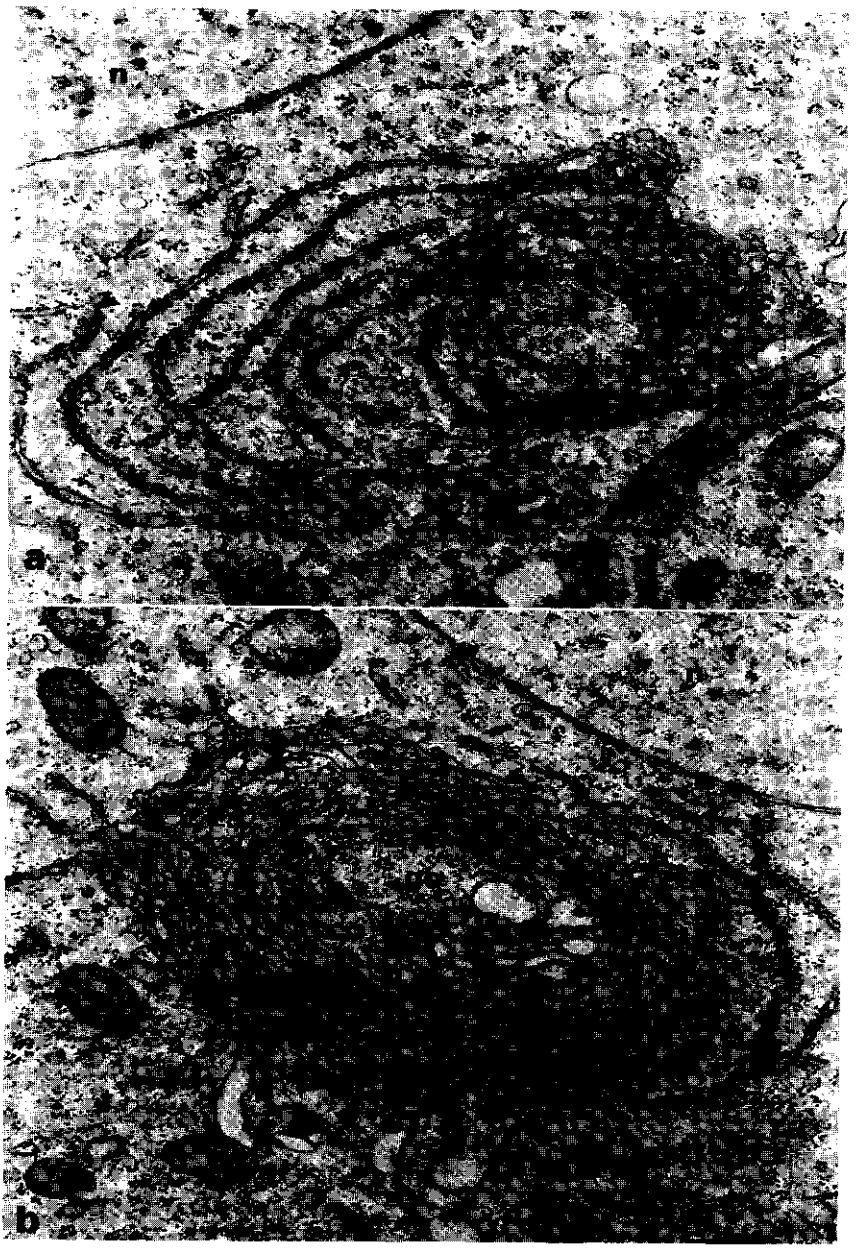

FIG. 46

FIG. 47. Corpus allatum.
a. 60-h-old queen larva (12 h in queen cell). Large SER whorl. 27500 ×
b. 72-h-old queen larva (24 h in queen cell). Active cell; SER whorl with dilated membranes. (detail of fig. 48). 12500 ×
c. 78-h-old queen larva. SER area with dense body. 35000 ×
d. Detail of c. The dense body is composed of granular substance; no membrane surrounds this structure. 35000 ×

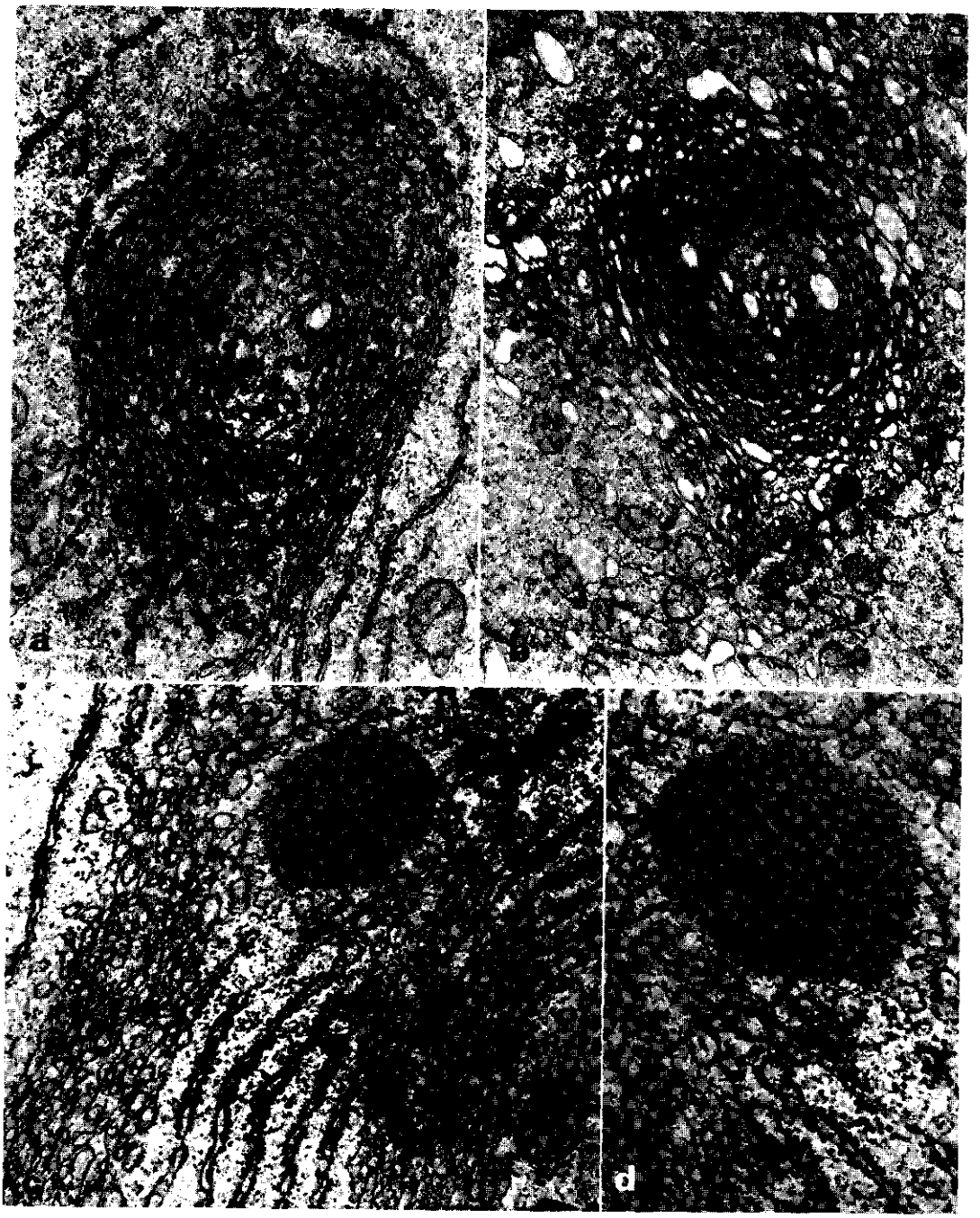

FIG. 47

FIG. 48. Corpus allatum.
72-h-old queen larva (24 h in queen cell). Overall picture of active and inactive cell. 6000 ×

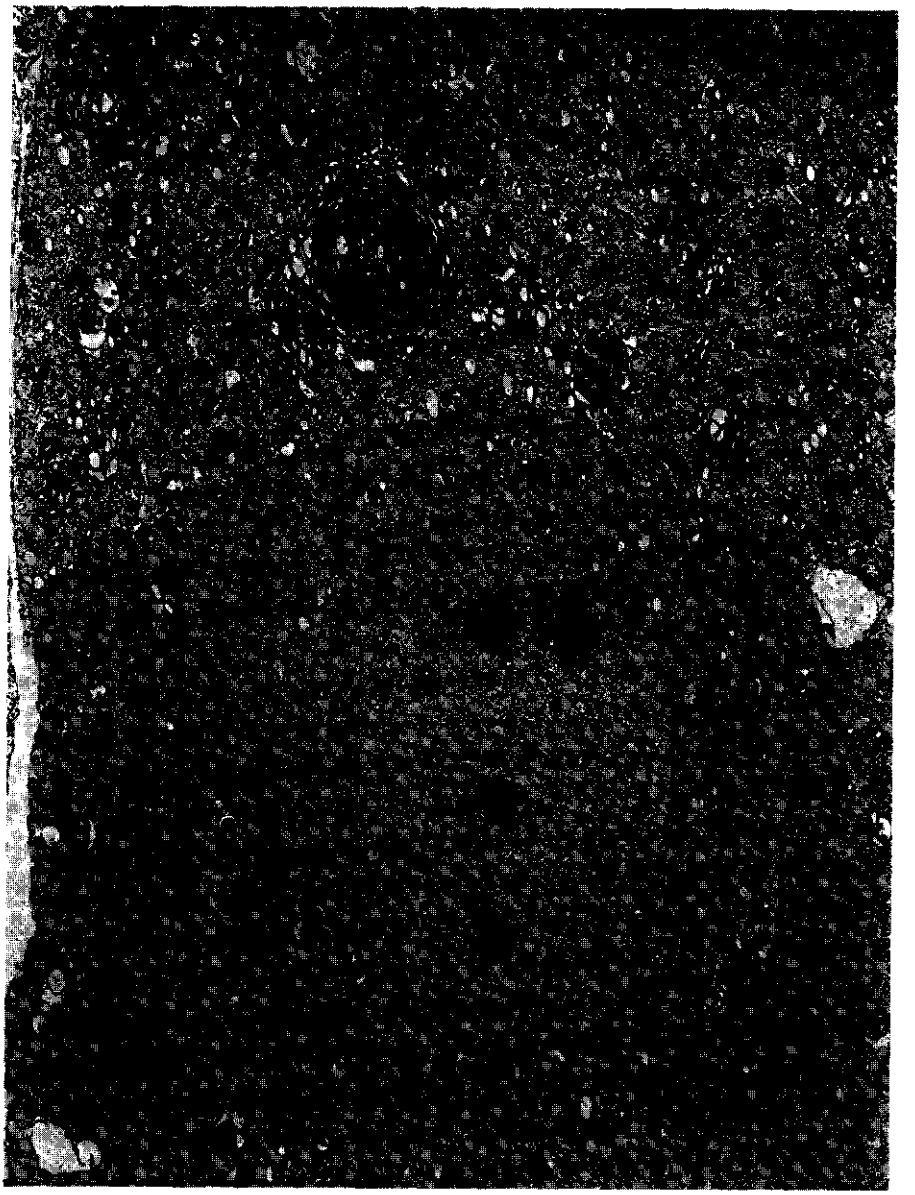

FIG. 48

FIG. 49. Corpus allatum.
84-h-old queen larva (36 h in queen cell). Much SER is seen near the nucleus, excluding most cell organelles. Note dense body in the SER (arrow). 6000 ×

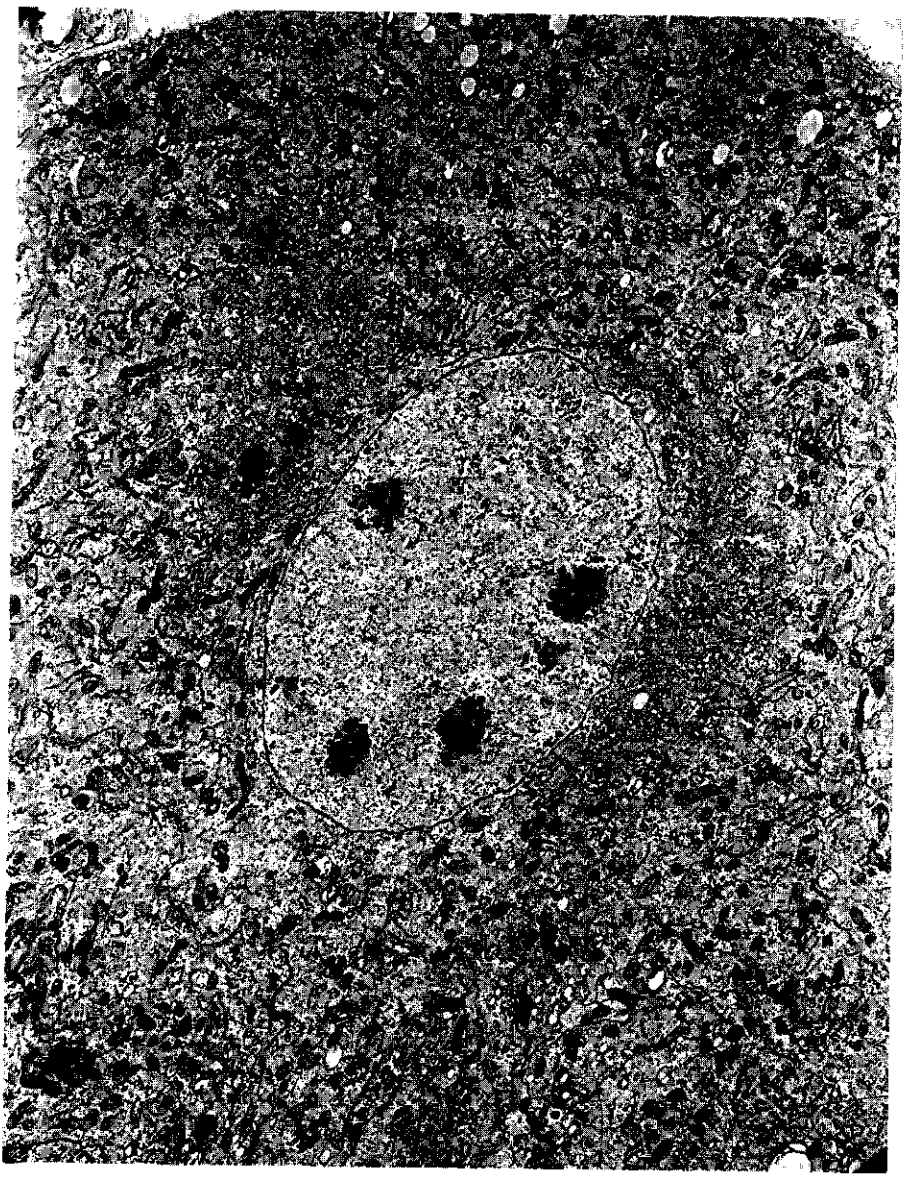

FIG. 49

FIG. 50. Corpus allatum.
a. 84-h-old queen larva (36 h in queen cell). SER area in close detail. 16000 ×
b. Same larva as a. Golgi complexes active; dense body in SER (arrow). 20000 ×

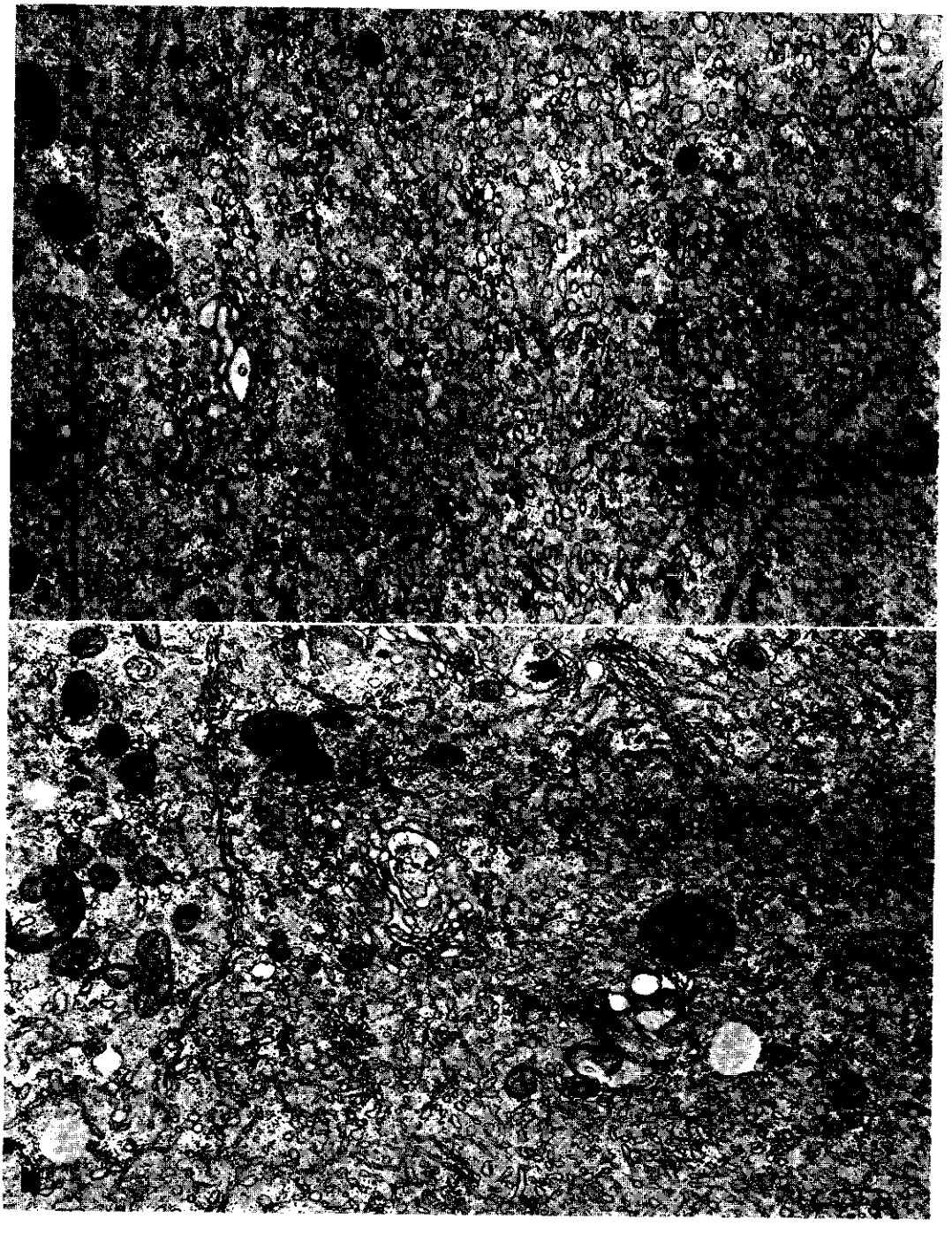

FIG. 50

FIG. 51. Corpus allatum.

a. 96-h-old queen larva (48 h in queen cell). Intercellular spaces dilated. Sometimes membrane bound vacuoles may be seen to fill up the space (arrows). 14000 ×

b. 96-h-old queen larva (48 h in queen cell). Jigsaw-like structure in the SER. 52000 ×

FIG. 51

FIG. 52. Corpus allatum.
a. 147-h-old queen larva (96 h in queen cell). Axon containing NS granules. CA cell containing lysosome-like bodies (L). Note similarity between NS and L granules. 30000 ×
b. Same larva as a. Numerous lysosome-like bodies in the cytoplasm. 16000 ×

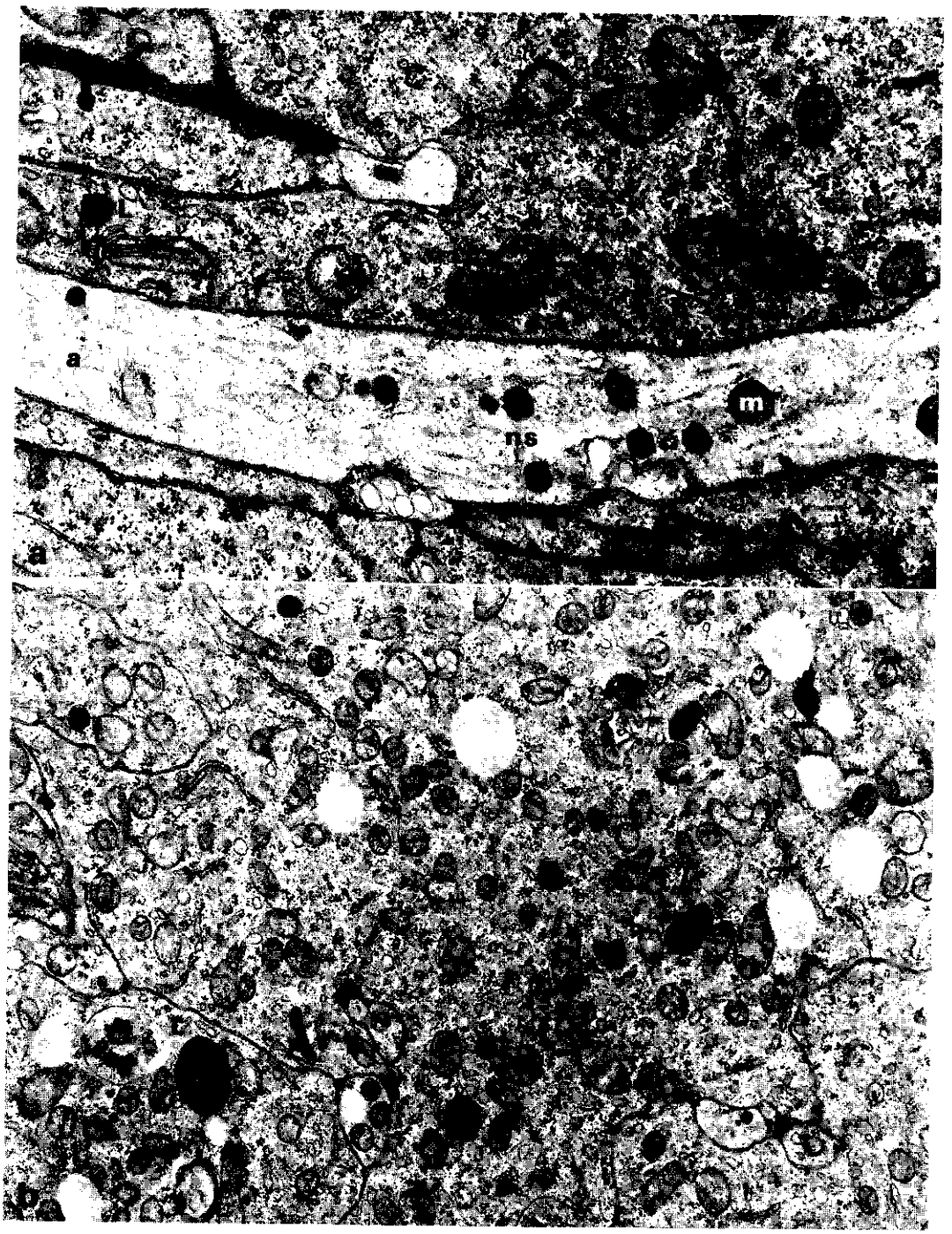

FIG. 52

FIG. 53. Corpus allatum.

a. 171-h-old queen larva (120 h in queen cell). Intercellular spaces dilated. At some places the space is filled up with membrane bound vacuoles. Numerous lysosome-like bodies in the cytoplasm. 22000 ×

b. 171-h-old queen larva (120 h in queen cell). Detail of lysosome-like bodies. 44000 ×

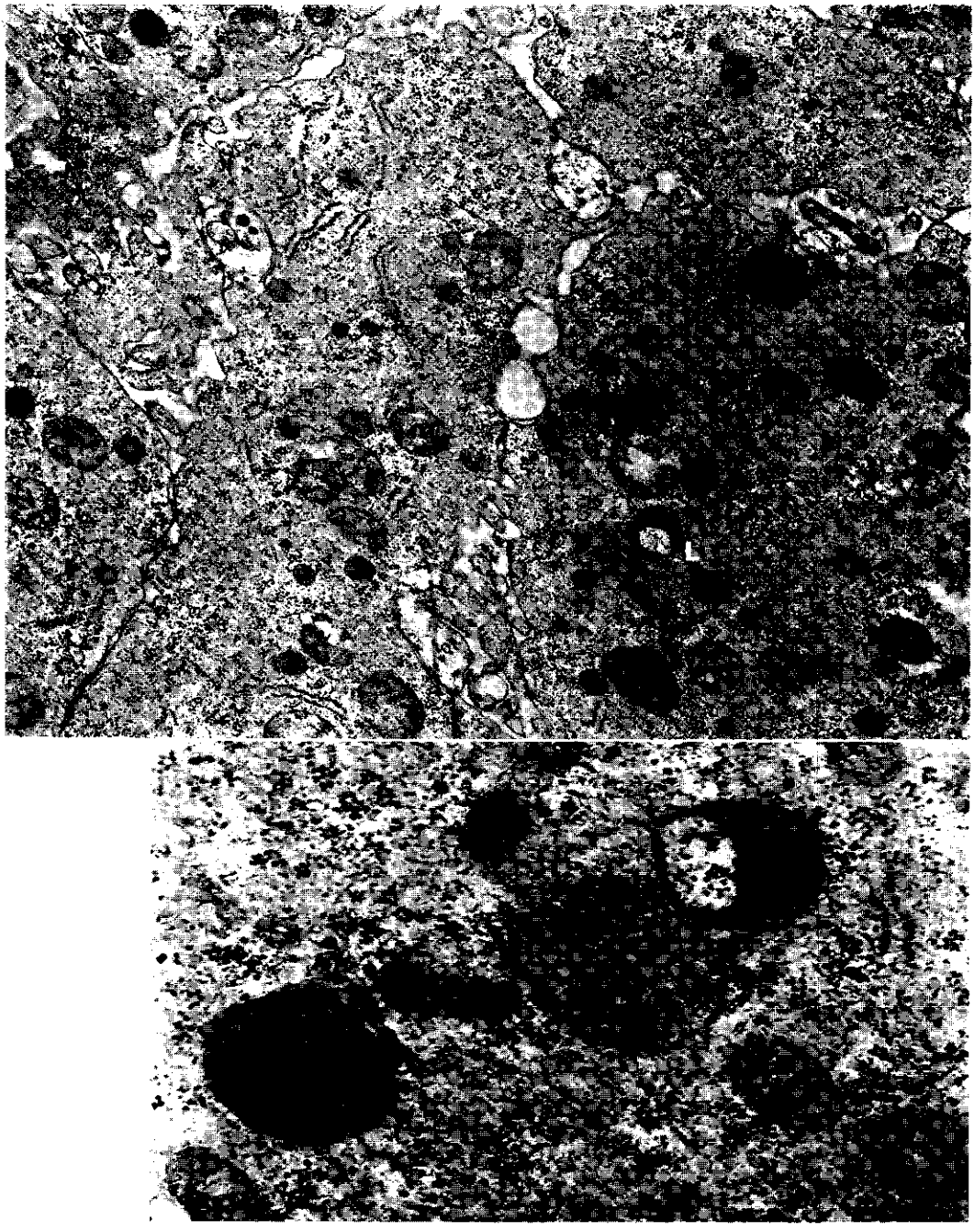

FIG. 53

FIG. 54. Topical application of JH.
a. Head of a normal queen.
b. Heads of queen-like adults from JH treated worker larvae.
c. Head of a normal worker bee.
d. Sting of a normal queen.
e. Sting of a queenlike adult from a JH treated worker larva.
f. Sting of a normal worker bee.

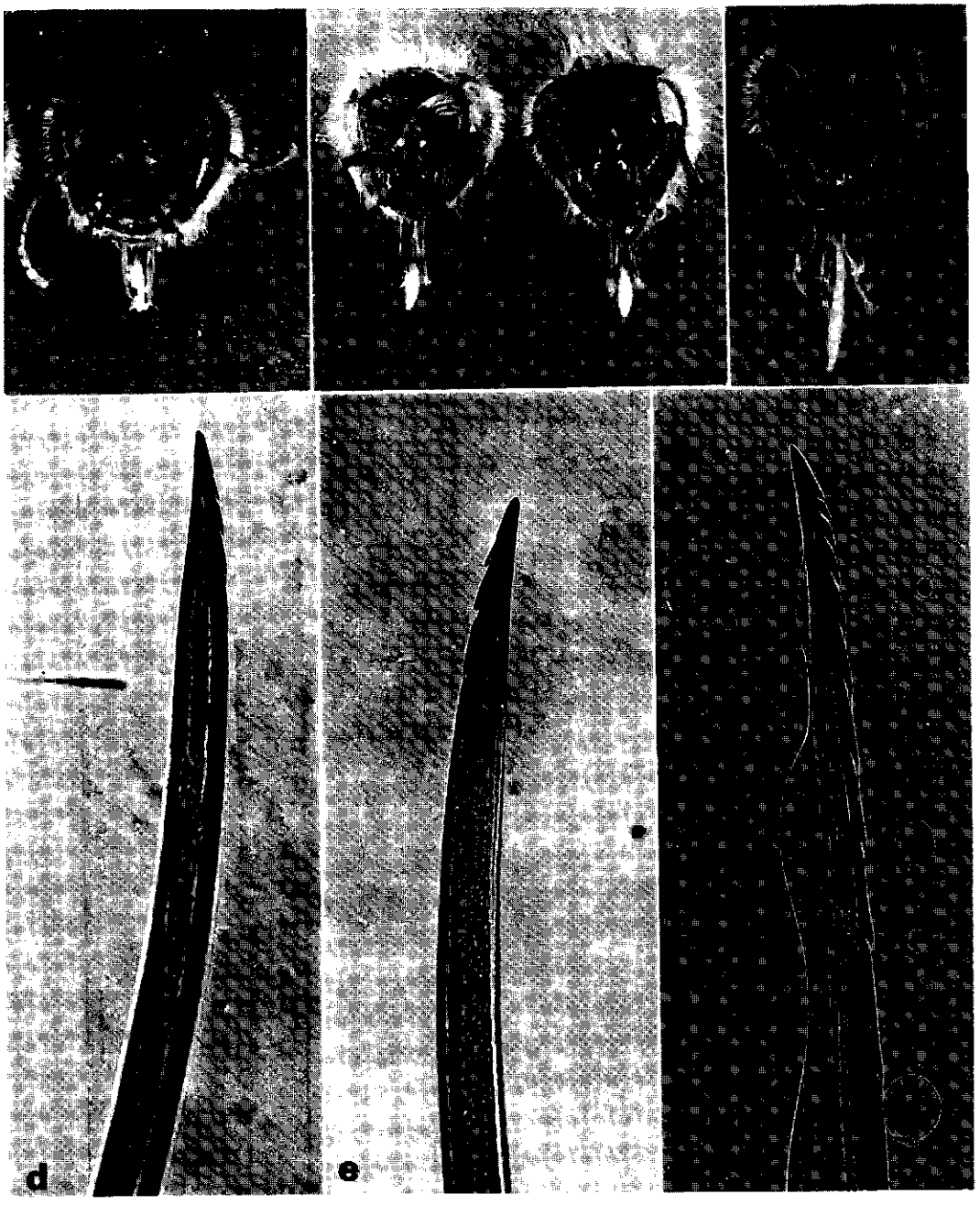

FIG. 54

FIG. 55. Topical application of JH.
a. Mandibles:
left: mandibles of a normal queen.
middel: (two pairs): mandibles of queenlike adults from JH treated worker larvae.
right: mandibles of a normal worker bee.
b. Mandibles of queenlike adults from JH treated worker larvae.
c. Metathoracic legs, outer side.
left: normal queen
middel: queenlike adult from a JH treated worker larva
right: normal worker bee (pollen basket).
d. Metathoracic legs, inner side.
left: normal queen.
middel: queenlike adult from a JH treated worker larva.
right: normal worker bee (pollen comb.).

FIG. 55