Oogenesis in *Tenebrio molitor*: Histological and autoradiographical observations on pupal and adult ovaries

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SUMMARY

(i) Nurse cells and oocytes in the telotrophic ovary of *Tenebrio molitor* L. (Insecta, Coleoptera-Polyphaga) are differentiated in the larval stadium.

(ii) During pupation DNA synthesis occurs in the nurse cells and is probably associated with polyploidy; some of them become multinucleate. The functional significance of these events is interpreted as preparation for subsequent massive RNA synthesis.

(iii) The oviducts incorporate [3H]thymidine and undergo elongation due to mitoses. This ceases at eclosion.

(iv) RNA synthesis in the pupal ovary is low, but increases in the nurse cells and follicle cells just prior to eclosion.

(v) In the adult ovary, once the growth phase has been initiated, a primary oocyte takes about six days to reach maturity.

(vi) The nurse cells, though apparently lacking nucleoli, synthesize much stable RNA which reaches each oocyte via a trophic cord.

(vii) The follicle cells undergo continuous DNA synthesis: some nuclei contain over 64 times the haploid amount of DNA. Replication is probably asynchronous within a nucleus: this may account for the phenomenon of simultaneous DNA and RNA synthesis in the follicle cells, which also lack nucleoli.

(viii) Oogenesis has been divided into nine developmental stages, three of which are vitellogenic.

(ix) The oocyte chromosomes are capable of RNA synthesis both when despiralized during early previtellogenesis and after karyosome formation, which occurs at stage 6.

(x) The protein content of the oocyte appears to have a dual origin: at least part of the ooplasmic proteins *form in situ*; while yolk proteins are derived from the haemolymph. The extra-ovarian protein reaches the oocyte via spaces which develop between the follicle cells.

(xi) The nucleoplasm becomes more heavily labelled with proteins than the ooplasm. With [3H]leucine, methionine and phenylalanine but not with tryptophane or arginine, there is an increased incorporation into the karyosome. It is suggested that this karyosome-associated protein may function in gene masking.

(xii) The significance of these findings is discussed with reference to the literature.

INTRODUCTION

The rapid advances in molecular biology in recent years have increasingly focused the attention of embryologists on the molecular events which underlie development. With an appreciation of the influence of egg constituents and

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their spatial arrangement on the future development of the embryo (Koch &
Heinig, 1968) a growing number of studies have been directed towards oogenesis
in both vertebrates and invertebrates (Raven, 1961; Davidson, 1968; Nørrevang,
1968; King, 1970; Bier, 1970; Mahowald, 1972b). It is becoming increasingly
evident that the clues to differentiation lie in the molecular architecture of the egg
itself.

Different types of development – classically designated as ‘mosaic’ versus
‘regulative’ or ‘determinate’ versus ‘indeterminate’ (concepts discussed by
Sander, 1971) – are reflected in different types of egg structure so that, as
pointed out by Krause & Sander (1962, p. 272), ‘we may expect different types
of oogenesis’.

Whereas the members of most classes of animals conform to one or other
developmental pattern, the insects are unusual in as much as they display the
whole spectrum from the so-called ‘mosaic’ to the ‘regulative’ type of develop-
ment within the class. Because of this natural variation it is important that many
different types of insect oocytes be investigated, if the later developmental
events are to be adequately interpreted. It is remarkable that even in closely
related insect species oogenesis can vary greatly (Urbani & Russo-Caia, 1969;
Urbani, 1970).

Oogenesis in insects occurs in two distinct types of ovary. The recognition of
additional categories is, at present, unjustified (see p. 203). In the panoistic ovary,
characteristic of the Exopterygota, the only nutritive tissue present is the folli-
cular epithelium which surrounds each oocyte. The metabolic activity of the
latter is similar to the much studied amphibian oocyte (Davidson, 1968):

\[ \textit{it develops lampbrush chromosomes during an extended diplotene (Kunz,}
\]
\[ \textit{1967b), synthesizes much RNA (Bier, Kunz & Ribbert, 1967) and possesses}
\]
\[ \textit{multiple nucleoli which reflect amplification of the ribosomal cistrons (Kunz,}
\]
\[ \textit{1969).}
\]

In the meroistic ovary, characteristic of the Endopterygota, the oocyte is
associated beside the follicular epithelium with a group of nurse cells or tropho-
cytes, the products of oogonal mitoses (King, 1970). There occurs a metabolic
division of labour, the synthetic functions of the oocyte being largely taken over
by the nurse cells, which produce RNA for transport to the ooplasm (Bier,
1963a, b, 1964; Pollack & Telfer, 1969; King, 1970).

The adephagid beetles (Urbani & Russo-Caia, 1964, 1969; Bier, 1965; Bier &
Ribbert, 1966; Bier et al. 1967; Ficq & Urbani, 1969), the black fly Simulium
(Zalokar, 1965), the crane flies (Lima-de-Faria & Moses, 1966) and the lacewing
Chrysopa perla (Gruzova, Zaichikovaz & Sokolov, 1972) provide notable
exceptions to this generalization. In these species although RNA is passed from
the nurse cells to the oocyte, as in other meroistic ovaries, much RNA is also
synthesized in the germinal vesicle on extrachromosomal DNA templates which
have been shown to represent amplification of the ribosomal cistrons (Gall,
1969; Gall, MacGregor & Kidstone, 1969; Lima-de-Faria, Birnstiel & Jaworska,
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1969). These insects thus show an interesting intermediate between the typically panoistic and meroistic conditions.

Transient RNA synthesis by the germinal vesicle has, however, also been demonstrated in some Dermaptera, Hymenoptera, Mecoptera, Lepidoptera, Diptera (see Engels, 1970, for summary) and Coleoptera (Büning, 1972), but in these species it appears not to be associated with extrachromosomal DNA.

Meroistic ovaries are further classified according to the arrangement of the nurse cells as either polytrophic or telotrophic. Most endopterygotes have ovaries of the polytrophic variety. In these each follicle contains an oocyte and a species characteristic number of nurse cells which communicate with the oocyte via cytoplasmic bridges or fusomes. The bugs (Hemiptera) and the polyphagous beetles (Coleoptera), on the other hand, possess telotrophic ovaries in which the nurse cells are aggregated terminally in the germarium. Each oocyte is connected to the germarium by a cytoplasmic extension, the trophic cord.


The telotrophic ovary in the Hemiptera (Bonhag & Wick, 1953; Mulnard, 1954; Wick & Bonhag, 1955; Bonhag, 1955a, b; Patchin & Davey, 1968; Huebner & Anderson, 1972a, b, c) and Coleoptera (Aggarwal, 1964, 1967; Schloottman & Bonhag, 1956) has been studied histologically, but few autoradiographic accounts exist which alone can give a dynamic view of metabolic events. Most of these investigations (Vanderberg, 1963; Bier, 1964; MacGregor & Stebbings, 1970; Mays, 1972) have been carried out on Hemiptera; the recent study of Bruchidius by Büning (1972) is the only autoradiographic account dealing with the telotrophic ovary of Coleoptera known to the author.

In the present study an attempt has been made to answer some of the questions left unsolved by the work of earlier investigators. Schloottman & Bonhag (1956) stated that the ‘functional significance of the binucleate and polynucleate forms of the trophocytes of Tenebrio is unknown’ and that the function of the ‘germinal vesicle during vitellogenesis is an enigma’. Furthermore, though nucleoli form a conspicuous feature of the hemipteran ovary, these organelles could not be detected in either the nurse cells or the follicle cells of Tenebrio. The metabolic events in the two orders were thus likely to be very different and to merit further investigation.
Fig. 1. Ventral view of the abdominal extremity of a male (A) and female (B) pupa, to show the differences.

Schlottman & Bonhag (1956), though noting the strong basophilia of the nurse cells, trophic cords and young oocytes were unable, with their methods, to demonstrate the passage of either DNA or RNA down the cords into the oocytes. Aggarwal (1964) also states that 'there seems to be no evidence of the contributions of any nutritive substance by the trophic tissue to the oocyte'. The nutritive function of the nurse cells thus remains unproven: an autoradiographic analysis therefore seemed likely to provide an answer to their functional significance.

In conclusion, the present study seeks to ascertain the following:

(a) the function of the nurse cells,
(b) the synthetic capabilities of the germinal vesicle,
(c) the origin of the ooplasmic basophilia,
(d) the origin of the protein yolk.

MATERIALS AND METHODS

(i) Culture

The Tenebrio molitor culture was maintained at room temperature as previously described (Ullmann, 1964). Sexing the adult beetles is difficult, but differences in the terminal abdominal segment in the pupae (Fig. 1) make separation of males from females at this stage an easy task. The sexed pupae were kept in separate containers and one day after eclosion, when the cuticle had tanned, the males were marked on the elytra with red paint. For routine investigations the females were mated three days after eclosion and the ovaries processed when the beetles were between 2 and 4 weeks old.

(ii) Histology and densitometry

The females were chloroformed, the wings removed and the ovaries dissected out in insect ringer (NaCl, 715 g; KCl, 0·35 g; CaCl, 0·21 g in 1 l. of distilled water) in a waxed dish. The ovaries were either fixed in Serra's solution (formol-
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acetic-ethanol 30:5:60) for 2 h; or in phosphate buffered glutaraldehyde for 4 h after which they were left in buffer overnight. Dehydration was carried out in a graded mixture of ethanol and butanol (Smith, 1940) to avoid hardening of the yolk. After 6 h impregnation in 54°C paraffin wax, the tissues were blocked and sectioned at 7 μm.

To observe the patency of the follicular epithelium the freshly dissected ovaries were immersed in a 1 % solution of Evans’ blue in insect ringer and viewed, by transmitted light, without a coverslip, as described by Pratt & Davey (1972).

To estimate the relative quantity of DNA in the follicle and gut nuclei, the cells concerned were stained by the Feulgen reaction and the density of the nuclei was then measured by a Barr & Stroud integrating microdensitometer (Richards, Walker & Deeley, 1956).

The follicular epithelium was stripped off the surface of large vitellogenic oocytes with the aid of a watchmaker’s No. 5 forceps and spread out flat on albuminized slides. The enlarged nuclei of the flattened epithelial cells (Fig. 9) were sufficiently distant from each other to allow measurements to be made on them individually. This could not, however, be achieved with younger follicles in which the cells were smaller and the nuclei thus placed closer together. The gut tissue was macerated and the cells spread out on the slide.

As the degree of staining is proportional to the quantity of DNA present, it was imperative to standardize the whole technique in order to ensure constancy of results. The method of standardizing the Schiff’s reagent by determining and adjusting its sulphur dioxide content and the staining procedure for the Feulgen reaction was that given by Symington (1969). The slides were mounted in DPX and covered with No. 00 coverslips. Measurements with the microdensitometer were made at 540 nm, using a ×10 eyepiece and ×45 objective.

(iii) Autoradiography

After light anaesthesia with chloroform, specimens of all ages were injected with 0·05 ml of the radioactive precursor, each dose containing 5 μCi. The solution was injected with a 1 ml disposable sterile syringe, 26 × 3/8 in., through the tergum into the abdomen. The animals were then killed after the desired period of in vivo incubation.

The following precursors were used:

- [3H]thymidine (sp. act. 18·6 Ci/mM), for incorporation into DNA;
- [3H]uridine-5-T (sp. act. 17·65 Ci/mM), for incorporation into RNA;
- and the amino acids [3H]leucine (sp. act. 13·8 Ci/mM);
- [3H]methionine (sp. act. 5·0 or 7·6 Ci/mM);
- [3H]phenylalanine (sp. act. 5·9 Ci/mM);
- [3H]arginine (sp. act. 8·7 Ci/mM); and
- [3H]tryptophane (sp. act. 4·2 Ci/mM).

The sections were mounted on subbed slides, dewaxed and rinsed in 5 %
trichloroacetic acid at 4°C for 10 min, in order to wash out unincorporated and soluble precursor molecules. The sections were covered with Kodak AR 10 stripping film and stored in light-tight boxes at 4°C for 5 weeks before development.

To demonstrate the specificity of the uridine labelling, some sections were treated with a 0.05 % solution of RNase (bovine pancreatic, 5 x crystallized, 9 k.u./mg Sigma) in Tris buffer, pH 7.5. The solution was heated to 80°C prior to use, in order to destroy any traces of DNA present. The slides were incubated at 37°C for 1 h and then rinsed in trichloroacetic acid. To demonstrate the specificity of [3H]thymidine labelling, sections were enzymically digested with a 0.05 % solution of DNase (2250 k.u./mg Sigma) in 0.05 M Tris buffer, containing 0.002 M MgSO4·7H2O, at pH 7.4 and 37°C for 2 h.

Control sections were treated similarly, but without the enzymes.

Autoradiographs were either left unstained, or stained in a 0.5 % solution of toluidine blue for 30–60 sec, to render them more distinct.

During the course of this work over 200 ovaries were examined.

**OBSERVATIONS**

(i) *Egg laying and oocyte growth*

In an attempt to ascertain the length of time taken by a primary oocyte to pass from the base of the germarium through the vitellarium until it is deposited, the egg laying habits of three dozen adult females were followed daily over a period of 4 months, from mid-June to mid-October. It was assumed that the period which elapsed between the deposition of two batches of eggs would represent the time taken by a primary oocyte to grow to maturation in the adult ovary.

Virgin females were permanently mated and each pair was kept at room temperature in a separate container which was lined with black paper. The white eggs, attached to the substratum by a sticky secretion of the collateral glands, were thus easy to collect (Ullman, 1964). A daily record was kept of any eggs laid.

Fig. 2 shows the pattern of egg deposition in three females. As can be seen, there is much variation, and a regular pattern is not discernible. There is, moreover, considerable variation in fecundity between individuals, the number of eggs produced per female during the four months observation period varying between 81 and 328 (Fig. 3). This is bound to be related to the variation in ovariole number found among specimens, as is shown in Fig. 4.

In *Tenebrio* the following developmental stages of the oocyte may be distinguished:

*Previtellogenesis*

*Stage 1.* Primary oocyte dormant, prefollicular and located in the posterior portion of the germarium. Dimensions similar to that of nurse cells. Chromosomes delicate, filamentous and synthetically inactive.
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Fig. 2. Laying performance of three females (specimens A, E and H in Fig. 3) reared under identical conditions. The pattern is arhythmical.

Fig. 3. Variable fecundity of 8 females which were pupated, hatched and mated on the same days; and subjected to identical environmental conditions, from 13 June 1969 to 8 October 1969, when they were killed. Selection has thus been for survival only.
Stage 2. Oocyte situated in neck region of ovariole, between germarium and vitellarium proper. Chromosomes now synthesize RNA. Often several oocytes found, side by side, surrounded by prefollicular tissue.

Stage 3. Oocyte enters transitional zone, where prefollicular cells become arranged around it to form a columnar follicular layer in which mitoses occur. Follicles aligned, one behind the other. Oocyte enlarges markedly and trophic cords become distinguishable.

Stage 4. There is a further increase in the size of the spherical oocyte. The nucleus transforms into a germinal vesicle, while the chromosomes contract to form a karyosome.

Stage 5. Period of intense euplasmatic growth, marked by the elongation of the oocyte. Germinal vesicle central. Follicle cells organized into a columnar epithelium.

Vitellogenesis

Stage 6. Early vitellogenesis. Yolk deposition at the lateral sides of the oocyte begins, and marks the start of a very rapid size increase. The large germinal vesicle is central. The follicle cells become cubical and spaces appear between them.

Stage 7. Midvitellogenesis. Maximum patency of follicular epithelium and yolk deposition. Germinal vesicle moves to mid-dorsal side. The trophic connection with the germarium is probably severed at this stage.

Stage 8. Late vitellogenesis. The follicle cells flatten, forming a squamous epithelium and the spaces between them virtually disappear. Yolk deposition ceases and the follicular epithelium commences to secrete the chorion. Breakdown of limiting membrane of germinal vesicle.

Stage 9. The mature oocyte, with fully formed chorion, is expelled from the follicle, via the pedicel, into the lateral oviduct. The empty follicle undergoes resorption.
Fig. 5. Major regions of the female reproductive system.

Fig. 6. Longitudinal section of an ovariole, showing oocytes in different developmental stages.
Table 1

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Fig. 7. Growth curve of the oocyte, as a function of time. The greatest increase in size occurs during the last 2 days of oogenesis. The beetles were kept at room temperature.

Normally there are only 3-4 follicular oocytes per ovariole: one at stage 2; one at stages 3 or 4; one at stage 5 and one at stages 6-8. There is only one oocyte in active vitellogenesis per ovariole at a time (Figs. 5 and 6).

As the development of oocytes in adjacent ovarioles is asynchronous in Tenebrio, the estimation of the speed of oocyte maturation by the time interval elapsing between batches of eggs laid proved impossible. A histological examination of the growth of the oocytes was thus undertaken. The ovaries of mated females were observed after immersion in Evans' blue and sectioned at intervals from eclosion until the 10th day; and the oocytes in the most advanced ovarioles were staged (Table 1) and measured (Fig. 7) during the first 6 days. It is evident that though the penultimate (T1) oocyte starts growth well before the terminal (T) oocyte has completed development, it is arrested in previtellogenesis until yolk deposition has ceased in the (T) oocyte.

Thus the primary oocyte appears to take 6 days to mature from the commencement of the growth phase. It is, however, likely that the eggs are retained
for some time, for specimens sectioned 8–10 days after eclosion often showed large numbers of eggs in the lateral oviducts. Such egg retention would constitute an additional reason for the irregularities observed in the pattern of egg deposition (Fig. 2).

(ii) Ovarian development in the pupa

(a) General structure

At the beginning of the pupal stadium, which at 25°C lasts 7–8 days, the ovaries are located in the fourth abdominal segment. They form compact oval structures embedded in the fat body and each ovary is composed of a number of ovarioles (p. 184). At this stage of development three major regions are distinguishable which correspond to (i) the terminal filament, (ii) the germarium and (iii) the pedicel and oviducal branch, of the adult ovariole. The oviducal branches on each side join to form the common lateral oviduct: these meet to form the single, median oviduct, or uterus (Fig. 5).

The terminal filament forms the most anterior tissue. In the newly ecdysed pupa it arises by a broad base from the germarium, tapering anteriorly into a fine thread. The elongate nuclei contain peripherally placed chromatin granules and are transversely orientated within the filament. At the tip of the filament the nuclei are more rounded (Fig. 8 A).

During the second day of pupation a constriction appears in the ovariole sheath, between the terminal filament and the germarium. As this extends inwards, it gives rise to the transverse septum which effectively separates the filament from the germarium. Septum formation is completed on the 3rd day. Occasionally rounded nuclei are found within the base of the filament. Structurally they resemble the nurse cells and probably represent stray cells which have got dislodged before septum formation.

Posterior to the terminal filament lies the cylindrical germarium, which comprises the bulk of the ovariole at this stage. The anterior five-sixths of the germarium contains the trophocytes or nurse cells. These are tightly packed and the spherical nuclei are conspicuous on account of the large, darkly staining chromatin patches they contain (Fig. 8 A, B).

The posterior sixth of the germarium is occupied by the primary oocytes. Histologically this zone may be distinguished by (a) its lack of basophilia; (b) the smaller nuclei of the oocytes; (c) the different configuration of the chromatin within them from that of the nurse cell nuclei (Fig. 8 B).

Scattered throughout the germarium are smaller nuclei embedded in the cytoplasm which ramifies between the nurse cells and oocytes. This interstitial tissue thus appears to be syncitial.

The branching lateral oviducts are already well-developed structures at the pupal ecdysis and the funnel-shaped distal end of each branch abuts onto the posterior end of a germarium. The walls of the ducts are composed of columnar epithelial cells whose oval nuclei are orientated at right angles to the axis, and
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are often seen in mitosis. The lumen of the funnel frequently contains cytoplasmic particles (Fig. 13).

During the sixth day of pupation development is initiated in the stage 1 oocytes at the base of the germarium and the terminal oocyte may reach stage 5 before eclosion occurs. This growth phase of the oocyte has been shown to be under the control of the gonadotrophic hormone of the brain (Laverdure, 1972a).

At this stage the trophic cords are very delicate structures and are easily missed as they traverse the follicular epithelium en route to the oocytes.

(b) DNA synthesis

Autoradiography after administration of tritiated thymidine indicates that DNA synthesis is rapidly taking place in the oviducal epithelium, throughout the pupal stadium. Labelling is already detectable 20 min after injection of the precursor, and with increasing time of incubation becomes very heavy (Fig. 13).

The only other cells in the ovary observed to synthesize DNA during the pupal stadium were the nurse cells and, occasionally, sheath cells (Fig. 12). In

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**Figures 8-11**

Abbreviations. CH, chorion; F, funnel shaped termination of oviduct; FB, fat body; FC, follicle cell; GV, germinal vesicle; K, karyosome; M, mitotic figure; N, nucleus; NC, nurse cell; NR, 'neck' region; Oo, oocyte; OV, oviduct; P, pedicel; PE, periplasm; SP, space; TC, trophic cord; TF, terminal filament; Y, yolk.

Fig. 8. Longitudinal section of the ovariole of a 1-day-old pupa. Heidenhain’s haematoxylin.

(A) Junction of germarium and terminal filament. Arrows point to coccidian parasites, sometimes found in the germarium.

(B) Base of germarium, showing nurse cells with large, heavily staining nuclei; young oocytes and the junction with the funnel-shaped end of the oviduct branch.

Fig. 9. Portion of follicular epithelium from an oocyte in midvitellogenesis, seen in surface view. Feulgen stained. The nuclear chromatin is condensed to a varying degree. Note the patency of the follicular epithelium.

Fig. 10. Surface views of vitellogenic follicular epithelium, after immersion in Evans’ blue.

(A) Midvitellogenesis. Note the spaces between the cells, into which the dye has penetrated.

(B) Late vitellogenesis. The follicle cells have altered shape, largely occluding the formerly occurring spaces between them. The dye can be seen penetrating the epithelium in restricted regions, at the corners of the cells.

Fig. 11. Longitudinal sections through the follicular epithelium, stained with Masson’s trichrome stain.

(A) Midvitellogenesis. Note the spaces between the cells, into which the dye has penetrated.

(B) Stage 9. No spaces occur between the follicle cells, which are now secreting the chorion.
contrast to the oviducal epithelium, however, the nurse cells were only observed labelled at the end of the 1st day of pupal development.

When 1-day-old pupae were injected with the precursor, allowed to metamorphose and the adult ovaries subsequently autoradiographed, some of the nuclei of the lateral oviducts, pedicels, sheath cells and nurse cells retained the label, even 15 days after injection of the precursor. However, none of the follicle cells were labelled.

(c) RNA synthesis

During the first 5 days of pupation the rate of RNA synthesis in the ovary is low, except in the ducts which do show a moderate labelling, especially at the funnel shaped end.

By the 6th day of pupation the oviducts are attaining their definitive lengths and have virtually stopped making autoradiographically detectable RNA. At the same time the first follicles are forming and from this time onwards the tissue most active in RNA synthesis is the follicular epithelium. The rate of RNA production in the nurse cells steadily increases as the imaginal moult is approached, while the nuclei of those oocytes which have entered the growth phase also show a diffuse labelling over the extended chromosomes.

Enzymically controlled toluidine blue staining shows the growing oocytes to be rich in RNA. In order to ascertain the origin and mode of accumulation of this RNA, 5- to 7-day-old pupae were injected with tritiated uridine and sacrificed at various times after injection (i.e. 1, 3, 4, 6, 8, 13, 17 and 21 days).

After 24 h incubation with the precursor the label was diffusely distributed over the nurse cells, follicle cells; and the karyosome; but none was observed in the ooplasm. Three days later the pattern was rather different. The labelling was

**Figures 12-16**

Fig. 12. Longitudinal section of the gerarium of a 1-day-old pupa, incubated with \[^3H\]thymidine for 2.5 h. Most of the nurse cells have incorporated the label. The base of the gerarium, where the oocytes are located, remains unlabelled.

Fig. 13. Longitudinal section through the base of the gerarium and its oviducal branch. Note the funnel shaped distal end of the duct, its heavily labelled nuclei; and the lack of incorporation into the oocytes. Six-hour-old pupa, incubated with \[^3H\]thymidine for 2 h.

Fig. 14. Longitudinal section through the follicular epithelium in midvitellogenesis. Note the heavily labelled chromatin. Some labelling can also be discerned over the follicular cytoplasm and the ooplasm. Incubated with \[^3H\]thymidine for 30 min.

Fig. 15. Longitudinal section through an ovariole, showing 1 vitellogenic and 3 previtellogenic follicles. Note that most of the follicular nuclei show DNA synthesis. Incubated with \[^3H\]thymidine for 30 min.

Fig. 16. Portion of follicular epithelium in midvitellogenesis, stained by the Feulgen reaction. Note the large and heavily stained chromatin patches (which probably represent chromosomes) distributed just below the nuclear membrane. Compare with Fig. 14. The variable nuclear size of adjacent cells is probably a reflection of their differing ploidy.
confined to the cytoplasm of the nurse cells, follicle cells and oocytes but was conspicuously absent from their nuclei. Evidently the nuclear RNA is transported to the cytoplasm where it accumulates in an apparently stable form, presumably as rRNA.

(iii) Autoradiography of the adult ovary

(a) DNA synthesis

Fifteen minutes after exposure to tritiated thymidine the majority of pre-follicular and follicular nuclei become labelled. After longer incubation times, which ranged from 30 min to 4 days, the labelling became more intense but did not alter in distribution (Fig. 15). As can be seen from comparing Figs. 14 and 16, the pattern of labelling in the nucleus corresponds exactly to that of the chromatin distribution as visualized by the Feulgen method, making allowance for the fact that Fig. 14 is a section and Fig. 16 a squash preparation. After 24 h incubation with the precursor a small amount of label was detectable, spread diffusely over the follicle cell cytoplasm, the yolk and ooplasm, but this amount fails to account for the massive synthesis of DNA by the follicle cells (Figs. 14 and 15).

Mitoses were rarely observed in the follicular tissue; and then only in very young follicles, thus confirming the observations of Schlottman & Bonhag (1956). The occurrence of rapid and extensive DNA synthesis in a virtually non-mitotic tissue could therefore have one of two explanations: (i) the nuclei could be synthesizing DNA for transport to other sites in the cell or ovary; (ii) the nuclei could be undergoing endomitosis or polyteny.

The negligible labelling in the cytoplasm of the follicle cells and oocytes has already been noted and the synthesis of DNA for transport elsewhere thus seems an inadequate explanation. The alternative possibility, that the cells are undergoing endomitosis, was therefore tested by measuring the DNA content of vitellogenic follicle cell nuclei and comparing them with gut cell nuclei. The measurements were made with the aid of an integrating densitometer.
Table 2. Summary of results with $[^3H]$thymidine

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Abbreviations
- nen, nurse cell nucleus; ncc, nurse cell cytoplasm; t, trophic cord; gv, germinal vesicle; ch, oocyte chromosomes; oo, ooplasm; y, yolk; fen, follicle cell nucleus; fec, follicle cell cytoplasm; ov.n., oviduct nuclei; ov.c., oviduct cytoplasm. +, incorporation; −, no incorporation; ±, light incorporation; o, very little incorporation of label.

Three separate measurements were made with the typical results shown in Fig. 17. It can be seen that the modality of gut cells contain the diploid quantity of DNA, as indicated by the peak in the histogram. Since the midgut epithelial cells are continually undergoing regeneration from mitotically active cells at the base of the crypts, it is not surprising to find a small number of cells with the tetraploid DNA value.

Measurements on follicle cells, however, suggest that the nuclei are highly polyploid. Indeed, diploid nuclei were not found at all in mature, vitellogenic follicles; whereas some nuclei reach a ploidy value of 64 (Fig. 17). Attempts to measure DNA content in successive follicles of the same ovariole have not been successful, owing to technical difficulties (p. 183).

In order to ascertain if there was a rhythm in DNA synthesis, autoradiographs were prepared of ovaries from the first to the fortieth day of adult life. No change in the pattern of DNA synthesis was observed: there was always a massive incorporation of tritiated thymidine.

(b) RNA synthesis

Exposure of the ovaries to tritiated uridine results in rapid labelling of the prefollicular, follicular, nurse cell and previtellogenic oocyte nuclei (Figs. 18–22). The labelling, detectable already after 15 min, increases in intensity with prolonged incubation and, after 3 h, it is also found in substantial amounts in the cytoplasm of the follicle and nurse cells. This cytoplasmic labelling, indicative of the extra-nuclear transport of RNA, is most marked in the previtellogenic follicles. The ooplasm and trophic cords, however, remain unlabelled; and the latter can sometimes be identified as pale streaks, as they traverse the heavily labelled follicular epithelium.

Ooplasmic labelling was first observed 24 h after exposure to the precursor, when it was found diffusely distributed over oocytes of all stages. By the end of the 2nd day the ooplasm becomes more intensely labelled and a definite gradient of distribution can be distinguished (Fig. 28). The greatest accumulation of label is found at the anterior end of the oocyte, in which region the trophic cord
Oogenesis in Tenebrio molitor makes contact with it. This differential distribution of RNA was only observed during a transient period in young vitellogenic oocytes and could not be detected at earlier or later stages of development (Figs. 27–30). In older vitellogenic oocytes, however, a new pattern of RNA distribution comes to supersede the previous anterior-posterior gradient. This is caused by the lateral accumulation of the yolk globules which thus displace centrally the RNA rich ooplasm (Fig. 29). By the end of vitellogenesis, however, with the permeation of the ooplasm by the yolk globules and its transformation thereby in the reticuloplasm, no obvious patterns of RNA distribution are any longer discernible (Fig. 30).

The trophic cords also become conspicuously labelled during the 2nd day of incubation. They can most clearly be seen as they traverse the base of the germarium where the, as yet synthetically inactive, stage 1 oocytes are located. The intensity of the label in the cords resembles that in the nurse cells, to which they can be traced (Figs. 24–26).

In stage 2 and 3 oocytes the chromosomes are extended and stainable by the Feulgen method. In such oocytes the autoradiographic silver grains are dispersed over the whole nucleus (Figs. 19, 20). In stage 4 oocytes (p. 186), the chromosomes contract to form a karyosome (Fig. 21). Autoradiographs of this and subsequent developmental stages show that the nuclear labelling is almost totally restricted to the karyosome, irrespective of the period of incubation, leaving the nucleoplasm but slightly labelled (Figs. 22, 29–31).

The germinal vesicle remains embedded in a mass of cytoplasm which is unevenly distributed around it (Figs. 29–31). This cytoplasm subsequently contributes to the maturation plasm in which the reduction divisions take place (Ewest, 1937). (See Table 3, p. 201.)

(c) Protein synthesis

Of the precursors used tryptophane and arginine appeared to be less readily incorporated into the tissues than were phenylalanine, methionine and leucine, and consequently yielded rather faintly labelled autoradiographs. The amino acid patterns in the trophic cords and ooplasm, the dispersed chromosomes of the oocyte and the follicular nuclei are labelled.

Figures 18–22

Adult ovary, incubated with [3H]uridine for 1.5 h.

Fig. 18. Longitudinal section through a portion of the germarium. The label is localized over the nuclei.

Fig. 19. Longitudinal section through the base of the germarium and the youngest follicle. The nuclei of the nurse cells, follicle cells and growing oocyte are labelled; the dormant oocytes at the base of the germarium and the ooplasm are unlabelled.

Fig. 20. Longitudinal section of previtellogenic follicle. In contrast to the trophic cord and ooplasm, the dispersed chromosomes of the oocyte and the follicular nuclei are labelled.

Fig. 21. Longitudinal section through a previtellogenic follicle. The chromosomes in the germinal vesicle have contracted to form a karyosome.

Fig. 22. Longitudinal section of an oocyte beginning elongation. Note the labelled karyosome in the centrally placed, largely unlabelled germinal vesicle.
Oogenesis in *Tenebrio molitor*

Acids were incorporated by all ovarian tissues, except by vitellogenic oocytes enclosed by the chorion (Figs. 32–36).

Faint, diffuse labelling, indicative of the presence of protein, was first observed 30 min after exposure to the precursor and it gradually increased in intensity in the following hours. Indications of a differential protein distribution emerged after 1 ½ h and by 6 h a distinct pattern was evident: the cytoplasm of the nurse cells, follicle cells and ooplasm were moderately labelled; while the most intense concentration of protein appeared in association with the periplasm of vitellogenic oocytes (Fig. 32B). In subsequent stages of development this protein becomes incorporated into the peripherally formed yolk spheres (Figs. 32C and 34). These newly formed yolk globules are smaller than those lying more centrally which probably form by coalescence; the latter are found scattered randomly among the unlabelled yolk spheres throughout the ooplasm (Fig. 35) as in some other meroistic species (Bier, 1970). The labelling in the germinal vesicle of previtellogenic oocytes exceeds that in the ooplasm (Figs. 33, 36); and after 10 h incubation the karyosome appears even more intensely labelled than the nucleoplasm. However, karyosome labelling was observed only with tritiated, methionine and phenylalanine (Figs. 33, 34).

As the germinal vesicle enlarges the labelling within it becomes fainter, so that in vitellogenic oocytes it is no longer above that of the surrounding cytoplasm (compare Figs. 33 and 36 with 34). This is presumably due to dilution of the nucleoproteins in the larger volume in which the nuclear sap is now contained. Occasionally a heavily labelled karyosome is encountered in vitellogenic germinal vesicles (Fig. 34).

Once vitellogenesis is completed, the flattened follicle cells undergo a functional change and begin to secrete the chorion. This forms a barrier to the further passage of macromolecules into the oocyte, so that it can no longer be labelled (Fig. 32). (See Table 4, p. 205).

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**Figures 23–27**

Specimens incubated with [3H]uridine for 48 h.

Fig. 23. Transverse section through the germarium. Most of the label has passed from the nuclei into the cytoplasm which is very heavily labelled.

Fig. 24. Longitudinal section through an ovariole. The cytoplasm of the nurse cells and follicle cells is heavily labelled in contrast to the ooplasm and ‘neck’ region, housing the prefollicular oocytes.

Fig. 25. Longitudinal section through the ‘neck’ region (junction between gerarium and vitellarium). The cytoplasm of the nurse cells is heavily labelled as is the trophic cord issuing from them. The prefollicular oocytes are virtually unlabelled.

Fig. 26. Enlarged portion of Fig. 24. Note the heavily labelled trophic cord issuing from the gerarium and traversing the lightly labelled ‘neck’ region.

Fig. 27. Previtellogenic follicle with follicle cells heavily labelled, the ooplasm but lightly. Compare with Figs. 19–22, where the label is still located in the follicle cell nuclei and the ooplasm is not labelled above background.
Table 3. Summary of results with $[^3H]$uridine in the adult

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Abbreviations as in Table 2. The chromosomes in previtellogenic stages were always labelled; the karyosomes in vitellogenic stages were variably labelled.

DISCUSSION

(i) General considerations: pupal development, follicular patency and egg laying

As mitoses were never observed in the pupal germarium, the final oogonial divisions must occur in the previous, i.e. larval stadium, but Saling (1907) makes no mention of this in his account of gonadal development in *Tenebrio*. By the beginning of pupation the oogonia are differentiated into functional oocytes and abortive oocytes, or nurse cells, much as they are in bugs (Wick & Bonhag, 1953; Masner, 1968). In the beetle *Bruchidius* (Bünning, 1972), though mitoses do not occur during pupation, the nurse cells and oocytes become fully differentiated only in the imago. The present observations in no way contradict the assumption of Schlottmann & Bonhag (1956) that the oocytes of *Tenebrio* remain arrested in the prophase of the first meiotic division throughout oogenesis.

FIGURES 28–31

Specimens incubated with $[^3H]$uridine for 48 h.

Fig. 28. Longitudinal section of oocyte in early vitellogenesis. Note the influx of labelled RNA at the anterior end, where the trophic cord enters the oocyte. The label is distributed in an antero-posterior gradient.

Fig. 29. Longitudinal section of oocyte in midvitellogenesis. As the yolk spheres form peripherally, the RNA rich ooplasm takes up a more central position in the oocyte. Note antero-posterior graded distribution of RNA; accumulation of ooplasm around germinal vesicle, which is dorsally displaced: and the labelled karyosome.

Fig. 30. Longitudinal section of stage 8 oocyte. The graded distribution of RNA is no longer conspicuous, obscured perhaps by the influx of yolk. Note the uneven layer of ooplasm surrounding the germinal vesicle.

Fig. 31. Longitudinal section of portion of vitellogenic oocyte, showing ellipsoidal germinal vesicle containing a labelled karyosome.
Oogenesis in Tenebrio molitor

It may here be mentioned that the recognition of a new type of insect ovary in Hydrophilus (Gundevia & Ramamurty, 1972) is believed by the author to be invalidated by the fact that most of the distinguishing features (binucleate nurse cells, lack of trophic core and mitoses in the germarium) occur also in Tenebrio, which clearly has telotrophic ovaries (Schlottmann & Bonhag, 1956; present investigation). Further, the absence of trophic cords in Hydrophilus needs confirmation (p. 208).

In Tenebrio the chief morphological events of ovarian development during the pupal stadium are the following: (a) the elongation of the oviducts; (b) the polyploidization and appearance of multinucleate nurse cells; and (c) the initiation of the previtellogenic growth phase of the oocyte, which is associated with the formation of the first follicles.

At the end of pupation the main respect in which the ovaries differ from the adult condition is in lacking vitellogenic oocytes. Lack of the corpus allatum hormone (JH), which is not secreted at this stage, is responsible for this (Lender & Laverdure, 1964, 1965).

If a vitellogenic follicle is immersed in stain (p. 183) or a piece of the follicular epithelium is stripped from the oocyte and examined in surface view, numerous gaps can be observed between the irregularly shaped cells (Figs. 9, 10). In

Figures 32–35

Fig. 32. Stages in protein yolk formation. Incubated with [3H]methionine for 5 h 45 min.

(A) Diffusely labelled follicular epithelium and reticuloplasm, representing in situ synthesis.

(B) Heavily labelled proteins, believed to be of extra-ovarian origin, accumulated at the oocyte periphery.

(C) The peripherally accumulated labelled proteins are incorporated into yolk spheres by the oocyte, and begin their inward migration. The follicular epithelium and reticuloplasm are relatively lightly labelled.

(D) Portion of mature oocyte. The chorion secreted by the follicle cells forms a barrier between the oocyte and regions outside it. Thus, while the follicular epithelium can still be labelled, the oocyte cannot.

Fig. 33. Stage 5 oocyte, incubated with [3H]phenylalanine for 10 h. Labelling of ooplasm and follicular epithelium is similar, and represents in situ synthesis. A zone of more intense labelling at the oocyte periphery heralds the onset of vitellogenesis: it is believed to represent haemolymph-derived proteins. The concentration of proteins in the germinal vesicle exceeds that in the ooplasm; while the karyosome is the most intensely labelled region of the oocyte.

Fig. 34. Section through a vitellogenic oocyte, incubated with [3H]methionine for 5 h 45 min. The enlarged germinal vesicle contains a labelled karyosome, but the labelling of the nucleoplasm no longer exceeds that of the ooplasm. Note the small protein yolk spheres, formed peripherally.

Fig. 35. Portion of interior of a stage 7 oocyte, incubated with [3H]phenylalanine for 10 h. The small peripherally formed yolk spheres probably coalesce to form these larger globules, which migrate interiorly individually and are to be found mingled at random with unlabelled yolk spheres.
younger follicles the cells fit closely together, and no intervening spaces occur. These interfollicular spaces are also evident in sections and are rendered conspicuous when stain lodges between the cells (Fig. 11).

Vitellogenesis in insects is under endocrine control (Telfer, 1965; Masner, 1968; Higham and Hill, 1969; Pratt & Davey, 1972), and the corpora allata in *Tenebrio*, also, have been shown to produce a hormone (JH) which allows yolk
Oogenesis in Tenebrio molitor

Table 4. Summary of results with $^3$H-amino acids

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Abbreviations as in Table 2. Arginine and tryptophane were less readily incorporated than leucine, methionine and phenylalanine. Labelled karyosomes were only found after incubation with methionine and phenylalanine.

deposition to occur (Lender & Laverdure, 1964a, b, 1965; Mordue, 1965a, b, c; Laverdure, 1972a). It has been established in the bug Pyrrhocoris (Masner, 1968) that the action of this hormone is gonadotropic and mediated via the follicular epithelium, in which it induces morphological alterations. The cells change shape, become binucleate and small spaces appear between them which permit access from the haemolymph to the oocyte surface. Pratt & Davey (1972), apparently unaware of Masner's work, come to a similar conclusion as a result of their study of oogenesis in the bug Rhodnius. They, too, implicate the gonadotrophic hormone of the corpora allata in bringing about patency of the follicular epithelium, which makes sequestration of proteins from the haemolymph possible.

Laverdure (1972a) has recently shown that the JH in Tenebrio has a metabolic action, bringing about synthesis and release of yolk proteins, stored in the fat body. She queries the gonadotrophic action of the hormone but does not present any evidence to exclude such an effect. Demonstration of the metabolic function on JH does not, in itself, exclude a simultaneous gonadotrophic action. It would be instructive to examine follicular patency in Tenebrio in the presence and absence of JH, as has been done in some bugs (Masner, 1968; Pratt & Davey, 1972).

In any case, the demonstration that yolk deposition in Tenebrio is dependant on JH, and likewise that follicular patency develops at this same period, provides strong circumstantial evidence that the events of vitellogenesis in this insect resemble those described for other species (Telfer, 1965; Bier, 1970; Bell & Barth, 1971). It is thus likely that, in Tenebrio, also, JH is implicated in follicular differentiation, though the cells never become binucleate.

Observations on egg laying behaviour and measurements on the developing oocytes (p. 184) indicate that (a) the ovarioles do not develop synchronously, a condition observed also in other insects (Meng, 1968; Pratt & Davey, 1972); and (b) that the oocytes take about 6 days to mature (p. 188). In Tenebrio, as in Rhodnius (Pratt & Davey, 1972), there is never more than one oocyte per ovariole in active vitellogenesis. Yolk deposition in the penultimate (T1) oocyte
is delayed in both insects, until chorion formation in the terminal (T) oocyte begins. However, in other insects (Mays, 1972) several oocytes undergo vitellogenesis simultaneously.

Though recent studies (Pratt & Davey, 1972; Laverdure, 1972b) have revealed a number of hormonal and feedback factors which regulate previtellogenic oocyte differentiation, the control mechanisms involved in the coordinated growth of insect oocytes within an ovariole are still not well understood.

(ii) DNA synthesis

Although the oocytes fail to incorporate [3H]thymidine at all during the developmental stages studied, the nurse cells do become labelled during a limited period (p. 191). This contrasts with the condition in flies (Nigon & Nonnenmacher, 1961; Muckenthaler & Mahowald, 1966; Basile, 1969; Engels & Ribbert, 1969); bugs (Vanderberg, 1963; Orlando, 1966; Mays, 1972) and some beetles (Urbani & Russo-Caia, 1969; Büning, 1972), where the nurse cells synthesize DNA in the adult ovary. In *Drosophila* the nurse cells incorporate [3H]-thymidine even when they are degenerating (Muckenthaler & Mahowald, 1966).

In the absence of mitoses or extra-nuclear transport in the pupal germarium of *Tenebrio*, this DNA synthesis must be the prelude to (a) cryptomitotic (amitotic) divisions, which give rise to the bi- and tri-nucleate nurse cells; and (b) the endomitotic divisions, which give rise to the polyploid nurse cells. In the nurse cells of the beetle *Dytiscus* repeated, asynchronous endomitoses have been described as well as autoradiographically detectable DNA in the cytoplasm (Urbani & Russo-Caia, 1969; Ficq & Urbani, 1969).

The DNA content of *Tenebrio* nurse cell nuclei has not been directly measured, but there is circumstantial evidence for their endopolyploid character. First there is an increase in size when prepupal and adult nurse cell nuclei are compared; secondly, although all the nurse cells appear to incorporate [3H]-thymidine in the pupa (Fig. 12), only a small proportion of them become multinucleate. Thus the DNA synthesis in the majority of the nurse cells does not result in nuclear division and so must be accounted for by endomitosis.

Endopolyploidy in nurse cell nuclei also occurs in Mecoptera (Ramamurty, 1963b, 1970), Dermaptera (Engels, 1970), and Diptera (Painter & Reindorp, 1939; Bier, 1957; Matuszewski, 1968; Engels & Ribbert, 1969; Basile, 1969) among others, and appears to be a general phenomenon in meroistic ovaries (Bier, 1970). Orlando (1966) observed DNA synthesis in the nurse cells of the bug *Megura viciae* but stated that 'thymidine ³H incorporation cannot be ascribed to continuous endomitotic divisions. It can therefore be assumed that the active synthesis which occurs in the nuclei does not concern genetically stable DNA but a metabolic DNA.' It thus seems likely that the DNA synthesis in *Megura* nurse cell nuclei is associated with replication of the ribosomal (rRNA) cistrons, as has been shown for *Calliphora* (Ribbert & Bier, 1969) where multiple nucleoli
Oogenesis in Tenebrio molitor

occur. In Tenebrio nurse cells, however, no nucleoli have been found, and it is not known if differential replication takes place.

The most active site of DNA synthesis during pupation in Tenebrio is the oviducal epithelium (Fig. 13), reflecting the rapid elongation of this structure. Prolonged incubation of 15 days or over with [³H]thymidine shows this nuclear label to be stable, though subject to dilution due to mitoses. As under these conditions none of the first formed follicles are labelled (p. 193), it appears that their precursors (the prefollicular nuclei) do not synthesize DNA during pupation. This is in great contrast to their subsequent behaviour, as is discussed in the following section.

It has been shown that the only tissue synthesizing DNA in the adult ovary is the follicular epithelium (p. 194). Schlottman & Bonhag (1956), on the basis of their histochemical examination of the adult Tenebrio ovary, suggested that 'the DNA content of the nucleus increases as the follicular epithelium undergoes differentiation' (p. 366). This has been confirmed by the present investigation which has demonstrated, by direct measurements, that the follicle cells have a variable and high DNA content (p. 195). This observation is interpreted as reflecting the increasing endopolyploidy of the follicle cells.

As indicated by Fig. 17, diploid nuclei are no longer to be found in vitellogenic follicle cells, some of which contain over 64 times the haploid amount of DNA. However, the expected peaks at the doubling points (i.e. those representing the 4C, 8C, 16C, etc., values) were not observed. Thus there appears to be a continuous and asynchronous replication of the follicle cell chromosomes, throughout their development. Orlando & Cremer (1968) come to a similar conclusion in their study of aphid ovaries, in which the follicular nuclei may reach a ploidy value of 64. Asynchronous replication, moreover, could account for the anomaly that the follicular nuclei are active in DNA and RNA synthesis simultaneously.

A possible alternative explanation to asynchronous replication to account for the continuous distribution of DNA values for follicle cell nuclei (Fig. 17) is suggested by Fox (1970). He has recently examined a number of insect somatic tissues and found the DNA values to be variable, but tissue specific. However, in locusts (but not in Dermestes) 'the peaks in the distributions do not conform with the members of a doubling series' and Fox suggests that this may be related to tissue differentiation by differential replication of the genome.

Thus while endomitosis must account for the bulk of the follicular DNA synthesis in Tenebrio — as suggested by the close correlation of Feulgen staining and [³H]thymidine labelling (Figs. 14 and 16) — the possibility of differential replication cannot be excluded and is consistent with the data presented in Fig. 17.

Endopolyploidy of the follicle cells has also been demonstrated in Gryllus (Durand, 1955); and Drosophila (Schultz, 1956) in which the nuclei may contain 8–16 times the haploid amount of DNA. Since cell size and ploidy are
related, the 100-fold increase in the size of the follicle cells of *Pyrrhocoris* (Masner, 1968) likewise indicates a high degree of polyploidization. The latter process appears to be characteristic of the differentiated insect follicular epithelium. The follicle cells in *Tenebrio* never become binucleate, as they do in many insects (Gross, 1903; Bonhag & Wick, 1953; Vanderberg, 1963; Masner, 1968), endopolyploidy alone equipping the cells for the exacting function of chorion formation.

Ooplasmic DNA has been reported in a few insects (Telfer, 1965; Mahowald & Muckenthaler, 1966) and in *Pyrrhocoris* (Mays, 1972) unspecific labelling of the ovarian tissues occurs, after prolonged incubation with $[^3H]$thymidine. There is some evidence for a DNA contribution to the oocyte by the follicle cells in *Tenebrio* (p. 194), but this needs further investigation.

(iii) RNA synthesis

(a) Nurse cells, follicle cells and oocyte

During pupation there is a low level of RNA synthesis which is restricted largely to the oviducts (p. 193). As eclosion is approached the nurse cells become more active, and this increased RNA synthesis gains momentum in the adult. The prefollicular nuclei and the follicular cells behave likewise.

RNA synthesis is very rapid in the nuclei and is later transported to the cytoplasm of both nurse cells and follicle cells (p. 195). However, not till the 2nd day of incubation does it accumulate in detectable quantity in the trophic cords and ooplasm. The intensity of labelling in the nurse cells and trophic cords which can be traced to them (Figs. 24–26), and the graded pattern of label distribution in vitellogenic oocytes (p. 195, Fig. 28) clearly indicate the passage of RNA from the nurse cells, via the trophic cords, to the oocytes.

In the bugs (Hamon & Folliot, 1969; MacGregor & Stebbings, 1970; Huebner & Anderson, 1972b, c) the trophic cords are much better developed than in the beetles, and they are richly endowed with microtubules between which large numbers of ribosomes occur. MacGregor & Stebbings (1970) suggest that the microtubules play a vital role in the transport of ribosomes from the trophic tissue to the oocyte. The trophic cords of beetles, on the other hand, are delicate and easily overlooked, as appears to have been done in *Callosobruchus analis* (Aggarwal, 1967); and probably *Hydrophilus* (Gundevia & Ramamurty, 1972) where they are said to be lacking. Ultrastructural studies are needed to substantiate these claims. Büning (1972), however, has demonstrated their existence in *Bruchidius obtectus*, and has also shown that very few microtubules occur in them.

In view of the fact that the passage of RNA down the tubes, from the germarium to the oocyte, has been demonstrated in both bugs (MacGregor & Stebbings, 1970; Mays, 1972) and beetles (Büning, 1972; present investigation), it would appear that the microtubules are inessential in this transport. It is thus
more likely, as suggested by Hamon & Folliot (1969), that their functional significance is cytoskeletal.

RNA contribution (as distinct from cytoplasm) by the nurse cells to the oocyte was first convincingly demonstrated by the autoradiographic studies of Bier (1963b). He found that in the housefly Musca domestica the labelled RNA which first entered the oocyte via the fusomes soon disappeared. Bier (1963) suggested that this may be due to messenger RNA turnover. The stable RNA which later accumulated in the oocyte, subsequent to the passage of nurse cell cytoplasm into it, he took to be ribosomal RNA (Bier, 1967).

Mays (1972) claims to be able to detect a similar biphased transportation of RNA in the telotrophic ovary of Pyrrhocoris, with a faster moving fraction preceding the bulk of a slower moving fraction composed, presumably, of ribosomal RNA.

In Tenebrio, however, such a biphased passage of RNA has not been detected, and the nurse cell derived RNA appears to be stable. Moreover, this RNA is not associated with the breakdown of nurse cells and the influx of their cytoplasm into the oocyte, as is the case in polytrophic ovaries. These findings are in agreement with those for Oncopeltus (Bier, 1964), Notonecta (MacGregor & Stebbings, 1970) and Bruchidius (Büning, 1972).

In Tenebrio there is a relatively tardy appearance of the label in the trophic cords and oocytes (p. 197) when compared with events in polytrophic ovaries, and this time sequence is comparable to that in other telotrophic ovaries (Engels, 1970; Mays, 1972). It seems that in the telotrophic ovary, where the oocytes are at some distance from the nurse cells, the accumulation of RNA by the oocyte takes longer than in the polytrophic, yet not so long as in the panoistic ovary (Bier, 1970). The intermediate position of Tenebrio in the insectan developmental series, postulated previously on embryological grounds (Ullmann, 1964), thus receives physiological support from comparative oogenesis. Büning (1970) reaches a similar conclusion for Bruchidius. In Tenebrio as in the great majority of insects (Telfer, 1965; Bier, 1970), there is no follicular RNA contribution to the oocyte, Panorpa (Ramamurty, 1970) being a notable exception.

The synthesis of rRNA is generally associated with the development of a nucleolus, and in the telotrophic ovaries of the bugs (Bonhag, 1955a; Vanderberg, 1963; MacGregor & Stebbings, 1970) and the beetles Collosobruchus (Aggarwal, 1967) and Bruchidius (Büning, 1972) the nurse cells and follicle cells contain conspicuous nucleoli. Systems rapidly synthesizing RNA, moreover, are often associated with multiple nucleoli, a situation which represents amplification of the ribosomal cistrons (Brown & Dawid, 1968; Ribbert & Bier, 1969; Gall, 1969).

In Tenebrio, however, no nucleoli are evident in either the nurse cells or the follicle cells – at least using conventional histological techniques (Schlottmann & Bonhag, 1956; and present investigation). Endopolyploidy, on the other hand, does occur (p. 195) and it may well involve differential replication without the...
liberation of DNA templates into the nucleoplasm, as occurs in forms with multiple nucleoli (Ribbert & Bier, 1969). Such restricted DNA synthesis has been described for Sciarid polytene chromosomes where ‘DNA-puffs’ occur (Pavan, 1965; Crouse & Keyl, 1968; Mattingly & Parker, 1968; Pavan & Da Cuhna, 1969); and is suggested by the work of Fox (1970), as already discussed (p. 207).

The functional significance of the multinucleate nurse cells, which so puzzled Schlottman & Bonhag (1956), is evidently that it enhances RNA synthesis by the cells which are thus endowed. But what factors induce division of some nurse cell nuclei only, remains obscure.

Harris (1970) claims that the nucleolus is essentially implicated in the extranuclear transportation of RNA, and that in its absence passage of RNA from the nucleus to the cytoplasm fails to occur. Although the evidence provided by embryonic and hybrid cells is persuasive, it is difficult to reconcile Harris’ views with the situation in Tenebrio, where the nurse cell chromosomes obviously synthesize large quantities of RNA for extracellular transport yet apparently in the absence of a nucleolus. An electron microscopic investigation of the nurse cell-oocyte interrelationship, coupled with a biochemical analysis of RNA synthesis during oogenesis is needed in Tenebrio, and is likely to prove rewarding.

(b) The germinal vesicle

RNA synthesis by the oocyte nucleus, so characteristic of panoistic ovaries, is also demonstrable in a number of meroistic-polytrophic species (p. 181). Transient RNA synthesis by the germinal vesicle occurs in Mecoptera (Ramamurty, 1963a), Neuroptera (Gruzova et al. 1972), adephagid Coleoptera (Bier et al. 1967) and Diptera (Zalokar, 1965; Bier, 1963a, b; 1967). Recently it has been shown to occur also in the telotrophic ovaries of phytophagous Coleoptera (Bünning, 1972; present investigation); and perhaps Hemiptera (Mays, 1972).

As has been shown (p. 197; Figs. 19–20), the dispersed chromosomes of the young oocytes are synthetically active and become rapidly labelled, simultaneously with the nurse cell and follicle cell nuclei. Karyosomes at developmental stages 4 and 5 are also labelled (Figs. 21 and 22) as are, frequently, those of vitellogenic oocytes (Figs. 29–31). In Chrysopa (Gruzova, 1966), however, karyosomes remain unlabelled.

In Drosophila RNA synthesis by the germinal vesicle takes place exclusively during vitellogenic stages 9–11, when a karyosome has formed and the nucleolus has disappeared; and the available evidence suggests that the RNA is non-ribosomal in nature (Mahowald & Tiefert, 1970). In Tenebrio, as has been seen, RNA is synthesized throughout the previtellogenic stages (p. 195), but the nature of this RNA is unknown; nor could it be established whether it was passed to the ooplasm.

Thus not only are some meroistic oocytes capable of a limited amount of RNA production, but this synthesis may, apparently, occur on chromosomes
Oogenesis in Tenebrio molitor contracted to form a karyosome. Karyosome formation therefore cannot be equated with complete or irreversible chromosome inactivation.

Bier et al. (1967) pointed out that the limited amount of label associated with the karyosomes of Musca and the beetle Pterostichus is not necessarily indicative of chromosomal RNA synthesis, and suggested that the label may be derived from the nurse cell RNA which attaches to the oocyte chromosomes and inhibits the latter from synthesis.

Although the present work does not allow one to distinguish between synthetic inhibition by exogenous RNA and genuine synthesis by the chromosomes in Tenebrio, yet the latter alternative is the more attractive. Apart from the lack of evidence for the above hypothesis, comparative studies of insect oogenesis indicate that the inactivation of the oocyte chromosomes in meroistic ovaries is a secondary phenomenon, and scattered instances of RNA synthesis are therefore to be expected.

(iv) Protein synthesis

In Tenebrio there is evidence for an ovarian and an extra-ovarian component of the oocyte proteins. The ooplasmic protein is apparently synthesized in situ because the label appears simultaneously in all the ovarian tissues. Eventually the nucleoplasm comes to be more intensely labelled than the cytoplasm (p. 199), from whence the nuclear label seems largely to originate. A uniform labelling of the ooplasm after short incubation times with tritiated protein precursors also occurs in the scorpion fly (Ramamurty, 1963b), Rhynchosciara (Basile, 1969), Pimpla (Meng, 1970) and Pyrrhocoris (Mays, 1972), indicating autonomous protein synthesis, while the passage of proteins from the ooplasm into the germinal vesicle has been described in both insect (Zalokar, 1965; Mays, 1972) and amphibian (Arms, 1968; Ficq, 1972) oocytes. In Tenebrio there is no evidence for the passage of labelled proteins from the nurse cells to the oocyte, as has been claimed for Pyrrhocoris (Mays, 1972).

The yolk proteins, on the other hand, are derived from extra-ovarian sources as in other insects (Telfer, 1965; Bier, 1970; Mahowald, 1972b); and Basile's (1969) comments on this matter are incorrect. Recently, however, evidence has been presented for an ovarian contribution to the protein yolk. First, in Rhodnius (Vanderberg, 1963) the follicle cell proteins become incorporated into yolk and this has also been demonstrated for the cecropia moth, utilizing autoradiography combined with short-term organ culture (Anderson & Telfer, 1969, 1970; Melius & Telfer, 1969). A similar follicular contribution cannot be excluded in Tenebrio, but the observations presented here are inadequate to distinguish between the ovarian and extra-ovarian components. Secondly, in the parasitoid hymenopteran Nasonia (King, Rafai & Richards, 1972) and in Drosophila (Mahowald, 1972a) observations suggest autonomous yolk synthesis by the oocyte. A dual origin for the yolk proteins has also been demonstrated in Crustacea (Kessel, 1968), Arachnida (Dumont & Everett, 1967; Jenni, 1971) and
vertebrates (Droller and Roth, 1966; Korfsmeier, 1968) but in these the ovarian contribution comes from the oocyte itself and not from the follicle cells.

The hormonal dependence of yolk deposition and its correlation with the development of follicular patency has already been discussed (p. 203). The morphology of the follicular epithelium at the time of vitellogenesis, the accumulation of labelled proteins at the oocyte surface and their subsequent inclusion in the peripheral yolk spheres justifies the conclusion that in Tenebrio, as in other insects studied (Ramamurty, 1963b; Telfer, 1965; Beams & Kessel, 1969; Melius & Telfer, 1969; De Loof, Lagasse & Bohyn, 1972) yolk proteins are sequestered from the haemolymph and reach the oocyte surface via interfollicular spaces.

What is the significance of the karyosome associated proteins? The data are not inconsistent with the hypothesis that they have a masking function, i.e. that they are involved in gene inactivation, becoming associated with the chromosomes during their contraction. The nucleoproteins would seem more likely candidates for this role than the postulated exogenous RNA (p. 211).

Although Bier et al. (1967) found that the oocyte nuclei incorporated tritiated amino acids intensely in both panoistic and meroistic ovaries, they could not detect an increased incorporation into the karyosphere; neither could Gruzova (1966).

Histones, which are basic proteins, have been implicated in the masking process (Paul, 1970). It is also known that the amino acid tryptophane does not occur in histones (Vendrely & Vendrely, 1966). By using a variety of amino acids (p. 183), including tryptophane, it was hoped to ascertain whether the karyosome-associated proteins were histones or not. The results obtained are inconclusive, because the amino acids were not utilized by the tissues with equal facility (p. 197). It is noteworthy, in this connexion, that in amphibian oocytes proteins labelled with [3H]leucine are preferentially incorporated into the chromosomes (Ficq, 1972). Thus the negative results obtained with tryptophane are not significant.

The endobody, a rounded organelle of obscure function, found in the germinal vesicle of a variety of insects and first described by Bier et al. (1967) has not as yet been located in Tenebrio. In Pimpla (Meng, 1970) the endobody incorporates tritiated protein precursors and resembles the labelled karyosome in Tenebrio. Much more work on the metabolism and ultrastructure of the germinal vesicle is needed before its role in the oogenesis of Tenebrio can be elucidated.

I am most grateful to Professor D. R. Newth for his stimulating interest and criticisms throughout the course of this work. I am also indebted to the following, whom I wish to thank: Professor J. M. Mitchison, for teaching me autoradiography; Dr J. O'Sullivan, for making available and teaching me to use the microdensitometer in the Pathology Department of the Royal Infirmary, Glasgow; Dr D. A. Ede, for reading the manuscript; Mrs C. Morrison, for histological and Mr P. Rickus for photographic assistance: and numerous colleagues who have benefited me by their discussions.
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(Received 1 February 1973)