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NUTRIENT TRANSFER DURING THE REPRODUCTIVE CYCLE IN GLOSSINA AUSTENI NEWST.: HISTOLOGY AND HISTOCHEMISTRY OF THE MILK GLAND, FAT BODY, AND OENO CYTES

ABSTRACT. Using thick sections cut from Epon-embedded tissues fixed in Karnovsky's fixative, the cytological changes visible in the light microscope were described for milk gland, fat body and oenocytes during the pregnancy cycle of the female of Glossina austeni. Histochemical procedures on paraffin sections were used to explore changes in nucleic acids, proteins, and tyrosine-containing proteins. The milk gland undergoes a cycle of secretion correlated with the pregnancy cycle and the results are consistent with the view that the protein component of the milk is synthesized in the secretory cells of the gland. Tyrosine is particularly abundant in the oenocytes which appear to play a crucial role in the metabolism of this amino acid.

Introduction

The genus Glossina comprises one of the few groups of insects in which, by virtue of adenotrophic viviparity, all of the nutrition necessary for the formation of a new fly is transmitted to the larva while it is within the reproductive tract of its mother. Thus, the female tsetse fly deposits, not an egg, but a mature larva which pupates without further feeding. The maturing larva is nourished in the uterus of the female by a 'milk' containing abundant protein and lipid (Cmelik et al., 1969). This material is a secretion of the milk gland, a branched tubular structure opening into the uterus. The tsetse fly is a blood feeder, taking large blood meals at irregular intervals. The metabolism of the female fly is, therefore, primarily directed towards the conversion of the blood meal into milk in order to nourish the maturing larva. We have investigated the flow of protein nutrients through the haemolymph during the pregnancy cycle of Glossina austeni using a variety of techniques. The present paper is the first of a series dealing with this subject, and describes the basic histological changes which occur in the milk gland, fat body and oenocytes of G. austeni during the first two cycles of pregnancy.

The milk gland was first described by Minchin (1905), Stuhlmann (1907) and Roubaud (1909). The structure has been re-investigated in G. palpalis more recently by Hoffmann (1954). These workers were in essential agreement on the morphology and cytology of the gland. The present work confirms their view of the gross morphology of the gland, but presents new
information concerning its cytology, development and functioning.

Materials and Methods

The flies were maintained as previously described (Tobe and Davey, 1971a). The cytological descriptions in this paper are based on the abdomens of flies which were opened and flooded with modified Karnovsky's (1965) fixative (Huebner and Anderson, 1972). The opened, fixed abdomens were processed by the methods of Huebner and Anderson (1972), small pieces of tissue dissected, cut and embedded in Araldite-Epon (Anderson and Ellis, 1965). Sections, 1–2 μ in thickness, were cut on an LKB ultratome using glass knives, transferred to microscope slides and stained with 1% toluidine blue in 1% borax at 60°C for 5 min. Sections were then mounted in immersion oil.

Histochemical procedures were carried out on paraffin sections cut at 4 μ from abdomens fixed in Baker's formalin (Humason, 1967) and dehydrated by the dioxane method (Miller, 1937). For protein, the mercury-bromophenol blue procedure was used (Mazia et al., 1953). For tyrosine-containing proteins, the diazotization-coupling procedure (Pearse, 1968) was used on sections and Millon's reaction (Humason, 1967) on whole dissected abdomens after fixation in Baker's formalin for 15 min. For nucleic acids, the methyl green pyronin ¥ method (Kumick, 1955) and the picric acid-Schiff-methylene blue procedure (Green, 1970) were both used with appropriate controls.

For experiments involving the uptake of peroxidase by various tissues, flies were injected with 50 μg of horseradish peroxidase Type II (Sigma Chemical Co.) in 1 μl of insect saline. Four hours after injection, the abdomens were opened and fixed as previously described for cytological investigations. After 2 hr wash in 0.1 M sodium cacodylate, the abdomens were exposed for 1 hr to the following solution: 40 mg of 3,3'diaminobenzidine (Sigma Chemical Co.), 0.8 ml of hydrogen peroxide, Gomori Tris-HCl buffer, 0.05 M, pH 7.2 to 100 ml. The abdomens were washed twice in 0.1 M phosphate buffer, and post-fixed in 1% osmic acid in 0.1 M phosphate buffer for 1 hr. The tissues were processed through Araldite-Epon as described above.

Results

The anatomy of the reproductive system

Fig. 1 is a diagrammatic representation of the female reproductive system of G. austeni approximately 3 days before the first larvalposition. At this time the uterus is swollen with a late second or early third instar larva, and the left ovary contains the largest oocyte, which is in the final stages of formation. The milk gland, which is at its maximum development at this time, consists of a ramifying system of blind tubules which meet to form the single common collecting duct. The common collecting duct passes under the oviduct and enters the dorsal wall of the uterus. The further fate of this duct within the uterus has been described in an earlier paper (Tobe and Davey, 1971b).

We confirm the observations of earlier workers such as Roubaud (1909) and Hoffmann (1954) that the milk gland consists of two portions which, it will be seen, are also distinguishable in terms of histological characteristics. We have preferred to designate these two regions as the distal and proximal milk glands. It is uncertain at which level of ramification the proximal milk gland is replaced by the distal milk gland.

The diameter of the milk gland as an index of physiological age

The milk gland undergoes dramatic cyclical changes which are correlated with the pregnancy cycle. While these changes will be described in greater detail in a later section of this paper, they are reflected in changes in the transverse diameter of the tubules which make up the gland. These changes can be seen in both proximal and distal portions, but are more obvious in the distal tubes.

Fig. 2 shows the changes in the transverse diameter of the distal milk gland from eclosion to the time of the second larvalposition. These measurements were obtained from serial sections of the abdomens of more than 200 flies fixed at various stages of development. The flies were of
Fig. 1. Diagram of the principal internal organs of reproduction in a female Glossina austeni. CD, common collecting duct; DMG, distal milk gland; LO, left ovary; OV, oviduct; PMG, proximal milk gland; RO, right ovary; SP, spermatheca; U, uterus.

known temporal age. The physiological age of the flies in the second cycle of pregnancy is easily fixed by the time of the first larviposition, which coincides with the second ovulation. The time of the first ovulation is not easily relatable to any parameter which precedes it in time except eclosion. Since the time from eclosion to the first ovulation is variable, we have related the events in this period to the first ovulation. The physiological age of flies in their first cycle of pregnancy has been
Fig. 2. The cross-sectional diameter of the distal milk gland as correlated with the physiological age of the female fly. Each point represents the mean diameter of the gland from up to five flies, and the maximum range of variation about each point does not exceed ±10%. Physiological age is expressed as days before larviposition, with time of larviposition representing day 0. L1 and L2 represent the first and second larvipositions, and O, the first ovulation.

The common collecting duct
Fig. 3 shows a transverse section of the common collecting duct of a female 3 days
before the first larviposition. The duct contains two channels, each lined by an elaborate chitinous intima, beneath which can be found a layer of epidermal cells. The duct is surrounded by helically arranged muscle fibres, elements of which penetrate between the epidermal layers surrounding each channel. Although the channels are distended with secretion at this time, in newly emerged flies they contain only scanty amounts of a more diffuse material (Fig. 4).

The proximal milk gland

The junction of the common collecting duct and the proximal milk gland is shown in Fig. 4. Each of the two channels in the collecting duct leads into one tubule of the proximal gland. Immediately distal to this point, each proximal tubule divides into two, giving a total of four tubules. The heavy muscular tissue which is characteristic of the common collecting duct is no longer apparent. The lumen of the milk gland bears a spinose intima which, in the early stages of the secretory cycle, is thrown up into a number of folds which allow for the distension of the lumen of the gland. The intima is presumably the product of the inconspicuous epidermal cells, the nuclei of which can sometimes be discerned just beneath the intima (Figs. 5, 7). Another layer of cells surrounding the epidermal layer can also be discerned. These are the secretory cells of the proximal tubule, which at this early stage are poorly developed.

Distal to the junction between the proximal gland and the collecting duct, the tubules decrease abruptly in diameter, so that the tubules of the proximal milk gland of a newly emerged female fly are very inconspicuous indeed, and little cellular detail can be seen (Fig. 6).

By the time of the first ovulation, about 10 days after eclosion, the proximal milk gland has increased markedly in diameter (Fig. 7). The nuclei of the secretory cells are now much larger, with prominent nucleoli, and the cytoplasm shows numerous vacuoles. The lumen is larger in diameter and the intima less folded.

At this time, a small vesicle can be discerned in the apical region of each secretory cell (Figs. 7-9). Penetrating to the region of this vesicle is a cylindrical opening, lined with cuticle, and confluent with the lumen of the gland. Each such ductule passes through an epidermal cell which is presumably responsible for its formation. As pregnancy progresses, and the milk gland enlarges, these structures become more obvious. Thus, by 11 or 12 days after eclosion (2 or 3 days after the first ovulation), the vesicles are larger and contain secretion and the ductules are obvious (Fig. 8). By 5 days before larviposition (Fig. 9), the vesicle is much swollen with secretion, and the nucleoli of the secretory cells are very prominent.

Although no secretion is obvious in the lumen of the proximal gland at this time, observations of the gut contents of larvae show that milk is being ingested, suggesting that the secretion is being transported along the gland very rapidly. At high magnification (Fig. 10), the ductule presents an unusual structure in that it appears to be plugged by a diffusely fibrous material.

Secretion continues to be elaborated by the proximal gland until just before larviposition, but by 1 day before larviposition, the cytological signs of secretion are beginning to wane (Fig. 11). Although secretion is present in the lumen, the secretory vesicles are shrunken, the nuclei are smaller and the nucleoli not so prominent. The diameter of the tubule has not changed, but the diameter of the lumen has decreased, reflecting a change in shape of the cells to a more columnar pyramidal profile.

By the end of the first day after the first larviposition, the diameter of the gland has decreased to half that of the tubule 1 day before larviposition. There is no secretion in the vesicle, or in the lumen, which is at least partially collapsed. The nucleoli have reorganized themselves into prominent structures (Fig. 12).

By 3 to 4 days before the second larviposition, the proximal gland has again increased in diameter and secretion is apparent in the vesicle and in the distended lumen of the gland. The nuclei are large with prominent nucleoli.

The distal milk gland

In a newly emerged fly, the tubules of the distal milk gland are poorly developed and have a transverse diameter of about 10 μ— even smaller than the proximal...
gland at this stage. Little cellular detail can be discerned (Fig. 13).

By 10 days after eclosion, at about the time of ovulation, the tubules have increased in diameter to about 35 μ, the nuclei have become large with prominent nucleoli and there are few vacuoles evident in the cytoplasm (Fig. 14).

After this stage, the distal milk gland undergoes rapid development, and by 5 days before the first larviposition (normally about 13–14 days after emergence), the details of the structure of the secretory cell, which is essentially similar to that of the proximal tubule, are apparent. Each secretory cell contains a large secretory vesicle.
which communicates with the lumen by a cuticle-lined ductule as previously described for the proximal gland. As can be seen from Fig. 15, the secretory vesicle is present and swollen, the nuclei are large with prominent nucleoli, and many vacuoles appear in the cytoplasm. Each vesicle communicates with the lumen via a cuticle-lined ductule. The cuticular intima of the distal gland is thinner than that of the proximal gland and has no spines associated with it. The epidermal cells which presumably secrete the intima are also present.

It should be emphasized that the transition from the type of cell characteristic of the proximal gland to that characteristic of the distal gland is not a sharp one, and intermediate forms are observed. Fig. 16 shows one such form taken from the same fly as Fig. 15. The gland is smaller in diameter and shows a larger lumen than the distal gland. It lacks the spines characteristic of the intima of the proximal gland, and has the thinner intima of the distal gland.

The distal tubules continue to increase in diameter until 3 days before the first larviposition. The secretory cells become enormously distended with secretion, especially near the blind tips of the tubules (Fig. 17). Fig. 17 reveals various other features of the cell particularly well. The extensive vacuolation characteristic of both the proximal and distal glands is very obvious. The contents of these vacuoles is not fixed by the techniques used. The cytoplasm of the secretory cells contains clumps of dense material. The secretion in the lumen of the gland contains a number of refractile bodies which appear to be crystalline. The nuclei and cytoplasm of the epidermal cells of the gland are most easily distinguished at this stage. Finally, the cells with enormously distended secretory vesicles also reveal the ductules leading to the lumen particularly well (Fig. 18).

From 3 days before to just after the first larviposition, the distal gland decreases in size, reaching a minimum after larviposition (Fig. 19). The secretory vesicles and lumen contain no secretion at this stage. By 4 to 5 days before the second larviposition, the tubules again increase in diameter, the secretory vesicles are becoming distended with secretion and the cytoplasm is vacuolate (Fig. 20). This growth continues for a further 1 or 2 days and is succeeded by a rapid decline attributable to the rapid transport of secretion out of the lumen, although the cells do not cease to elaborate secretion until about 1 day before larviposition.

The uptake of peroxidase by the milk gland Because of the very rapid growth of the gland, and rapid elaboration of the secretion, the obvious possibility that part of the secretion originates in exogenous protein from the haemolymph was tested by determining whether injected horseradish peroxidase entered the lumen of the gland.

The results of such an experiment are represented by Figs. 21 and 22. It can be seen that even in glands from flies injected 3 days before larviposition, when secretory activity is maximal, no peroxidase appears in the lumen of the gland, although it penetrates between muscle cells surrounding the common collecting duct (Fig. 21) and is prominent on the surface of the gland. Traces of activity were sometimes seen between the cells of the gland (Fig. 22). It is clear, therefore, that the secretion of the gland does not contain exogenous protein.

The fat body and oenocytes The fat body of the adult female of G. austeni occupies much of the abdomen and morphologically consists of a series of spherical structures covered by a thin tunic which joins the spheres together in long chains like strings of beads. Interspersed among these fat body cells are smaller, denser spheres. Because of this close association with the fat body, these cells are presumed to be oenocytes, although they do not resemble the oenocytes described in higher Diptera (Perez, 1910; Wigglesworth, 1965).

Both oenocytes and fat body exhibit cyclical changes during the post-emergence growth of the fly and during subsequent cycles of pregnancy, but these changes are not as obvious and predictable as those found in the milk gland. The fat body spheres undergo changes in both size of lipid droplets and in cross-sectional diameter. The changes in cross-sectional diameter are shown in Fig. 23,
Fig. 17. Longitudinal section of the blind tip of the distal milk gland 3 days before the first larviposition. The vesicles (v) are distended with secretion, the cytoplasm contains clumps (c) of dense material and the lumen contains many refractile particles (r). e, epidermal cell. × 750.

Fig. 18. As for Fig. 17, but at increased magnification. d, ductule; i, intima; v, vesicle. × 1800.

Fig. 19. Transverse section of distal milk gland of a female just after the first larviposition. The vesicles (v) are small. l, lumen. × 750.

Fig. 20. Transverse section of a distal milk gland 4–5 days before the second larviposition. The vesicles (v) are distended with secretion and the lumen (l) also contains secretion. × 750.

Fig. 21. Transverse section of the common collecting duct of a female 3 days before the first larviposition, after treatment with the peroxidase procedure. Peroxidase is present between the muscle cells (arrow), but is absent from the lumen (l). × 750.

Fig. 22. Longitudinal section of a distal milk gland 6 days before the second larviposition. While traces of peroxidase activity can be discerned on the surface of the gland and between the cells, there is no activity in the secretory vesicles (v) or in the lumen (l). × 750.

Fig. 24. Fat body (fb) and pupal fat body (pfb) of a newly emerged female. × 750.
and these changes presumably reflect changes in the size of the lipid droplets and hence fat deposition. The physiological time scale in Fig. 23 has been fixed by measurement of the diameter of the milk gland in each fly used. While small variations in the cross-sectional diameter of the fat body are apparent from day to day, an overall trend is clear. There is a very gradual increase in diameter from eclosion until the time of the first ovulation followed by a steeper increase until the middle of the first pregnancy cycle. A period of variation ensues followed by a rapid decline to a minimum at the time of larviposition. A second period of growth occurs during the first 5 days of the second cycle of pregnancy, followed by a decline as the second larviposition approaches. Although there is variability in this curve, it is clear that the maximum lipid reserves are present between 4 and 5 days before the larviposition.

The changes described by Fig. 23 can also be visualized in sections taken at various times during the cycle. The adult fat body in newly emerged flies has small lipid droplets (Fig. 24). At this stage, large irregular masses of tissue with large vacuoles and abundant protein granules are also apparent. These protein-rich tissues (Fig. 24) are presumed to be the remains of the larval and/or pupal fat body (Perez, 1910; Evans, 1935; Wigglesworth, 1965). This material usually disappears by the fourth day after emergence.

The differences in the size of the lipid droplets at ovulation (Fig. 25), and at various times during the cycle of pregnancy can be seen in photographs included primarily to document the changes which occur in oenocytes (Figs. 27, 28, 30), or to illustrate the uptake of peroxidase (Figs. 31, 32).

The nuclei of the fat body cells are indistinct and only occasionally visible. It has not been possible to distinguish cell borders within the spheres. Protein granules appear in the cytoplasm of the fat body at various times (Figs. 25 and 27), and these granules are most apparent about the middle of the pregnancy cycle.

It is possible that there may be more than one type of fat body cell. Occasionally spheres are observed which differ from the majority of spheres in that they contain, in addition to lipid droplets, numerous protein granules and exhibit prominent nuclei (Fig. 28).

The cells designated as oenocytes have large, presumably polyploid, nuclei and dense cytoplasm, both characteristic of oenocytes (Perez, 1910; Wigglesworth, 1965).

Fig. 25. Oenocyte (oe) and fat body (fb) of a female at the time of the first ovulation. × 750.

Fig. 26. Oenocyte from a female at the time of eclosion. × 750.

Fig. 27. Oenocyte (oe) and fat body (fb) from a female 4 days before the first larviposition. Note protein granules in the fat body (arrow). × 750.

Fig. 28. Oenocyte (oe) and two types of fat body (fb) of a female 7 days before the first larviposition. Note the prominent nucleus and abundant protein granules (arrow) in one type of fat body. × 750.

Fig. 29. Oenocyte from a female at the time of the first larviposition. × 750.

Fig. 30. Oenocyte (oe) and fat body (fb) from a female 4 days before the second larviposition. The fat body contains some protein granules. × 750.

Fig. 31. Fat body of a female 6 days before the first larviposition treated by the peroxidase method. Only a few granules exhibit activity (arrows). × 750.

Fig. 32. Fat body of a female at the time of the first larviposition treated by the peroxidase method. Granules exhibiting activity (arrows) are more abundant. × 750.
In all developmental stages the oenocyte spheres possess a densely staining acidophilic cytoplasm, eight or more large nuclei with prominent nucleoli and no visible cell boundaries. The spheres do not change significantly in size during either of the first two pregnancy cycles observed, remaining at a maximum cross-sectional diameter of about 50–60 μ.

On the other hand, qualitative changes do occur during this period. In newly emerged flies, the cytoplasm of the oenocytes contains a few deeply staining inclusions (Figs. 6, 13). These inclusions become more prominent during the period between eclosion and ovulation (Figs. 25, 26) and remain large during the first cycle of pregnancy (Figs. 27, 28). About 7 days before the first larviposition, vacuoles appear in the cytoplasm and persist throughout the first cycle (Figs. 27, 28, 29), but these are virtually absent during the second cycle (Fig. 30).

**Peroxidase uptake by the fat body and oenocytes**

The possibility that exogenous protein is sequestered by these tissues was tested by the injection of peroxidase. In general, oenocytes do not take up this protein in detectable form at any time during the first two cycles of pregnancy, although the reaction is detectable in the tunic surrounding the oenocytes.

On the other hand, fat body appears to sequester peroxidase in small amounts.

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**Table 1. The staining characteristics of the distal milk gland (DMG), oenocytes (OE) and epidermal cells (EP) during the first two pregnancy cycles of G. austeni. The horizontal lines opposite each test indicate periods of pronounced staining of the tissue.**

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<tr>
<th>Staining method</th>
<th>Physiological age (days)</th>
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<tr>
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<td>6 3 0 6 3 L1 6 3 L2</td>
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<tr>
<td>Methylene blue (RNA)</td>
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<td>Pyronin Y (RNA)</td>
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<td>Bromophenol blue (protein)</td>
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<td>Millon reaction (tyrosine-protein)</td>
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<td>Diazotization-coupling (tyrosine-protein)</td>
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DMG: Distal milk gland
OE: Oenocytes
EP: Epidermal cells
throughout the first two cycles of pregnancy (Figs. 31, 32). There is some indication that the uptake of peroxidase near the time of larviposition is greater than at other times (Fig. 32). This corresponds with the period of minimum fat body diameter and it is reasonable that the fat body, which may be low in metabolites, should sequester haemolymp proteins at this time.

Histochemical studies
Table I represents the staining characteristics of various tissues during the first two pregnancy cycles when sections of abdomen, or, in the case of Millon's reaction, whole mounts of tissues, were subjected to histochemical procedures designed to reveal RNA and protein. Fat body is not included in this figure because the cytoplasm of the fat body cells at all stages was stained only lightly by the agents used. When protein granules were present in the fat body, however, these were stained by both bromophenol blue and the diazotization-coupling method, indicating significant quantities of tyrosine-containing protein in these cells.

Table I reveals that the distal milk gland stains positively for RNA and protein from about the time of the first ovulation until about 1 day before the first larviposition. By 1 day after larviposition the milk gland is again active and remains so until about 1 day before the second larviposition. The first appearance of large quantities of RNA and protein in the cells at the time of ovulation corresponds closely with the timing of the period of rapid growth as revealed by the increase in diameter of the gland. It should also be noted that the secretion of the milk gland stains intensely by both the Millon reaction and the diazotization-coupling procedures whenever it is present.

Table I also shows that the oenocytes stain positively for RNA and protein during all stages observed. They stain particularly intensely with both the Millon reaction and the diazotization-coupling method, indicating large quantities of protein rich in tyrosine.

The epidermal cells stain for RNA and protein for a short period just before and after the first ovulation, and again for a very short period at the time of the first larviposition.

Discussion
It is the primary purpose of this paper to provide a structural background for more detailed studies on nucleic acids and protein synthesis to follow. Certain features of the fat body, oenocytes and milk gland, however, permit us to make some preliminary statements about their mode of functioning.

It is clear from both the measurements of the diameter of tubules and the histological studies that the milk gland undergoes a period of intense secretory activity which is correlated with the presence of an embryo or larva in the uterus. Thus, the size of the tubule, the histology of the cells, the presence of RNA as revealed by two procedures, and the staining of protein by three procedures, are all distinctly cyclical in nature, with minima coinciding with ovulation or larviposition. These results imply that, as far as the protein component of the milk is concerned, the cells of the milk gland, in particular those of the distal tubules, are involved in the synthesis of the product. The possibility that some of the protein which appears in the milk is synthesized outside the gland is rendered unlikely by the failure of horseradish peroxidase to appear in the secretion. The results in this paper do not bear on the lipid which is known to occur in the milk (Cmelik et al., 1969).

The gland clearly secretes during most of the pregnancy cycle in both the first and second cycles, with secretion ceasing only shortly before the larviposition. This interpretation differs from that of Hoffmann (1954) who maintained that secretion during the second and subsequent cycles terminated before the middle of the cycle. Our observations confirm the suggestion of Roberts (1971) that the secretion is produced throughout the larval instars.

The elaboration, secretion and transport of the milk must be an extremely rapid process. During the 9 days that the larva is in the abdomen, it increases in weight by about 25 mg (Tobe and Davey, 1972). The milk gland alone is the source of the nutrient which brings about this increase. We will be concerned with the synthesis of
the secretion in future papers, but the rapidity of the transport of the secretion along the lumen of the gland raises the question of the mechanism by which this movement is effected.

It is clear that the mechanism of transport does not rely solely on the supposed pressure gradient resulting from the build-up of secretion. If this mechanism were important, we would expect always to find secretion in the duct. In fact, the duct is empty of secretion at about the time of larviposition. It is possible that the secretion could be squeezed out along the ducts by pressure resulting from muscle contractions. However, there is no evidence of a muscular sheath around the upper part of the gland. Milk may be moved into the uterus as a consequence of 'sucking' actions on the part of the larva. Several observations suggest that this is unlikely. Firstly, the milk gland duct forms a narrow U-shaped channel in the dorsal wall of the uterus (Tobe and Davey, 1971b), and this channel is usually collapsed in histological sections, suggesting that a negative pressure such as induced by the hypothetical sucking might have the same effect. Secondly, the mouth of the larva is not directly attached to the papilla, and the secretion appears to be passed freely into the uterus. Unless there is a perfect seal between the head of the larva and the uterine wall, air would enter the uterus through the genital opening. Even if the seal were perfect, we would expect spermatozoa to be sucked out of the spermathecae. Thirdly, when the uterus is distended by a nearly mature larva, the pressure from the developing larva would prematurely operate to further collapse the portion of the duct of the gland which lies within the uterine wall.

It is proposed, therefore, that the heavy, spirally arranged musculature of the common collecting duct is important in the transport of the secretion. Contractions of these spiral muscles will cause a twisting and shortening of the duct, forcing secretion into the uterus. The spines and projections within the common collecting duct prevent the backflow of the secretion into the gland. Relaxation of the muscles produces a negative pressure in the lumen of the common collecting duct. The narrow, collapsed lumen and tortuous path of that part of the duct which traverses the uterine wall, prevents the backflow of the secretion. The secretion thus moves from the lumen of the gland into the common collecting duct and the cycle is repeated. Observations reported in detail elsewhere (Tobe, 1972) demonstrate that the hypothesized movements of the collecting duct can be observed in fresh dissections, and that the muscles are innervated.

The fact that the post-emergence growth of both the milk gland and fat body is relatively slight until the time of the first ovulation, when the growth accelerates sharply, once again focuses attention on the first ovulation as a critical event in the physiology of the female tsetse. We have already shown that at the time of the first ovulation, the size of the blood meal taken by the female increases and post-emergence cuticle deposition ceases (Tobe and Davey, 1972).

It is also clear from the histochemical procedures that tyrosine plays an important role in the metabolism of the larva. The milk is known to contain large quantities of tyrosine (Cmelik et al., 1969). A number of abdominal tissues contain large quantities of tyrosine-rich proteins. Of particular relevance is the conspicuous staining of the oenocytes by both Millon's reagent and the diazotization-coupling method, demonstrating that the oenocytes are particularly rich in tyrosine. The significance of this observation will be considered in detail in later papers. It is worth pointing out here, however, that the apparent requirement for large quantities of tyrosine-rich proteins in the milk poses certain problems for the female tsetse. Since the milk gland does not take up exogenous protein, it is unlikely that it is taking up the tyrosine as a haemolymph protein. It is equally unlikely that the amino acid is being synthesized by the milk gland, since no other insects have been shown to synthesize the aromatic amino acids (Brunet, 1963; Gilmour, 1965). The milk gland must, therefore, obtain its tyrosine from the haemolymph in the form of the amino acid or as a small peptide.

Tyrosine is sparingly soluble in aqueous solutions, and it would be impossible to store large quantities of this amino acid in the haemolymph. Several alternative mechanisms for the storage of tyrosine are available: (i) it could be stored in the form
of a tyrosine-rich protein as in *Calliphora* (Munn et al., 1967; Munn and Greville, 1969; Munn et al., 1971); (ii) it could be stored as a more soluble peptide such as the β-alanyl-L-tyrosine of *Sarcophaga* (Bodnaryk and Levenbrook, 1969; Levenbrook et al., 1969); (iii) it could be stored as the more soluble phenolic phosphate ester as in *Drosophila* (Mitchell and Lunan, 1964) or *Sarcophaga* (Seligman et al., 1969).

It is, of course, possible that the tyrosine may be stored in sites other than the haemolymph. Thus abdominal tissues may store the tyrosine and release it as required. The fat body does not seem to be involved in such a process as its activities appear to be directed towards lipid metabolism. The oenocytes would appear to be better candidates for such a function. In any case the detailed analysis of the relative importance in tsetse flies of these several alternatives will have to await a description of the dynamics of tyrosine-containing proteins.

The epidermal cells also undergo cyclical activity which is correlated with the cycle of pregnancy. We have already described some of the activities of the epidermal cells up to the first ovulation: these activities were associated with the deposition of endocuticle which occurs between emergence and the first ovulation (Tobe and Davery, 1972). However, the fact that the epidermal cells undergo a second, somewhat shorter, burst of activity at the time of the second ovulation suggests that these cells may be in some way involved with the reproductive cycle. The significance of these changes awaits further investigation.

**References**


