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Digestive System Morphology and Salivary Enzymes of the Potato Leafhopper, *Empoasca Fabae* (Harris)

LORNA CHUMLEY BERLIN², AND EDWIN T. HIBBS

Abstract. The digestive tract of the potato leafhopper *Empoasca fabae* (Harris) was very much like descriptions of the tracts of other *Empoasca* and closely related Cicadellidae. Major morphological differences: (1) no filter chamber was present in *E. fabae*, (2) the Malpighian tubules were joined at their distal ends which lie free in the body cavity, (3) and the fourth Malpighian tubule branched from one of the other tubules.

The principal salivary gland of *E. fabae* was composed of four pairs of lobes which varied in histological and cytological detail as well as in their staining reaction with toluidine blue, a metachromatic dye. Each accessory gland was attached at one point to the body wall by a muscle.

An invertase (sucrase) and amylase (diastase) were active in salivary gland macerates but were not demonstrated in media fed upon by the leafhoppers. Protease activity was found in whole salivary glands. No lipase was detected in histochemical sections of the salivary glands.

INTRODUCTION

The hypothesis has been advanced that toxic salivary secretions are injected into the host plant by feeding potato leafhoppers, *Empoasca fabae* (Harris), and that the physiological alterations induced result in the eventual death and desiccation of leaf cells.

Publications concerning the interrelationships of the potato leafhopper and its host plants reported that this leafhopper fed from the plant phloem (Medler, 1941; Peterson and Granovsky, 1950; and Saxena, 1954a). Inserted stylets became enveloped in a viscous gel early considered of plant origin; however, Smith (1932) and Storey (1939) found that the feeding sheath was of insect origin. It clearly marks the site of feeding. Histological studies of leaves fed upon by the potato leafhopper showed disorganization and granulation of cell plastids, enlargement of nucleoli, and complete disorganization of the phloem region (Granovsky, 1930; and Medler, 1941) and led Medler to propose a single compound injected by the leaf-

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hopper as the causal agent of hopperburn. Symptoms similar to hopperburn have followed plant injections of gross leafhopper macerates in distilled water (Eyer, 1922; Fenton and Hartzel, 1923; Granovsky, 1926; Martin, 1930; Medler, 1940; and Peterson, 1949), a fact presented to support the theory of the insect origin of stimuli inducing hopperburn.

The literature revealed little information either to help characterize the natural salivary secretions of the potato leafhopper or to describe the morphology of the digestive system. However, a basic anatomical pattern of the salivary glands among homopterans presented a lobulated principal gland from which two ducts originated. One duct led to the salivary pump region and the other terminated in the accessory gland (Dobrosky, 1931; Quadri, 1949; Willis, 1949; Saxena, 1954b, 1955a; and Nuorteva, 1954, 1956a, 1956b).

In the investigations reported in this paper a morphological basis for investigating the salivary glands and their secretions has been formed through describing the anatomy and histology of the digestive system, particularly the salivary glands, of the potato leafhopper. Digestive enzymes of salivary gland macerates were qualitatively explored. Third to fifth instar potato leafhopper nymphs or adults collected from field-grown Irish Cobbler potatoes, or from greenhouse cultures maintained on broad bean, *Vicia Faba L.*, were used.

PROCEDURE, RESULTS AND DISCUSSION

Morphology of the Digestive Tract

The digestive tracts of third to fifth instar potato leafhopper nymphs were exposed for observation in situ. The insects were dissected in a drop of insect Ringer's solution (Thompson, 1952). The head was grasped at the level of the compound eyes with a forceps and the ventral abdominal integument was slit along the lateral margins with a sharpened needle. The sternites were then peeled off, one by one, leaving the digestive tract visible in the body cavity.

The sections for histological study were fixed in Smith's (1926) modification of Carnoy's fixative, dehydrated in an ethyl alcohol series, infiltrated with a 2 per cent collodium solution, hardened in chloroform, infiltrated with paraffin (m.p. 52-54°C), cast into blocks and cut at 10 μ (Davenport, 1960). The sections were stained in Delafield's haematoxylin for 15 minutes, destained in acid alcohol (three drops of concentrated hydrochloric acid in 50 per cent ethyl alcohol), and counterstained for 2 minutes with alcoholic eosin. The sections were cleared in xylene and mounted in Fisher Permount.

Regions of the digestive tract of the potato leafhopper were

the stomodaeum, mesenteron, proctodaeum and associated structures (Fig. 1).

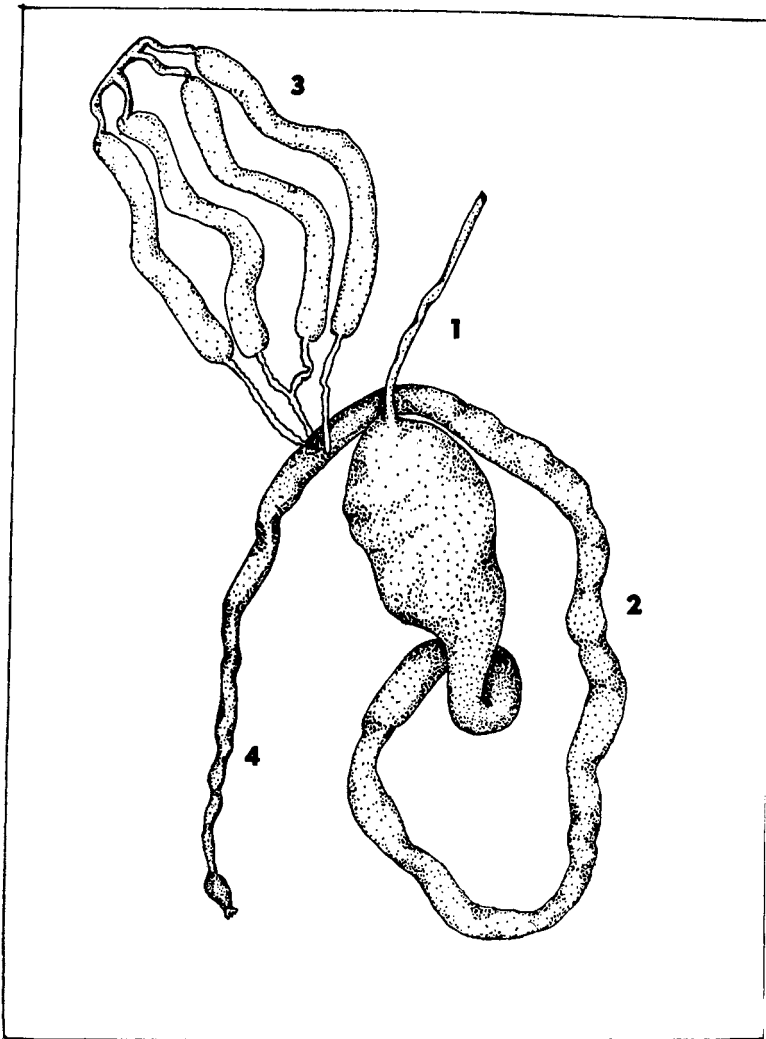


Figure 1. Morphology of the digestive tract of the potato leafhopper (70x).

1. stomodaeum
2. mesenteron
3. Malpighian tubules
4. proctodaeum

Stomodaeum. The stomodaeum was divisible into a pharynx and esophagus. In gross morphology the pharynx was a short, oblong organ located in the head cavity. The wall of the pharynx was composed of a thin chitinous intima, an irregular layer of epithelial cells, a layer of longitudinal muscle fibers, and an

outer layer of circular muscle fibers. The epithelial cells were irregular in shape with indistinct cell boundaries.

The esophagus (Fig. 2) led from the pharynx to the mesenteron. In the proximity of the mesenteron, the chitinous intima of the esophagus became progressively more folded and the epithelium more thin. There was no layer of longitudinal muscle fibers in the esophagus.

The esophageal fold (Fig. 2) was located at the junction of the esophagus and the mesenteron and involved the projection of the epithelial cells and a chitinous lining into the lumen of the mesenteron.

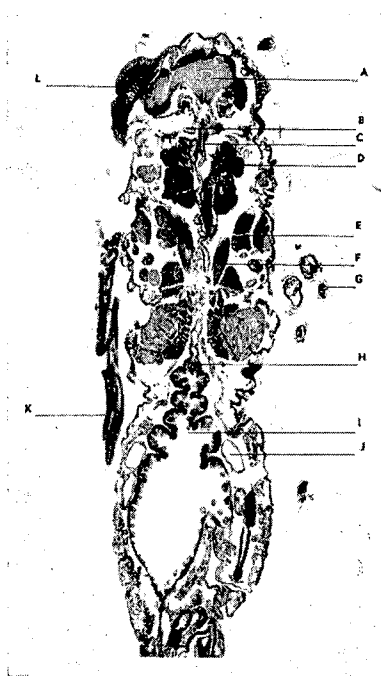


Figure 2. Longitudinal section of the potato leafhopper (73x).

- | | |
|------------------------|--------------------|
| A. brain | H. esophageal fold |
| B. accessory gland | I. ventriculus |
| C. esophagus | J. proctodaeum |
| D. salivary gland | K. wing |
| E. circular muscle | L. compound eye |
| F. longitudinal muscle | |
| G. leg | |

Mesenteron. The mesenteron was composed of an elongate, dilated pouch (ventriculus) and a tubular portion. The ventriculus and proximal tubular mesenteron formed the descending loop of the mesenteron while the distal part looped up and lay close to the ventriculus. In many Homoptera a filter chamber occurs in this area.



Figure 3. Cross-sections of the potato leafhopper gut. Cellular modifications functioning to facilitate the diffusion of liquids from the dilated ventriculus (right) into the distal tubular portion (left) would be apparent if a filter chamber were functional. In addition, the two areas would be held appressed by connective tissue. Top 160x. Bottom 400x.

The functional filter chamber may be composed of the closely associated anterior and posterior mesenteron and proctodaeum bound together by membranous connective tissue and muscle. The occurrence of a filter chamber in the Cicadellidae has been debated in the literature. Muir (1926) and Licent (1911a, 1911b, and 1912) reported that the Cicadellidae possess a filter chamber, but Quadri (1949) reported that the Cicadellidae did not. Saxena (1955a) divided the Cicadellidae into two groups, those with a filter chamber and those without. He stated, however, that the presence or absence of a filter chamber must be demonstrated in serial sections as a histological entity, and formed with a basement membrane and external musculature as described by Kershaw (1913). On this basis, a morphological filter chamber was not present in *E. fabae* (Fig. 3).

The wall of the ventriculus was composed of an inner epithelial layer, a layer of circular muscle and a thin, outer serous membrane. No chitinous intima was present in the ventriculus. The columnar epithelial cells of the ventriculus were variable in shape (Fig. 3). The distal parts of the epithelial cells were free from each other and projected into the lumen. The epithelial cell cytoplasm contained granules and was convoluted in the portion proximal to the nucleus. The cytoplasm appeared to be arranged lineally in the portion of the cell extending into the lumen. The cell walls were not distinct. The free cell border was striated.

The epithelial cytoplasm was filled with vacuoles differing in size and shape. In some cells the vacuoles were small and few, in others they increased in relative size and number. In some cells the vacuoles appeared to be released into the lumen by rupture of the cell. Day and Powning (1949) reported that cytoplasmic vesicles released into the lumen were products of cellular breakdown rather than of secretion, but Saxena (1955b) reported that these structures were sites of physiological activity.

Small groups of 'nidi', the regenerative or replacement cells, at the base of epithelial cells were scattered throughout the entire mesenteron.

In some living preparations a red pigment was noted in the ventriculus. This pigment was localized in the more distal area of the ventriculus and was insoluble in water; insect Ringer's solution; clove oil; cold, dilute hydrochloric acid; Lugol's iodine; and chlorine water. The red pigment was doubly refractive in polarized light.

The tubular mesenteron (Fig. 4) was composed of an epithelium with a striated free border, a circular muscle layer and an outer serous membrane. The epithelial cells were more wave-

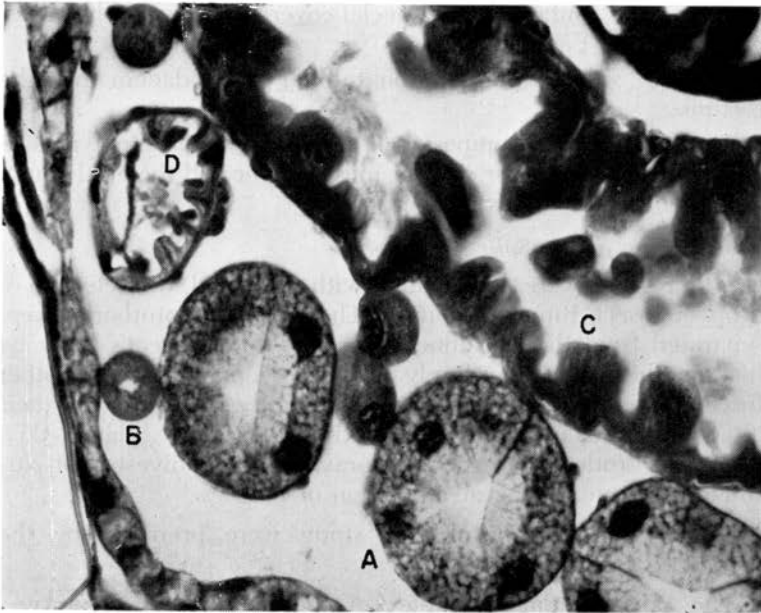


Figure 4. Tubular mesenteron and Malpighian tubules (600x).
 A. glandular portion of Malpighian tubule
 B. tubular portion of Malpighian tubule
 C. tubular mesenteron
 D. rectum

like and less like the projecting fingers noted in the ventriculus. Many vacuoles and secretory granules were present in the epithelial cytoplasm.

Four Malpighian tubules arose at the junction of the mesenteron and proctodaeum. Of the four tubules, two arose independently and two had a common point of origin (Fig. 1).

Each Malpighian tubule consisted of three distinct regions, a tubular proximal portion with a beaded appearance (Fig. 4); a broader glandular portion (Fig. 4); and a short, narrow tubular part joined with the other tubules to form a continuous lumen. The proximal portion was composed of narrow, uninucleate and binucleate cells surrounding a central lumen. The cytoplasm of these cells contained granules and vacuoles. The inner surface of the proximal and distal portions was of the brush-border type. The glandular portion was composed of very large cells with reticular cytoplasm. The nuclei were small and discrete. The histological structure of the distal region was much like that of the proximal region.

Proctodaeum. The proctodaeum consisted of a tubular section and a distal expanded area, the rectum. The tubular proctodaeum was composed of a chitinous intima, a thin epithelial layer and an outer layer of circular muscle. The epithelium was

syncytial and contained large nuclei covered by a very thin layer of cytoplasm (Fig. 2).

A rectal valve separated the tubular proctodaeum and the rectum.

The rectum was composed of an extra longitudinal muscle layer between the outer circular muscle layer and the epithelium. A chitinous intima was present.

Morphology of the Salivary Glands

The insects were inactivated with CO₂ and dissected in a drop of insect Ringer's solution. The head and prothorax were separated from the remaining segments of the insect's body by holding the metathorax firmly with a forceps and with another forceps gently pulling the head and prothorax free from their attachments. The translucent multilobate salivary glands then visibly protruded from the prothorax and were divested of surrounding tissue by the manipulation of needles.

The sections for histological study were prepared by the methods described above.

The salivary gland complex of the potato leafhopper was composed of principal and accessory glands. The principal glands were located in the anterior part of the prothorax, one on each side of the esophagus (Fig. 5). Each gland was composed of four lobules joined together at the hilus of the gland. The salivary duct originated at the hilus and joined with the other salivary duct en route to the salivary syringe where it terminated. Each salivary duct became bifurcate, one branch looping anteriorly to the head and then widening into an accessory gland or sac. Pigmentation did not appear to be a general characteristic of the salivary glands.

An aqueous 0.5 per cent solution of the metachromatic dye, toluidine blue, was used to help differentiate parts of the salivary gland complex. The metachromatic colors of toluidine blue are pink or purple. Nuclei stain deep blue. The salivary glands (Fig. 5) stained so that each pair of lobes appeared a different hue: pair one stained light blue; pair two, pink; pair three, dark blue; and pair four, blue-green; and the accessory gland, the salivary and accessory ducts all stained pink. The many-hued staining reaction of the salivary gland suggested chemical differences among the various lobes of the salivary gland and their secretions.

A histological study clearly indicated differences in the structure of paired lobes. The principal gland was composed of large cells with a granular cytoplasm. The nuclei were either ramified or discrete and stained darkly with haematoxylin. Each

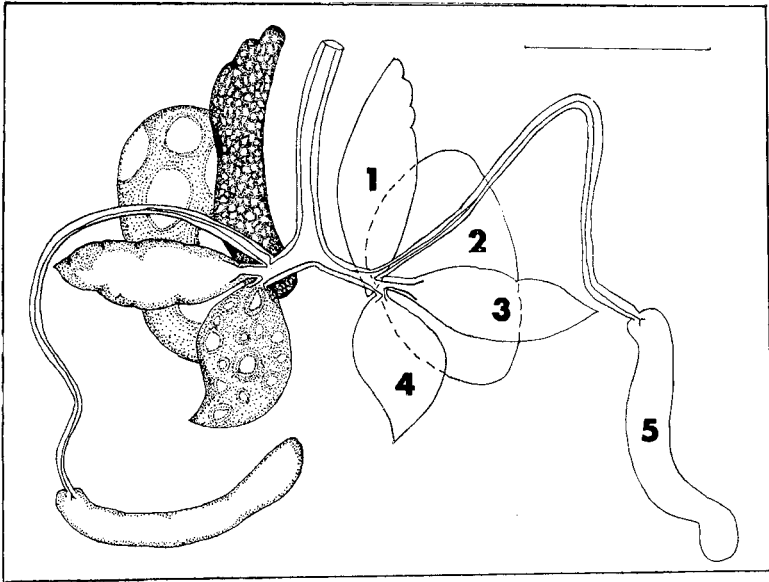


Figure 5. Morphology of the salivary gland complex of the potato leafhopper (296x).
1-5. lobes of the principal gland
5. accessory gland

pair of lobes of the principal gland were composed of cells distinct from the cells in the other pairs (Fig. 6).

A composite drawing of the cell types (Fig. 6) was prepared to indicate four distinct tissue types. Type 1 was composed of a cellular border arranged about a central collecting area ultimately leading to the hilus of the gland. The cytoplasmic ground stained evenly and lightly with haematoxylin and eosin, but the glandular secretions and the borders of each secretory area stained a bright red with eosin. Two types of secretory products were present in the collecting area: serous and granular. The nuclei were small and discrete and arranged about the outer border of the collecting area.

The cytoplasmic ground of type 2 was composed of small four- to six-sided bundles containing varying numbers of granules. Under 100X magnification, these bundles merged into each other to present a dark background of minute collecting areas and canals in the cytoplasm. The nucleus was unevenly indented and deeply stained with haematoxylin.

Type 3 was penetrated by a ramifying network of small canals leading to the hilus of this lobe. The cytoplasmic ground was homogeneous and deeply stained with haematoxylin and eosin. A fine-textured, purple-staining substance was present in the canals. The nuclei of the cells stained deeply with haematoxylin and were slightly granular in texture.

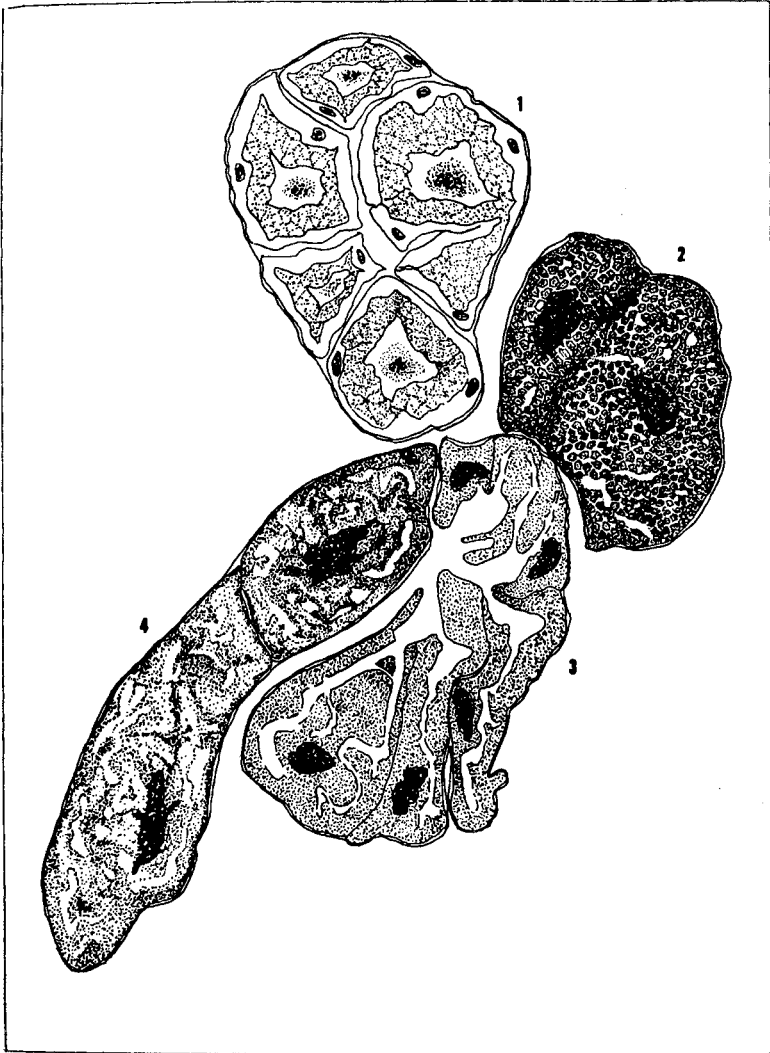


Figure 6. Histological structure, types 1, 2, 3 and 4, of lobes of the principal salivary gland (970x).

Type 4 was composed of large cells with ramified nuclei. A system of canals, smaller than that in the third type, branched throughout the cytoplasm. The cytoplasmic ground was slightly reticular and contained some darker-staining areas.

The accessory gland (Figs. 1 and 2) was composed of an outer serous membrane and a thin epithelium. The epithelium was composed of flattened, elongate, cuboidal cells with brush borders. The accessory duct also contained flattened cells with brush borders.

Salivary Enzymes

The presence of an enzyme can be detected by incubating the suspected source with a suitable substrate and testing for the presence of reaction products.

Invertase. Tests for invertase (sucrase) were conducted with salivary gland macerates by the method of Bronskill et al. (1958). Because of potential variation from either innate differences among glands or minor inequities in dissection techniques, from eight to fourteen salivary glands were combined in each macerate. Salivary glands obtained from third to fourth instar nymphs were ground in a Cenco-Potter homogenizer with .05 ml 2.5 per cent sucrose solution per gland. The macerate was incubated at 37°C for two hours. After incubation, .05 ml of Benedict's qualitative solution were added (Hawk et al., 1954, p. 1322). The mixture was placed in a boiling water bath for eight minutes. The resulting colors were noted and recorded. A blue color denoted a negative test, a green or yellow-green color denoted a weakly positive test. Invertase (sucrase) activity was observed by this method.

Tests for invertase in solutions fed upon by the potato leafhopper were made by the method of Medler (1940). His procedure was modified so that six to ten leafhoppers were confined in a gelatin capsule separated by a Parafilm membrane from 2.5 per cent sucrose solution. After allowing the insects access to feed from 24 to 96 hours the solutions were diluted, one to one, with Fehling's solution, boiled for eight minutes and the color noted. The development of a red precipitate should have denoted a positive test. No red precipitate was evident.

Further experimentation was made utilizing 1 ml of 2.5 per cent sucrose solution accessible to the feeding of 20 leafhoppers for 96 hours. The solution was tested for reducing sugars with Benedict's qualitative solution. Negative results were obtained consistently.

In none of these tests was there any qualitative evidence for the action of invertase in sucrose solutions accessible to the feeding of potato leafhopper nymphs.

Amylase. The method of Nuorteva (1954) was utilized to detect amylase activity in whole salivary glands or gland macerates. Starch (2 per cent)-agar (4 per cent) plates were made up freshly each day and stored at 4°C until ready for use. When two salivary glands were laid on the surface of the starch-agar plate and incubated at room temperature (25°C) for 1½ hours, cleared areas appeared when a dilute iodine (IKI) solution flooded the surface of the plate. A drop each of insect Ringer's solution and human salivary amylase solution were used as con-

trols. Although this method only crudely indicated the action of an amylase, it prompted further experimentation to detect the presence of this enzyme. Amylase activity was confirmed by iodine reaction tests on incubated salivary gland macerates (.05 ml .004 per cent soluble starch solution per gland, 2½ hrs at 37°C) and also by testing similar incubated macerates for reducing sugars with Benedict's qualitative reagent.

Tests for amylase in solutions fed upon by the insect were conducted by a modification of Medler's (1940) method described above. Thirty leafhopper nymphs were used per test. Potato starch solution, .004 per cent, was accessible to feeding for 24 to 96 hours. Tests of the solutions with Fehling's and Benedict's qualitative reagents were negative.

Protease. The method of Nuorteva (1954) was used in which salivary glands dissected in insect Ringer's solution were placed whole, or crushed with needles, in a drop of buffer solution, pH 6.4, or an unexposed Eastman Lantern slide plate. The plate previously had been cleared in a 20 per cent solution of sodium thiosulfate, washed in distilled water, dried and stored at 4°C. The plate was incubated at 25°C in a moist chamber for 6 to 12 hours. After incubation the plate was washed in running water, fixed in 4 per cent formaldehyde to inactivate the enzyme, and stained in acid fuchsin or Delafield's haematoxylin for 15 minutes; cleared areas, ranging from 1 to 5 mm diameter, denoted protease activity. No attempts were made to detect the presence of protease in solutions fed upon by the potato leafhopper.

Proteases and amylases have been demonstrated in the macerated salivary glands of homopterans by Davidson (1923), Saxena (1955b) and Nuorteva (1954, 1956b, 1958a, 1958b, and 1958c). Nuorteva studied a great number of Hemiptera-Homoptera species and concluded that the absence of these enzymes indicated that an insect was a phloem-feeder. Conversely, the presence of an amylase and protease indicated that an insect was a mesophyll-feeder. Unless further investigations alter the present view that *E. fabae* (Harris) feeds from the phloem, this insect appears to be an exception to Nuorteva's generalization.

Lipase. A histochemical method was used. Whole nymphs or adults were fixed, infiltrated and embedded by the method of Salkeld (1959). The Tween method of Gomori (1945) was used to detect sites of lipase activity. Although some activity was observed in midgut tissues, there did not appear to be any reaction in salivary gland tissues.

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