

# CELLULAR AND SECRETORY PROTEINS OF THE SALIVARY GLANDS OF *SCIARA COPROPHILA* DURING THE LARVAL-PUPAL TRANSFORMATION

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## ABSTRACT

The cellular and secretory proteins of the salivary gland of *Sciara coprophila* during the stages of the larval-pupal transformation were examined by electrophoresis in 0.6 mm sheets of polyacrylamide gel with both SDS-continuous and discontinuous buffer systems. After SDS-electrophoresis, all electrophoretograms of both reduced and nonreduced proteins from single glands stained with Coomassie brilliant blue revealed a pattern containing the same 25 bands during the stages of the larval-pupal transformation. With the staining procedures used in this study, qualitative increases and decreases were detected in existing proteins and enzymes. There was no evidence, however, for the appearance of new protein species that could be correlated with the onset of either pupation or gland histolysis. Electrophoretograms of reduced samples of anterior versus posterior gland parts indicated that no protein in the basic pattern of 25 bands was unique to either the anterior or posterior gland part. Electrophoretograms of reduced samples of secretion collected from either actively feeding or "cocoon"-building animals showed an electrophoretic pattern containing up to six of the 25 protein fractions detected in salivary gland samples, with varied amounts of these same six proteins in electrophoretograms of secretion samples from a given stage. Zymograms of non-specific esterases in salivary gland samples revealed a progressive increase in the amount of esterase reaction product in one major band and some decrease in the second major band during later stages of the larval-pupal transformation.

## INTRODUCTION

The dipteran salivary gland has been extensively studied as a model system for the analysis of chromosome morphology and physiology (2, 8, 32). A number of cytogenetic and radioautographic studies of salivary gland cells in diverse dipteran species have focused on the polytene chromosomes of these cells and their puffing patterns during larval development. In vivo and in vitro studies from many laboratories (3, 7, 20, 24) have provided evidence in support of the hypothesis that the puffing phenomenon is a cytological manifestation of genetic regulation in

these cell systems (7, 43) and that a correspondence exists between chromosomal puffing activity and biochemical events within these tissues (3, 6, 9, 19). To date, only one study has indicated a possible relationship between a specific puff site and an electrophoretically defined protein (20). There is, however, no conclusive evidence as to whether a puff site encodes information for several proteins, one protein, or necessarily any protein (46).

An electrophoretic analysis of the salivary

glands of *Sciara coprophila*<sup>1</sup> during the larval-pupal transformation is of special interest because of the maximal activity of the polytene chromosomes in these cells during this period, with specific puff sites showing a selective synthesis of either RNA or DNA (14, 17, 35). Although RNA puffs are a regular feature of dipteran polytene chromosomes throughout larval development, DNA puffs are exclusive to the sciarids and are found only during the larval-pupal transformation (31). Several investigators have suggested that major changes in protein synthesis may occur as a result of this period of intense puffing activity during late fourth instar, especially in response to proposed template activity of the DNA at the DNA puff sites (13, 27, 31).

The present paper is a report on our analysis by two types of electrophoresis of the cellular and secretory proteins of salivary glands of *Sciara*. This work is but a first step toward our eventual goal of understanding the patterns of protein synthesis in this tissue and the functional relevance of particular types of chromosomal puffs that characterize sciarid salivary gland cells during certain stages of the larval-pupal transformation. As reported elsewhere (5), our vertical microsystem for electrophoresis in thin sheets of polyacrylamide gel allows the use of minute tissue samples containing less than 5  $\mu\text{g}$  of protein. A major advantage of this particular micromethod is the ease with which electrophoretograms from many different tissue extracts, such as individual *Sciara* salivary glands or gland parts, may be compared with one another on a single sheet of polyacrylamide gel.

## MATERIALS AND METHODS

### *Culture and Staging of Animals*

All specimens of *Sciara coprophila* used in this study were from an inbred stock line (NN) maintained in

<sup>1</sup> Although appropriate taxonomic designation of this species is now *Bradysia coprophila* (Lintner), according to the recent generic revision of North American Sciaridae by Steffan (42), there is an appreciable cytogenetic literature where the same species appears as *Sciara coprophila* (13, 28, 41). We have elected to retain the older name here to encourage comparisons between our present findings on the salivary gland proteins of this animal with previous data from studies concerned primarily with the formation of DNA-puffs by salivary gland chromosomes of *Sciara* during late larval life (14, 17, 35).

our laboratory for more than 60 generations by single pair matings. Putative sexual phenotype of larvae in this species can be conveniently predicted through use of the sex-linked, dominant genetic marker, *wavy*, a wing character expressed in adult females and carried on the X' chromosome of this monogenic species. A wavy-winged female (XX') characteristically gives rise to female progeny, whereas her straight-winged sibs (XX) produce exclusively male offspring (12, 28). Animal rearing and feeding followed procedures essentially like those previously described (41, 42). Larvae were routinely cultured at 17°C in a constant temperature Freas Model 805 incubator (Precision Scientific Co., Chicago, Ill.).

A number of different morphological criteria in addition to the chronological age of a given culture were used for precise staging of larval or pupal development in *Sciara*. These criteria are based on the definitions of Rasch (35) and span a period of approximately 6–8 days. Data presented in this paper are based on samples of female tissues, except for one series of experiments specifically designed to compare tissues from male and female animals. The following abbreviations will be used to designate different stages of sciarid development during the larval-pupal transformation: early eyespots (EES); mid-eyespots (MES); late eyespots (LES); pharate pupa or prepupa (PP); pupae within 1–24 hr after molt (P<sub>1</sub>); pupae within 24–48 hr after molt (P<sub>2</sub>).

### *Sample Preparation*

**TISSUE-DISRUPTED SAMPLES FOR SDS-ELECTROPHORESIS:** Salivary glands from individual animals were dissected in insect saline (4) and stripped of all adhering fat. After several rinses in fresh saline, each set of glands was placed on the tip of a microliter syringe needle. The needle tip was then lowered to the bottom of a 0.1 ml test tube (Microchemical Specialities Co., Berkeley, Calif.) and the piece of tissue was dislodged from the needle tip into 3  $\mu\text{l}$  of 0.1 M phosphate buffer, pH 7.0, containing either 1% SDS (dodecyl sulfate, sodium salt, Serva, Heidelberg, Germany) or 1% mercaptoethanol and 1% SDS (45). Tissues were disrupted by drawing the buffer and tissue fragments up and down into the bore of the syringe at least five times or vibrating the tube as high speed in the holder of a Cenco "Whirlmix" (Cenco Instruments, Breda, The Netherlands). Samples prepared with 1% mercaptoethanol were reduced for 2 hr at 37°C. All samples in 1% SDS were prepared on the same day as electrophoresis and, before and after reduction, held at room temperature. 1  $\mu\text{l}$  of a 40% sucrose solution was added to each sample before electrophoresis.

**SECRETION SAMPLES FOR SDS-ELECTROPHORESIS:** One, two, or three larvae of a given stage were placed in approximately 10  $\mu\text{l}$  of insect saline

and allowed to secrete in water for 15–30 min. As the animals secreted in saline, their viscous secretion product (largely insoluble in insect saline) was collected on the tip of a needle. The secretion was then placed in 3  $\mu$ l of 1% mercaptoethanol-1% SDS buffer. The secretion immediately lost its stickiness and could easily be removed from the needle by shearing the tip against another needle. Samples were reduced for 2 hr at 37°C; 1  $\mu$ l of a 40% sucrose solution was added to each sample before electrophoresis.

**POOLED-HOMOGENIZED-CENTRIFUGED (PHC) SAMPLES FOR DISCONTINUOUS ELECTROPHORESIS:** Salivary glands were dissected in chilled insect saline (4), stripped of adhering fat, and held in a depression slide containing chilled saline until the total number needed was collected (10–40 gland pairs depending on the design of an experiment). The glands were placed on the tip of a microhomogenizer pestle and lowered into a 1 ml glass microhomogenizer (Macalaster Scientific Corporation, Cambridge, Mass.) containing 50–20  $\mu$ l of chilled extraction buffer (0.1 M Tris-HCl, pH 6.7, plus 0.005 M ethylenediaminetetraacetate (EDTA), 0.005 M mercaptoethanol (23), and 20% sucrose). Tissues were homogenized by hand for 1 min in an ice bath. The homogenate was collected with a 5  $\mu$ l syringe and transferred to a 0.1 ml test tube. The pestle and mortar were scraped with the syringe needle and rinsed with extraction buffer so as to adjust the final net sample volume to be equivalent to two pairs of glands per  $\mu$ l of buffer extract, for example, 5  $\mu$ l = the extract from 10 pairs of glands. Homogenized samples were stored at –8°C. Before electrophoresis, homogenate were thawed at room temperature and centrifuged at 2700 *g* for 5 min in a Misco Electric Microcentrifuge (No. 5500, Microchemical Specialties Co.). The supernatant was transferred to a chilled 0.1 ml test tube and samples of this soluble protein fraction were used for electrophoresis. After centrifugation, representative samples of five pairs of salivary glands (MES female) prepared in this manner contained approximately 37  $\mu$ g of protein in the supernatant (which was applied to the gel pocket) and 15  $\mu$ g of protein in the pellet (25).

### *Electrophoresis*

As described elsewhere (5), a Plexiglas apparatus, modified from the design of Reid and Bielewski (37), was used for both discontinuous and SDS-continuous electrophoresis with 0.6 mm polyacrylamide gel sheets and up to 15 sample pockets per sheet. Gel sheets for discontinuous electrophoresis (16, 30) were cast as layers of varying acrylamide concentration (3%, 8% and 12%) to obtain optimal resolution of both fast-moving and slow-moving anionic proteins. 8% polyacrylamide gel sheets were used for SDS-electrophoresis (45). In both systems, samples were applied with

a microliter syringe (Hamilton Co., Whittier, Calif.) by layering the sample at the bottom of a pocket containing electrophoresis buffer. The standard time of discontinuous electrophoresis (Marquette University, Milwaukee, Wis.) was 40 min at 1550 v and 15 ma supplied by an Eico constant voltage regulator (Allied Electronic Corp., Chicago, Ill.). The standard time of SDS-continuous electrophoresis (Max Planck Institut für Biologie, Tübingen, Germany) was 2 hr at 50 v and 37 ma supplied by a Beckman Duostat set for constant voltage regulation (Beckman Instruments, Inc., Fullerton, Calif.).

### *Staining Methods*

Before staining, the proteins in a gel sheet were fixed for 15 min at 4°C in 5% trichloroacetic acid. Gel sheets were subsequently placed for 18–72 hr at 4°C in a freshly prepared solution of 25% methanol, 7% acetic acid, and 0.1% Coomassie brilliant blue 250R (Colab Laboratories, Inc., Glenwood, Ill.). Gel sheets were destained in multiple rinses of 7% acetic acid. Selected gel sectors were stained overnight in a solution of 0.7% Alcian blue (Chroma, Stuttgart, Germany) in 3% acetic acid and destained in alternate rinses of 7% acetic acid and tap water (27). For the periodic acid-Schiff (PAS) reaction, gel sheets were fixed for 15 min at 4°C in 7% acetic acid and then transferred to freshly prepared 1% periodic acid for 1 hr at 4°C. After a 15-min wash in tap water at room temperature, gels were stained for 1 hr in Schiff's leucofuchsin reagent. After three 10-min rinses in 1% sodium metabisulfite and five additional rinses in tap water, gels were stored overnight at 4°C in a large volume of distilled water. For zymograms of nonspecific esterase activity (discontinuous electrophoresis system), gel sheets or sectors were incubated for 1 hr in a reaction mixture containing 1 mg/ml  $\alpha$ -naphthol acetate and 0.2 mg/ml fast blue RR (Dajac Laboratories, Philadelphia, Pa.) in 0.1 M phosphate buffer, pH 6.5 (21). Zymograms for acid and alkaline phosphatase activity (discontinuous electrophoresis system) were developed according to Hubby and Lewontin (21), with some procedural modifications necessitated by our thin gel sheet system. After development, zymograms were routinely fixed for 1 hr in 7% acetic acid, evaluated, and photographed within 24 hr.

### *Evaluation of Electrophoretograms*

Electrophoretograms, stained with Coomassie brilliant blue and other stains, or zymograms after treatment in an enzyme reaction mixture, were evaluated by visual examination and measurements of the original gel sheets. Some diagrams were prepared from measurements of band mobilities on negative images or prints made of enlargements of individual electro-

phoretograms. Selected electrophoretograms were scanned at 560 nm with the low power optics of a Leitz microspectrophotometer (15) (E. Leitz, Inc., Rockleigh, N.J.). Details on the photography of gel sheets and densitometric scanning of electrophoretograms after discontinuous electrophoresis are given elsewhere (5). After SDS-electrophoresis, electrophoretograms were photographed with a Polaroid 3 M camera, using Polaroid 4 × 5 Land Film, Black and White, Type 55 P/N (Polaroid Corp., Cambridge, Mass.).

## RESULTS

### *The Salivary Gland during the Larval-Pupal Transformation*

A salivary gland pair from either a male or a female *Sciara coprophila* is composed of approximately 350–360 cells. A gland from a female larva at late fourth instar, when fully extended, is approximately 1 cm long, and consists of an elongate, tubular sac which is formed by a single layer of cells surrounding a narrow lumen. The proximal secretory duct of each gland member is continuous with the lumen, and the two ducts join to form a single secretory duct which enters the mouth of the animal. Each gland is divided into an anterior and posterior region by the “neck” cells which occur distal to the flattened, rather platelike array of some 14–15 pairs of cells that comprise the proximal, or anterior region of the gland. Using the Lowry procedure (25) to estimate amounts of total protein, we find that pairs of salivary glands from females of *Sciara* show a maximum protein content during the LES stage of the larval-pupal transformation (Fig. 1).

The main product of the salivary gland is a clear, viscous, mucous-like secretion which serves at least two separate functions during the latter portion of late fourth instar. From hatching through the EES and MES stages, actively feeding animals secrete a lubricant with which they coat food particles and the cuticle of their own and other larval bodies. Larvae stop feeding at the early LES stage, but continue to secrete a viscous substance up to the late LES stage. As the animals secrete this material, they bind straw and other detritus in the culture vessel around themselves into a type of cocoon. The animals usually continue their pupal development within this structure, and the viscous material remains somewhat pliable as long as the culture vessel is moist. After the salivary gland ceases secretory function, gland

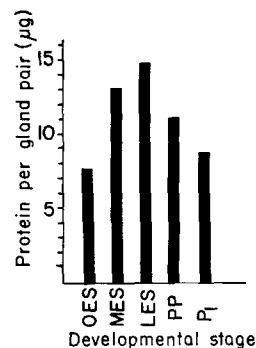


FIGURE 1 Protein content (Lowry) of salivary glands from females of *Sciara coprophila* during the larval-pupal transformation. Each value represents the average from 10 or more replicate determinations on individual pairs of glands. Stage designations include: OES, late fourth instar, just before appearance of imaginal eyespots; MES, or mid-eyespots; LES, or late eyespots; PP, pharate pupa or prepupa; P<sub>1</sub>, pupa within 24 hr of pupal molt.

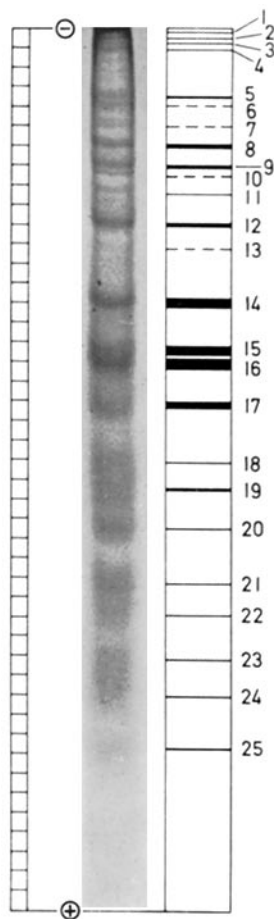
cells remain intact for an additional 3–4 days, with little or no apparent function other than preparation for eventual gland histolysis during pupal metamorphosis (36).

### *SDS Electrophoresis of Tissue-Disrupted Samples*

ELECTROPHORETIC PATTERN OF THE SALIVARY GLAND AND SECRETION: 25 bands were routinely seen in Coomassie brilliant blue-stained electrophoretograms of reduced and unreduced samples of single salivary glands after electrophoresis in 0.1% SDS (45). Fig. 2 shows a photograph of a typical electrophoretogram of a salivary gland sample and includes a diagram with band numbers for clarification and discussion of the banding pattern.

In SDS electrophoresis, protein fractions are separated according to their molecular weights (40, 45). As shown in Fig. 3, the distribution of proteins in electrophoretograms according to their approximate molecular weights was determined by comparing, on the same gel sheet, the electrophoretic mobilities of the proteins in *Sciara* samples with the electrophoretic mobilities of four reference proteins (45). Five of the *Sciara* protein bands were located within the mobilities of cytochrome c (11,700) (22) and chymotrypsinogen (25,700) (45), and seven of the bands between chymotrypsinogen and serum albumin

(68,000) (22). 10 of the protein bands fell within the range of serum albumin and thyroglobulin (335,000) (22). Three protein bands, just entering the gel and distinct from each other only under low power of a dissecting microscope, migrated less than thyroglobulin. These three large proteins, represented by bands 1, 2, and 3, all stained for acid mucopolysaccharide with Alcian blue (26). Band 1 was also PAS positive (26). These bands, therefore, may have molecular weights lower than the weights indicated by their mobility in SDS-gels (40). Except for these bands and an unde-



**FIGURE 2** Photograph of a typical electrophoretogram of the proteins from a reduced sample of a single salivary gland stained with Coomassie brilliant blue after electrophoresis in 0.1% SDS (45). A diagram of the electrophoretic pattern is included with band numbers for the discussion of the protein bands in SDS-electrophoretograms. Each of the divisions of the scale at the left of this figure is equivalent to 1 mm.

defined zone encompassing the fastest migrating proteins and peptides, no other protein bands stained with Alcian blue or the PAS reaction. This zone may also contain lipids and polysaccharides.

Fig. 4 presents photographs of an electrophoretogram of a reduced secretion sample collected from actively feeding animals (A) and a salivary gland electrophoretogram (B), with band numbers affixed to those loci which appear to correspond to the six bands regularly detected in electrophoretograms of secretion samples. In electrophoretograms of secretion samples from both actively feeding and cocoon-building animals, the intensity of staining in certain bands varied somewhat among individual samples collected from different animals at any given stage. Profiles showing a combination of several, or all, of the same six bands, however, constitute a reproducible feature of these samples. In some electrophoretograms of salivary gland samples, an increase in the intensity of staining of band 7 suggested higher than usual concentrations of this protein fraction in the gland cells and in the lumen. Electrophoretograms of secretion collected from LES animals sometimes contained a very prominent protein band, apparently corresponding to band 11, that was only lightly stained in electrophoretograms of secretion from actively feeding animals. An undefined zone, encompassing the fastest migrating fractions, was the only area that stained with either Alcian blue or the PAS reaction in electrophoretograms of secretion samples.

Electrophoretograms of single gland samples from animals representing the stages during the larval-pupal transformation (EES-P<sub>1</sub>) were compared on the same gel sheet and from one gel sheet to another for evidence of reproducible increases and decreases in the staining properties of any of the 25 bands shown in Fig. 2 and for the presence of new protein bands at any of the stages. The electrophoretic pattern of the salivary gland proteins remained basically the same up to the onset of gland histolysis during stage P<sub>2</sub>. As can be seen in the photographs of Fig. 5, representing electrophoretograms of samples from four developmental stages, some increases in staining can be seen in bands 13 and 25. We have found no evidence, however, for the appearance of any new protein bands that could be unequivocally associated with the onset of puparium formation or with the beginning of gland histolysis.

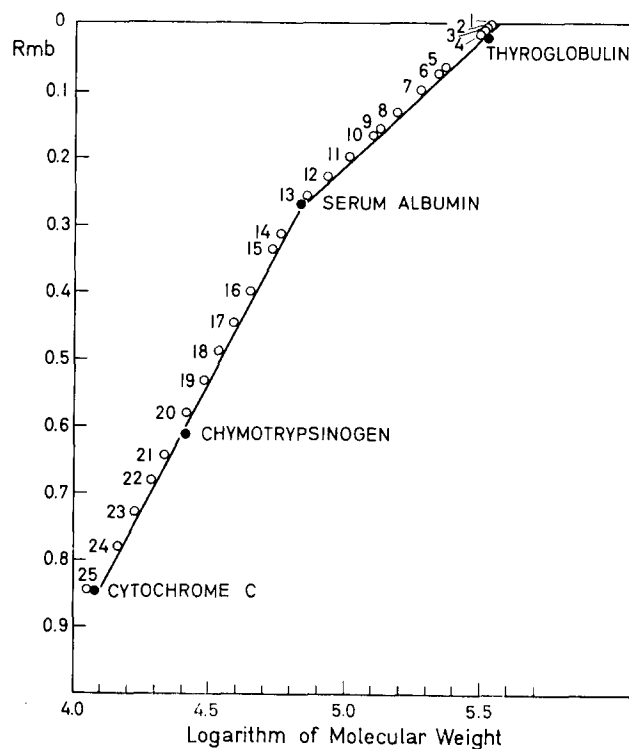


FIGURE 3 A comparison of the relative mobility of the proteins in electrophoretograms of samples of *Sciara* salivary glands with electrophoretograms of proteins with a known molecular weight (45). Solid circles represent proteins of known molecular weight. Open circles represent salivary gland proteins numbered according to the diagram in Fig. 2. The ordinate represents relative mobility (Rmb) in SDS-electrophoretograms. The abscissa represents the logarithm of the molecular weight of the samples of known molecular weight.

**ELECTROPHORETOGRAMS OF ANTERIOR AND POSTERIOR GLAND PARTS:** Several investigators have noted cytological differences along the anterior and posterior axis of the *Sciara* salivary gland (17, 33, 34, 36). Fig. 6 shows the almost identical electrophoretograms of samples of anterior and posterior gland parts from several stages during the larval-pupal transformation. Although no proteins were exclusive to either gland part, secretory proteins from the posterior part pass through the anterior part in order to be secreted. Therefore, any differences between the two parts may be reduced since our samples contained both cellular proteins and the secretory contents of the gland lumen.

Further studies are now in progress using spectrophotometric scans of electrophoretograms of anterior and posterior gland parts to evaluate the significance of quantitative differences in certain protein fractions. As shown in Fig. 6, for

example, most of the bands in electrophoretograms E and F are stained with equivalent intensities. Bands 8 and 9, however, appear more intensely stained in sample F than they do in sample E.

*Discontinuous Electrophoresis of Pooled, Homogenized, and Centrifuged (PHC) Samples*

**ELECTROPHORETIC PATTERN OF THE SALIVARY GLAND:** Electrophoretograms of salivary gland extracts were initially studied in a discontinuous electrophoresis system that was designed for obtaining maximal sharpness of banding detail and enzyme studies (5). Major features of the electrophoretic pattern found for pooled, homogenized, and centrifuged (PHC) samples, with up to 27 bands resolved by staining with Coomassie brilliant blue, remained basically unchanged during the larval-pupal transforma-

tion. Stage-specific increases and decreases in the relative amounts of particular protein bands were, however, consistently observed. These increases and decreases in the staining intensity of

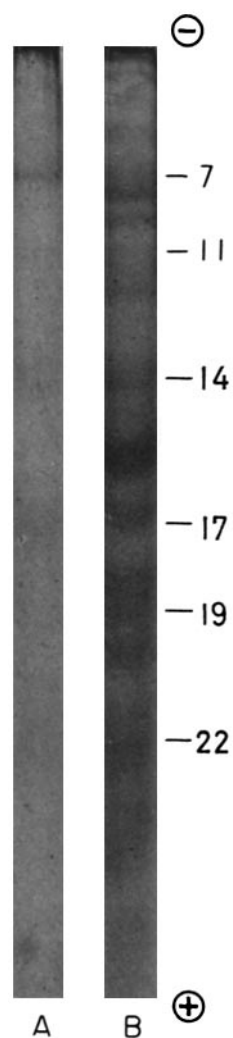


FIGURE 4 Electrophoretogram (A) of a reduced sample of secretion from actively feeding animals. Protein bands stained with Coomassie brilliant blue after electrophoresis in 0.1% SDS. This sample represents the secretion collected in saline from three animals in 30 min. Numbers affixed to the bands of the salivary gland electrophoretogram (B) are those which correspond to protein bands in the electrophoretogram of the secretion sample (see also Fig. 2). In A, the bands corresponding to bands 19 and 22 in salivary gland electrophoretograms are very faint, but these bands were clearly visible in the electrophoretogram on the original gel sheet. A, Secretion collected from EES (early eyespots) or actively feeding animals; B, one salivary gland from an EES (early eyespots) animal.

certain bands are clearly evident in the densitometric scans of electrophoretograms from EES, PP, and P<sub>1</sub> gland samples shown in Fig. 7. Fig. 8 presents a diagrammatic representation of Fig. 7 with assigned band numbers for further clarification. Because of the two methods of extraction and electrophoresis, no correspondence is intended between band numbers in Fig. 2 and Fig. 7. Protein bands 6, 7, and 8 increased in stainability from EES through P<sub>1</sub>. In contrast,

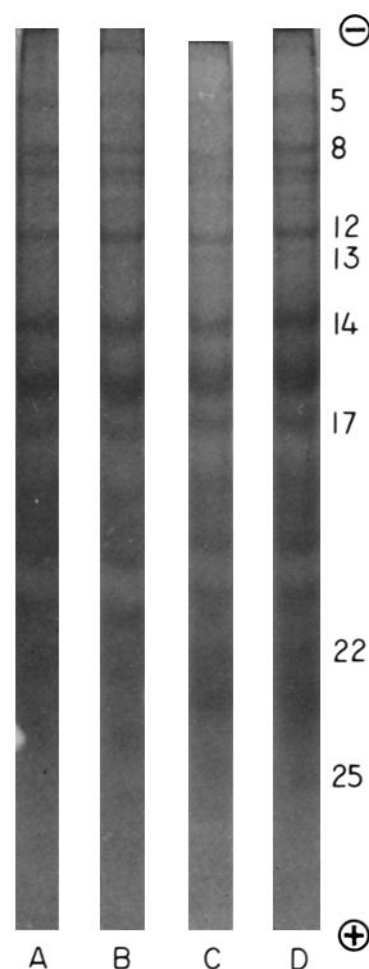


FIGURE 5 Electrophoretograms of samples of salivary glands from animals at four different stages during the larval-pupal transformation stained with Coomassie brilliant blue after electrophoresis in 0.1% SDS. All electrophoretograms are taken from the same gel sheet. A, One salivary gland from an EES (early eyespots) animal; B, one salivary gland from a LES (late eyespots) animal; C, one salivary gland from a PP (pre-pupa) animal; D, one salivary gland from a P<sub>1</sub> (pupa-1) animal.

bands 19, 20, and 21 decreased during this period. Bands 2, 4, 10, and 5 showed fluctuations in staining densities apparently unrelated to specific stages of gland differentiation. As shown in Fig.

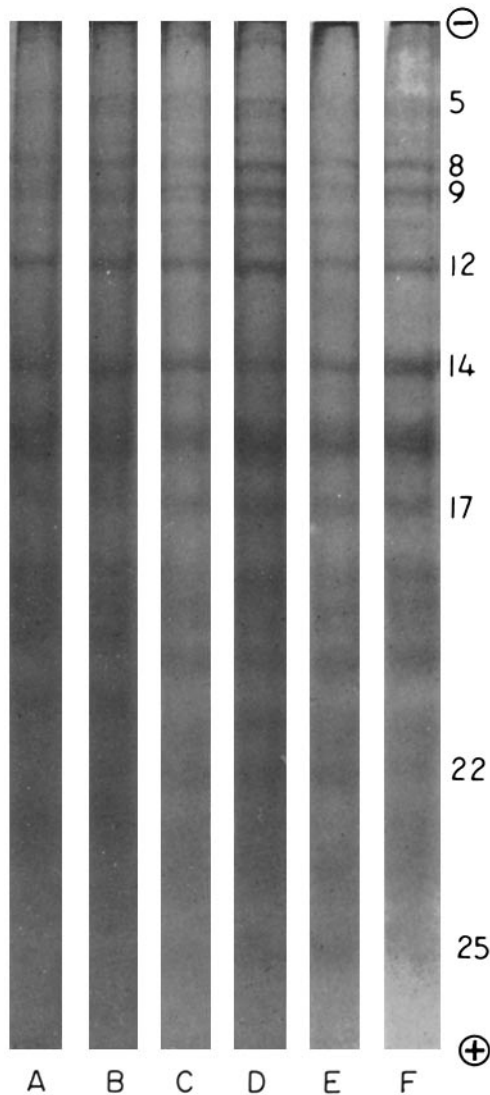


FIGURE 6 Electrophoretograms of reduced samples of anterior and posterior parts of the *Sciara* salivary gland stained with Coomassie brilliant blue after electrophoresis in 0.1% SDS. Electrophoretograms A and B are taken from the same gel sheet and C, D, E, and F from another gel sheet. A, Two anterior gland pairs from EES (early eyespots) animals; B, two posterior glands from the same animals as A; C, two anterior gland pairs from PP (prepupa) animals; D, two posterior glands from the same animals as C; E, two anterior gland pairs from P<sub>1</sub> (pupa-1) animals; F, two posterior glands from the same animals as E.

8, seven of the fine bands in the PP electrophoretogram (and also in the adjacent LES electrophoretogram not shown here) were not present in either the EES or MES electrophoretograms. However, the electrophoretograms on the gel sheet used to construct the diagram showed exceptionally fine resolution of banding detail. Additional electrophoretograms of gland samples from these stages indicated that these bands were not a regular feature of LES and PP samples in this electrophoresis system.

**MALE AND FEMALE SALIVARY GLANDS:** Electrophoretograms of gland samples from male and female animals at three different stages (EES, PP, and P<sub>1</sub>) were compared to determine if any changes in the banding pattern could be attributed to the sex chromosome complement of females (XX) and males (XO) in this highly inbred sciarid species. No sex differences were detected in electrophoretograms of male and female salivary gland samples. Electrophoretograms of hemolymph samples from male and female animals, on the other hand, showed some differences in banding pattern (unpublished results).

**ZYMOGRAMS OF SALIVARY GLAND SAMPLES:** In *Sciara* (36, 44) and other dipterans (10, 29, 39), increases in the activity of a number of enzyme systems preceding gland histolysis have been detected through both biochemical and histochemical procedures. Zymograms of *Sciara* gland samples developed to show non-specific esterase activity (Fig. 9) indicated changes in the amount of reaction product in the two major bands during the larval-pupal transformation. As is obvious in Fig. 9, there is a progressive increase in the amount of reaction product in the lower band and some decrease in the upper band. These data suggest that one form of esterase is more active during the period of active feeding and another form in the period preceding gland histolysis.

For the demonstration of either acid or alkaline phosphatase activity in zymograms of *Sciara* gland samples, it was necessary to use 1-5% Triton X-100 in the extraction buffer (1). After extraction with Triton, electrophoretograms of these samples contained a diffuse zone that gave rise to various distortions in the mobility and form of the bands showing phosphatase activity. In spite of the technical difficulties encountered with the use of Triton, zymograms of gland



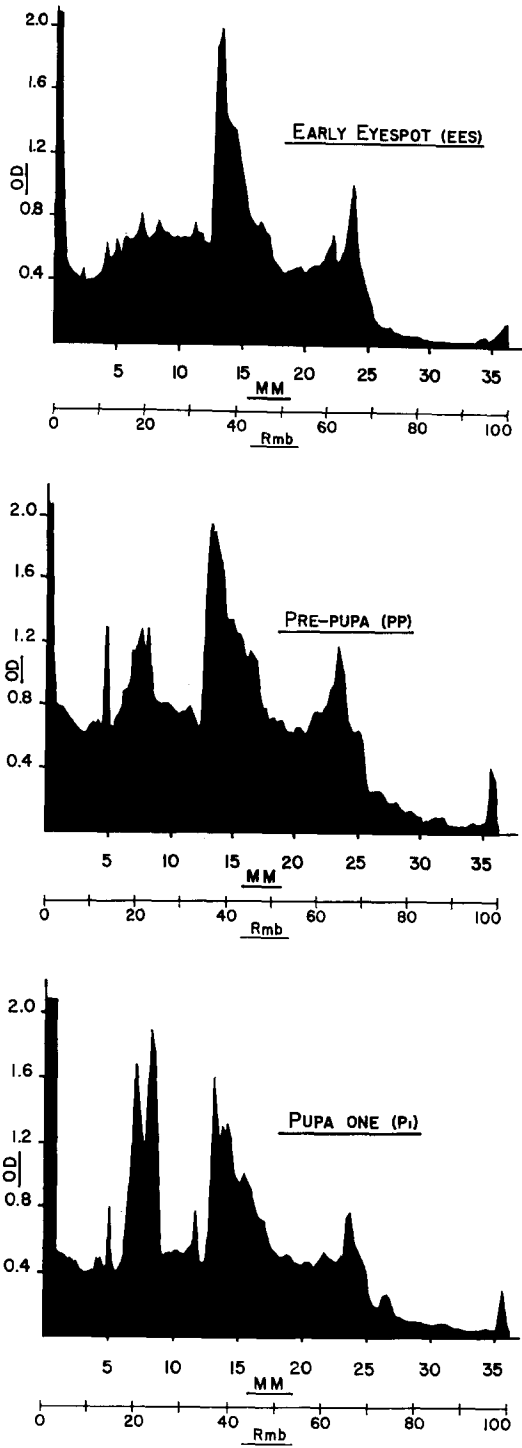


FIGURE 7 Densitometric scans of electrophoretograms of protein samples extracted from five *Sciara* salivary gland pairs by the pooled, homogenized, and centrifuged method. All electrophoretograms used for

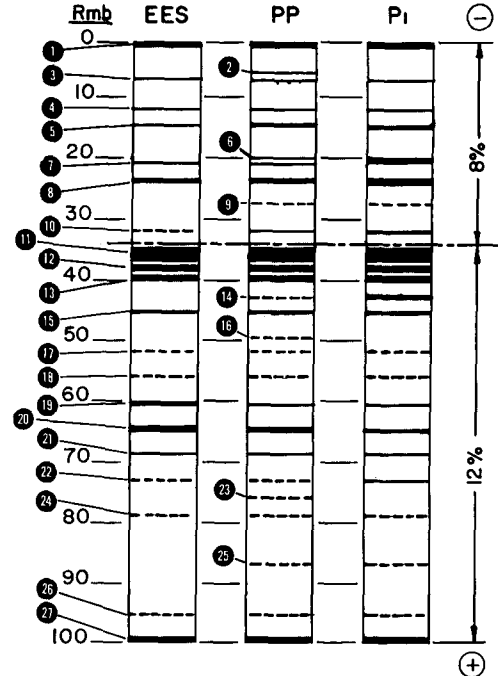


FIGURE 8 Summary diagrams of the original electrophoretograms and the densitometric scans shown in Fig. 7, for samples representing five salivary gland pairs from EES, PP, or P<sub>1</sub> stages of female *Sciara*. In addition to showing limits of the 8% and 12% separation gel layers, this figure includes a scale for relative electrophoretic mobility (Rmb) like that given in Fig. 7 and specific band numbers for the text discussion of protein patterns in electrophoretograms using discontinuous electrophoresis.

samples from PP, P<sub>1</sub>, and P<sub>2</sub> showed a progressive increase in the amount of reaction product in assays for either acid or alkaline phosphatase activity.

the particular scans shown were from the same gel sheet after its staining with Coomassie brilliant blue. The point of sample entry into the separation gel phase is shown at 0 on the abscissa. The anode is at the right of each figure. Note accumulation of stained peptides marking the glycinate front, shown here at 36 mm from the interface between the spacer gel and the separation gel layers. In this figure, stages of *Sciara* development are designated as follows: EES, larvae with faint imaginal eyespots; PP, pharate pupa or prepupa; P<sub>1</sub>, within 24 hr after pupal molt. A scale for relative electrophoretic mobility (Rmb) has been included on each of the figures for convenience in comparing profiles depicted as densitometric scans or as banded electrophoretograms (cf. Fig. 8).

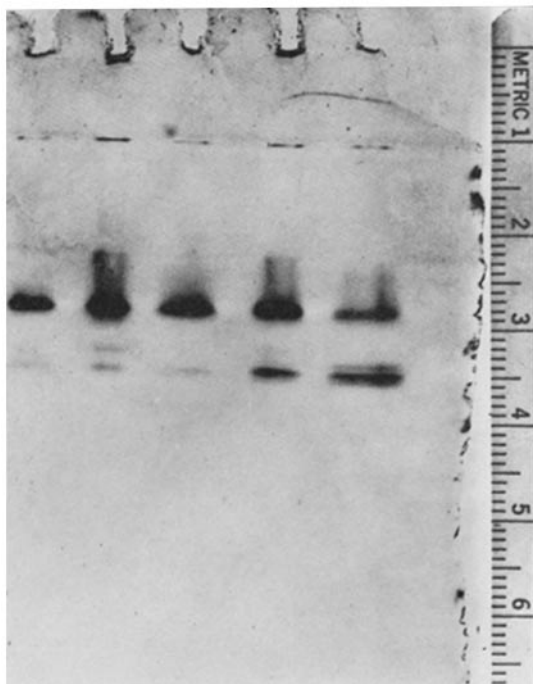


FIGURE 9 Zymograms from a single gel sheet, showing multiple zones of nonspecific esterase activity in samples prepared from 10 pairs of *Sciara* salivary glands by the pooled, homogenized, and centrifuged method. From left to right: EES or early eyespots; MES or mid-eyespots; LES or late eyespots; PP or prepupa; and P<sub>1</sub> or pupae within 24 hr of pupal molt. Each of the smallest divisions of the scale shown at the right of this figure is equivalent to 1 mm.

#### DISCUSSION

The electrophoretic patterns of proteins extracted from the *Sciara* salivary gland, and from separated anterior and posterior gland parts, remained basically unchanged during the larval-pupal transformation. Some of the observed variations in the staining properties of certain bands in electrophoretograms of both salivary gland and secretion samples from a given stage were probably related to fluctuations in both secretory activity and concentration of certain protein fractions in the secretion. With both SDS-electrophoresis and discontinuous electrophoresis, there were no qualitative changes (all-or-none differences) in the patterns of electrophoretograms of salivary gland samples stained with Coomassie brilliant blue that could be correlated either with the onset of pupation or gland histolysis. Zymograms developed for nonspecific esterase activity, how-

ever, indicated a considerable increase in the deposition of reaction product in one band as a concomitant of pupation. Our study has detected increases and decreases in the staining intensity of existing protein fractions and in the activity of certain enzymes in salivary gland samples from the period of larval-pupal transformation. We find no evidence, however, to suggest simple, temporal correlations between observed changes in the appearance of protein electrophoretograms and the occurrence of both RNA and DNA puffs along the giant polytene chromosomes of *Sciara* salivary glands during this same developmental period. From the data presently available, we can only conclude that no "new" protein bands appear, nor do any "old" protein bands disappear in any of the comparisons of salivary gland extracts analyzed here.

Although very low molecular weight proteins and peptides, a majority of the enzymes, and other nonproteinaceous materials were excluded from this study by our methodology, we consider that electrophoretic analysis of a broad spectrum of major cellular and secretory proteins serves as a reasonable index of changes in the status of *Sciara* salivary gland proteins at particular developmental stages. Our present data, of course, represent analysis of patterns of protein *accumulation* rather than protein *synthesis* in the salivary gland. Radioautography of electrophoretograms of both gland and secretion samples from animals injected with <sup>14</sup>C-labeled protein hydrolysate would provide a more sensitive index of changes in the genetic activity of salivary gland cells. Data demonstrating an enhanced, differential synthesis of certain salivary gland protein fractions will be published elsewhere (Been and Grossbach, in preparation).

The main function of the salivary gland during late fourth instar is the secretion of a viscous, mucous-like substance for the coating of food particles and lubrication (EES, MES), and during LES, the secretion of a similar appearing substance for the construction of a type of cocoon. A reproducible electrophoretic pattern with secretion samples was obtained only after solubilizing the secretion in a buffer containing 1% SDS and 1% mercaptoethanol and reducing the sample for 2 hr at 37°C. This suggests that the *Sciara* secretion product, upon secretion by the animal, represents a high molecular weight complex. We have no information about the secretory proteins in the gland lumen because it was im-

possible to collect secretion from the long narrow lumen of the gland without contamination by cellular proteins. Since samples extracted with 1% SDS in the absence of mercaptoethanol showed only an occasional band in electrophoretograms, 1% mercaptoethanol was probably necessary to split the disulfide bonds of the secretory protein complex and thereby free its protein subunits.

Three, presumably different, types of secretory granules were identified in an electron microscope study of the anterior cells of the *Sciara* salivary gland during late fourth instar (33). These granules, which varied in number at a given stage and from stage to stage, may be a morphological indication of certain proteins of the secretion product which varied in their stainability in electrophoretograms of secretion samples from this same period (bands 7 and 11 in Fig. 4, A). Further studies are in progress to determine the differential synthesis of the protein fractions that are detected in electrophoretograms of secretion samples.

Since DNA puffs appear exclusively in salivary gland cells during stages of the larval-pupal transformation (RNA puffs are present throughout fourth instar), some comments on our data in relation to this phenomenon seem warranted. Template activity has been suggested for this amplified DNA (13, 27, 31), but it is not yet known if the amplified DNA at DNA puff loci is transcribed for protein synthesis (27). Molecular hybridization experiments of DNA puff loci have indicated that amplified DNA does not code for ribosomal RNA (18). A recent study has suggested that DNA puffs are not essential for normal development of sciarid salivary glands (38). We have as yet found no evidence of qualitative changes in fractions that would suggest a compelling need for selective amplification of DNA templates for protein synthesis in these cells during the onset of metamorphosis or later, during gland histolysis. A possible correlation between amplified DNA and protein synthesis, mediated through either short-lived or long-lived mRNA's (11), cannot be excluded, however, and could feasibly be investigated through studies on protein synthesis in the sciarid salivary gland after temporary inhibition of DNA synthesis by the injection of hydroxyurea, shown to be a selective inhibitor of DNA puff formation when injected at the appropriate stage of larval development (38).

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