THE MECHANISM OF SECRETION BY THE SALIVARY GLAND OF THE COCKROACH *PERIPLANETA AMERICANA* (L.)

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Summary

The two main cell types of the cockroach salivary gland, the ductule-containing cells and the zymogenic cells, are together responsible for the secretion of a powerful amylase and a mucoid substance. The evidence presented suggests that the precursors of both these materials are elaborated in the zymogenic cells and are passed to the ductule-containing cells for excretion. The morphology of the secretory ducts suggests that they also play a part in the elaboration of the saliva.

I. Introduction

The salivary glands of insects are of special importance in the transmission of protozoal, bacterial, and viral diseases of animals and plants. However, their physiology has been little studied, and, although many functions have been attributed to their secretions, there is comparatively little evidence upon which to base conclusions. As a preliminary to the investigation of more complex salivary glands, those of *Periplaneta americana* (L.) have been examined. Lebedeff (1899) studied these organs in some detail and collated previous data on them, but almost nothing new on the histology of the glands has since been added. He described two types of cells in the glands and concluded that one type produced mucin whereas the other produced the digestive enzyme, and this opinion has never been questioned. Later, Wigglesworth (1927) characterized the enzyme as amylase and found the saliva extremely active in the hydrolysis of starch. He reported invertase to be absent, although it was found in the salivary glands of the cockroach, *Blattella germanica* (L.). Using histochemical methods, Day (1949*b*) confirmed Lebedeff's statement concerning the presence of a mucoid substance in the glands and suggested that the amylase and the mucoid materials were secreted together.

In a consideration of the function of the salivary gland it is necessary to ascribe the secretory products to the correct cell type. But the problem is not as simple as indicated by Lebedeff's report, and an investigation of it is the subject of this paper.

II. Morphology of the Salivary Glands

The morphology of the glands, their ducts, and reservoirs of the related *Blatta orientalis* was well described by Miall and Denny (1886), and that of *P. americana* less accurately by Bordas (1897, see his Plate 4, Fig. 3). The

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principal features are illustrated in Figure 1. The acini show an extraordinary superficial resemblance to the salivary glands of vertebrates. The clusters of secretory acini surround the oesophagus and occur at the ends of branching ducts which join with the ducts of the reservoirs and discharge into the salivarium at the base of the hypopharynx. No muscles surround the ducts except the sphincter at the base of the hypopharynx. Nor do the reservoirs have in-

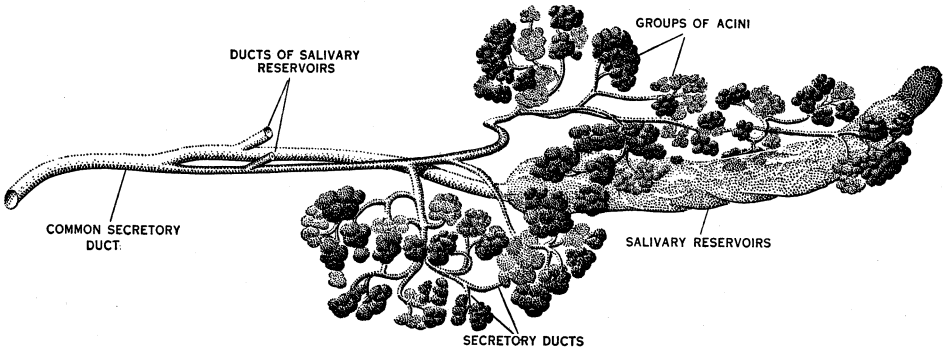


Fig. 1.—Principal features of salivary glands.

trinsic musculature, and so they must be filled by back pressure of those secretory products which are not discharged into the buccal cavity. Similarly, they must be emptied by the pressure of the haemolymph upon their elastic walls. The histology of these is peculiar (Plate 2, Figs. 1 and 2). The ability to expand is provided by the greatly folded epithelium. The wall of the reservoir appears to be composed of the two closely appressed but distinct layers of cells, the outer layer having vacuolated cytoplasm and the inner layer having a prominent chitinous lining. In the dilated reservoir these two layers become almost indistinguishable. This interpretation of the histology of the epithelium of the reservoirs would seem unquestionable, except for one disturbing observation. In the epithelium of the contracted reservoirs, nuclei appear to migrate from one layer to the other and, in fact, some may be constricted in the middle and appear to be partly in both layers. The significance of this is not clear.

III. Histology of the Glands and their Ducts

The excretory ducts of the salivary glands and those of the reservoirs both have a thin epithelium, and a taenidia-like lining (Plate 2, Fig. 3). The ducts of the reservoirs retain this structure throughout their length. A few small cells of a fat-body-like tissue surround them over part of their length. The ducts of the glands soon become thick-walled and remain so to the acini themselves (Plate 2, Fig. 4). The cytoplasm of the larger epithelial cells is dense and packed with mitochondria. Characteristic striations occur in the cytoplasm towards the lumen border where the taenidia-like thickening is still distinct, though less marked than in the excretory duct. The nuclei of the epithelial

cells are centrally located. Occasional peripheral nuclei belong to cells of the tracheae which penetrate between the epithelial cells. Intracellular tracheae and tracheoles are very abundant.

Between the acini may be found groups of cells which give every indication of being groups of haemocytes. However, they are of such compact structure that they may be mistaken for definitive organs. A similar structure in another species of cockroach is illustrated by Day (1950, Plate 4, Fig. 24). A study of orcein-stained whole mounts of salivary glands suggests that the acini are surrounded by a connective tissue sheath. In addition, the acini are closely bound together by tracheoles, their extensive innervation and their duct system. An attempt was made to check the shapes of the constituent cells in the glands by dissociation either in Goodrich's (1942) solution, or in hyaluronidase prepared from bull testis by the method of Madinaveitia (1941). The acini are bound together so completely that both these fluids are ineffective.

The acini of the glands are composed of cells of three main types (Plate 1, Fig. 1). The first are the small, centrally located cells of the intercalated ducts. The other two cell types are larger. One type contains striking intracellular ductules. These are Lebedeff's (1899) "peripheral cells" and are homologous with the "parietal cells" of the grasshopper described by Beams and King (1932). The ductules (especially of *Blattella*, but also of *Periplaneta*) are clearly differentiated by soaking the gland in 1 per cent. silver nitrate overnight in the dark, then teasing in glycerine and exposing to sunlight. The ductules always occur in pairs (Plate 1, Fig. 1) and connect with multicellular intercalated ducts in the acinus and eventually with the secretory ducts. The cytoplasm of the ductule-containing cells is finely granular, basophilic, and relatively constant in appearance. The ductules consist of a swollen terminal portion with a striated lining and a narrow neck portion directed centripetally.

The third cell type is the "mucous cell" of Lebedeff or preferably the "zymogenic cell" (Beams and King 1935). The nuclei of these cells are larger than those of the ductule-containing cells and easily distinguished from the other nuclei in the gland. These cells undergo striking changes during secretion, well shown in Bouin-fixed sections stained in Mallory's triple stain. In the non-secreting phase (*a*), which is most marked in individuals in which the glands have been denervated but is also found in starved specimens, the cytoplasm is sparse, acidophilic, and finely vacuolated (Plate 1, Fig. 2A). It gives a weak Bismarck brown stain for mucoids. In the secreting phase (*b*) the cytoplasm first becomes granular, intensely basophilic and gives a strong stain with Bismarck brown (Plate 1, Fig. 2*B*). Later the basophily is again replaced by material staining with orange G in Mallory's stain and which is apparently discharged as the secretory product (phase *c*) (Plate 1, Fig. 2 *C* and *D*). In the final depleted phase (*d*), most of this acidophilic material is discharged and the basophilic substance is produced again. No regenerative cells are present in the acini, so it is clear that complete destruction of cells in the course of secretion does not occur. It will be noted that secretion is asynchronous.

SALIVARY GLAND OF THE COCKROACH

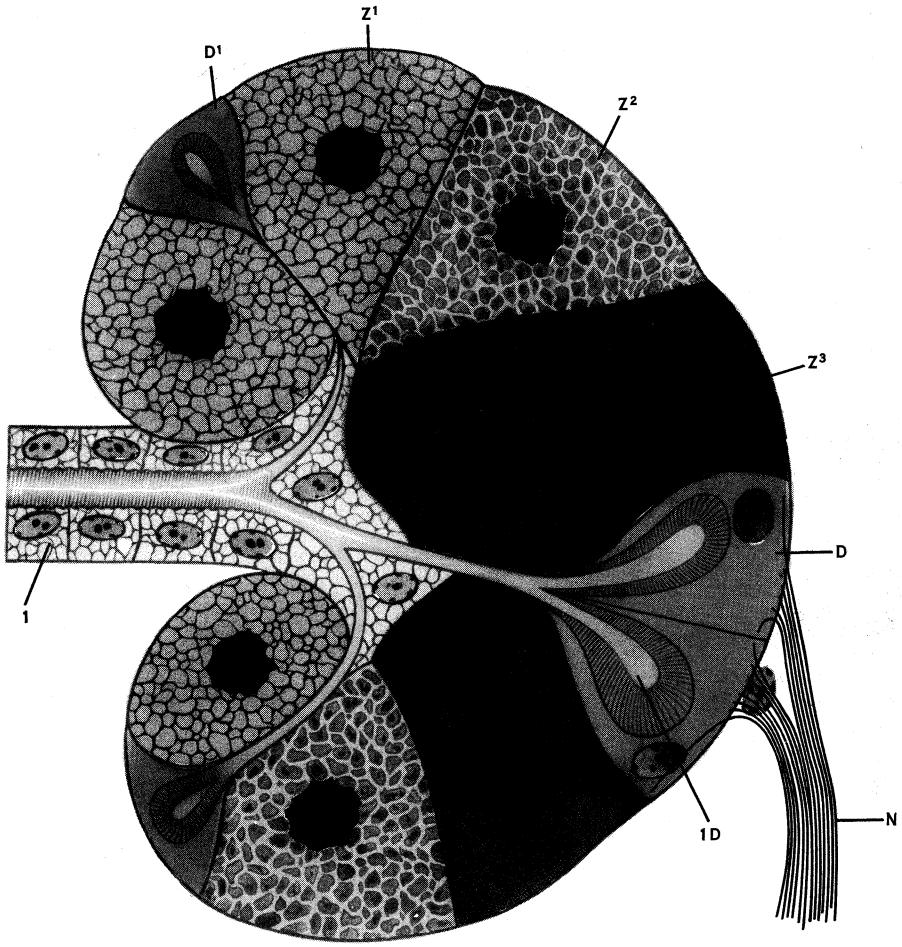


Fig. 1

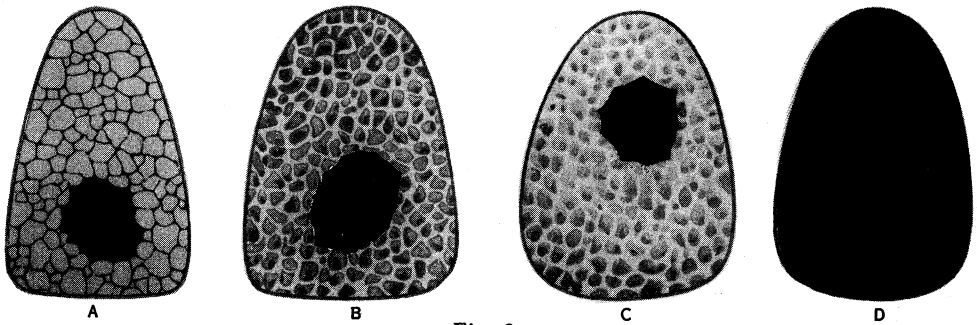
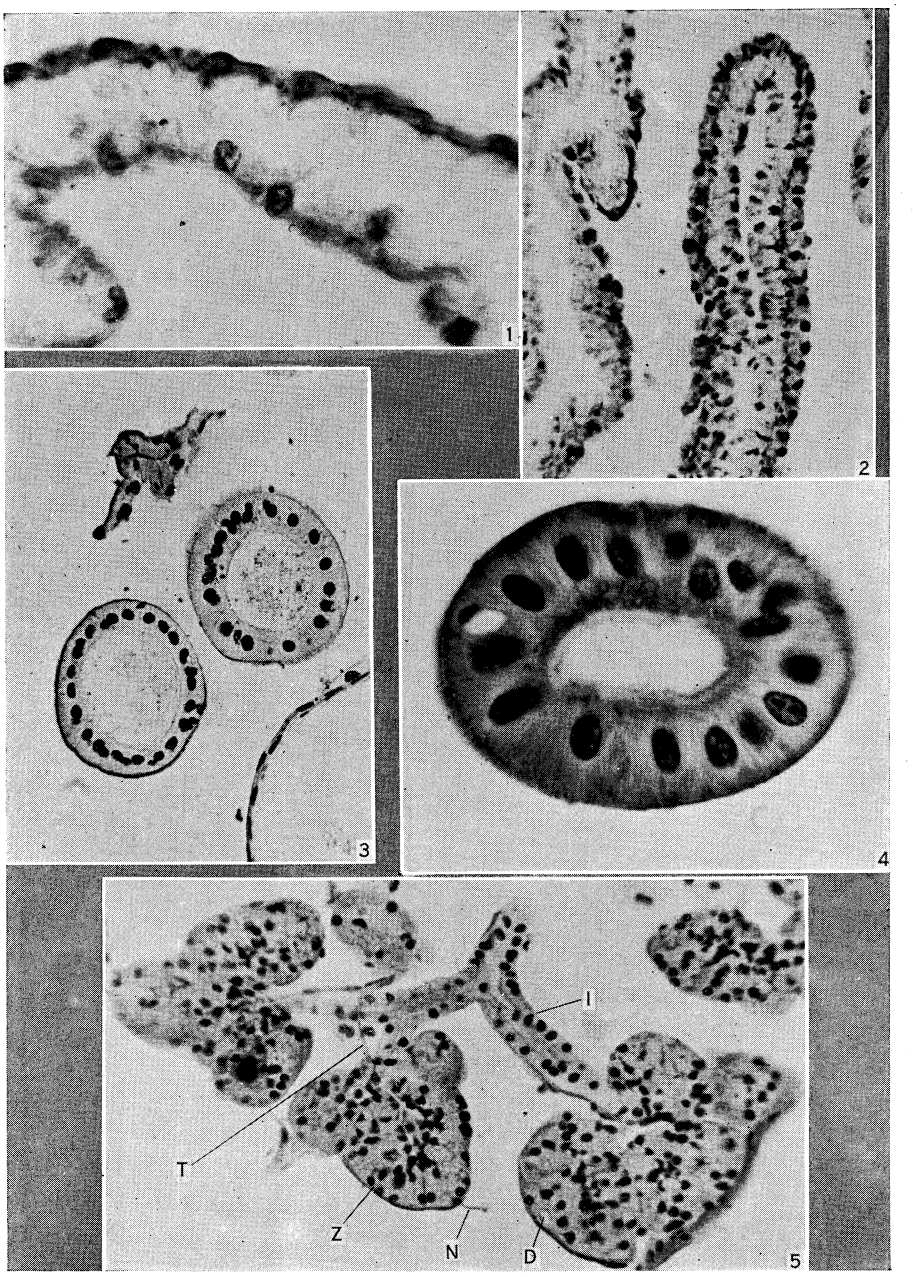


Fig. 2

SALIVARY GLAND OF THE COCKROACH



The histology of the salivary glands of adult *Periplaneta* submitted to a variety of experimental conditions has been studied. Following almost all treatments, some cells can be found in each of the four phases of secretion, but the proportion of cells in each phase varies markedly. The gland of insects starved ten days is mainly in the non-secreting stage. Upon feeding, phases *b, c,* and *d* become abundant. Injection of 0.1 ml. of 1:1000 pilocarpine into a *Periplaneta* adult produces characteristic tremors, hesitant gait, and continuous movements of the mouth-parts (cf. Roeder 1939). It produces both cytological evidence of secretion and reduction in the amylase content of the gland. Neither histamine (0.1 ml. of 1:1000 for 30 minutes) nor electrical stimulation from an inductorium of the *nervus recurrens posterior,* the nerve supplying each gland (Nesbitt 1941), produced comparable changes.

An attempt was made to watch the progress of secretor· changes by placing dissected glands, either with their innervation intact or severed, in a 1:1000 pilocarpine in Ringer solution under the high powers of the microscope. No changes in the cells were observed either by normal transmitted light or under phase contrast.

The innervation of the acini as seen in methylene blue preparations is very thorough (Day and Powning 1949). Intercellular nerves are well demonstrated by the Boeke technique, and nerve endings are found abundantly on the ductule-containing cells (Plate 1, Fig. 1). If each *nervus recurrens posterior* is severed the insect lives apparently normally for more than 7 days, but the glands become small and consist almost entirely of cells in the non-secreting phase.

IV. Enzyme Production

The cytological observations reported above have been related to the phase of secretion of enzyme by performing amylase determinations on one gland of which the other member of the pair was used for cytological study. Only males were employed in these experiments, and the reservoirs and most of the secretory ducts were discarded; also treatments prior to the enzyme estimation were maintained as constant as possible in an attempt to reduce variability in the results, which, in spite of all precautions, was considerable.

Amylase was determined by the Linderstrom-Lang and Holter technique as described by Day and Powning (1949). Arbitrary enzyme units were selected so that the activity of a single gland, from a normal adult male, in 8 ml. of buffer was equivalent to 100 units.

The amylase content of each of the glands from a series of insects was determined separately. It was found that the content of one gland was identical with that of the other member of the pair within the limits of accuracy of the method ($\pm$ 5 units). (The cytological structure of each of the glands from one insect was likewise similar, although difficult to measure quantitatively.)

Data from an experiment on the effects of various treatments on amylase content are summarized in Table 1. A cytological estimate of the relative number of cells in all secretory phases shows beyond doubt that there is no

correlation between amylase activity and the number of cells in any secretory phase. High or low enzyme activity may be associated with marked acidophily or basophily of the cytoplasm of the zymogenic cells. However, high enzyme content was always associated with marked cytological activity of the cells in phases ($b + c$), suggesting that the zymogenic cells were responsible for the production of amylase. These conclusions were checked with a larger series of 75 insects of both sexes submitted to a variety of treatments (pilocarpine, histamine, starvation, denervation, etc.).

TABLE 1

AMYLASE CONTENT AND CYTOLOGY OF SALIVARY GLANDS OF ADULT MALE *PERIPLANETA*

Treatment	Amylase Units	Cytological Appearance No. of Cells out of 10 in: Phase *a*	Phase *b*	Phase ($c + d$)
Starved 7 days	37	3	1	6
	50	4	5	1
	46	4	5	1
	50	4	3	3
Fed only water 7 days	36	4	2	4
	20	4	1	5
	40	3	2	5
Fed only starch 7 days	100	1	5	4
	32	3	1	6
	37	4	1	5
	140	4	1	5
Fed normal diet	112	2	3	5
	180	3	1	6
	120	3	3	4
	95	6	1	3

In both of these series, some slides were stained with Bismarck brown and some with Mallory's triple stain. Comparison of these shows clearly that the basophilic substance of phase *b* and the acidophilic substance characteristic of phase *c* both give the test for mucoid substances. Further confirmation of the nature of these materials was obtained by incubating acetone-fixed slides in hyaluronidase (Dempsey *et al.* 1947). Some slides so treated were used as a source of enzyme for the digestion of starch solutions. There was considerable loss in activity due to the treatment but sufficient remained to give a marked difference between the amount of amylase extracted from control slides and from similar slides incubated for 30 minutes at 37°C. in a preparation of hyaluronidase. Subsequently both series were stained in Mallory's triple stain. The acidophilic substance, and only this, was noticeably depleted by the treatment with hyaluronidase. The contents of the zymogenic cells give a positive Hotchkiss test for polysaccharides (periodate-Feulgen reaction). The evidence suggests, therefore, that the zymogenic cells produce the mucoid substance.

Thus, of the several lines of evidence presented, none is in disagreement with the conclusion that the mucoid substance and the amylase are both elaborated by the zymogenic cells. If this hypothesis is correct, the function of the ductule-containing cells requires consideration. The distribution of alkaline phosphatase in the glands suggests an explanation. This enzyme was found to be confined in the salivary glands of *Blattella* to those parts of the zymogenic cells surrounding the neck portion of the ductules (Day 1949*a*). Its distribution in *Periplaneta* is the same. In view of the suggested action of alkaline phosphatase in the transference of materials across cell boundaries, and because the distribution of the enzyme in many insect tissues is explained by such an action, it seems reasonable to assume that such is its function in the acinus. This evidence, together with that on the morphology and innervation of the ductule-containing cells, points to their dominant role in the excretion of the secretory products.

V. The Role of the Secretory Ducts in the Formation of Saliva

Neither the saliva from the mouth-parts nor the contents of the reservoirs give the same staining reactions as the contents of the cells of the acinus. It is reasonable to suggest, therefore, that the ducts perform a role in the production of the saliva. The following facts support this contention:

(1) The histology of the ducts, particularly the cytoplasmic striations and the intracellular striations, certainly indicates some function other than a purely mechanical one of transporting the saliva from the acinus to the mouth-parts or reservoir. This is clearly suggested by a comparison between the structure of the ducts of the glands with those of the reservoirs. The latter apparently function only in conduction.

(2) The presence of many mitochondria in the epithelial cells of the ducts suggests that they perform a secretory function.

(3) A non-specific esterase, although completely absent from the cells of the acini, is demonstrable in low concentration in the ducts by the histochemical method of Nachlas and Seligman (1949). Although its function is problematical its presence suggests again some function other than a purely mechanical one.

There is very little information on the composition of the saliva as ejected over the mouth-parts. It is a clear liquid containing an active amylase. On evaporation, conspicuous crystals are deposited. These give a strong positive test for chloride. The saliva is not viscid, nor does it give any histochemical test for mucoid substances.

VI. Discussion

The hypothesis of the secretion of saliva which emerges from the observations and experiments mentioned above is as follows. The secretory products consist of a mucoid substance and a powerful amylase. (The possibilities that they are one and the same or that the mucoid substance is the enzyme precursor are worth considering.) Precursors of both mucoid and enzyme originate

in the zymogenic cells. They, or products elaborated from them, are passed through the cell walls to the ductules and thence through the intercalated ducts to the secretory ducts, where further changes occur, until the completed saliva is either excreted on to the mouth-parts or passed to the reservoir.

This hypothesis does not suggest the mechanism whereby nerves innervating the ductule-containing cells cause them to excrete their contents or replenish them from the zymogenic cells. Nor is the possibility eliminated that the ductule-containing cells contribute some substances to the saliva. The work of Beams and King (1935) on the grasshopper salivary glands suggests that the homologous cells in these insects do, in fact, produce a component of the salivary secretions.

The histochemical demonstration that the zymogenic cells produce the mucoid substance is in agreement with the original suggestion of Lebedeff (1899). However, the assignment of the amylase precursors also to the zymogenic cells is not in agreement with Lebedeff's views and the evidence for this conclusion is therefore summarized, as follows:

(1) The amylase is the most significant component of the saliva and it is reasonable to suggest that it is produced from the dominant cells of the acinus.

(2) The cytological activity of the zymogenic cells is marked when high enzyme concentration is recorded.

(3) The reduction in enzyme following pilocarpine is correlated with changes that were more marked in the zymogenic cells than in the ductule-containing cells.

(4) The reduction of material in the zymogenic cells by the action of hyaluronidase was accompanied by loss of amylolytic activity.

It is appreciated that none of these lines of evidence is conclusive, but together they strongly suggest that the zymogenic cells are the source of amylase of the saliva, and thus confirm the opinion of Hofer (1887).

The action of pilocarpine in causing secretion in *Periplaneta* is noteworthy. This parasympatheticomimetic drug causes salivary secretion in vertebrates and also in a number of insects (Lebedeff 1899; Oka 1930). The superficial resemblance between the salivary glands of vertebrates and some insects mentioned above is thus matched by some, perhaps fortuitous, physiological similarities.

VII. Acknowledgments

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EXPLANATION OF PLATES 1 AND 2

PLATE 1

Fig. 1.—Semidiagrammatic section of acinus of salivary gland of *Periplaneta. I*, intercalated duct; Z^1-Z^3, zymogenic cells in phases of secretion; *D*, ductule-containing cell; *ID*, Intracellular ductule; *N*, nerve.

Fig. 2.—*A-D*, phases in secretion of zymogenic cells; *A*, regenerating or non-secreting phase. Note acidophilic, finely vacuolate cytoplasm; *B*, early secreting phase. Cytoplasm granular and intensely basophilic; *C*, late secreting phase. Basophilia partly replaced by acidophilic cytoplasm; *D*, excreting phase. Cytoplasm completely acidophilic.

PLATE 2

All figures are photomicrographs of the salivary glands and associated organs of *Periplaneta americana* (L.), taken with a Leica photomicrographic attachment.

Fig. 1.—Section of the dilated salivary reservoir.

Fig. 2.—The same in the contracted condition.

Fig. 3.—Section of excretory ducts.

Fig. 4.—Section showing secretory ducts. Note taenidia-like thickenings, striated peripheral cytoplasm, central nuclei, dense cytoplasm, and intercellular trachea.

Fig. 5.—Section of acini of gland showing non-secreting resting phase. Note cytoplasm of zymogenic cell (*Z*), ductule-containing cell (*D*), intercalated ducts (*I*), tracheae (*T*), and nerves (*N*).