

DNA Content and Synthesis in Several Tissues and Variation of Moulting Hormone-Level in *Gryllus bimaculatus* DEG (Ensifera, Insecta)

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The mode of growth of several tissues in *Gryllus bimaculatus* was investigated during postembryonic development by cytophotometric methods. In contrast to the situation in holometabolous insects, the tissues growing by endomitosis reach only moderate levels of polyploidy. In this case the growth of tissues is achieved by mitotic divisions of small cells with subsequent polyploidization.

The time courses of DNA synthesis were measured within the 3rd and, for comparison, the 8th larval instar by incorporation of labelled thymidine followed by autoradiography. Hemocytes, cells of the regeneration crypts of the midgut, gonads and nervous tissue showed a continuous incorporation rate; by contrast, DNA synthesis in other tissues was confined to a given time within the moulting cycle.

The changes in moulting hormone titre of the 3rd larval instar were investigated. The quantities of ecdysone and 20-OH-ecdysone were estimated by radioimmunoassay, and the hormones were identified by high pressure liquid chromatography (HPLC). The titre changes in time with at least 2 distinct maxima. The DNA synthesis periods are correlated with the hormone peaks, that of epidermis and tracheae with the first peak, and that of pylorus, ileum, rectum, Malpighian tubules and fat body with the second. DNA synthesis in prothoracic glands and oenocytes exhibits a time course that is the inverse of the hormone-secretion time course. The question whether moulting hormones have an influence on DNA synthesis is discussed.

Introduction

Growth and development in insects are not continuous processes because of the rigid exoskeleton; they occur in steps, in the course of several moults. Only the laying down of a new exoskeleton and the shedding of the old allow a new spurt of growth. Moults are induced by secretion of the moulting hormone ecdysone, which is produced by prothoracic glands, oenocytes and other not yet identified organs [1–6]. In the early days of hormone physiology [1], a single hormone titre maximum was supposed.

More recent investigations, however, have shown that multiple maxima of the hormone occur within a single moulting period [7, 8]. With the elaboration of new techniques (bioassay, high pressure liquid chromatography, radioimmunoassay) it is now pos-

sible to determine the hormone content of single animals and also the titre course within a moulting cycle.

The occurrence of somatic polyploidy in insects complicates the situation. Growth in this case takes place without interconnected mitosis. One goal of the present investigation was to clarify the mode of growth and the DNA content of variously differentiated tissues during postembryonic development. The other goal was to describe the pattern of DNA synthesis independent of DNA values within well defined stages of development. These were the 3rd and, for comparison, the 8th larval instars.

Another question was to ask whether growth, in this case DNA synthesis, is correlated with the changes of the hormone titre. Several studies have provided good arguments for an influence of the increasing moulting hormone titre on the initiation of DNA-synthesis [9–13]. These studies were done mainly with imaginal disks; only a few papers deal with the influence of moulting hormones on the other tissues of an insect in the course of a moulting cycle [14, 15].

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Materials and Methods

Animals

The crickets were taken from the stock of the Zoological Institute of the University of Mainz. The animals were kept at 30 °C and a relative humidity of 80–90%. Food consisted of rolled oats, dried milk powder and a daily presentation of salad. Under these conditions the 3rd larval instar lasted 75–80 h, the 8th 135–140 h.

The adult period is reached after the 9th instar. For exact age determinations newly hatched larvae were removed from mass cultures at 2-h intervals and kept as singles or in small groups.

Photometry

For determination of DNA values in animals of the different larval instars all tissues available were dissected. As a rule, larvae of the several stages were sacrificed 35 h post molt to avoid the phase of massive DNA synthesis. The tissues were spread out with a micro-scalpel on a slide, air dried and fixed in neutralized formalin (8–10%) for 12 h. The fixation in neutralized formalin resulted in a homogeneous distribution of the DNA; this procedure avoids the inhomogeneous distributions that occur following fixation in Carnoy. Hydrolysis was performed for 8 min in 1 N HCl at 60 °C. Slides were incubated for 2 h in Schiff's reagent, rinsed, dehydrated in the usual way and finally mounted in Caedax having the same refractive index as glass and immersion oil.

Determinations of DNA were performed with a microspectrophotometer (Leitz-Wetzlar). The voltage of the light source was equilibrated by a stabilized low-voltage transformer. Monochromatic light was obtained via an interference filter with a transmission maximum at 550 nm. The magnifying lens system at the input to the photomultiplier was adjusted to give measurement areas with diameters of 1.7, 2.8, 5.1 and 9.9 μm . Thus the DNA content of nuclei having different sizes could be determined.

The measurements were performed on whole nuclei [16, 17]. This was possible after fixation in neutralized formalin giving a homogeneous Feulgen reaction. Small variations in the staining intensity within several batches were corrected by normalization with respect to diploid ganglion cells. About 50 nuclei of each tissues and larval instar were measured.

The DNA content, measured by microspectrophotometry, is given in *c* values (e.g. 2 *c*, 4 *c*, 8 *c*...) by contrast with the chromosome number of polyploid cells which is counted exactly and expressed in *n* (e.g. 2 *n*, 4 *n*, 8 *n*...). It is assumed that cells having *c* values of 4 or more are polyploid. The possibility that these cells are resting in G_2 is only given for 4 *c* cells.

Autoradiography

The rate of DNA synthesis was worked out mainly within the 3rd instar. For comparison with these results animals of the 8th larval instar were also tested. [^3H]thymidine (Amersham-Buchler, spec. activity 15–30000 mCi/mmol) was used as a precursor. Injection intervals of 5 h were employed during the 3rd larval instar; each specimen received 1/3 μCi thymidine. The injection in the 3rd instar larvae was performed with a Leitz micromanipulator. The injection intervals within the 8th instar were 24 h, the amount of label being about 1 μCi /animal. The tracer was allowed to be incorporated for 30 min. Thereafter the specimen were fixed in Bouin-Dubosq-Brasil for 24 h, rinsed in 70% ethanol, embedded in paraffin and cut in sections 6 μm thick. Preparation of slides was done as proposed by Ruthmann [18]. After the sections were mounted on slides they were covered by Kodak AR 10 stripping film and exposed in darkness for 4 weeks. The development was performed with Kodak X-ray developer D19b. The autoradiographs were stained in a solution of toluidine blue (0.25%) or in Ehrlich's acid hemalum. The label-index was obtained by counting 1000 cells per tissue. In those tissues having less than 1000 cells (corpora cardiaca, corpora allata, oenocytes) the index was normalized so as to be comparable to the 1000 cell counts. 5 animals per age group were injected.

Hormone-titre estimation

Larvae of the 3rd instar were homogenized in quartz sand and extracted by perchloric acid [19]. Specimens for hormone analysis were taken at 3-h intervals throughout the moulting cycle. Successive pairs of samples were averaged to give 6-h hormone-titre estimates. Extracts with a reduced volume were given on precoated silica gel plates (Merck-Darmstadt). 1/3 was cochromatographed with authentic ecdysone and 20-OH-ecdysone. The other 2/3 of the plates were used to determine the

amount of these ecdysteroids. Thus a clear-cut separation of ecdysone and 20-OH-ecdysone was possible. The eluates of the distinct regions were estimated in a radioimmunoassay [20]. 23, 24, ^3H -labelled ecdysone was a gift of the Deutsche Forschungsgemeinschaft by Prof. Karlson, Marburg.

High-pressure-liquid-chromatography

Some samples of the 3rd instar larvae were tested by HPLC to confirm the identity of the extracts with authentic ecdysone and 20-OH-ecdysone. The larvae were homogenized in quartz sand with 70% aqueous methanol. The purification of the extracts was performed as proposed in [21]. The final extracts were analyzed by an equipment of Pye-Unicam (pump LC-XPS, flow rate 1.0 ml/min, pressure ca. 2000 psi, 50% methanol, isocratic conditions, column: μ -Bondapak C_{18} Waters Ass., wave length of the UV-spectrometer 242 nm).

Results

Postembryonic growth of several tissues

A survey of the development of polyploidy patterns in several tissues is given in Fig. 1.

Nervous system: The great majority of the neurons remain diploid. Only some giant neurons achieve higher degrees of polyploidy. Hence the mode of growth is mitotic.

Epidermis: Great parts of the epidermis have large inclusions of pigment. As far as DNA estimations could be made, the bulk of the cells measured 2 *c* or, immediately before the mitotic period, 4 *c*. The development of the polyploidy pattern in bristles was not investigated.

Oenocytes: The oenocytes of *Gryllus bimaculatus* are restricted to the abdomen. The great majority are found in the neural sinus near the ventral cord. Single cells or groups of them are surrounded by fat cells. Some of the oenocytes are also found in the parietal fat body of the visceral sinus. Immediately after leaving the egg, the oenocytes show a differentiated pattern of polyploidy extending from 4–32 *c*. From the beginning of the 3rd larval instar to the adult a shift of the polyploidy spectrum to 16–128 *c* is observed. To produce a vigorous increase of the larval body size within this period, new oenocytes must be differentiated. They come from the pleura near the stigmata and are outgrowing from the formation of epidermal cells. A

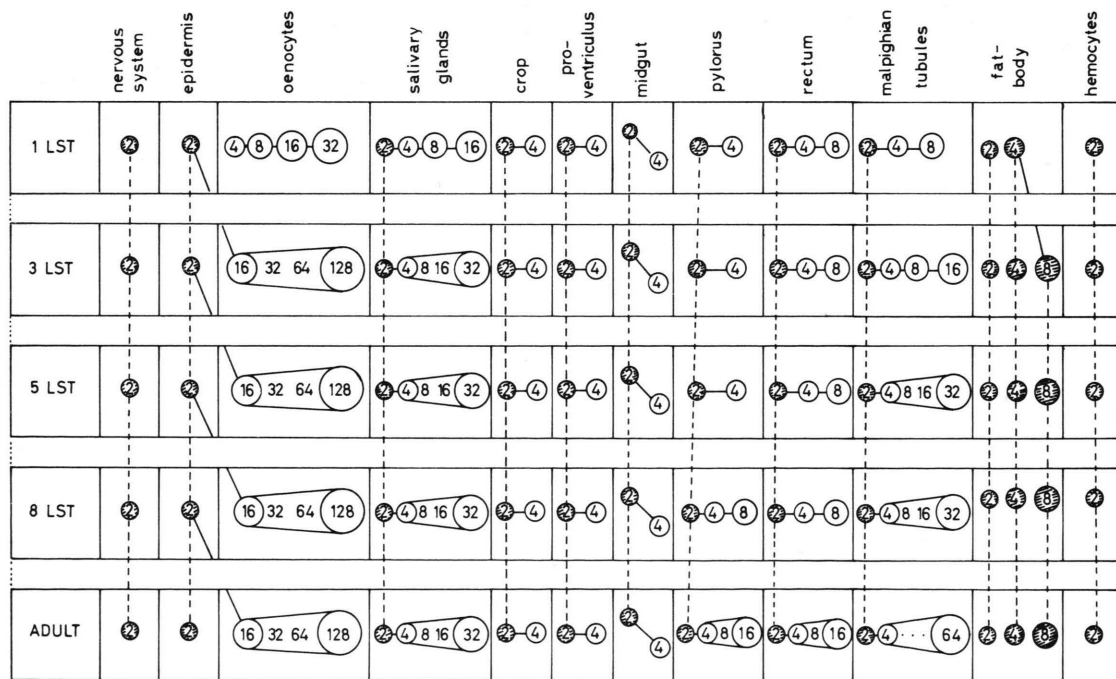


Fig. 1. Polyploidy spectra of several tissues in *Gryllus bimaculatus* within the postembryonic development. Dark circles represent mitotic cells, they are connected by broken lines. Open circles represent cells growing by endomitosis. Numbers in circles show the DNA content. 2 *c*, DNA content of a diploid cell.

series of DNA synthesis steps must have taken place, before they are recognized as clearly differentiated oenocytes.

Salivary glands: Like the oenocytes, the salivary glands show a broad spectrum of polyploidy levels in the range 2–16 *c*. In the course of postembryonic development the range is enlarged only to 32 *c*. The growth of the salivary glands starts from small diploid cells which increase their DNA content.

Crop: Most of the nuclei in the crop are diploid. In young larvae 4 *c* nuclei are found occasionally. The main growth of the crop involves diploid cells that divide by mitosis.

Proventriculus: DNA values in the proventriculus are nearly the same as those found in the crop.

Midintestine: In differentiated epithelia of the midgut, cell divisions are not found. All nuclei have a DNA content of 4 *c*. New cells are produced in regeneration crypts. These cells have DNA values of 2 *c*. The differentiation of new functional epithelial cells involves one step of DNA synthesis not followed by a mitosis.

Pylorus: At the beginning of the larval period two DNA classes (2 *c* and 4 *c*) are observed. This pattern is extended only in older larval stages, reaching 2–16 *c* in adults. The ability to undergo mitosis is confined to 2 *c* cells.

Rectum: A polyploidy pattern ranging from 2–8 *c* is measured at the beginning of larval development. It is extended only in adults to 16 *c*. It must be pointed out that the cells of the rectal papillae do not show mitotic stages, and that growth and differentiation of new cells takes place in regions lying at the edges or between the papillae.

Malpighian tubules: These show a broad pattern of polyploidy ranging from 2–8 *c* at the beginning of the larval period, and from 2–64 *c* at the end of it. Notice that the tubules have a zone of growth at their distal ends comprising small cells (2 *c*). In the differential parts of the tubules these small cells are not seen. Thus the growth of MT takes place by providing diploid cells with a subsequent period of polyploidization that results in characteristic DNA levels.

Fat body: Shortly after hatching the fat body shows a constant distribution of DNA classes consisting of 2 *c*- and 4 *c*-cells. This pattern is enlarged to 8 *c* in older larval stages. It is a characteristic feature of fat body that polyploid cells undergo cell divisions during a mitotic period within each larval

instar. Two S-phases must be performed within one instar requiring two cell divisions in sequence.

Hemocytes are diploid (2 *c*) throughout development.

Endocrine system: The changes of polyploidy of prothoracic glands, corpora allata and corpora cardiaca were not investigated for the entire postembryonic development. DNA measurements were only made for the last-but-one larval instar and for the adult. According to these determinations prothoracic glands show a very pronounced pattern (with 2 *c*, 4 *c*, 8 *c*, and 16 *c*), whereas the nuclei of corpora allata show 2 *c* and 4 *c*. In corpora cardiaca of larvae 2-, 4- and 8 *c* were found. In the adult however only 2 *c* was observed. Therefore, with the change to the adult period, the large cells in corpora cardiaca seem to degenerate.

DNA content in *muscles* and *trachea* was difficult to estimate as the nuclei of these tissues are strongly flattened. Because of the small size of these cells the DNA content will, as a rule, not exceed 2 *c* or 4 *c*.

Titre course of ecdysteroids within the 3rd larval instar

Ecdysone: Immediately after ecdysis the concentration of ecdysone in the whole animal is about 200 ng/g (Fig. 2). It remains at this level until 20 h post ecdysis and rises then to the 1st maximum. This first maximum lasts only for 5–10 h. The elevation to the second maximum occurs at the age of 40 h. In animals 55 h old the titre falls back to 100–200 ng/g and does not change until the end of the 3rd instar. The greatest value observed was 1400 ng/g at the age of 42 h.

20-OH-ecdysone: At the beginning of the 3rd larval instar an average of 430 ng/g 20-OH-ecdysone was found. The single values show a rather large variation. 10–20 h post ecdysis they reach 200 ng/g and rise to the first maximum between 20 and 30 h. During the next 10 h the titre falls and then rises to the main maximum between 40 and 60 h post ecdysis. The majority of the values lie near 1500 ng/g. Concentrations that exceed 2000 ng/g are rare. After the reduction of the concentration in larvae older than 60 h a slight elevation of the content of 20-OH-ecdysone is observed. Most characteristic of the titre course is the baseline value of about 200 ng/g to which the titre returns towards the end of the 3rd larval instar only for ecdysone. In particular, note that changes of the titre course of

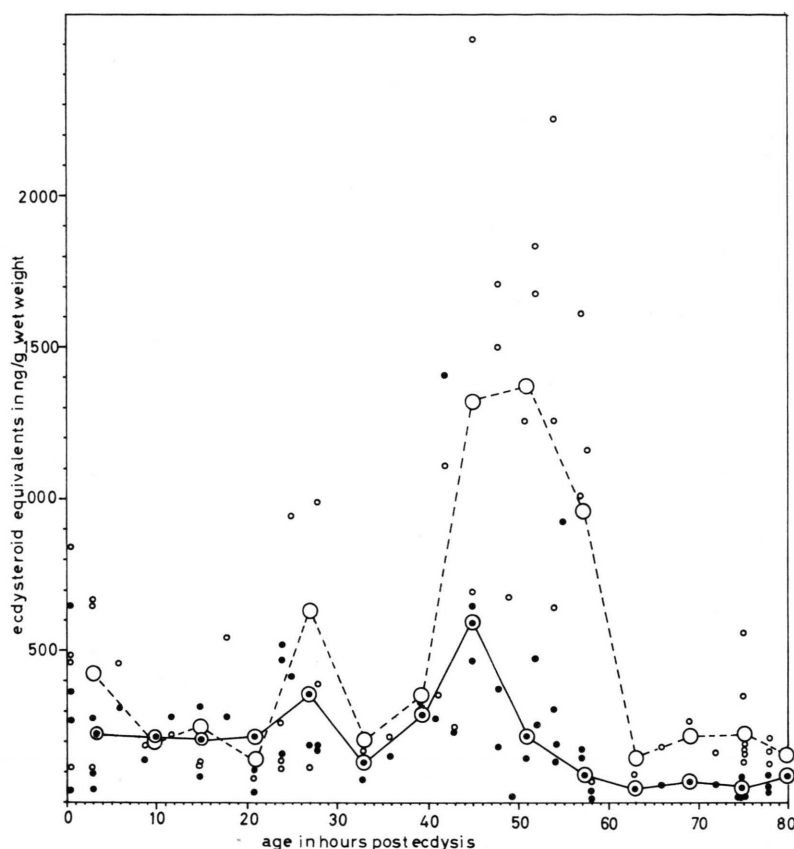


Fig. 2. Changes of the moulting hormone titre within the 3rd larval instar of *Gryllus bimaculatus*. Dark circles, ecdysone, open circles 20-OH-ecdysone. Little circles, single determinations, great circles, average of 6 h intervals.

20-OH-ecdysone are preceded by changes in ecdysone.

In comparison to the 3rd larval instar the titre course of the 8th instar shows 3 maxima; the first at 20 h, the 2nd between 60 and 70 h and the 3rd between 90 and 120 h post moul. The titre course of the 8th larval instar will be published in detail elsewhere.

The hormones isolated were further identified by HPLC. At the time of hormone titre maximum (56 h post moul) a distinct peak of 20-OH-ecdysone is to observe (Fig. 3). Ecdysone could not be found at this time in larger quantities; this is in good agreement with the findings of the hormone estimation by radioimmunoassay (Fig. 2).

DNA synthesis based on autoradiography (Fig. 4a-d)

Epidermis: Counts of ^3H -labelled nuclei were done from tissue of the front part of the abdomen. The incorporation rate is low within the first

10–15 h of the moulting cycle. The main DNA synthesis starts near 30 h and is terminated near 40 h. Although there are some replicating nuclei in older larvae their number is less than 1%.

Oenocytes: The oenocytes are cells derived from the ectoderm. In *Gryllus* new cells are differentiated within each larval instar. In our observations, this happens within the second part of each moulting cycle. Immediately after ecdysis the greatest percentage of synthesizing cells is found (more than 30%). The DNA synthesis continues until 35 h. The labelled nuclei are observed in regions having only differentiated cells. Obviously they contribute the higher levels of the polyploidy pattern. The low replication rate in larvae older than 40 h is confined to cells that are differentiated at this moment (Fig. 6b).

Prothoracic glands: With respect to DNA synthesis, the prothoracic glands differ from other tissues in that they begin to synthesize DNA at the

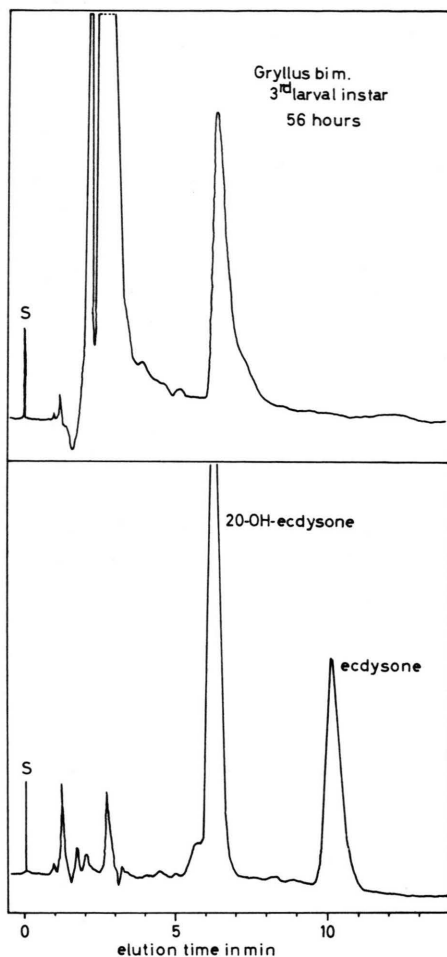


Fig. 3. HPLC of larvae 56 h old, 20-OH-ecdysone is the predominant component. At this time of the moulting cycle nearly no ecdysone is apparent. Lower part of the diagram: Elution times of authentic ecdysone and 20-OH-ecdysone.

end of the larval instar. The synthesis is continued during ecdysis and lasts into the following instar. It is surprising that, within the period of hormone secretion, no thymidine incorporation occurs.

Tracheal system: In the branches of the tracheal system the first nuclei labelled were observed 15 h post ecdysis; the synthesis period lasts until 45 h. In the maximum more than 70% of the cells incorporate thymidine simultaneously; this means that the replication rate within the whole synthesis period is more than 100%. Thus the tracheal system, compared with other tissues, grows more rapidly than the other tissues within the 3rd instar. In com-

parison to DNA synthesis a reduced mitotic index is observed so that some of the cells must grow by endomitosis. In animals older than 45 h only few labelled nuclei were found. The maximum of DNA replication runs parallel to the development of the first moulting hormone-titre peak (Figs. 4a, 6d).

Salivary glands: DNA synthesis in salivary glands occurs nearly within the whole 3rd instar. Maxima are observed at 30 and between 50 and 60 h. They are correlated to the hormone titre course.

Crop and proventriculus: Both have extended DNA synthesis periods in the course of which the crop shows a main incorporation rate between 40 and 50%. The synthesis lasts until 60 h after ecdysis (Fig. 4b).

Pylorus, ileum, rectum: The pylorus, the first part of the proctodaeum, has a clear-cut maximum of synthesis between 45 and 60 h. The number of replicating cells exceeds 50%. The other parts of the hindgut, ileum and rectum, have more enhanced synthesis periods occurring between 40 and 60 h. The main activity in the rectum is not found in the papillae but in the intermediate zones having small, probably diploid cells (Fig. 6c). These intermediate zones seem to be the regions where new cells for the rectal papillae are formed, because mitosis was never found in differentiated papillae. The only purpose of DNA synthesis in the papillae is to enlarge the degree of polyploidy.

Malpighian tubules are very numerous in *Gryllus bimaculatus* and they grow in each larval instar of the postembryonic development. The pattern of polyploidy, as shown above, remains constant. At the distal ends of the tubules there is a proliferation region with very small cells showing DNA synthesis, during the whole instar (Fig. 7d). These little cells are the main regions in which cells and tubules are growing. The differentiated parts of the tubules perform their DNA synthesis between 45 and 65 h (Fig. 7c). At the age of 60 h more than 50% of the cells show thymidine incorporation.

Fat body: With regard to the replication rate, the fat body exhibits temporal patterns similar to those of Malpighian tubules. Main synthesis takes place between 50 and 60 h (Fig. 4c, 7f). Within the remaining periods very few cells are found to be replicating.

Nervous system: As a sample of the nervous system the ganglion of the 8th abdominal segment was investigated. The cells show a continuous incor-

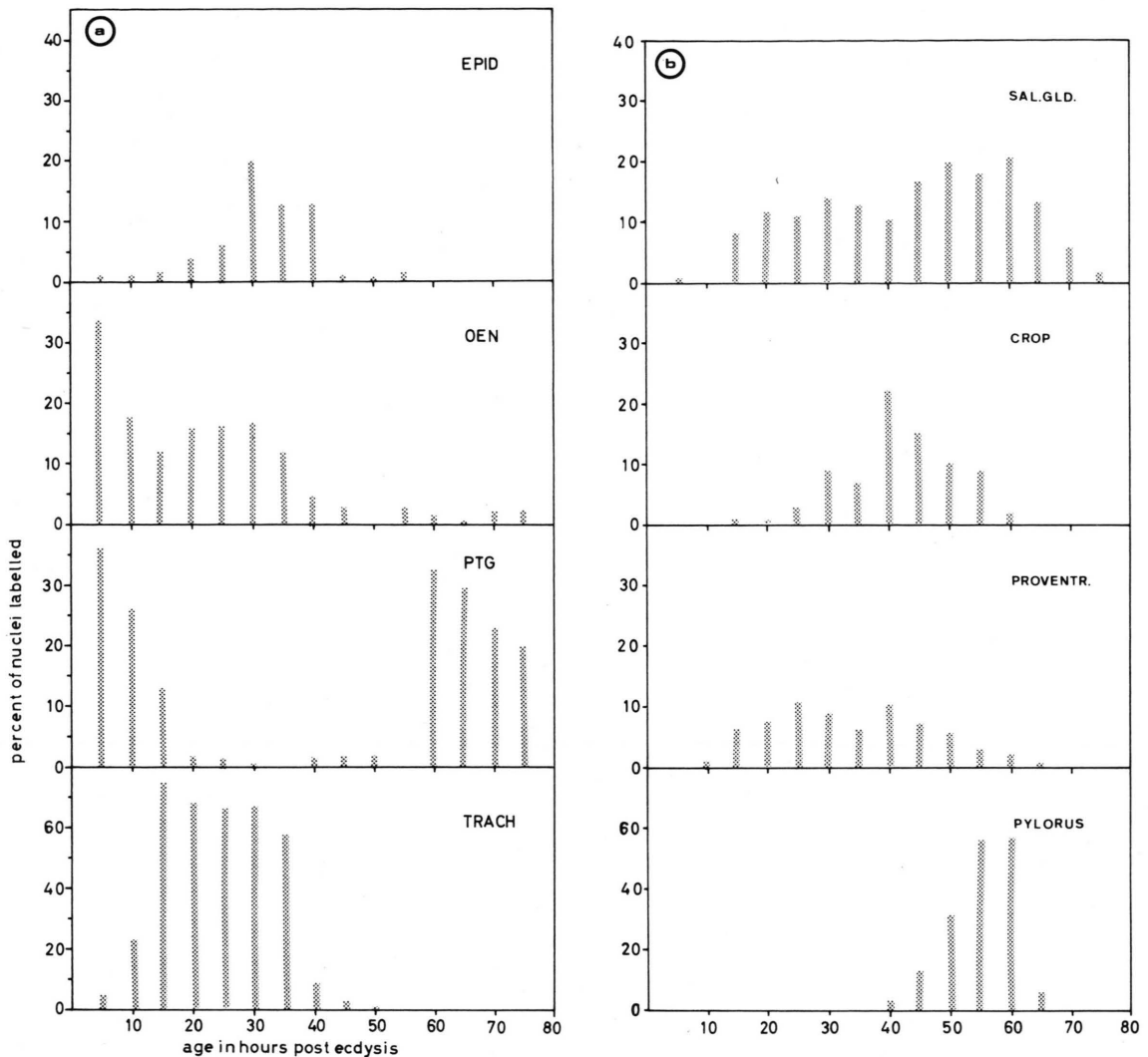


Fig. 4a-d. DNA synthesis based on autoradiography in several tissues of the 3rd instar of *Gryllus bimaculatus*. The values of nuclei labelled are given in percent with exception of ganglion VIII, they are indexed per thousand. Results below 1% are not taken into consideration.

poration of thymidine varying between 1 and 4% (Fig. 4d). Obviously the incorporation rate goes parallel to the variations of the hormone titre. The division capacity seems to be restricted to ganglion stem cells.

Corpora cardiaca: As a result of the low cell number the exact percentage of labelled nuclei could not be determined. However, the autoradiographs show that this tissue type synthesizes DNA throughout nearly the whole moulting cycle, with a maximum between 15 and 25 h. Animals 30 and

75 h old did not show any label. Within the second titre maximum an elevated DNA incorporation rate was not found.

Corpora allata: Within the first half of the 3rd larval instar no mitotic activity was observed. DNA replication shows elevated values at the end and, surprisingly, at the beginning of the instar (cf. prothoracic glands).

Muscles: The mode of replication was investigated in muscles of the prothorax and in those of the proventriculus. Exact counts are not possible

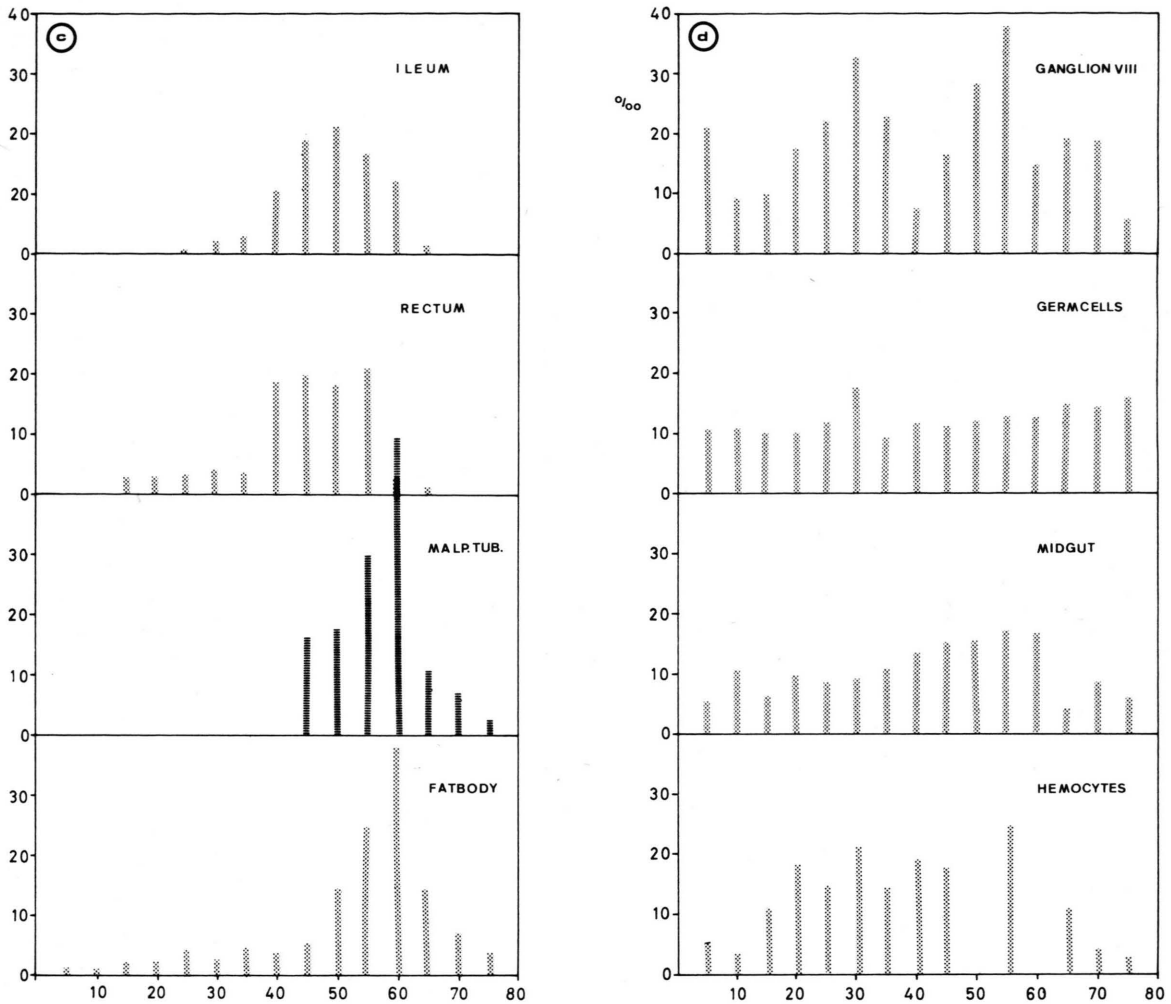


Fig. 4c, d

because the nuclei are very distorted. Therefore only rough estimates were obtained. DNA synthesis in muscles of the prothorax was observed between 20 and 55 h. In muscles of the proventriculus these are restricted to a period between 40 and 50 h. Consequently DNA synthesis in muscles can be co-ordinated only with the 2nd hormone titre maximum.

Hemocytes: The rate of DNA synthesis was measured only in peripheral cells. The incorporation rate within the hematopoietic organ was not considered. A typical feature of hemocytes is their continuous DNA incorporation. At the beginning and at the end of the moulting cycle, the incorporation rate is reduced. It shows slight elevations between 20

and 30, and between 40 and 60 h (Fig. 7b). Altogether, two little maxima are superimposed to a constant level. These maxima are correlated with the rising hormone titre.

DNA synthesis within the 8th instar

Because of the large body size of 8th instar larvae, only cross sections of the abdomen were investigated. Therefore the number of tissue types in which DNA synthesis was measured is smaller (Fig. 5). The time intervals between successive injections were 24 h.

The time pattern of DNA synthesis in epidermis and in trachea are the same as in the 3rd instar.

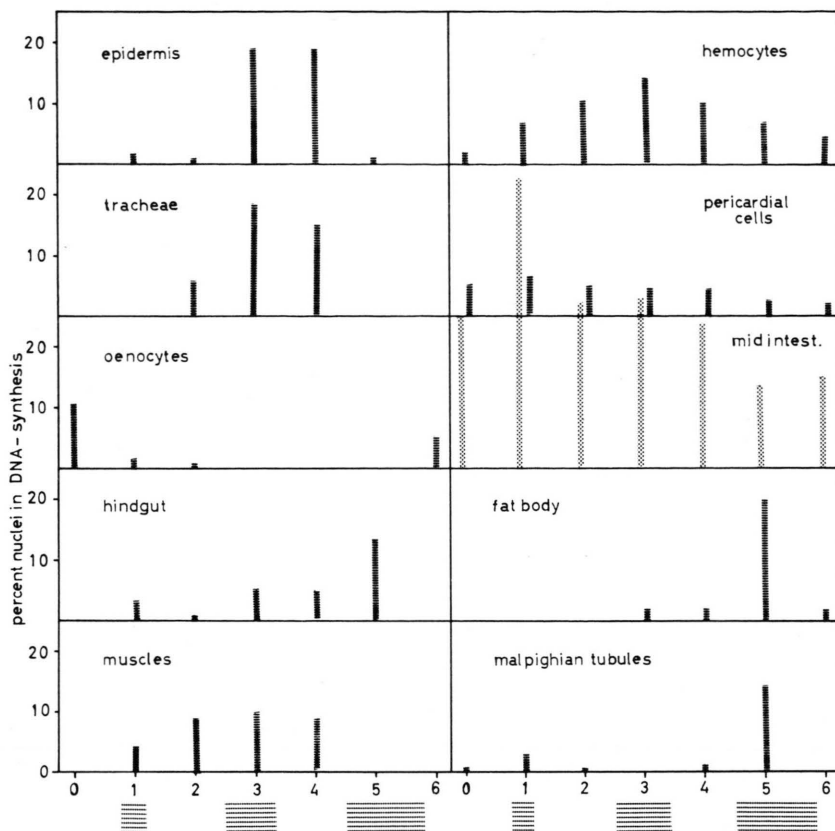


Fig. 5. DNA synthesis spectra of several tissues of the 8th larval instar. Dark bars beyond the abscissa represent the times of elevated hormone levels.

They are correlated with the hormone titre maximum at day 3. The maxima of DNA incorporation in hindgut, fat body and Malpighian tubules are correlated with the main hormone maximum at day 5. The oenocytes show the same pattern as was found within the 3rd instar. Hemocytes, regeneration crypts of the midgut and pericardial cells were labelled during the whole instar. Regeneration cells of the midgut showed a maximum on day 1. With the exception of regeneration cells of the midgut, the index of labelled cells does not exceed 20%. This means, that the rate of growth in tissues as a whole is smaller in the 8th than in the 3rd instar.

Discussion

Polyploidy patterns in Gryllus in comparison with other insects

Polyploidization by endomitosis was discovered in *Gerris lateralis* [22]. In insects, however, all tissues do

not follow this mode of growth. On the contrary, one can distinguish several types:

- 1) mitotic growth
- 2) endomitotic growth
- 3) combinative growth (the latter was called "fakultativ endomitotisches Wachstum" by Risler). In this mode, mitotic and endomitotic growth are observed in the same tissue and/or in the same cell.

To date, systematic investigations, including those of postembryonic development, have been made in Lepidoptera [24], Hymenoptera [16, 23], Nematocera [25], Coleoptera [17], Hemiptera [26], and Brachycera [22]. These investigations showed that the growth of most tissues starts with a low polyploidy class. The latter is extended in the course of each molt by 1 to several steps. Either a great part of the tissues undergoes disintegration during metamorphosis (Hymenoptera, Lepidoptera and Diptera) or they are

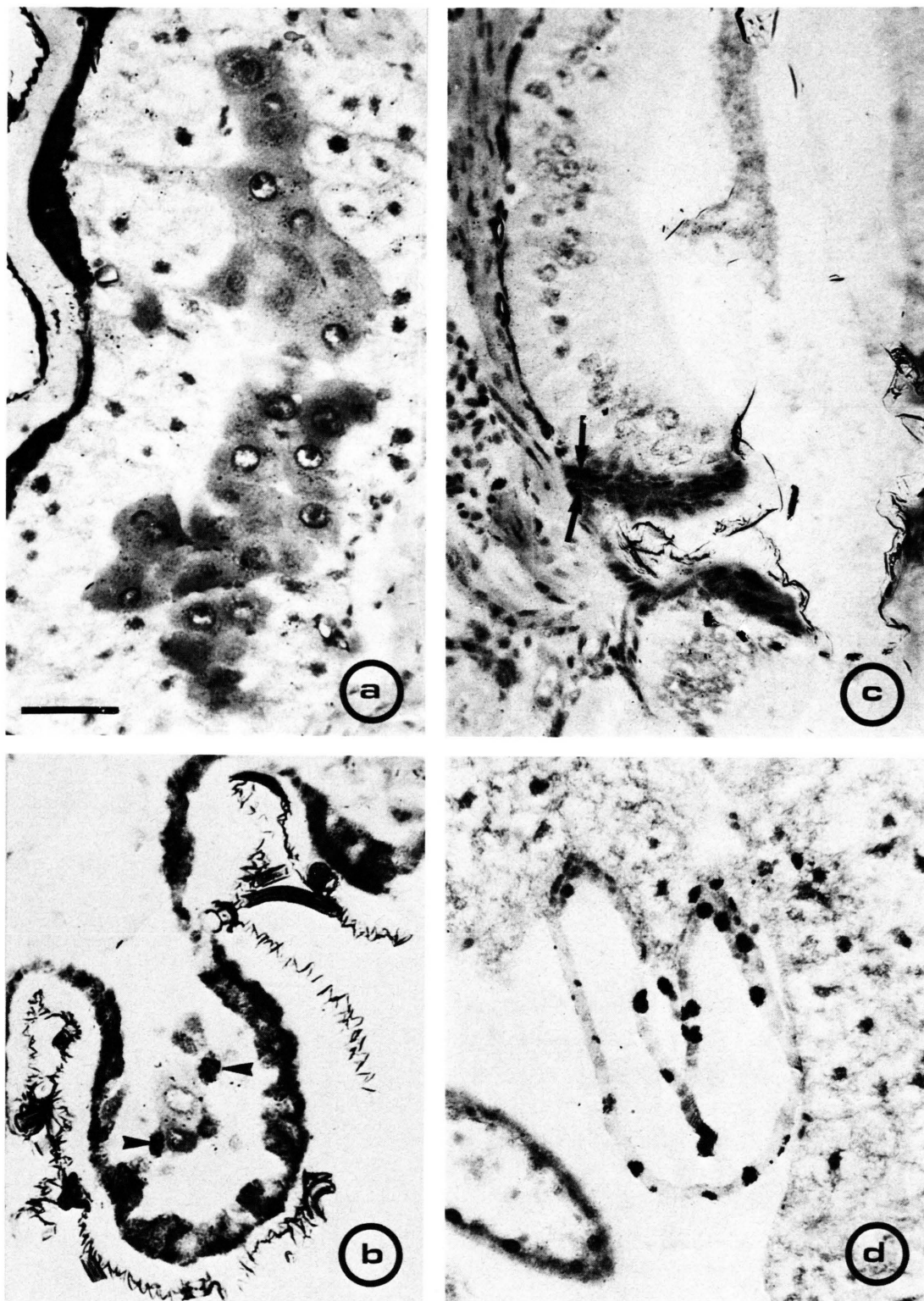


Fig. 6. a) Group of differentiated oenocytes of an 8 instar larva; b) oenocytes that are on the way of differentiation near the pleura, where they come from; they are still very small and show DNA synthesis (arrows); dark parts in the epidermis are due to pigmentation; c) rectal papilla, differentiated cells show no label, whereas some cells at the edge of the papilla are in DNA synthesis (arrow); d) massive DNA incorporation into cells of the tracheal system. Barr indicates 50 μ m.

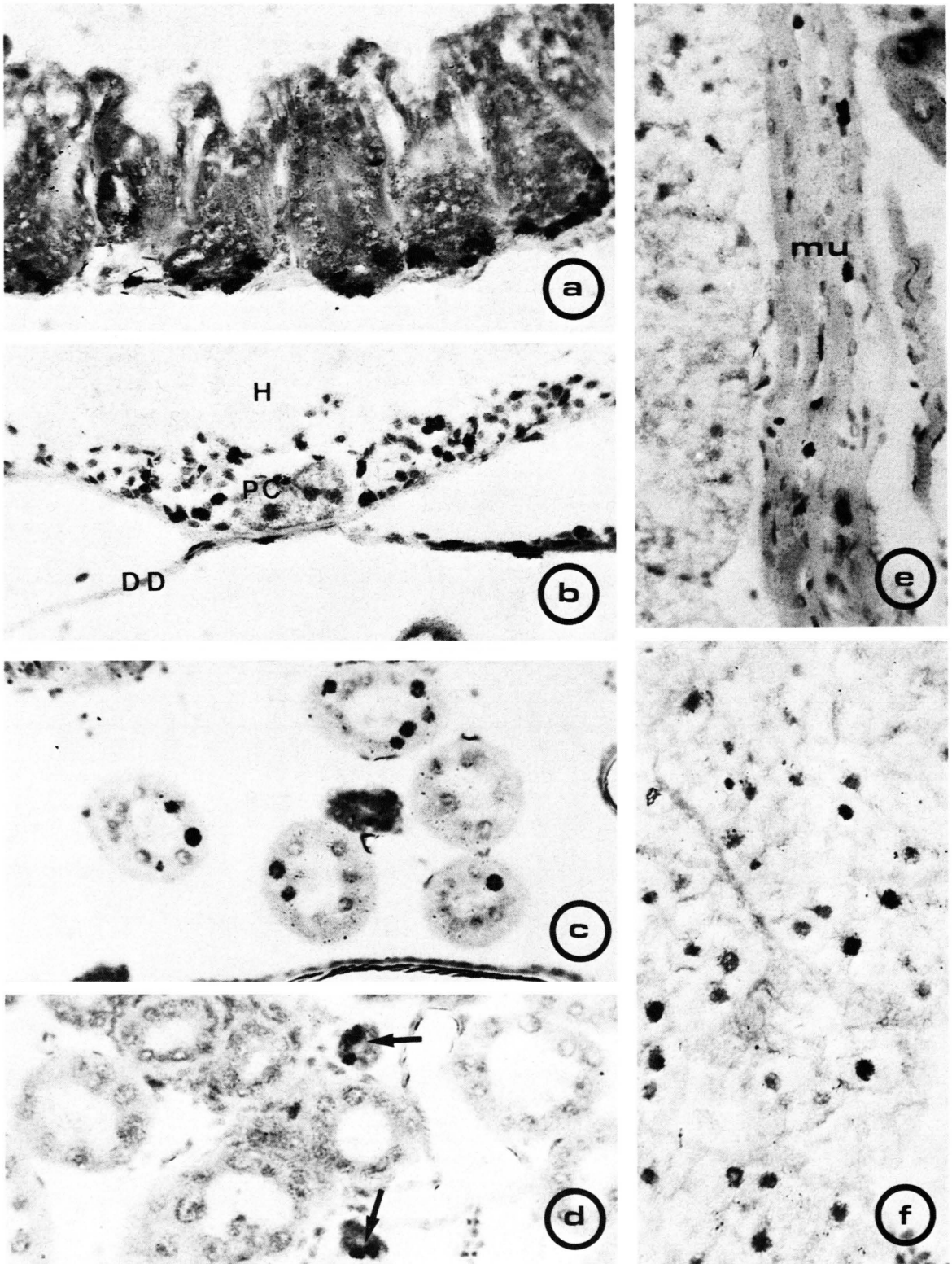


Fig. 7. a) Cross section of the midgut. Incorporation of thymidine only into cells of regeneration crypts. b) Cross section of the heart. A lot of hemocytes are labelled. PC, pericardial cells, DD, dorsal diaphragm; c) DNA synthesis in differentiated parts of the Malpighian tubules; d) DNA synthesis only in growing zones of Malpighian tubules. None of the cells in the differentiated parts is labelled; e) dorsoventral abdominal muscle with synthesizing nuclei; f) fat body in DNA synthesis.

transferred to the adult with maintenance of polyploidy level attained (Coleoptera).

In *Gryllus*, which is a typical hemimetabolous insect, the mode of growth is different from that in the other insects mentioned. First, at the end of embryonic development some tissues have already a distinct pattern of polyploid cells (e.g., oenocytes, salivary glands, midintestine, rectum Malpighian tubules, fat body). Comparable findings were made in Heteroptera [28] and Hymenoptera [29, 30]. In other tissues most cells are diploid and show mitotic growth (epidermis, nervous system, regenerative crypts and hemocytes). In oenocytes, salivary glands, crop, proventriculus, pylorus and Malpighian tubules, there is – in addition to the diploid cells – a pattern of polyploid cells typical of each larval instar. In contrast to the situation in other insects investigated, endopolyploid cells do not elevate their degree of polyploidy with each moult; rather a polyploidy pattern, once established, is maintained by adjusting cells having a lower degree of polyploidy. In other words, the growth necessary for an enlargement of volume within a tissue begins with diploid cells that reach their degree of polyploidy typical of this tissue.

Mode of growth and pattern of polyploidy in the fat body of *Gryllus* are similar to those in Coleoptera [17]. 3 polyploidy classes are observed, unaltered from the 3rd larval instar to the adult; they undergo cell divisions in each instar at rather restricted periods.

The polyploidy patterns of adults of *Gryllus* are nearly identical to those of older larvae. In contrast to observations in the holometabolic insects (Hymenoptera, Lepidoptera, Diptera) the majority of the cells are transferred to the adult.

In *Schistocerca gregaria* the mode of growth of epidermis, fat body and Malpighian tubules was investigated [31]. In this species, closely related to *Gryllus*, the epidermal cells normally showed 2c, some of them being in G₂-phase 4c. The DNA content in fat body cells, mainly 4c, was interpreted as a result of cells resting in G₂-phase. Immediately after mitosis they pass a new S-phase. A similar observation was made in the epidermis of *Tenebrio* [32]. As far as *Gryllus* is concerned, we believe that in the fat body there is a true pattern of polyploid cells. They are still able to divide, having a long G₁- and a short G₂-phase. The interval between DNA synthesis and mitosis nevertheless amounts to some hours. But S-phase does not take place immediately after mitosis.

Changes of the moulting hormone titre and DNA synthesis

For small animals like those of the 3rd larval instar the content of moulting hormones is readily estimated with the technique of the radioimmunoassay. A most striking feature of the titre course is the high base-line level, which does not go below 200 ng/g. This base-line may be due to the extraction by perchloric acid [19]. In other hormone determinations the extraction was made with 70% methanol. In other investigations with other species 3 maxima in moulting hormone titre were found [7, 8]. In the present study 2 maxima, the first between 20 and 30, the second between 40 and 60 h were detected. Perhaps the short duration of the 3rd instar and the variation of the values measured near the beginning of the instar obscure the first peak, which was found in the 8th larval instar.

For the interpretation of moult initiation it is necessary to point out that ecdysone occurs always before the maximum of 20-OH-ecdysone. When ecdysone shows its maximum a certain percentage is already converted to 20-OH-ecdysone. An exchange of the two hormones is not possible, because each sample was cochromatographed with authentic ecdysone and 20-OH-ecdysone. An analysis by HPLC of some samples confirmed the results obtained by radioimmunoassay following TLC. This result is in accordance with an investigation of the changes of epidermis, sense organs and moulting hormone titre within the last larval instar in *Gryllus bimaculatus* [33].

A comparison of the periods in which DNA is synthesized during the moulting cycle leads to 3 different categories of the tissues examined:

- tissues not showing any correlations with ecdysone maxima. These are nervous system, the cells of regeneration crypts in the midgut, gonads and hemocytes. The number of synthesizing cells in hemocytes seems to be influenced slightly by the elevation of the hormone titre. Comparable findings were reported for Lepidoptera [14] and Coleoptera [15].
- Tissues showing short periods of synthesis that are related to the several hormone maxima. To this category of cells belong epidermis, tracheae, crop, pylorus, ileum, rectum, the differentiated parts of Malpighian tubules and fat body. Synthesis in epidermis and tracheae is correlated

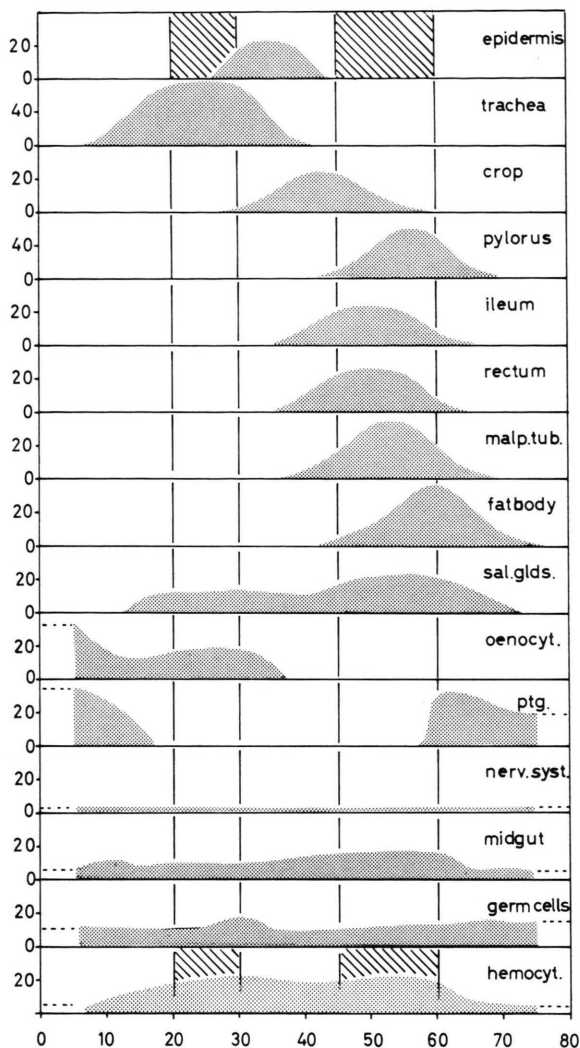


Fig. 8. Survey of DNA synthesis in the 3rd larval instar of *Gryllus bimaculatus* combined with hormone titre maxima (diagonal stripes). Abscissa, age of the animals post ecdysis. Ordinate, number of labelled cells in percent. sal. glands, salivary glands; ptg., prothoracic glands; nerv. syst., ganglion VIII.

with the first hormone maximum between 20 and 30 h post moul. Synthesis in the other tissues mentioned is linked to the second hormone titre maximum. The synthesis in crop however seems to be induced by the first maximum. The responses of the several tissues to the rising hormone titre obviously occur with different delays; the tissues are not synchronized completely (compare ileum and rectum).

c) Tissues that show an inverted time course as compared to the hormone titre course (prothoracic glands and in parts the oenocytes). DNA synthesis in these tissues occurs when the production of moulting hormones is at the lowest level.

In summary a large fraction of the tissues of the insect body show a rhythmic DNA synthesis. The periods of thymidine incorporation are linked in most tissues to the second hormone-titre maximum. The findings reported here do not demonstrate a causal relation but rather a parallelism of two events: the variations of the hormone titre on the one hand and the synthesis of DNA on the other. It would of course be very difficult to show such causal relationships for all tissues of an insect. In some cases however, the conditions for inducing DNA synthesis have been investigated. The results of these papers are partly controversial, and are briefly discussed here.

In cultivated imaginal disks of *Galleria* [9] DNA synthesis could be initiated by ecdysone, whereas 20-OH-ecdysone and 22-isoecdysone had an inhibitory effect. Accordingly, in the present investigation new hormone synthesis should be started by an increasing hormone level. Shortly after ecdysone appears in *Gryllus* a conversion to 20-OH-ecdysone is observed, so that both hormones are present at the same time in the insect body. The inhibition of DNA synthesis by 20-OH-ecdysone found in *Galleria* wing disks, therefore, can not occur in *Gryllus*. Every initiation of DNA synthesis must be blocked within few hours. The findings in *Gryllus* argue against the results reported of *Galleria*.

A stimulating effect of inokosterone (an isomer of 20-OH-ecdysone) on thymidine incorporation was reported in isolated embryonic legs of *Blaberus* [34]. The influence of the hormone lasts for some days and can not be repeated by new gifts of hormone.

A strong dependence of DNA synthesis on the presence of 20-OH-ecdysone is reported for the wing disks in *Antheraea* [12]. Comparable findings are described for the same tissue in *Manduca sexta* [10]. In this case the hormone titre maximum in pupae is followed by an increase in DNA content of whole wing disks. An increase of DNA synthesis is also described in *Calliphora* after prepupae had left their wet environment to migrate in dry sand [35]. The DNA synthesis at this stage is initiated by an injection of 20-OH-ecdysone.

The rate of mitosis in regeneration crypts of *Aeshna* was elevated markedly by application of ecdysone as well as by 20-OH-ecdysone. The frequency of cell divisions is not reduced until the sixth day after injection [13].

A more differentiated pattern of DNA-synthesis induction seems to exist in the epidermis of the last instar in *Manduca* [36]. Two maxima of [³H]thymidine incorporation were observed. The first of the two is not controlled of molting hormones because it occurs when ecdysone is not present, whereas the second is obviously under the control of the first hormone peak. The second and by far greater ecdysone maximum has no influence on DNA synthesis in epidermis. This situation corresponds well with the present findings in the epidermis of *Gryllus*. The second hormone titre peak in *Manduca* is involved in pupal programming or pupal commitment [37].

An interesting parallel to these results in *Manduca* was described in *Bombyx* [38] where two DNA synthesizing periods have also been found. Only the latter of the two is hormone sensitive.

A completely opposite result was obtained in cultivated *K_c*-cell-lines of *Drosophila* [39]. 20-OH-ecdysone in physiological concentrations acts on cells by arresting them in the G₂-phase for several days. No increase of DNA was observed within this period.

In the present investigation the influence of juvenile hormone (JH) was not taken into consideration because the titre of JH is not yet known in *Gryllus bimaculatus*. However, it is of great interest that in the adult fat body of *Schistocerca* polyploidization – and with it, DNA synthesis – is controlled by JH or the JH-analogue ZR 515 [40].

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