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Synthesis and Secretion of Mucoprotein Glue in the Salivary Gland of *Drosophila melanogaster*

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Summary. The processes of involved in the accumulation and secretion of adhesive mucoprotein ("glue") in third instar larvae of *D. melanogaster* are described. The RNA templates for glue protein seem to be synthesized within 72-85 hours after oviposition, because treatment of larvae at this age with actinomycin D inhibits the accumulation of the secretion. The secretory granules appear in the salivary gland cells of 90 hour larvae and are discharged into the gland lumen of 118 hour larvae.

Secretion of mucoprotein material by salivary gland cells into the gland lumen depends on high ecdysone concentration. Mucoproteins are synthesized, but not discharged, by these cells in ecdysone-deficient homozygous $l_{(2)}gl$ larvae as well as in normal larvae isolated from ecdysone by ligatures or transplantation of the salivary glands into adult abdomens. Ecdysterone injection into normal 100 hour larvae, 7 day $l_{(2)}gl$ larvae or into adult imaginal abdomens into which salivary glands have previously been stimulates mucoprotein secretion into the gland lumen.

Determination of total protein content in the salivary gland by Lowry's method has shown that "glue" proteins make up 21–32% of the total protein content in the gland.

The glands of the $l_{(3)}tl$ mutant do not give a PAS-positive reaction, nor are the granules of the secretion observed under the phase contrast microscope.

An efficient tool helping to study the establishment and development of a character as well as the activity of genes controlling this character is the *D. melanogaster* salivary gland, because at the end of third instar mucoprotein secretion is produced intensely in these glands (Fraenkel, 1952; Fraenkel, Brookes, 1953; Rizki, 1967; Gaudecker, 1972; Lane *et al.*, 1972) and polytene chromosome puffs are very active (Becker, 1959; Ashburner, 1967, 1972; Zhimulev, 1974).

This study was undertaken with a view to providing additional data on the formation of mucoprotein "glue" and to relate this process with puffing pattern at the end of the third larval instar.

Material and Methods

Stocks. Larvae of the wild strains *Oregon*-R, *Canton*-S, *Batumi*-L and mutants $l_{(2)}gl$ and $l_{(3)}tl$ were used. In the mutant $l_{(2)}gl$ strain, the ecdysone-secreting function is impaired (Hadorn, 1937; Korochkina, 1972; Scharrer and Hadorn, 1938) and ecdysone-stimulated puffs do not develop (Becker, 1959; Ashburner, 1970b). The $l_{(3)}tl$ mutant strain fails to pupate and its chromosomes are very short with changed banding pattern (Kobel and Breugel, 1968; Zhimulev *et al.*, 1974).

The cultures were maintained at 25° C. The development of normal larvae was synchronized by selecting larvae during the second moulting (69–72 hrs after oviposition) as described in a previous paper (Zhimulev, 1974). Pupation occurred at about 120 hours. All the developmental stages are expressed in hours or days after oviposition.

$L_{(2)}gl$ homozygotes were obtained using Ashburner's technique (Ashburner, 1970a) and $l_{(3)}tl$ homozygotes as described elsewhere (Zhimulev *et al.*, 1974).

Histochemical Technique. The histochemical identification of mucoprotein secretion was carried out using three methods:

(1) *PAS-staining of whole glands.* The dissected salivary glands were fixed in a 3:1 alcohol-propionic acid mixture, washed in water, immersed in 1% HIO_4 for 1–2 min, washed in water, stained with Schiffreagent for 5–7 min, washed in water and embedded in glycerol. The preparations obtained were examined under the light microscope.

(2) *Second Method.* The glands were fixed in a 3:1 alcohol: propionic acid micture for 5 min and then squashed in 50% propionic acid on a slide. The coverslip were removed after immersion in liquid nitrogen. The slides were passed through three changes of alcohol, rinsed in water, stained with PAS as described above, then treated with increasing alcohol concentrations and embedded in balsam.

(3) *Method of Precipitation of Mucoprotein Substance Into Granules after Long-Term Fixation at* 0° C. It is known that when secretion-filled glands are placed in 95° alcohol, the secretion immediately hardens (Kodani, 1948). On the other hand, after longterm storage at low temperature the isolated secretion material becomes insoluble in organic solvents, acids and bases (Perkowska, 1963). For this reason, we tried to precipitate the mucoproteins directly in the cells. To achieve this, the dissected glands were fixed in a 3:1 alcohol:propionic acid mixture and stored at 0° C for 2–4 weeks. During this period the glue precipitates and large dark granules in the cytoplasm are observed under the phase contrast microscope. This method makes possible the simultaneous establishment of secretion and analysis of chromosomes in the same cells.

Ligatures, Transplantations and Injections. Rubber ligatures were placed at the level of the 4th segment of 100 hour larvae according to Anderson *et al.* (1966). 24 hours later, salivary glands were dissected in those larvae whose anterior part was pigmented and posterior part was still white.

The salivary glands of 69–72 hour larvae (second moulting) were transplanted into the abdomen of 3–5 day old females using the method of Ephrussi and Beadle (1936). The glands were dissected and fixed 24–168 hours or 24 days after transplantation.

Ecdysterone was dissolved in Ephrussi-Beadle medium (Ephrussi and Beadle, 1936) to a final concentration of 2.5 mg/ml, 0.01 µl of solution was injected into the abdominal segment of a larva at the level of the gonads or into an adult abdomen. The glands were fixed at different time intervals after ecdysterone injections. Control injections consisted of 0.01 µl Ephrussi-Beadle solution.

Assay of Protein Content. Total protein content was measured in the salivary gland of third instar larvae according to Lowry's technique (Layne, 1957). A solution of crystalline bovine albumin (Koch-light laboratories, LTD, England) was used as standard. The protein content per gland was estimated from an average of two independent determinations. At each developmental stage protein was assayed in samples containing 12 and 20 pairs of glands. In order to express protein content in the secretion as percentage of total protein in the gland, two secretion-filled glands from a 120 hour larva were separated; the secretion was evacuated by needle puncture from one gland, the other gland being left intact. Then protein content was measured in samples of 20 pairs of glands.

In addition, protein content in the secrete was determined by comparing values obtained just before puparium formation (120 hour larvae) and after glue discharge (2 hour prepupae).

Actinomycin D Administration. Actinomycin D ("Reanal", Hungary) was used in experiments on the inhibition of the accumulation of mucoprotein material in the salivary glands. Third instar larvae (at 72–90 hours of development) were placed for 2 hours in saline to which 20 γ/ml actinomycin D was added. The layer of solution was thin so that larval spiracles had access to air. The larvae were incubated with actinomycin D in darkness at 25° C, then transferred to tubes with food. The salivary glands were dissected from larvae and fixed at 100 hours. Preliminary experiments have shown that the placement of larvae in pure saline for 2 hours does not affect the formation of mucoprotein in the salivary gland. For this reason, normally developing larvae whose salivary glands were fixed at 100 hours were used in these experiments as controls.

Results

Accumulation of Mucoproteins in the Salivary Glands of Third Instar Larvae. Weak PAS-positive staining of the salivary glands is observed as early as 72 hours (second moulting). The proximal and distal parts do not differ in staining intensity (Figs. 1a, b). Phase contrast microscopy has shown that the salivary glands of 72–90 hours larvae are devoid of granules (Fig. 2a). Staining intensity of the adjacent fat bodies is highest at this point and much deeper than that of the distal part of the gland (Fig. 1b).

From 95 hours onwards, heavy staining of the glands starts from the distal cells and proceeds towards the duct. Small granules may be visualized in single cells of the salivary glands at 95 hours (Fig. 2b) and by 100 hours the distal part is packed with PAS-positive substance and differs very much in colour from the proximal part (Fig. 1c). Numerous granules fill all distal cells (Figs. 2c, d). Staining intensity of the fat body gradually decreases up to the time of pupation (Fig. 1c). Starting from 118 hours, large globules of secretion appear in the lumen (Fig. 2e). At the time of puparium formation (about 120 hours) the secretion fills the gland lumen (Figs. 1d, 2f). As with 72 hours larval cells, prepupal gland cells are also still weakly PAS-positive two hours after spiracle eversion (Fig. 1e).

Mucoproteins in the Salivary Glands of $l_{(3)}tl$ Mutant. The whole salivary gland of $l_{(3)}tl$ mutant gives a PAS-negative reaction (Figs. 1f–h) and only few scattered cells are PAS-positive (Fig. 1g). In this case, granule-filled cells are readily distinguished from non-secretory cells (Fig. 2g). Large bulky patches of "silk secrete", which have been described earlier (Zhimulev, 1973a), in the salivary gland lumen of the $l_{(3)}tl$ mutant give a very weak PAS-staining. The fat body adjacent to the salivary gland is particularly intensely stained (Figs. 1f, g).

Actinomycin D Sensitive Stages of Secrete Accumulation. Treatment of larvae with actinomycin D at 72–85 hours significantly inhibits the formation of mucoprotein secretion (Table 1). During this period actinomycin D partially or totally inhibits the accumulation of secretion in 79–100% of larvae. Actinomycin D treatment at 90 hours has no marked influence on the accumulation of PAS-positive material in the salivary glands.

Changes in Total Protein Content in the Salivary Glands of Third Instar Larvae. The determination of total protein content in salivary gland cells has shown that

Table 1. Actinomycin D effect on the accumulation of PAS-positive substances salivary glands

Age of treated larvae (hours after oviposition)	Glands fixed at 100 hours			Number of glands studied (100%)
	Filled with secretion	Half-filled with secretion	Empty	
72	2 (7%)	5 (19%)	17 (74%)	27
76	2 (5%)	12 (28%)	29 (67%)	43
80	7 (21%)	11 (33%)	15 (46%)	33
85	0 —	4 (40%)	6 (60%)	10
90	30 (77%)	6 (15%)	3 (8%)	39
Glands of 100 hour larvae (no actinomycin D treatment)	20 (87%)	3 (13%)	0	23

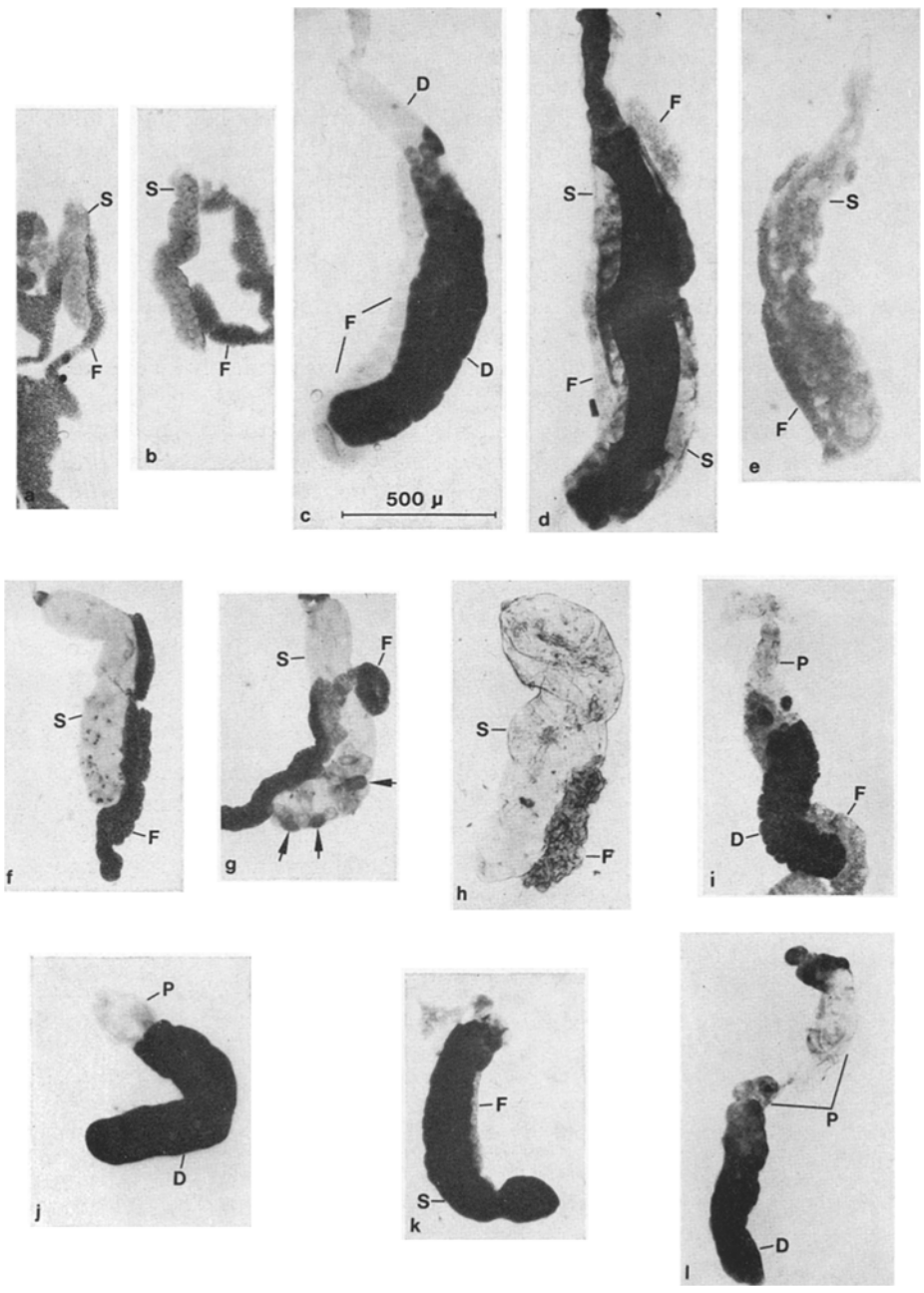

Fig. 1a—l. PAS-positive material in the salivary glands during normal development (Batumi-L larvae) (a—e), in $l_{(3)}tl$ mutants (f—h), in $l_{(2)}gl$ mutants (i), after *in vivo* cultivation (j, l), and in ligatured larvae (k). (a) 80 hours, (b) 90 hours, (c) 100 hours, (d) 120 hours after oviposition; (e) 2 hours prepupa; (f) PAS-negative salivary gland, 168 hours; (g) PAS-negative salivary gland with some PAS-positive cells (arrows), 168 hours; (h) 17 days after oviposition; (i) 168 hours; (j) three days of incubation in the adult abdomen, the gland was transplanted with fat body; (l) the same, the salivary gland transplanted without fat body; (k) gland of ligatured larva. *S* salivary gland, *P*, *D* proximal and distal parts, *F* fat body

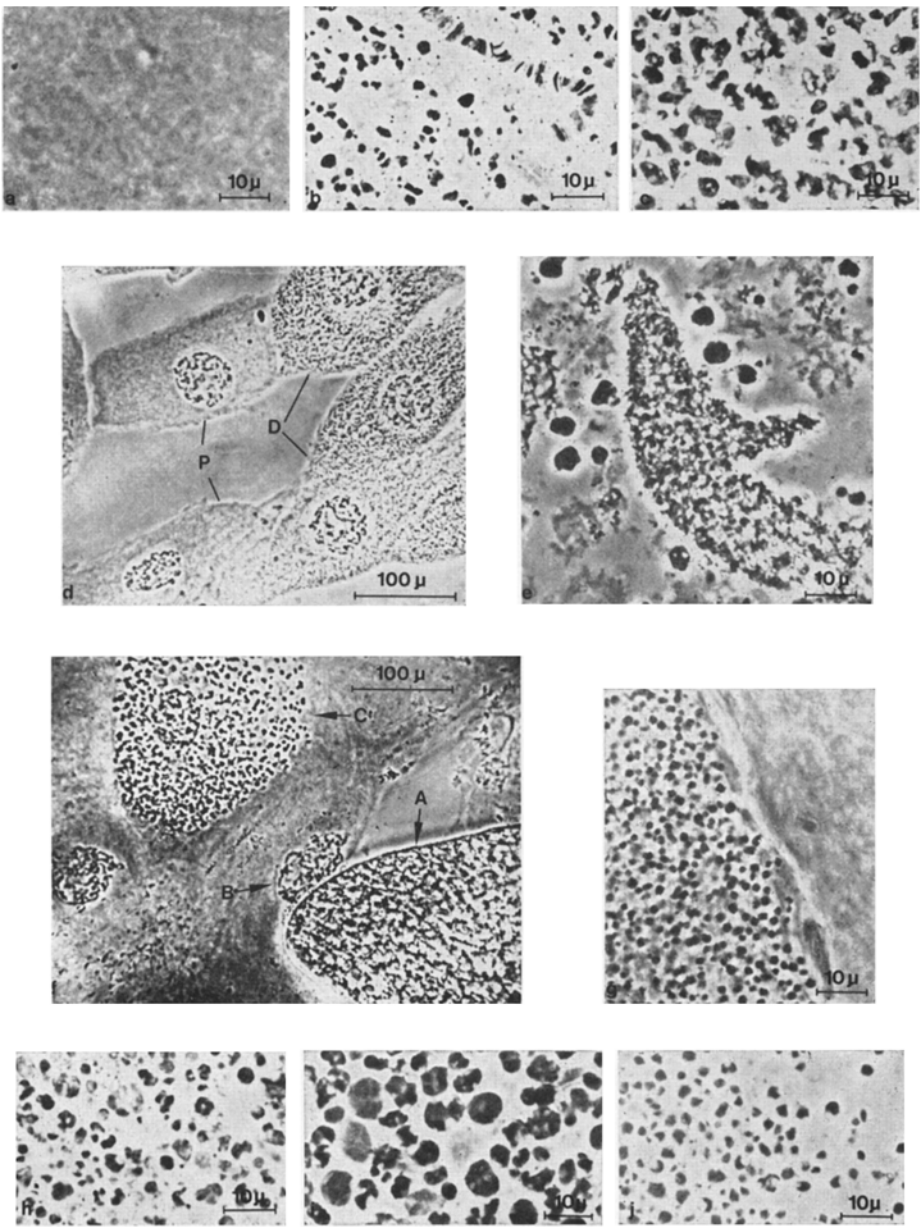

Fig. 2a—j. Mucoprotein granules in the salivary gland cytoplasm during normal development of Batumi-L larvae (a—f), in $l_{(3)}tl$ (g) and $l_{(2)}gl$ (h) mutants, in ligatured larvae (i) and after transplantation into the adult abdomen (j). Squash preparations, phase contrast. (a) 90 hours, (b) 95 hours, (c) 100 hours. (d) 100 hours, border between proximal (*P*) and distal (*D*) cells; (e) globule of secretion (arrow) in the salivary gland lumen, 118 hours; (f) salivary gland of 120 hours larva just before spiracle eversion. Arrows: *A* patch of mucoprotein in the salivary gland lumen, *B* cell without mucoprotein granules, *C* cell with mucoprotein granules

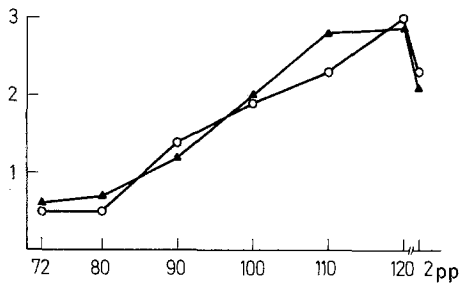

Fig. 3. Changes of protein content in the salivary glands of third instar larvae. Abscissa: Time from oviposition (hours), 2 pp-hours after spiracle eversion. Ordinate: Total protein content (μg per gland). o——o Batumi-L larvae; ▲——▲ Oregon-R larvae

Table 2. Relative proportion of "glue" protein in the salivary glands of larvae just before spiracle eversion

Strain	Total protein per salivary gland (μg)		Proportion of "glue" protein (%)	Total protein per salivary gland (μg)		Proportion of "glue" protein (%)
	Before "glue" discharge (120 hour larvae)	After "glue" discharge (2 hour prepupae)		Secretion filled gland	After experimental evacuation of secretion	
Batumi-L	3.0	2.3	23	3.3	2.6	21
Canton-S	3.7	2.5	32	3.4	2.4	28
Oregon-R	2.9	2.1	28	3.0	2.1	30

during the observation period, from second moulting (69–72 hours) to the point of pupation, protein content in the gland rises more than fivefold and attains maximum values at about 120 hours (Fig. 3). The dynamics of protein accumulation is similar in both strains studied. The comparison of total protein content in the salivary gland cells of 120 hour larvae (before release of secretory material) and in 2 hour prepupae (after its release) has revealed that protein secretion makes up 23–32% of the total protein in the salivary gland. After experimental evacuation of all the secretion from the gland at 120 hours the values for protein content were comparable 21–30% (Table 2).

Hormonal Regulation of Glue Secretion. The secretion starts to appear in the lumen of 118 hour larvae (Fig. 2e). Analysis of chromosome puffs indicates that this pattern corresponds to PS-7, when mucoproteins are released into the lumen (Table 3). Quite obviously, mucoprotein is secreted from cells into the gland cavity at the time when ecdysone concentration is high. In an attempt to elucidate whether ecdysone is necessary for secretion, 100 hour larvae were injected with ecdysterone and glands were fixed 8 hours later. Before steroid injection the PAS-positive granules are evenly arranged in the cytoplasm (Fig. 4a), whereas 8 hours after it, PAS-positive patches appear in the lumen (Fig. 4d). Most cells are devoid of PAS-positive material, although single cells contain huge granules

Table 3. Ecdysone control of mucoprotein secretion

Experiments	No. of larvae with secretion in gland lumen		Total No. of larvae studied
	Absent	Present	
Normal development of Batumi-L larvae, 115 hours, PS-5	65	0	65
Normal development of Batumi-L larvae, 118 hours, PS-7	0	52	52
Ecdysterone injection into 100 hour Batumi-L larvae and fixation after 8 hours	1	29	30
$L_{(2)}gl$ larvae, 7 days	72	2	74
$L_{(2)}gl$ larvae, 11 days	30	2	32
Ecdysterone injection into 7 day $l_{(2)}gl$ larvae and fixation after 7 hours	4	28	32
100 hour Batumi-L larvae ligatured; fixation at 124 hours	20	0	20
Transplantation of 72 hour Batumi-L salivary glands into adult abdomen, fixation 2–7 days later	63	2	65
The same, time of incubation 24 days	10	0	10
Transplantation of 72 hour Batumi-L salivary glands into adult abdomen, ecdysterone injection after 3 days and fixation 10 hours later	5	17	22

at apical poles (Fig. 4b, c). Almost all larvae react similarly to ecdysterone injection (Table 3). Thus, under the influence of ecdysterone, mucoprotein secretion occurs 10 hours earlier than in normally developed *D. melanogaster*.

The salivary glands of 7 days old larvae of the lethal $l_{(2)}gl$ strain, i.e. two days after the puparium formation of their heterozygous siblings, show intense PAS-positive staining with very distinct proximal and distal parts of the gland (Fig. 1i). No patches, nor globules of mucoproteins were found in the gland lumen (Figs. 2h, 5a). The secretion is not discharged into the gland lumen even in 11 day old larvae (Table 3). Seven hours after hormone injection mucoprotein is released into the lumen (Fig. 5b). It should be noted that hormone injection into $l_{(2)}gl$ larvae does not always induce such a clear-cut reaction. In some individuals PAS-positive material accumulates in the cell apex, but is not released. In most larvae, however, ecdysterone induces discharge of secretion into the gland lumen (Table 3).

Studies of salivary glands which were isolated from hormone by ligatures have shown that the secretion fills the cells, but is absent in the gland lumen (Figs. 1k, 2i and Table 3).

Mucoprotein secretion was also studied in salivary glands of 69–72 hour larvae that were transplanted into adult abdomens which contain no ecdysone (Novak, 1966). The two experimental designs were as follows: I. Transplantation of salivary glands and adjacent fat bodies; II. Transplantation of salivary glands only. As the results are similar irrespective of the experimental design, they are analysed

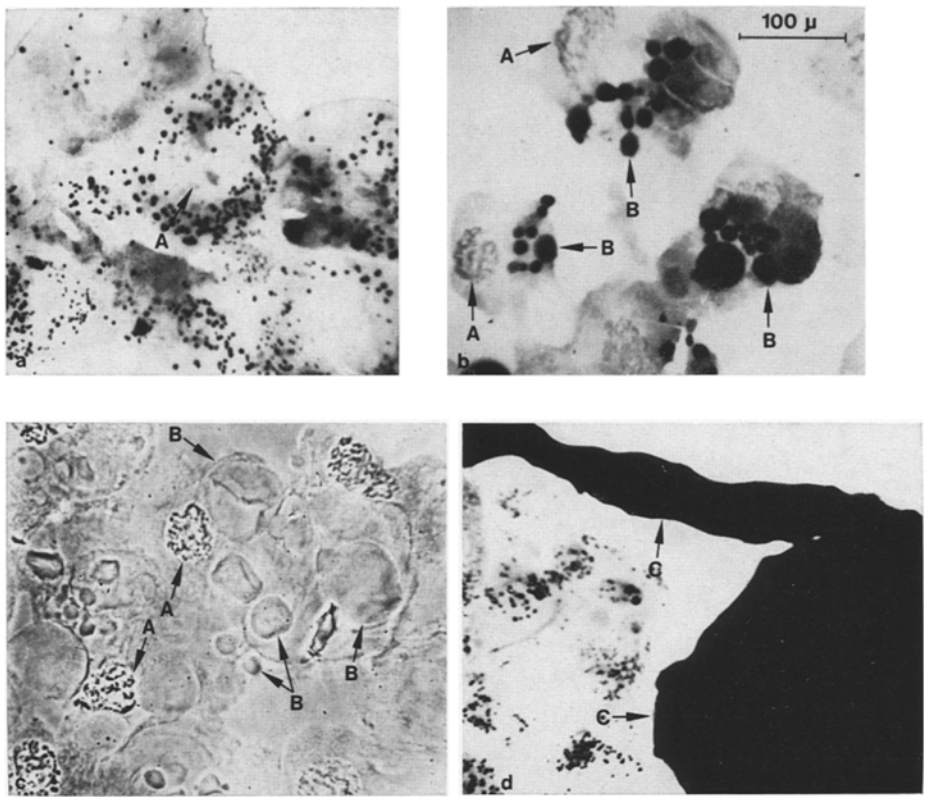

Fig. 4a—d. Mucoprotein granules and patches in the salivary glands of 100 hour Batumi-L larvae (a) and after ecdysterone injection (b—d). (a, b, d) PAS-staining of squash preparations; (c) phase contrast. Arrows: *A* nucleus, *B* droplets of secretion, *C* patch of mucoprotein in the salivary gland lumen

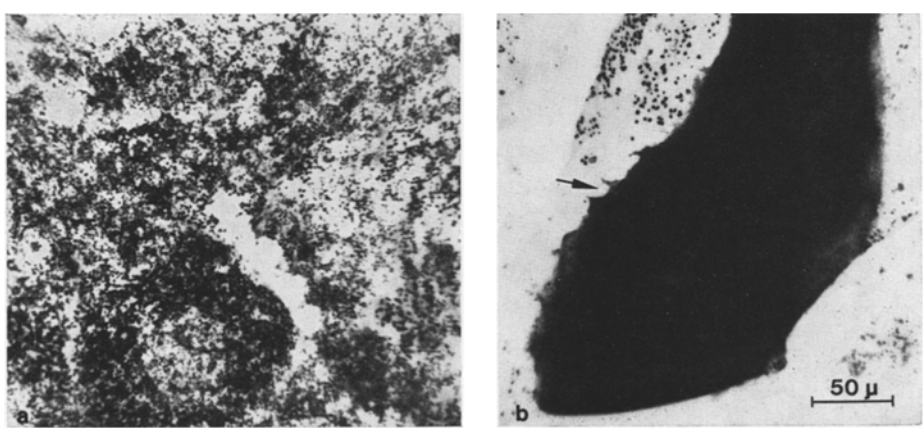

Fig. 5a and b. Mucoprotein in $l_{(2)}gl$ salivary gland cells before (a) and after (b) ecdysterone injection. PAS-staining of squash preparations. (a) granules of secretion in cytoplasm, (b) patch (arrow) of secretion in the gland lumen

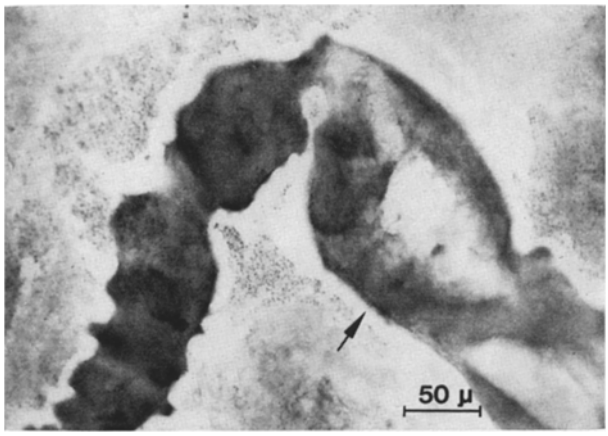

Fig. 6. Patch of secretion (arrow) in the transplanted salivary gland after ecdysterone injection into the adult abdomen

together. In salivary glands transplanted into the abdomens of adults, secretion accumulates in the distal part of the glands with a delay of 24 hours as compared with normal development. With longer incubation of salivary glands the cells swell with secretion and the intensity of PAS-staining increases (Figs. 1j, l). However, 24 days after incubation there is not a single gland lumen containing mucoprotein secretion (Figs. 1j, 2j, Table 3). Ecdysterone injection into the abdomens results in mucoprotein secretion into the gland lumen (Fig. 6, and Table 3).

Discussion

One of the main products of the salivary gland of *Drosophila* third instar larvae is the so-called "glue" required for the fixation of the pupa to the substrate (Fraenkel, 1952; Fraenkel, Brookes, 1953). The present results indicate that the mucoproteins make up 21–33% of the total protein content in the salivary gland prior to puparium formation. This is similiar to the values obtained for *Sciara* (Been, Rasch, 1972).

It has been demonstrated that this glue may be a mucoprotein because it contains carbohydrate derivatives and heterogeneous proteins (Kodani, 1948; Perkowska, 1963). Glue is 70% protein and 30% carbohydrates (Ashburner, Blumental, cited in Ashburner, 1970b).

Fig. 7 is a diagram of the dynamics of mucoprotein formation and of some related processes in a salivary gland. Treatment of larvae with actinomycin D at 72–85 hours interferes with secretion. This may be interpreted as indicating that m-RNA synthesis occurs during this time interval and not solely during the second moulting as suggested by Lane *et al.* (1972). Unfortunately, the method used does not permit us to establish whether m-RNA of the secretory material is synthesized later than 85 hours after oviposition. What is observed, however, is that the protein content rises until immediately before discharge of protein into the lumen (Fig. 3). It may thus be suggested, that protein is synthesized either on the templates

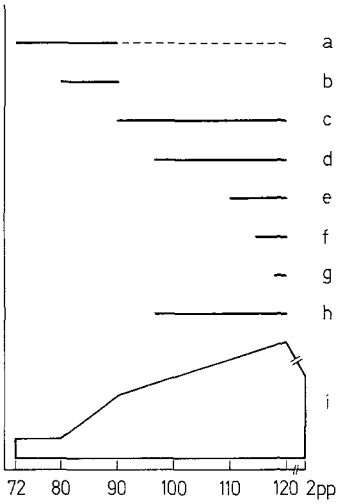

Fig. 7. Schematic sequential representation of processes associated with the synthesis and secretion of mucoprotein. Abscissa: time after oviposition (hours), 2 pp–2 hour prepupa. Black lines indicate time intervals of: (a) Actinomycin D sensitive period, (b) PAS-positive staining of fat body, (c) PAS-positive staining of salivary gland, (d) Appearance of granules under phase contrast in the cytoplasm, (e) High ecdysone concentration (Becker, 1962; Ashburner, 1967), (f) Appearance of ecdysone—stimulated puffs (Becker, 1959, Ashburner, 1967, 1972; Zhimulev, 1974), (g) Discharge of mucoproteins into salivary gland lumen, (h) Developmental stage at which puffing patterns were described (Ashburner, 1972; Zhimulev, 1974; Belyaeva *et al.*, 1974), (i) Change of total protein content in salivary gland

which are synthesized *de novo* until puparium formation, or on stable templates synthesized during the period of 72–85 hours of development. Such stable templates have been described for mucopolysaccharides of *Chironomus* salivary gland secretion (Clever, 1969; Clever *et al.*, 1969; Wobus *et al.*, 1972). If m-RNAs for protein secretory material were actually synthesized during the limited time-interval of 72-85 hours, it would follow that puffs active during the last 10 hours of larval development, 110–120 hours (Ashburner, 1967, 1972), cannot be related to mucoprotein synthesis. On the other hand, were m-RNAs synthesized continuously starting from 72 hours of larval development to 120 hours, then there would be reason to assume that genes coding for secrete proteins are located in small continuously functioning puffs (Belyaeva *et al.*, 1974; Zhimulev, 1974). It is impossible to study puffs in 72–85 hour larvae because of the small chromosome size.

Eighty hours after oviposition the protein content in the gland begins to rise sharply; this may be due to gland growth and to accumulation of protein components of the secretion (Fig. 7i).

Starting from 100 hours, elevation of total protein content in the gland seems to be due mainly to increasing protein content in the secretion, since after discharge of the secretion (2 hour prepupae) the protein level is the same as in 100 hour larvae (Figs. 3, 7i).

From 95 hours onwards the secretion in the salivary gland cells may be identified both histochemically and by means of phase contrast microscopy

(Figs. 7c, d). Electron microscopy has demonstrated that the activation of the Golgi complex and droplets of the secretory material are first observed in salivary cells 21–25 hours after the second moulting, i.e. about 90–95 hours after oviposition (Rizki, 1967; Gaudecker, 1972; Lane *et al.*, 1972).

The results obtained have shown that in *Drosophila melanogaster* as well as in *Drosophila hydei* (Poels, 1970, 1972; Poels *et al.*, 1971) ecdysone accelerates mucoprotein release from the cells into the salivary gland lumen. It has also been found that in the absence of this hormone "glue" is not secreted at all in salivary glands of normal larvae transplanted into adult abdomens, in the salivary glands of ligatured larvae, and in $l_{(2)}gl$ larvae.

In the course of normal development ecdysone content starts to rise from 110 hours (Becker, 1962; Ashburner, 1967). When its titre attains a high level, secretion is discharged into the lumen. Large globules of the secretion (Fig. 2e) start to appear at 118 hours, i.e. at PS-7, although secretion starts somewhat earlier, at PS-5 (Lane *et al.*, 1972). Hence, the results obtained lend further support to the idea that the process of mucoprotein secretion in *Drosophilidae* is under hormonal control (Poels, 1970). Furthermore, ecdysone most probably stops glue synthesis *de novo* (Poels, 1970). Fig. 4b shows that after secretion mucoproteins do not reappear in cell cytoplasm.

The recent experiments of Poels (1972) have demonstrated that an ecdysone-stimulated synthesis of RNA and proteins is required for mucoprotein secretion. This prompted the suggestion that the RNA required may be synthesized in ecdysone-stimulated puffs. If this were so, these puffs, which may be associated with mucoprotein secretion, would be specific only to the distal part of the gland where the mucoprotein secretion is synthesized (Berendes, 1965; Gaudecker, 1972). The results of comparisons of puffing in the proximal (PAS-negative) and distal (PAS-positive) parts of *Drosophila melanogaster* salivary glands was disappointing and did not reveal any puffs specific to this part of the gland (Zhimulev, 1973b). A single small specific puff, 27EI-2, just slightly more active in the distal cells, has been identified,but is present during the entire period of prepupal development (Ashburner, 1972) and for this reason could not be ascribed to the secretory activity of this part of the gland.

Concerning the relation between puffing activity and the mechanism controlling secretion it may be speculated that:

1. Direct visual analysis of puffing is not sufficient to characterize chromosome activity. Many RNA-synthesizing regions do not show puff-characteristic features and, in spite of this, incorporate ^{3}H-uridine (Berendes, 1965; Zhimulev, Belyaeva, 1975). These small puffs are presumably activated while under the effect of ecdysone and code for the proteins required for mucoprotein secretion.

2. There is a regulation of genome activity at the translation level. In this case, the ecdysone-stimulated puffs (or puff) responsible for mucoprotein secretion would arise in functionally inactive tissues such as the proximal portion of the salivary gland and RNA, though synthesized, would be degraded subsequently and no puff products would be transported into the cytoplasm.

There is no comprehensive picture allowing us to locate the site where the components of mucoprotein secretion are synthesized. Transport of different components of the secretion into the gland from the haemolymph is a widely

discussed possibility (Laufer, 1965; Doyle, Laufer, 1969; Bianchi *et al.*, 1973). Two observations are pertinent: a definite periodicity in the appearance and release of PAS-positive material in the fat body and salivary gland (Figs. 1b, 7b). The fat body is PAS-positive earlier than the salivary gland (80 hours), and subsequently fat body staining intensity decreases. From Figs. 1a—d of Lane's paper (Lane *et al.*, 1972) it is also clear that PAS-positive material starts to accumulate in the fat body first and then in the salivary gland, although this sequence is not explicitly mentioned in the paper.

In this connection, it is of interest that in the $l_{(3)}tl$ mutant the fat body shows exceptionally intense staining (Figs. 1f, g) with a PAS-negative salivary gland. On the other hand, in the salivary gland lumen of $l_{(3)}tl$ larvae thick, faintly PAS-staining patches of secretion are observed. These patches have a somewhat different appearance under the phase contrast microscope (Zhimulev, 1973a).

It is known that the fat body in insects is the central organ of intermediate metabolism of fat and carbohydrates, the site where many proteins are synthesized (Kilby, 1963; Martin *et al.*, 1970; Wyss-Huber and Lüscher, 1972; Peled and Tietz, 1973). In this connection, it is conceivable that the fat body participates in the formation of the salivary gland secretion either directly or through haemolymph transport and thus provides some of the components required for the synthesis of mucoprotein substance. It is possible that in $l_{(3)}tl$ mutants the transport of the carbohydrate component from the fat body to the gland is impaired. "Silk secrete" (Zhimulev, 1973a) seems to be a protein component with slightly diluted PAS-positive material which accumulates in single cells (Fig. 1g).

However, the idea (appealing as it may seem) that the carbohydrate component of the secretion is synthesized in the fat body and then transported to the salivary gland is difficult to reconcile with the results obtained with the transplantation of salivary glands devoid of the fat body into imaginal abdomens (Fig. 1l).

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References

Anderson, R., Hancock, M., Burdette, W. J.: A simple method for ligating and injecting *Drosophila* larvae. Dros. Inform. Serv. **46**, 198 (1966)

Ashburner, M.: Patterns of puffing activity in the salivary gland chromosomes of *Drosophila*. I Autosomal puffing patterns in a laboratory stock of *Drosophila melanogaster*. Chromosoma **21**, 398–428 (1967)

Ashburner, M.: Patterns of puffing activity in the salivary gland chromosomes of *Drosophila*. V. Responses to environmental treatments. Chromosoma, **31**, 356–376 (1970a)

Ashburner, M.: Function and structure of polytene chromosomes during insect development. in: Adv. Insect. Physiol. London-New York **7**, 1–95 (1970b)

Ashburner, M.: Puffing patterns in *Drosophila melanogaster* and related species in: Results and Problems in Cell Differentiation (W. Beermann ed.) **4**, 101–151 (1972)

Becker, H. J.: Die Puffs der Speicheldrüsenchromosomen von *Drosophila*. I. Mitt. Beobachtungen zum Verhalten des Puffmusters im Normalstamm und bei zwei Mutanten, giant und lethal-giant-larva. Chromosoma **10**, 654–678 (1959)

Becker, H. J.: Die Puffs der Speicheldrüsenchromosomen von *Drosophila melanogaster*. Die Auslösung der Puffbildung, ihre Spezifität und ihre Beziehung zur Funktion der Ringdrüse. Chromosoma **13**, 341–384 (1962)

Been, A. C., Rasch, E. M.: Cellular and secretory proteins of the salivary glands of *Sciara coprophila* during the larval-pupal transformation. J. Cell. Biol. **55**, 420–432 (1972)

Belyaeva, E. S., Korochkina, L. S., Zhimulev, I. F., Nazarova, N. K.: Puff characteristics in the X-chromosome of *Drosophila melanogaster* females. Tsitologia (USSR) **16**, 440–446 (1974)

Berendes, H. D.: Salivary gland function and chromosomal puffing patterns in *Drosophila hydei*. Chromosoma **17**, 35–77 (1965)

Bianchi, A. G. de, Terra, W. R., Lara, F. J. S.: Formation of salivary secretion in *Rhynchosciara americana*. I. Kinetics of labelled amino acid incorporation. J. Cell Biol. **58**, 470–476 (1973)

Clever, U.: Chromosome activity and cell function in polytenic cells. II. The formation of secretion in the salivary glands of *Chironomus*. Exp. Cell Res. **55**, 317–322 (1969)

Doyle, D., Laufer, H.: Sources of larval salivary gland secretion in the dipteran *Chironomus tentans*. J. Cell Biol. **40**, 61–78 (1969).

Ephrussi, B., Beadle, G. B.: A technique of transplantation for *Drosophila*. Amer. Naturalist **70**, 218–225 (1936)

Fraenkel, G.: A function of the salivary glands of the larvae of *Drosophila* and other flies. Biol. Bull. **103**, 285 (1952)

Fraenkel, G., Brookes, V. J.: The process by which the puparia of many species of flies become fixed to a substrate. Biol. Bull. **105**, 442–449 (1953)

Gaudecker, B. von: Der Strukturwandel der larvalen Speicheldrüse von *Drosophila melanogaster*. Ein Beitrag zur Frage nach der steuernden Wirkung aktiver Gene auf das Cytoplasma. Z. Zellforsch. **127**, 50–86 (1972)

Hadorn, E.: An accelerating effect of normal "ring-glands" on puparium formation in lethal larvae of *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **23**, 478–484 (1937)

Kilby, B. A.: The biochemistry of insect fat body. In: Advances in Insect Physiology. Academic Press, London-New York **1**, 112–174 (1963)

Kodani, M.: The protein of the salivary gland secretion in *Drosophila*. Proc. Nat. Acad. Sci. USA **34**, 131–135 (1948)

Kobel, H. R., Breugel, F. M. A., van: Observation on ltl (lethal tumorous larvae) of *Drosophila melanogaster*. Genetica **38**, 305–327 (1968)

Korochkina, L. S.: Change of the ring gland in post-natal development of *Drosophila melanogaster* Canton-S and in $l_{(2)}$gl mutant. Dros. Inform. Serv. **48**, 74–75 (1972)

Lane, N. J., Carter, J. R., Ashburner, M.: Puffs and salivary gland function: the fine structure of the larval and prepupal salivary glands of *Drosophila melanogaster*. Wilhelm Roux' Archiv, **169**, 216–238 (1972)

Laufer, H.: Chromosomal puffing and its relation to cell function and development. Arch. d'Anat. microscop. **54**, 648–651 (1965)

Layne, E.: Spectrophotometric and turbidimetric methods for measuring proteins. in: Methods in Enzymology **3**, 447–500, New York: Academic Press 1957

Martin, M. D., Kinnear, J. F., Thomson, J. A.: Developmental changes in the late larva of *Calliphora stygia*. IV. Uptake of plasma protein by fat body. Austr. J. Biol. Sci. **24**, 291–299 (1971)

Novak, V. J. A.: Insect Hormones. London: Methuen and Co LTD 1966

Pelled, Y., Tietz, A.: Fat transport in the locust *Locusta migratoria*: the role of protein synthesis. Biochim. et biophys. Acta **296**, 499–509 (1973)

Perkowska, E.: Characteristics of *Drosophila* salivary gland secretion. Exp. Cell Res. **32**, 259–271 (1963)

Poels, C. L. M.: Time sequence in the expression of various developmental characters induced by ecdysterone in *Drosophila hydei*. Develop. Biol. **23**, 210–225 (1970)

Poels, C. L. M.: Mucopolysaccharide secretion from *Drosophila salivary* gland cells as a consequence of hormone induced gene activity. Cell Differ **1**, 63–78 (1972)

Poels, C. L. M., Loof, A. de, Berendes, H. D.: Functional and structural changes in *Drosophila* salivary gland cells triggered by ecdysterone. J. Insect Physiol. **17**, 1717–1729 (1971)

Rizki, T. M.: Ultrastructure of the secretory inclusion of the salivary gland cell in *Drosophila*. J. Cell Biol. **32**, 531–534 (1967)

Wobus, U., Popp, S., Serfling, E., Panitz, R.: Protein synthesis in salivary gland. Mol. Gen. Genet. **116**, 309–321 (1972)

Wyss-Huber, M., Lüscher, M.: In vitro synthesis and release of proteins by fat body and ovarian tissue of *leucophaea maderae* during the sexual cycle. J. Insect Physiol. **18**, 689–710 (1972)

Scharrer, B., Hadorn, E.: The structure of the ring gland (corpus allatum) in normal and lethal larvae of *Drosophila melanogaster*. Proc. Nat. Acad. Sci. USA **24**, 236–242 (1938)

Zhimulev, I. F.: Description of a new type of secrete in the larval salivary gland of *Drosophila melanogaster*. Dros. Inform. Serv. **50**, 46 (1973a)

Zhimulev, I. F.: Comparison of puffing patterns in proximal and distal parts of the salivary gland in *Drosophila melanogaster*. Dros. Inform. Serv. **50**, 96–97 (1973b)

Zhimulev, I. F.: Comparative study of the function of polytene chromosomes in laboratory stocks of *Drosophila melanogaster* and the $l_{(3)}$tl mutant (lethal tumorous larvae). I. Analysis of puffing patterns in autosomes of the laboratory stock Batumi-L. Chromosoma **46**, 59–76 (1974)

Zhimulev, I. F., Belyaeva, E. S.: ^{3}H-uridine labelling patterns in *Drosophila melanogaster* salivary gland chromosomes, X, 2R, and 3L. Chromosoma **49**, 219–231 (1975)

Zhimulev, I. F., Belyaeva, E. S., Lychev, A. V.: Changes of banding patterns in the polytene chromosomes of $l_{(3)}$tl mutant of *Drosophila melanogaster*. Genetica (USSR) **10**, N 10, 35–50 (1974)

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