THE SECRETORY ACTIVITY OF THE PROVENTRICULUS OF DROSOPHILA MELANOGASTER[1]

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NINE FIGURES

INTRODUCTION

The proventriculus (cardia) of the larva of *Drosophila melanogaster* is a pear-shaped region of the anterior midgut which secretes the peritrophic membrane. This membrane, a continuous lining of the midgut and hindgut, encloses the food in the gut and is excreted in the form of a casing around the faeces. Structural details of the larval proventriculus of *D. melanogaster* have been presented by Strasburger ('32). In a study of the distribution of Golgi-material-and-secretion complexes in various epithelial cells of the midgut of *D. melanogaster* larvae (ebony mutant), Siang-Hsu ('47) suggested that the anterior portion of the epithelial wall of the proventriculus secretes the material forming the peritrophic membrane. He found that cells in this region of the proventriculus contained Golgi bodies and secretory granules which were not present in the remaining cells of the proventriculus.

During an examination of slides which had been stained by the periodic acid-Schiff reaction to study the polysaccharides of the larval cuticle of *Drosophila willistoni* (Rizki, '55), it was noted that the peritrophic membrane and a ring of epithelial cells of the proventriculus stained darker than the

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other proventricular cells. The present report deals with the role of these heavily staining cells in the formation of the peritrophic membrane. *D. melanogaster* was considered a more desirable species for this investigation since it is larger, relatively easier to handle, and has been more widely used in developmental genetics than *D. willistoni.*

MATERIALS AND METHODS

The Ore-R wild strain of *D. melanogaster* and a sex-linked lethal, 1(1)48J, have been used for the present investigation. Adult flies were placed in bottles containing paper spoons with cream of wheat-molasses food coated with a mixture of yeast and honey. The spoons with eggs were removed every 30 minutes or hourly and replaced with fresh spoons when eggs were timed for fixation. The flies were permitted to lay eggs on the same spoon for longer periods when larvae were desired; newly hatched larvae were collected from these spoons every hour, after an initial removal of all untimed larvae, and raised in stender dishes or evaporating dishes (50 × 90 mm) covered with petri dishes. The optimum density of the larvae on the food was standardized as one larva /cm² area of food. Eggs and larvae were raised at 25°C.

Embryos were fixed every two hours beginning eight hours after egg laying until the time of hatching (22 ± ½ hr.). First, second and third instar larvae were examined; the larval age given in this paper begins from the time of eclosion. Most of the material was fixed in Carnoy (1 part acetic acid: 3 parts 95% alcohol). Less satisfactory results were obtained with Bouin or formalin fixation. Several punctures were made in the larvae after they were immersed in the fixative and the mouth parts were immediately removed to allow better penetration of the fixative as well as the paraffin wax (54–56°C.). Some material was also fixed in 2% osmic acid adjusted to pH 7.2 for 15 minutes, transferred to 2% formalin at pH 7.2 for 15 hours and embedded in paraffin. Sections were cut from 3 to 12 μ.

The staining method used most extensively in this study is the periodic acid-Schiff reaction (PAS) as described by Hotchkiss ('48). By this procedure 1,2-glycol groups of polysaccharides are oxidized to form aldehydes which then combine with the Schiff reagent to give a colored product. The acetylation techniques of McManus and Cason ('50) have been used to verify the specificity of the PAS reaction for 1,2-glycol groups of polysaccharides. Campbell's ('29) modification of the chitosan-iodine test was used to demonstrate chitin in the peritrophic membrane. Tyrosine protein was stained by the cytochemical adaptation of the Millon reaction as given by Pollister ('50). Schrader and Leuchtenberger ('50) stained basic groups of proteins with a 0.1 % solution of fast green in 0.1 N HCl. Azure B (0.2 mg/cm^2) buffered with potassium acid phthalate to pH 4.0 has been used to stain ribonucleic acid following the technique of Flax and Himes ('52).

In order to study the lipid content of the cells of the proventriculus, larvae were dissected in 6% formalin and allowed to remain in this fixative for 24 hours at room temperature. These small pieces of tissue were then embedded in a mixture of polyethylene glycol waxes (Rinehart and Abu'l Haj, '51) 1500 and 4000. Sections were cut at 8 μ and stained by the propylene glycol-Sudan black B method of Chiffelle and Putt ('51). Extraction of lipids was accomplished as follows: Larvae were fixed in weak Bouin and extracted with pyridine according to Baker's ('46) method. Sections mounted on slides were re-extracted with pyridine for 24 hours at 56–60°C. Sections of embryos fixed in weak Bouin were also extracted by refluxing for 24 hours in a Soxhlet apparatus with hot acetone and for 24 hours in hot chloroform/methanol (Pearse, '53).

Various enzymes have been used in conjunction with some of the above staining procedures. Slides were incubated in 1% α-amylase for one hour at 37°C. Control slides for this experiment were placed in the buffer solution consisting of 0.2 M KH_2PO_4, 0.2 M Na_2HPO_4 and 0.2 N NaCl at pH 6.2

(Summer and Somers, '53). The procedures given by Pearse ('53) were followed for the digestions with pepsin, trypsin and ribonuclease with the exception that the concentration of the solution of pepsin was 1 mg/cm³ in 0.01 N HCl. Slides were treated with saliva for 30 minutes at room temperature before staining.

Attempts to interfere with the formation of the peritrophic membrane either by starvation for 24 hours or by feeding larvae on food containing M/500 2,4-dinitrophenol for 24 hours have been unsuccessful. However, in both instances the effects of starvation on the general growth of the larvae as compared to their respective controls were evident. The details of experiments concerning the regeneration of the peritrophic membrane in isolated proventriculi will be given in a separate section.

RESULTS

The observations on the proventriculus of the larvae of *D. melanogaster* agree with the description given by Strasburger ('32). The oesophagus is invaginated into a layer of foregut cells which in turn are surrounded by a layer of midgut epithelium (fig. 1). A haemocoel containing haemocytes is present between the invaginated oesophagus and the inner layer of proventricular cells. A solid cellular plug is formed by the latter cells; the large lumen pictured in figure 1 is a result of fixation shrinkage. An optical section of

Fig. 1 Camera lucida drawing of a sagittal section of the proventriculus of a *D. melanogaster* larva (72 hrs.). Section stained with fast green. APR, cells anterior to the peritrophic membrane-forming ring; B, striated border; H, haemocoel with haemocytes; I, cuticular intima of the foregut invagination; IR, imaginal ring; O, oesophagus; P, peritrophic membrane; PPR, region of the midgut posterior to the peritrophic membrane-forming ring; PR, cells which secrete the peritrophic membrane-forming substance; S, sphincter; V, vault; W_1, wall of the oesophagus with a cuticular lining; W_2, layer of the recurrent section of the invaginated foregut with alveolar cells; W_3, wall of the midgut, cardia. la outlines the anterior-posterior course of the foregut-midgut in the proventriculus corresponding to the right half of figure 1. The lower half of the invaginated region of the foregut shaped like a manubrium gives a probing action through the orifice of the gut at the level of the sphincter.

a freshly dissected proventriculus shows a close fit of this plug of cells against the midgut epithelium. There is an intercalary ring of imaginal cells at the junction of the fore- and midgut. These cells are strongly basophilic, in this re-

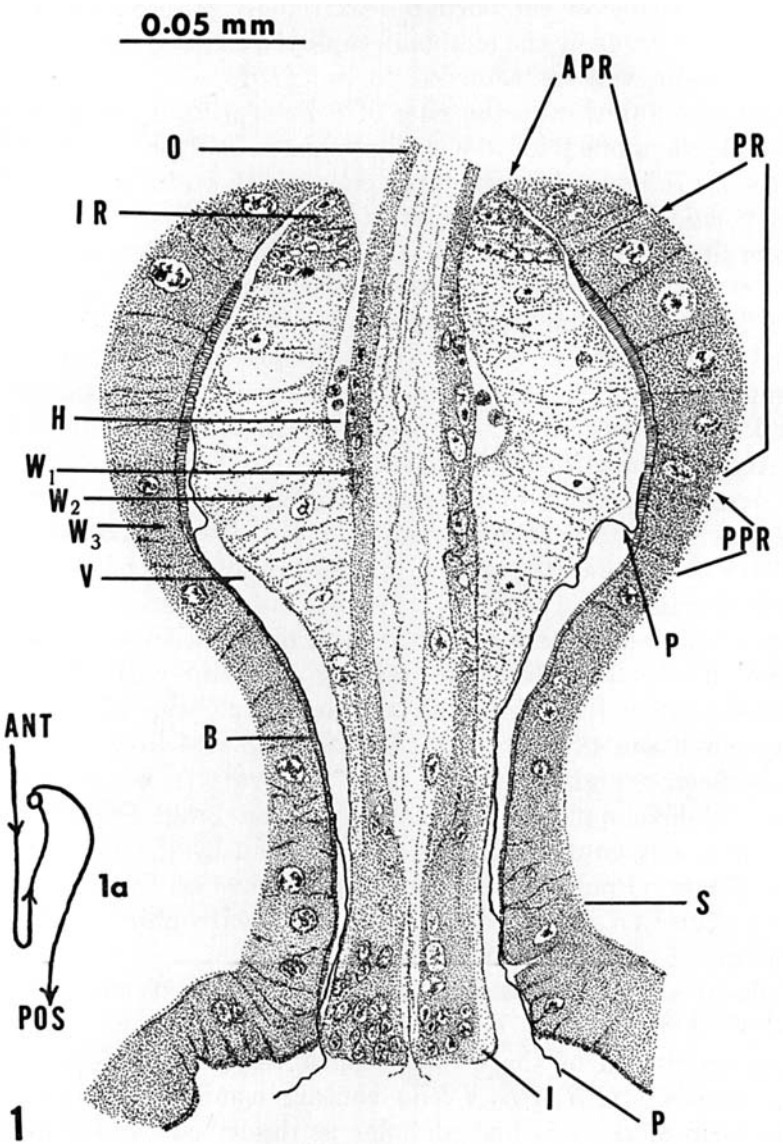

Figure 1

spect resembling the cells of the other imaginal disks. The two anteriormost cells of the midgut (cardia) following this imaginal ring are morphologically different from the subsequent cells of the midgut epithelium; they either possess no striated border or the border is extremely reduced so that it is not detectable by the methods employed in the present study. These cells will be referred to as *APR* cells, or the cells which are anterior to the ring of cells secreting the polysaccharide component of the peritrophic membrane. The *APR* cells are followed by four large cells which stain heavily with the periodic acid-Schiff reagent (PAS). Removal of glycogen from the larva by digestion with saliva or amylase increases the staining difference between these four cells, labelled *PR* cells, and the other proventricular cells as can be seen in a striking manner in figures 4 and 5. Whole mounts of the proventriculus which have been treated with amylase followed by PAS demonstrated very well that this ring which has such a strong affinity for PAS is four cells in width (figs. 2 and 3). Tangential sections of the proventriculus may contain more or less than four deeply stained cells. Two or three cells posterior to the *PR* cells (*PPR* cells) are morphologically indistinguishable from the *PR* cells, but the intensity of the PAS reaction in these cells is comparable to that of the *APR* cells. These differences in staining intensity with the PAS reaction after digestion with amylase have been observed in embryos from 18 hours and throughout larval life. Embryos have been examined at two hour intervals from the eighth hour of development until the time of hatching. Structurally the proventriculus is a well-formed organ by 12 hours of development (Poulson, '50), but no evidence of the PAS-positive secretion in the *PR* cells or the peritrophic membrane was noted until 18 hours of development.

Material fixed in osmic acid gave the most satisfactory cytoplasmic details. In unstained, 3 μ sections of the proventriculus, all of the cells in the anterior three-fourths of the cardia (*APR, PR, PPR*) contain osmiophilic bodies in the form of threads and globules as described by Siang-Hsu

('47). With phase contrast the cytoplasm of these cells shows many large inclusions while the cytoplasm of the remaining proventricular cells is smooth in appearance (figs. 6 and 7). When these sections are placed in turpentine oil to remove the osmic acid, then treated with α-amylase and the PAS reagent, the *PR* cells stain heavily in contrast to the *APR* and *PPR* cells. Observed under phase contrast this same section demonstrates the presence of numerous cytoplasmic inclusions in the *APR, PR* and *PPR* cells.

Although the osmic acid-fixed material was excellent for preserving cytoplasmic detail as well as the morphological appearance of the entire proventriculus, the faint brownish background which proved unremovable is not desirable for cytochemical staining. For all of the staining reactions discussed in the succeeding section Carnoy-fixed material has been used.

There is an accumulation of a homogeneous, PAS-positive substance at the striated borders of the *PR* cells. The first appearance of peritrophic membrane in the gut of *Drosophila* is found at the anterior level of the *PPR* cells. The PAS-positive peritrophic membrane is continuous with the secretory product of the *PR* cells, a morphological picture suggestive of the formation of peritrophic membrane from the latter. The peritrophic membrane in *Drosophila* gives a positive chitosan test demonstrating the presence of chitin. Since purified chitin is a polymer of N-acetylglucosamine residues joined by β-glycosidic ether linkages, no reaction with the periodic acid-Schiff reagent would be expected. The consistent parallel positive PAS reaction with a positive chitosan test in cuticle (Rizki, '55) and peritrophic membrane indicates that there may be other nonacetylated polysaccharides associated with chitin, or isolation and fixation of chitin alter the native state of this structural polysaccharide. Since the chitosan test requires treatment of the material to be tested with boiling saturated KOH, no cellular detail is preserved. Therefore this reaction cannot be used to identify the nature of the PAS-positive material in the *PR* cells or

TABLE 1

Staining reactions of the proventriculus [1]

TREATMENT [2]	PERITROPHIC MEMBRANE [3]	PR CELLS	APR AND PPR CELLS
Periodic acid-Schiff reagent (PAS)	vs	vs	s
PAS control	—	—	—
Acetylation + PAS	—	—	—
Acetylation + KOH + PAS	vs	vs	s
Amylase control + PAS	vs	vs	s
Amylase + PAS	vs	vs	vw
Saliva + PAS	vs	vs	vw
Pepsin control + PAS	vs	vs	s
Pepsin + PAS 1 mg enz./ml in 0.01 N HCl at 37°C. for 1 hr.	vs	vs	w
Pepsin control + fast green	w	vs	vs
Pepsin + fast green	w	w	w
Pepsin control + Millon	?	s	s
Pepsin + Millon	?	vw	vw
Trypsin control + PAS	vs	vs	s
Trypsin + PAS 0.1 mg enz./ml 0.05 M phosphate, pH 6, 37°C. for 1 hr.	vs	vs	w
Trypsin control + fast green	?	vs	vs
Trypsin + fast green	?	s	s
Ribonuclease control + azure B	?	s	s
Ribonuclease + azure B 0.2 mg enz./ml for 2 hrs., 56°C.	—	—	—
Sudan black B (SBB)	—	w	w
Lipid extraction + SBB	—	—	—
Lipid extraction + PAS	vs	vs	w
Lipid extraction + amylase + PAS	vs	vs	vw

[1] vs, very strong; s, strong; w, weak; vw, very weak; —, negative.

[2] Control is the buffer solution without enzyme. Ribonuclease obtained from the Worthington Biochemical Laboratory, Freehold, N. J.; other enzymes from the Nutritional Biochemical Corp., Cleveland, Ohio.

[3] In the vicinity of the *PPR* cells.

to study the secretion found in the lumen beside these cells. Under these circumstances other cytochemical stains have been employed to further explore the nature of the secretory activity of the *PR* cells.

The results of the enzymatic digestions and staining techniques are summarized in table 1. The *PR* cells are differentiated from the *APR* and *PPR* cells only by the PAS reaction; none of the other procedures which are specific for proteins, lipids and ribonucleic acid demonstrated any differences between these groups of cells. The PAS-positive material in the *PR* cells is not removed by saliva, amylase, pepsin or trypsin. Furthermore, removal of lipids from sections of embryos did not affect the PAS staining of the *PR* cells. The PAS-positive material of the peritrophic membrane in the vicinity of the proventricular cells and the larval endocuticle, which is a chitinous structure, behaved the same as the secretion of the *PR* cells.

Although the Millon reaction was not detectable in the peritrophic membrane at the level of the *PPR* cells and in the vault of the proventriculus, it shows a positive Millon reaction below the level of the sphincter and in the rest of the midgut. Similarly the membrane stains with Sudan black B in regions of the gut other than the proventriculus. The staining differences between the peritrophic membrane in the proventriculus and in the subsequent regions of the gut may be due to protein and lipid inclusions during digestion.

Lethal 1(1)48J

Hemizygous 1(1)48J embryos show abnormalities of the muscles and midgut as well as other structures. This sex-linked embryonic lethal, roughly localized between the *cut* and *vermillion* loci, has been studied by Dr. D. F. Poulson in detail (personal communication). The abnormality of the proventriculus of 1(1)48J embryos is of especial interest to the present study. Sections of embryos 22–24 hours old were examined. In all of the lethal embryos an enormous mass of PAS-positive material was found in the lumen of the proven-

triculus specifically in the vicinity of the *PR* cells (figs. 8 and 9). PAS-positive granules can be seen in the *PR* cells of those embryos and the *PR* cells can readily be distinguished from the neighboring cells by their secretory contents. In some embryos peritrophic membrane is secreted and extends a short distance in the gut, while in other instances no sign of peritrophic membrane is found. The membrane when formed is unusually irregular and thick as compared to the normal. Melanosis of the peritrophic membrane and the secretory mass often occurs in late embryos.

One aspect of the 1(1)48J syndrome is an upset in the mechanism leading to the formation of normal peritrophic membrane. The accumulation of the substance forming peritrophic membrane in the lethal embryos is clearly attributable to the *PR* cells. However, the continuous formation of peritrophic membrane involves other activities of the proventriculus as will be discussed later.

Experiments on the regeneration of peritrophic membrane

The morphological observations on the proventriculus of *D. melanogaster* demonstrate that the PR cells secrete a polysaccharide component of the peritrophic membrane, but they do not exclude the possibility that the secretion of the proventricular cells anterior and posterior to this region also participates in the process of peritrophic membrane formation. The isolation of small segments of the proventriculus and other regions of the anterior midgut and implantation of these tissues into adult hosts using the method of Bodenstein ('43) would be a desirable approach to localize the secretory centers participating in the formation of the peritrophic membrane. Unfortunately attempts to implant and recover tissue fragments consisting of *PR* or *PPR* cells alone proved technically difficult. Therefore as an alternative the alimentary tracts of early third instar larvae were removed in Waddington's solution and divided into two sections by cutting the proventriculus at the anterior level of the sphincter. Thus

one segment included the anterior proventriculus with the *PR* and *PPR* cells while the other segment consisted of the remaining proventricular cells, the caecae and the conical portion of the anterior midgut. These pieces of the gut were then implanted into adult males (2–3 days old). The implants were allowed to remain in the hosts from 48 to 72 hours, at the end of which the hosts were etherized and the implants removed and fixed in Carnoy. Paraffin sections were treated with amylase and stained with PAS. Whole mounts of some implants were also prepared (29 implants were recovered from a total of 70 paired implants).

The peritrophic membrane was carefully removed from the alimentary tract by gentle pulling before the gut was cut in two parts. To test for complete removal of peritrophic membrane 10 similar operations were performed and the guts immediately fixed. Examination of paraffin sections stained with amylase-PAS confirmed the complete removal of peritrophic membrane in these experiments.

An accumulation of PAS-positive material was found in the lumen of all of the implanted anterior pieces of the midgut. In addition to this accumulation several implanted proventriculi contained peritrophic membrane. The *PR* cells retain a strong affinity for PAS and often the PAS-positive secretion can be seen flowing out of these cells into the lumen of the proventriculus. The implants devoid of anterior proventricular cells failed to show any accumulation of PAS-positive material in the lumen or any peritrophic membrane.

DISCUSSION

There are two principal modes of peritrophic membrane formation as described by Wigglesworth ('30, '50). Peritrophic membrane may be the result of delamination of the surface of the midgut epithelium of different regions, or, as in the case of the *Drosophila* larva, it may be a continuous tube secreted by cells in the anterior midgut epithelium. Wigglesworth ('29) described the latter type of membrane formation in the proventriculus of the adult tsetse-fly by an elab-

orate mechanism involving the structure of this organ. The peritrophic membrane-forming substance is poured into the lumen of the proventriculus by a pad of epithelial cells. This secretion is then pressed to form a thin membrane as it is forced through a press mechanism consisting of the closely apposed walls of the invaginated foregut and the midgut. A similar mechanism of membrane formation has been found in many orders of insects, the most specialized occurring in the Diptera (Wigglesworth, '30). Differentiation of the cells secreting the peritrophic membrane-forming substance is clearest in species with a highly efficient press.

The peritrophic membrane in the *Drosophila* larva is produced by analogous structures involving the activities of a secretory region and a press mechanism displaying muscular contractions of the proventricular walls. Reduced basophilia is found in the muscles of 1(1)48J (Poulson, unpublished). The abnormal accumulation of PAS-positive substance in the proventriculi of the lethal embryos may be due to a failure of the musculature of the press mechanism. Occasional short pieces of peritrophic membrane occurring in some of the lethal embryos are imperfectly formed and not uniform in thickness, a further indication of an inefficient press mechanism. Similar difficulties are apparently encountered in the implanted proventriculi which invariably accumulate the peritrophic membrane-forming substance but lack continuous peritrophic membrane formation. Since the isolated proventriculi are no longer anchored by attachment to the foregut and midgut, the mechanical advantage resulting from peristalsis of the gut is lost to the proventricular press. Peristalsis apparently also adds a pulling action on the membrane passing out of the proventriculus. Furthermore in isolated proventriculi the sphincter of the midgut has been removed and the probing of the formed membrane through the orifice of the midgut sphincter by the foregut invagination is no longer in operation. Nevertheless the implantation experiments clearly demonstrate that the faculty of peritrophic

membrane formation resides in the proventriculus of the *Drosophila* larva.

Chitin has been reported in all examples of peritrophic membrane which have been investigated (Waterhouse, '53; Wigglesworth, '30). It is a long chain polysaccharide chemically defined as anhydro-N-acetylglucosamine. Taking this chemical composition as a clue a logical procedure would be to seek a cytological source of polysaccharide in the region where peritrophic membrane apparently originates, and then establish the identity of this cellular polysaccharide with the membrane. The periodic acid-Schiff reagent (PAS) can be utilized with proper precautions to stain intracellular chitin-polysaccharide or its immediate precursors, where the chitosan test is not applicable. PAS-positive material is of wide occurrence in the *Drosophila* larva; after the removal of glycogen, a strong positive reaction is found in the striated borders of the midgut epithelium, ingested material in the lumen of the gut, endocuticle, peritrophic membrane, and a ring of cells (*PR*) in the cardia. The likelihood of the striated borders of the midgut epithelium as a source of peritrophic membrane-forming substance is ruled out by the implantation experiments which demonstrate that this substance is found only in the presence of *PR* cells regardless of the inclusion of striated borders in the implanted tissue. *In situ* accumulation of PAS-positive material is found in the proventricular lumen corresponding to the level of the ring of *PR* cells and the peritrophic membrane emerges from this material immediately following these cells.

In addition to these morphological relationships there is a parallel cytochemical behavior of the intracellular secretion of the *PR* cells and the peritrophic membrane in this vicinity. The PAS-positive staining of these structures is not due to glycogen, protein or ribonucleic acid since it is not removable with α-amylase, saliva, pepsin, trypsin or ribonuclease. The intensity of the PAS reaction was unaffected by lipid extractions, thus excluding the presence of a lipoprotein. If the 1,2-glycol groups stained by PAS were associated propor-

tionately with proteins, nucleic acid or lipid, a differentiation of *PR* cells from the neighboring cells might be expected with some of the stains which have been employed (Millon, fast green for proteins; Azure B for ribonucleic acid; oil red O, Sudan black B for lipids). We may conclude that the PAS staining indicates the presence of a polysaccharide in the *PR* cells and the peritrophic membrane. Since the latter is a chitinous structure (chitosan) as mentioned earlier, in this instance the PAS is staining a chitin-polysaccharide. The immediate precursors of this chitin-polysaccharide are the secretion in the lumen and the secretory material in the *PR* cells. The intracellular PAS-positive material (Carnoy and osmic fixation) is granular, and the precursor in the lumen is a homogeneous plastic substance which is then molded and condensed into a membrane, the polymerized form.

In the absence of any knowledge about the biosynthesis of insect chitin, no suggestions can be made regarding the chemical structure of the intra- and extracellular precursors which are polymerized to form peritrophic membrane. Leloir and Cardini ('53) have reported the presence of an enzyme complex in extracts of *Neurospora crassa* whereby acetylglucosamine-1-phosphate can be synthesized from hexose-6-phosphate. Theoretically anhydro-N-acetylglucosamine can result from the polymerization of the former compound. Such a synthetic chain requires a phosphorylating mechanism similar to the one in glycogenesis: glucose-1-phosphate $\rightleftharpoons$ glycogen + phosphate. The secretory activity noted in the *APR* and *PPR* cells may be correlated with the enzymes participating in the polymerization of the peritrophic membrane.

SUMMARY

1. In *Drosophila melanogaster* a ring of cells located in the anterior region of the proventriculus (cardia) begins to produce the peritrophic membrane at 18 hours of embryonic development and continues to secrete the substance forming this membrane throughout larval life. These cells (*PR* cells) stain more heavily by the periodic acid-Schiff (PAS) reagent

for polysaccharides than the other proventricular cells, but are not differentiated by stains for proteins, lipids or ribonucleic acid. The PAS-positive material in these cells, in the secretion in the lumen of the proventriculus, and in the peritrophic membrane itself is not removable by amylase, saliva, pepsin, trypsin, ribonuclease or lipid extraction.

2. Since the peritrophic membrane contains chitin (chitosan test), it may be concluded that the PAS-positive intra- and extracellular secretions of the *PR* cells are immediate precursors of chitin-polysaccharide.

3. The implantation of isolated proventriculi and adjacent regions of the midgut into adult hosts has further demonstrated that the faculty of peritrophic membrane production resides in the proventriculus, and the presence of the *PR* cells is necessary for the secretion of polysaccharides forming this membrane.

4. The above conclusions are further supported by the observations on lethal 1(1)48J embryos which accumulate PAS-positive secretion from the *PR* cells in the lumen of the proventriculus. Abnormalities in the formation of the peritrophic membrane can be assigned to disfunction of the muscles and the press mechanism to mold the plastic secretion before polymerization into a uniform membrane.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

(Photomicrographs)

2 Whole mount of the anterior midgut of a Drosophila larva (early third instar) fixed in Cranoy, treated with α-amylase, and stained with the PAS reagent. The region of the proventriculus, PR, which is heavily stained is the ring of cells secreting the substance forming the peritrophic membrane. The negative-PAS reaction of the nuclei facilitates counting of the cells. This band is 4 cells in width; the anteriormost cells are out of focus due to the curvature of the proventriculus. In the surface view the ends of these spindle-shaped cells are wedged together; thus depending upon the plane of the cut in section, different numbers and different parts of the cells are seen. The peritrophic membrane and the foregut invagination have been removed from this specimen.

3 Optical section of a whole mount of a proventriculus (Carnoy-amylase-PAS). In addition to the deeply stained PR cells, there is a strong PAS reaction of the peritrophic membrane, P, in the lumen delimited by the reflected surface of the foregut invagination and the midgut epithelium. The peritrophic membrane is folded as it passes through the narrow orifice of the sphincter into the midgut which has been severed from the proventriculus. The light region anterior to the PR corresponds to the APR cells, and the proventricular lumen in this region is devoid of peritrophic membrane. One gastric caecum is attached, the other three having been removed.

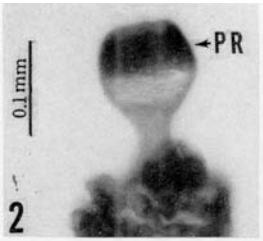
4 Frontal section of a third instar larva with the proventriculus in sagittal view (Carnoy-8 μ section-saliva-PAS). Note the strong staining reaction of the PR cells and the cuticle, C. Most of the tissues are outlined by a heavier reaction than the general background staining, but the striated borders give a strong PAS reaction as can be seen in sections of various regions of the gut. The deeply stained bodies in the lower region of the foregut invagination are ingested yeast cells.

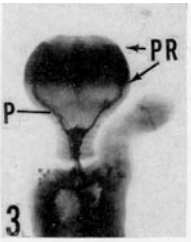
5 A region of the proventriculus in figure 4 under oil immersion to show the differences in intensity of the PAS reaction in the PR, APR, and PPR cells. Note the heavily stained granules in the PR cells and the peritrophic membrane, P. The intercellular spaces are due to shrinkage of the cytoplasm resulting from fixation; the anuclear segments of the PR cells are the ends of the spindles as shown in figure 2.

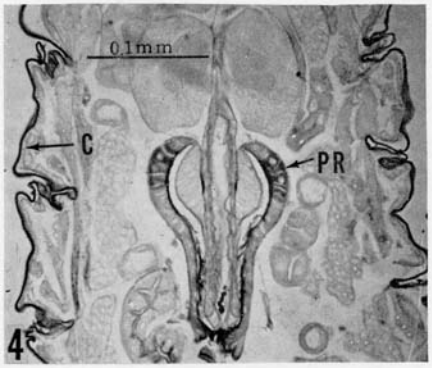
6 Region of a sagittal section of the proventriculus fixed in osmic acid-formalin. 3 μ, unstained section mounted in refractive index oil 1.460.

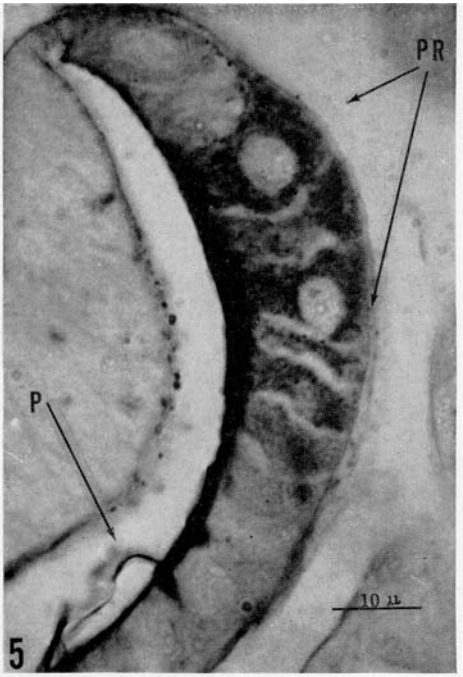
7 The section in figure 6 under phase contrast. The cytoplasm of the APR, PR, and PPR cells contains large granules in contrast to the smoother appearance of the remaining proventricular cells. Thus the anterior region of the proventriculus, designated by arrows, can be differentiated from the posterior region on the basis of secretory activity as described by Siang-Hsu ('47). This region is subdivided into three sections by the PAS reaction, the middle section alone, PR, secreting the polysaccharides incorporated in the peritrophic membrane.

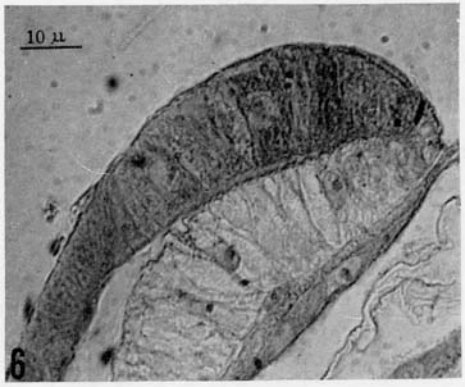
8 and 9 Consecutive sections (oblique) through the proventriculus of a 1(1)48J mutant embryo showing the accumulation of PAS-positive material in the lumen and the heavily stained PR cells. In figure 9 the secretion can be seen flowing out of the PR cells, X.

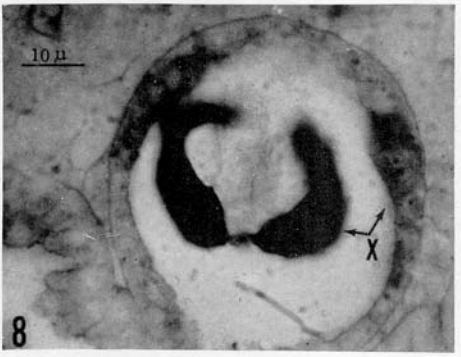

0.1mm
PR
2

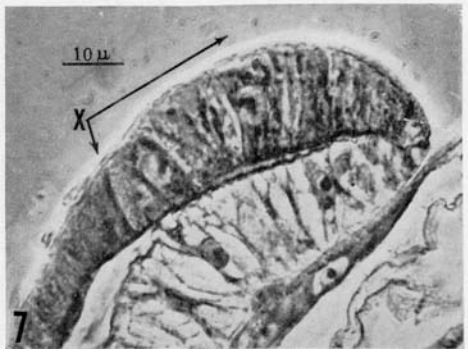

PR
P
3

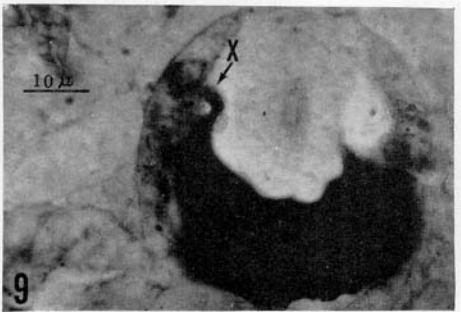

0.1mm
C
PR
4


PR
P
10 µ
5


10 µ
6


10 µ
X
8


10 µ
X
7


X
10 µ
9