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STRUCTURE AND DEVELOPMENT OF THE ALIMENTARY CANAL OF THE SOUTHERN
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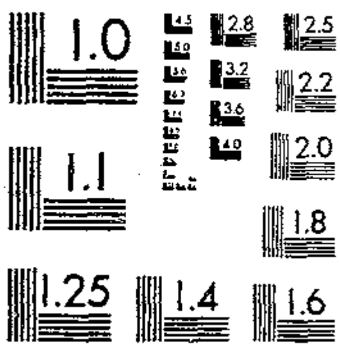

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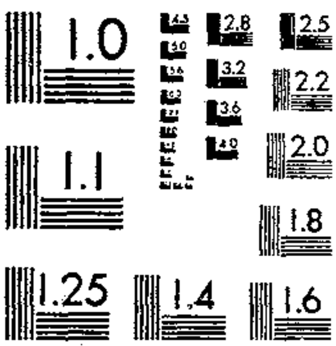

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Structure and Development of the Alimentary Canal of the Southern Armyworm Larva

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INTRODUCTION

Knowledge of the internal anatomy and physiology of insects is essential in the investigation of toxicological problems. In preparation for such work the present study was undertaken to learn the structure, microscopic anatomy, and postembryonic development of the alimentary canal of the larva of the southern armyworm (*Prodenia eridania* (Cram.)). Cytological studies and more detailed descriptions will be included in future reports on the special problems with which such studies are particularly concerned.

The southern armyworm larva is well suited as a generalized insect for experimental purposes. It breeds readily in small cages and is easily reared in large numbers throughout the year on its natural food plants. It feeds on a variety of plants and has a fairly rapid development. In size it is convenient for dissection and observation.

This larva is used extensively in investigational work on digestion in leaf-eating insects and the effects of various insecticidal compounds on the digestion and absorption of foodstuffs, which is carried on as a special research problem. It is essential that definite information as to the morphology of the digestive tract of the normal insect be available if the results of this investigational work are to be correctly interpreted. This study was therefore made as a part of the work on digestion and translocation of foodstuffs in insects.

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The southern armyworm is a typical lepidopterous larva and one of a heterogeneous group of noctuid larvae known as armyworms. The group has been reviewed by Crumb (*8, 9*).[1] A technical description, by Dyar, of the egg and larval stages is included in a paper by Chittenden and Russell (*7*). The species occurs in the lower Austral faunal area and is probably of tropical origin. It is destructive to various field and truck corps in the southern part of the United States (*7, 31*).

The larvae are about 1.2 mm. long at hatching. Mature larvae range in length from about 39 to 46 mm., and in breadth from about 6 to 7.5 mm. The average weight of such larvae is about 960 mg. About half of this weight is due to ingested material within the alimentary canal. The larvae develop in six stadia, requiring about 17 days at 27° C.

An examination of the few detailed accounts of the anatomy of the alimentary canal of lepidopterous larvae reveals a diversity of structure even among closely related species. Especially helpful is a comparative study of the morphology of the alimentary canal of lepidopterous larvae, including species of Noctuidae, given by Dauberschmidt (*10*). Bordas (*5*) described the alimentary canal and the Malpighian tubules of the larva of each of several representative species of Lepidoptera, including four species of the family Noctuidae, and Snodgrass (*24, 25*) has described the alimentary canal of noctuid species. Shinoda (*23*) made a comparative study of the histocytology of the midgut of several species of Lepidoptera. There is no report in the literature on the alimentary canal of the larva of the southern armyworm. In its general features it is similar to the alimentary canals of other lepidopterous larvae that have been described.

MATERIALS AND METHODS OF STUDY

The larvae used in this study, with the exception of the late sixth instars, were reared on fresh turnip foliage in an incubator at 27° C. The late sixth instars (60 or more hours past the fifth molt) were reared on the foliage of living turnip plants in a greenhouse insectary. Actively feeding larvae were selected and conserved for a time on fresh, clean leaves in a clean culture dish, in order that sand grains or other hard objects that may have been present in the lumen of the gut would be eliminated.

For the study of tthe microanatomy the paraffin method was employed. Larvae for study were selected and fixed during and immediately after the hatch, before and immediately after each molt, at 3, 12, 24, 36, and 48 hours after each molt, and at other suitable times. Larvae of the first four instars were killed and fixed in Carnoy and Lebrun's mixture. Larvae of the fifth and sixth instars were killed by immersion in hot water (at 60°–70° C.) and immediately dissected, and the desired tissues were removed and dropped into Bouin's picro-formol-acetic fixative. Serial sections of whole larvae and of isolated gut tissues were cut from 3 to 10μ in thickness, in both the transverse and the longitudinal planes. A thickness of 5 to 7μ was usually most satisfactory. The sections were stained

[1] Italic numbers in parentheses refer to Literature Cited, p. 27.

with Ehrlich's hematoxylin and counterstained with eosin or erythrosin.

Studies of the gross anatomy were made on living and freshly killed larvae, and on living dissections in normal saline. The study of the musculature was facilitated by maceration of the material in MacCallum's macerating fluid.

The illustrations were drawn at suitable magnifications with the aid of a camera lucida. Microscopic measurements were made with an ocular micrometer in a 10× or 15× ocular, and with a 1.8-mm. oil-immersion objective. The terminology used by Snodgrass (*25*) is employed. The histological methods are those of Lee (*18*) and Guyer (*14*).

GENERAL DESCRIPTION OF THE ALIMENTARY CANAL

The alimentary canal (fig. 1) is a straight tube extending from the mouth directly through the head, thorax, and abdomen to the anus. Since the larva is a continuous feeder, the canal is normally distended by ingested materials and may occupy most of the cavity of the thoracic and abdominal regions. The walls of the canal are

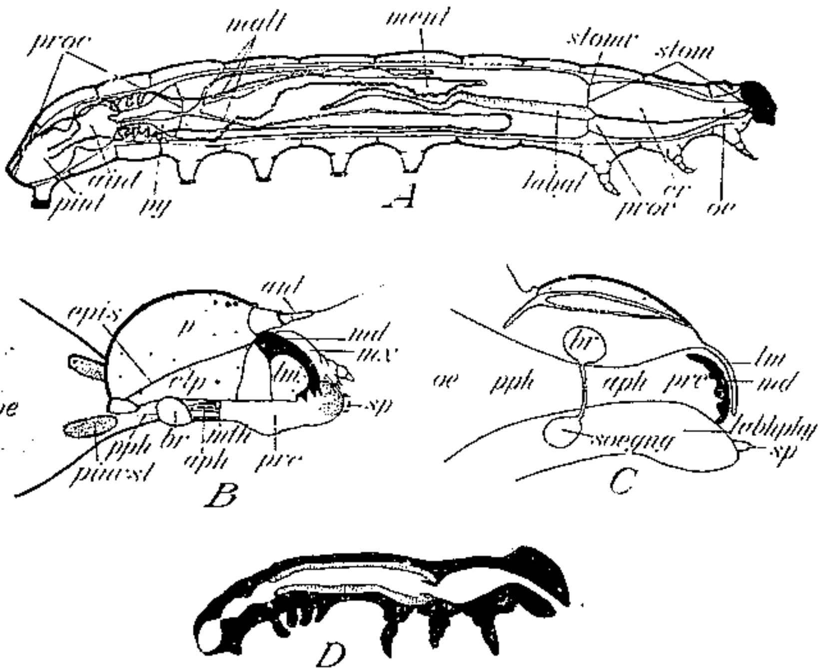

Figure 1.—*A*, Lateral view of late sixth-instar southern armyworm, showing the location of the alimentary canal, the labial glands, and the Malpighian tubules in relation to the body segments, ×2. *B*, Outline of head of late sixth instar, dorsal view, with the right half of the cranium and the thoracic integument cut away to show the relation of the cephalic stomodaeum to the head parts, ×10. *C*, Outline of head of late sixth instar in median longitudinal dorsoventral section, to show the relation of the cephalic stomodaeum to the head parts, ×10. *D*, Median longitudinal dorsoventral section of first instar, age 9 hours, showing the alimentary canal, ×40.

elastic, permitting the configuration and dimensions of the tube to vary according to the quantity and distribution of the ingested material that is present in the lumen. The wall may become distended in either the circumferential or the longitudinal direction, according to the region, but the distension is generally in the circumferential direction. Except for constant folds in certain regions, the distended wall is smooth. The collapsed wall is wrinkled crosswise or lengthwise.

The alimentary canal (fig. 1, *A*) consists of three primary divisions, the foregut or stomodaeum (*stom*), the hind-gut or protodaeum (*proc*), and the midgut or mesenteron (ventriculus) (*vent*). These divisions are easily distinguishable by the unaided eye. The midgut is thick-walled, whereas the foregut and the hind-gut are thin-walled and nearly transparent. The junction of the midgut with the hind-gut is well above the point of attachment of the Malpighian tubules in the posterior part of the pylorus. Under the microscope the junction of the midgut with each of the other two divisions is seen to be marked precisely by the abrupt cessation of the characteristic epithelium of the midgut and the beginning of that of the other divisions. Both the musculature and the intima also vary in the different regions.

The dimensions of the alimentary canal and its divisions vary according to the individual, the degree of its extension, and the quantity of material in the lumen, as well as with the stage of development. The length of the canal of a full-fed and moderately extended larva is approximately 44 mm. The average lengths of the primary divisions are, approximately, 9 mm. for the foregut, 27 mm. for the midgut, and 8 mm. for the hind-gut. It is seen that in the mature larva the midgut is approximately three times as long as either the foregut or the hind-gut. In the early instars (fig. 1, *D*) the three divisions are more nearly equal in length. Usually the midgut is about one-fourth longer than either of the other two divisions.

The foregut and the hind-gut are divided into several regions. Those of the foregut are difficult to delimit even under the microscope. The regions of the hind-gut, on the other hand, are easily distinguishable by the unaided eye.

In histological section the wall of the alimentary canal presents, in order, (1) an enveloping membrane of connective tissue, (2) one or two layers of muscle fibers or bundles, (3) a basement membrane, (4) a single layer of epithelial cells, and (5) an intima in the foregut and in the hind-gut, and a sheath of detached material in the midgut known as the peritrophic membrane. The functional and structural characteristics of the musculature, the epithelial cells, and the intima vary greatly according to the region.

The cytoplasm of all the muscle fibers of the alimentary canal is delicately cross-striated. The striations are clearly visible without staining or other special preparation.

The basement membrane is usually indistinguishable from the substance of the bases of the epithelial cells that rest upon it. In places where it is detached enough to be seen as a separate structure, it appears as a distinct, sharp, dark, and continuous line approximately 0.3μ thick and adhering closely to the epithelial sheet (fig. 5, *A*, *bmb*).

MOUTH PARTS AND LABIAL GLANDS

The mouth parts (fig. 1, *B* and *C*) have the well-known biting and chewing type of structure usually found in lepidopterous larvae.

The wall of the preoral cavity resembles that of the body wall of which it is a continuation. The tall columnar type of epithelial cell, which is characteristic of the body wall, ends abruptly at the point where the wall of the preoral cavity becomes that of the foregut and is replaced by a very different type of epithelial cell to be described as characteristic of the anterior pharynx.

The labial glands (fig. 1, *A*, *labgl*) are two simple, elongate tubes, which lie in the body cavity alongside the alimentary canal and extend usually from the metathoracic segment into the abdomen as far as the fourth abdominal segment. In the late sixth instar they are about 0.87 mm. in diameter at the large anterior end and taper gradually throughout their length of about 16 mm. to a diameter of about 0.25 mm. at the posterior end. A duct proceeds from the large anterior end of each gland through the thoracic cavity and into the neck region of the larva, where the two ducts unite to form a common duct, which is prolonged into the head and opens in the spinneret borne distally by the prementum.

FOREGUT (STOMODAEUM)

The general structural characteristics of the foregut, or stomodaeum, are constant throughout larval life. A great increase in size occurs. The following description is based on the late sixth instar except where specifically stated otherwise.

The foregut (fig. 1, *A*, *stom*) extends through the head and thorax and joins the midgut usually in the region of the third thoracic segment (metathorax). The total length increases from approximately 0.3 mm. in newly hatched larvae to 9 or 10 mm. in mature larvae. The diameter of the lumen of the cephalic portion in mature larvae is approximately 0.5 mm. The walls of the entire portion in the thoracic region are elastic and capable of great distention. This part may occupy almost all the free space within the thorax when the lumen is filled with food materials, and in this condition it may have a diameter of approximately 5 mm. When not distended the walls fold inwardly. The diameter of the tube of larvae that have not recently ingested food, and especially of starved larvae, becomes rather small, similar to that of the portion in the head. The walls are thin and transparent, permitting easy observation of the food materials in the lumen.

The foregut is clearly differentiated into several regions by structural characteristics and functional specializations. These regions are designated as the mouth, the buccopharynx, the eosophagus, the crop, and the proventriculus. The actual points of demarcation are not well defined, but the nature of the musculature and the character of the epithelium, the intima, and the internal structures serve to differentiate the parts structurally. Separate regions discharge the functions of ingestion, conveyance, and storage of the food materials, and their subsequent conduction into the midgut.

The wall of the foregut is continuous with that of the preoral cavity.

A characteristic of the epithelium throughout the foregut is the increase in size of the constituent cells with increase in size of the foregut during larval growth. No evidence was found of increase in numbers through division, or through development from regenerative cells as in the midgut. Henson (*17*) found that in *Vanessa urticae* (L.) (Lepidoptera) the foregut grows by increase in size of the cells but not by increase in cell numbers. Trager (*39*) found that in *Aedes aegypti* (L.) (Diptera) the foregut grows by increase in cell size.

Mouth and Buccopharynx

The mouth lies between the bases of the mandibles and is the anterior opening of the foregut.

The most anterior region of the foregut is the pharynx. The buccal cavity is structurally undifferentiated from the pharynx.

The posterior limit of the pharyngeal region of lepidopterous larvae is a matter of question. The portion of the foregut immediately posterior to the nerve ring may be regarded as a part either of the oesophagus or of the pharynx (*13*, *25*). The fact that dorsal dilator muscles arising on the vertex of the head are inserted in this region indicates that by definition (*25*) it is a part of the pharynx, and it is so regarded in this discussion. The portion of the foregut anterior to the nerve ring is designated as the anterior pharynx (fig. 1, *B* and *C*, *aph*) to distinguish it from the portion posterior to the nerve ring and designated therefore as the posterior pharynx (*pph*). For convenience in description the pharynx will be regarded as delimited posteriorly by a group of large and widely spaced muscles that encircle the canal in the neck region and probably function as a sphincter. They are here designated as the pharyngeal sphincter.

The pharynx as defined is located within the head and neck of the larva. In late sixth instars it is approximately 1.5 to 2.0 mm. long, narrow in the anterior or head part, and widened in the extreme posterior part (fig. 1, *B*, *C*). The pharynx is characterized by its musculature, the insertions in its wall of dorsal dilator muscles originating on the frontal and dorsal areas of the head wall, a distinct type of epithelial cell in the anterior part, six internal longitudinal ridges bearing small teeth, and two large internal structures in the region of the pharyngeal sphincter, which are formed as invaginations of the wall and bear numerous large teeth.

The pharynx is heavily muscled. The muscle fibers are connected by numerous small branches. The measurements given below apply to the late sixth instars (fig. 2). The muscle fibers of earlier stages are smaller. The several large, closely placed circular muscle bands of the anterior pharynx (*A*, *C*, *cmcl*), in fixed and stained preparations, are approximately 30 to 48μ broad. An extra large muscle over the buccal cavity is about 65μ broad. The muscles are arranged in plaques, and the ends are attached to the intima. The muscles lying nearest the nerve ring in the posterior pharynx (*B* and *C*) are narrower (17 to 36μ) than those in the anterior pharynx. The largest and most posterior circular muscle of the pharyngeal-sphincter group (*D*), in fixed and stained preparations, measures about 64 to 120μ in width. It gives off numerous large longitudinal branches posteriorly to the oesophagus and crop, and many smaller

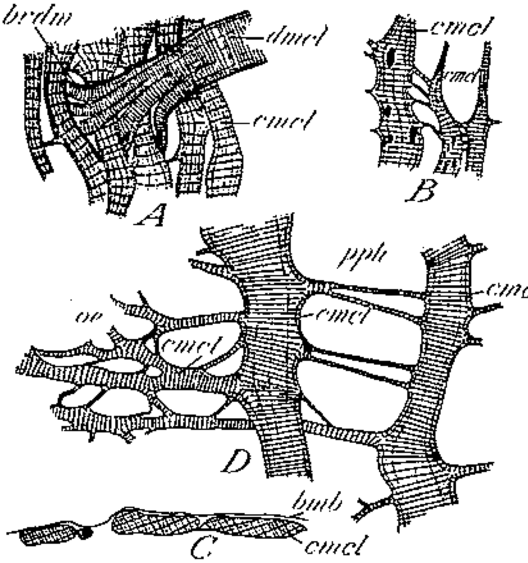

FIGURE 2.—Circular muscle fibers of pharynx of late sixth instars of the southern armyworm. *A*, Circular muscle fibers of anterior pharynx, showing also branches of a dilator muscle and entrance of the branches between the circular muscle fibers, ×65. *B*, Circular muscle fibers of posterior pharynx, ×65. *C*, Transverse sections of much flattened circular muscle fibers of posterior pharynx, ×180. *D*, Large circular muscle fiber marking posterior termination of pharynx, with the second circular muscle fiber of the pharyngeal sphincter and longitudinal branches given off to the oesophagus, showing branching and uniting of fibers on oesophagus, ×65.

branches to the pharynx. Anterior to this muscle are about four smaller circular muscles (25 to 54μ broad), which are irregular in direction although roughly parallel to the largest circular muscle. These muscles are widely spaced, the five extending over a distance of approximately 750μ. They are connected by numerous small strands of fibrillae. Underlying the layer of circular muscles is a layer of longitudinal fibers, which originate as branches of the circular muscles. They are more numerous in the posterior pharynx. The entrance of the several branches of a dilator muscle between the circular muscles of the anterior pharynx is illustrated in figure 2, *A*, and attachments of two branches on the intima of the wall of the anterior pharynx are illustrated in figure 3.

The anterior pharynx possesses a distinct and characteristic type of epithelium. The cells are comparatively large, measuring in late first instars approximately 7.4μ in greatest diameter, and in late sixth instars up to 50μ or more in diameter. They are much enlarged distally and frequently detached from one another basally. The cytoplasm is granular and stains uniformly. The nuclei are distinct, centrally located, and large, measuring 3 to 4μ in diameter in late first instars and up to 21μ in late sixth instars. Typical cells are seen in figure 3, *epthc*. The epithelial cells of the posterior pharynx are much smaller (7 to 16μ high in mature larvae). The nuclei are distinct. The cells are closely packed in the epithelial layer, and the cell boundaries are indistinct or absent.

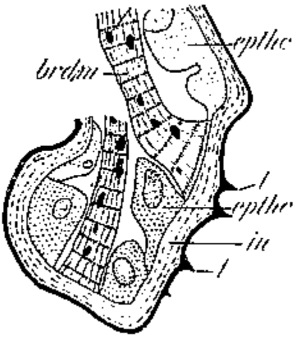

FIGURE 3.—Transverse section of ridge in anterior pharynx of late sixth-instar southern armyworm, ×290.

The intima of the pharynx of late sixth instars (fig. 3, *in*) ranges in thickness from about 10 to 16μ near the mouth and from about 5 to 8μ in the rest of the pharynx. In the first instar the intima of the posterior pharynx is approximately 1.0 to 2.0μ thick. The comparatively thick external zone of the intima lying next to the epithelial layer is finely striated and laminated. The internal zone next to the gut lumen is thinner, more compact, and hyaline in appearance. Numerous small, fine teeth are supported by the intima and project outward into the lumen. Some of these teeth are illustrated in saggital section in figure 3, *t*. The teeth in this region average about 5.3μ in length.

The wall of the pharynx is disposed in six longitudinal ridges, which project into the lumen and extend from the buccal cavity to near the posterior end of the pharyngeal region. They are observable in freshly killed larvae as well as in sections, and appear to be of constant form, although they may allow for expansion of the wall as the lumen becomes distended. One ridge is located dorsad, one ventrad, two dorsolaterad, and two ventrolaterad. They are most prominent near the anterior end. As they are prolonged in the posterior pharynx, they become gradually less prominent and disappear as the pharynx widens in the neck region. The extreme posterior region of the pharynx is wrinkled transversely. Figure 3 is a transverse section of the anterior pharynx and shows a part of a ridge with the epithelial cells (*epthc*), attachments of branches of a dilator muscle (*brdm*), the intima (*in*), and teeth (*t*) on the ridge.

Two large invaginated structures (fig. 1, *B*, *pinvst*) bearing numerous teeth are found internally on the ventrolateral wall in the region of the pharyngeal sphincter. They have essentially the same structure in all instars. Viewed dorsally they are elongate-oval in shape and are orientated with their longitudinal axes lengthwise of the canal. The sphincter as a whole embraces these structures during contraction. These structures may easily be observed in the intact pharynx merely by slitting and spreading the dorsal integument of the neck and thorax and looking through the transparent wall of the pharynx immediately back of the head. Each of the structures is an invagination of the wall of the pharynx. The basal part is narrow and forms a peduncle supporting an enlarged distal part. The distal part in late sixth instars is about 640 to 750μ long and about 150 to 200μ broad. The structure projects upward from the rest of the wall to a height of about 300 to 400μ. The height probably varies greatly according to the physiological condition of the structure. The peduncle is heavily muscled. The large and numerous muscle bands are inserted in the intima of each side of the structure. Longitudinal muscles are present near the base. The epithelial cells of the distal part are large, the cell boundaries are distinct, the cytoplasm is reticular and stains uniformly, and the nuclei are large and distinct and stain deeply (fig. 4, *A*, *epthc*). The epithelium of the peduncle is narrow and otherwise similar to that of the rest of the wall of the posterior pharynx. The intima is thin, measuring about 2.1μ in thickness in both the peduncle and the distal part. The intima (*in*) of the distal part of the structure supports numerous large

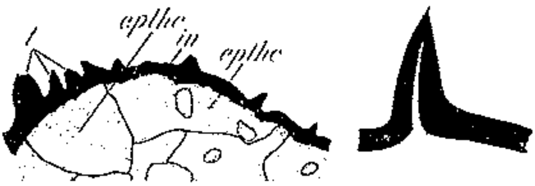

FIGURE 4.—*A*, Horizontal section through one corner of distal part of an invaginated postpharyngeal structure of late sixth-instar southern armyworm, ×220. *B*, Longitudinal section of a tooth located on the anterior end of an invaginated postpharyngeal structure of late sixth-instar southern armyworm, ×700.

teeth (*t*), which project into the lumen. Figure 4 shows, *A*, several of these teeth as they appear in a horizontal plane, and *B*, a longitudinal section of a tooth. A lumen extends from the underlying cytoplasm to near the tip of the tooth. The structure appears to be a direct continuation of the intima. The teeth are variable in size, the larger ones in sixth instars being 16 to 22μ high.

The function of the structures is problematical. From their position and structure it appears likely that they assist the sphincter muscles in forming a valve for retaining the solid particles of food materials passed back to the oesophagus by the pharynx. The structures with their backward-projecting teeth may form an effective barrier, since they may partially occlude the lumen when it is restricted in size by the contraction of the sphincter muscles. Observations of the region in feeding larvae tend to confirm this view. Because of the transparency of the wall of the foregut, the structures and food material are easily observed in the intact organ. In recently fed larvae the crop and oesophagus may be distended by food materials. Although packed tightly up to the region of the pharyngeal sphincter and these pharyngeal structures, the lumen anterior to the sphincter is usually free of ingested material.

To test the effectiveness of the pharyngeal sphincter as a valve, the head of a well-fed sixth instar was cut off immediately back of the occipital foramen, and just anterior to the sphincter. The region immediately became constricted and held back the food material in the crop and oesophagus, even under extra pressure.

The larvae may be induced to regurgitate by applying pressure on the thorax. The regurgitated material is a fluid containing plant pigments and suspended chloroplasts, but no solid particles of the size ordinarily bitten off by the insect.

From these observations it is concluded that the pharyngeal sphincter, probably assisted by the invaginated structures, forms an effective barrier against the return of food materials from the oesophagus. If the teeth are provided with nerves, they may transmit stimulating impulses to the underlying muscles, and perhaps also to those on the wall of the pharynx. The writer has found no description of corresponding structures in the literature he has reviewed.

Oesophagus

From the pharynx the oesophagus gradually enlarges until it joins the crop. Functionally the oesophagus and the crop appear to be undifferentiated. The musculature is similar and continuous over both regions. A group of three to five large, circular muscles is regarded as marking the division between the two. As defined, the oesophagus of the late sixth instar is approximately 1 to 2 mm. in length.

The musculature of the oesophagus and crop is a network. It originates as posteriorly directed branches from the pharyngeal sphincter muscles, which, by dividing and reuniting, form a plexus over the wall (fig. 2, *D*). In general the character of the musculature in the two regions is very similar, but it may differ somewhat in the direction of the main branches and in the shape of the meshes. In the oesophagus the main branches tend to run longitudinally, and the meshes tend to be narrowed. In the distended crop there is less tendency toward a longitudinal direction, and the meshes are larger and more rounded. Longitudinal branches of the circular muscle fibers of the proventriculus unite with the muscle fibers of the crop. The large, circular muscle fibers between the oesophagus and the crop give off small branches that connect with the underlying plexus of muscle fibers. They differ from the muscle fibers of the pharyngeal sphincter in being smaller and lying closer together. The individual fibers in stained preparations from late sixth instars measure 30 to 54μ in breadth.

The epithelium and the intima of the oesophagus are similar to those of the crop (described below).

Crop

The crop, or ingluvies, is between the oesophagus and the proventriculus. It is the largest region of the foregut, and in late sixth instars extends approximately 3.0 mm. in length. The musculature of the crop is a continuation of that of the oesophagus, which has been described. The wall (fig. 5) through the oesophagus, crop, and proventriculus is composed of a comparatively thin epithelial sheet (*epth*) and a thick intima (*in*). The epithelium is homogeneous and has the character of a syncytium. The cytoplasm is granular and takes stain uniformly. The nuclei are distinct and ovoidal. In stained preparations the thickness of the epithelial layer of late sixth instars is usually between 3 and 6μ, although it may vary between 2 and 17μ. The intima in the same preparations usually varies between 7 and 9μ, but may vary between 5 and 15μ.

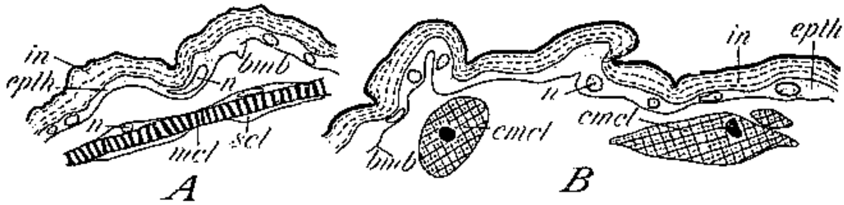

FIGURE 5.—Longitudinal sections through walls of crop and proventriculus of a late sixth-instar southern armyworm. *A*, Crop. *B*, Proventriculus. ×290.

PROVENTRICULUS

The proventriculus is simple in structure and probably functions in regulating the passage of food material into the midgut and in preventing its return to the crop. In living preparations of the alimentary canal the wall of the proventricular region may be observed, under certain conditions, to constrict violently and regularly as food material is forced from the crop into the midgut.

When both the crop and the proventriculus are distended by food materials, the two regions are indistinguishable by the unaided eye. The proventriculus of the late sixth instar is approximately 2.4 mm. in length and is characterized by widely spaced circular muscles. The epithelium and the intima (fig. 5, *B*) are similar to the corresponding structures of the oesophagus and crop.

The musculature of the proventriculus (fig. 6) differs markedly from that of the other regions of the foregut. It consists of about 15 to 20 large, circular muscle fibers connected by numerous small branches. The fibers give off branches which extend in all directions, especially longitudinally. In fresh preparations of late sixth instars the muscle fibers range in width from about 21 to 53μ or more. They may lie adjacent to one another, or they may be separated by distances of 60 to 200μ or more (fig. 5, *B*). Figure 6 shows parts of two circular muscle fibers with their interconnecting strands and branching. The underlying longitudinal muscle fibers are numerous and irregular in direction.

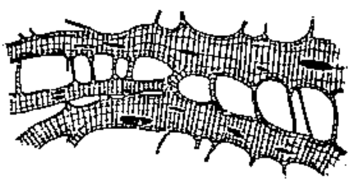

FIGURE 6.—Circular muscle fibers of proventriculus of late sixth-instar southern armyworm, showing branches and cross connections. ×65.

Numerous small muscle fibers that arise as branches of the muscle fibers of the proventriculus extend longitudinally external to the circular muscle fibers and are attached at the junction of the proventriculus with the midgut. Dilator muscle fibers orginating at the junction of the first and second thoracic segments of the body wall also pass forward over the foregut to be inserted on the midgut.

Stomodaeal Valve

The structure usually called the stomodaeal valve is formed as a simple invagination of the posterior end of the foregut into the anterior end of the midgut. The wall of the midgut is continuous with that of the foregut. The two lamellae formed by the invagination of the foregut wall remain together in fresh preparations, although separation is easily accomplished by teasing with needles under a binocular microscope, and they are free from one another as observed in histological sections.

In the empty or partially filled lumen the invaginated portion hangs loosely. Since, however, the lumen is normally filled with food material moving in the caudal direction, the wall is usually distended and the invaginated portion of the foregut is pressed against the wall of the midgut.

The epithelial layer and the intima of the invaginated wall are in general similar to those of the proventriculus. Near the junction with the midgut the wall is formed in a small loop. The epithelial layer in the loop is made up of well-defined and comparatively large cells, which may function (*17*) to regenerate the epithelium of the foregut during metamorphosis.

The muscular layer is absent from the invaginated wall. The muscle of the proventriculus, and likewise of the midgut, terminates at the junction with no special development at that point. The function of retaining the food material in the midgut against pressure appears to be borne entirely by the well-developed muscle fibers of the proventriculus.

MIDGUT (MESENTERON)

The midgut, or mesenteron, changes little in general features, except in size, during larval growth. The length increases from approximately 0.3 mm. in newly hatched larvae to 27 mm. in late sixth instars. The cellular constituents of the epithelium undergo changes that will be described below. The description of the gross anatomical features applies particularly to the late sixth instar.

The midgut is a large, undifferentiated tube of approximately uniform diameter throughout its length. It extends from its junction with the foregut near the posterior end of the thoracic region, throughout the first six abdominal segments, and joins the hind-gut usually in about the sixth abdominal segment. In feeding larvae the wall of the tube is distended by food material in the lumen, making the tube almost completely fill the abdominal cavity. The wall is of similar structure throughout, but in consequence of its elasticity it adapts its configuration to the contained ingested material. This sometimes gives a superficial appearance of a differentiation into regions.

The junction of the midgut with the hind-gut is marked by an abrupt cessation of the simple musculature and the comparatively thick epithelium of the midgut and the beginning of the more complex musculature, the comparatively thin epithelium, and the intima of the pylorus. There is no special muscular development or structure that might be regarded as a valve.

MUSCULATURE

The musculature of the midgut (fig. 7) has essentially the same character throughout the length of the division. It is much simpler than that of the foregut or the hind-gut, and consists of an outer layer of fibers extending longitudinally and an inner layer of fibers extending circularly. In addition to the regular longitudinal muscle fibers there are six large longitudinal bands, one pair located dorsally, one pair ventrally, and a single band on each side.

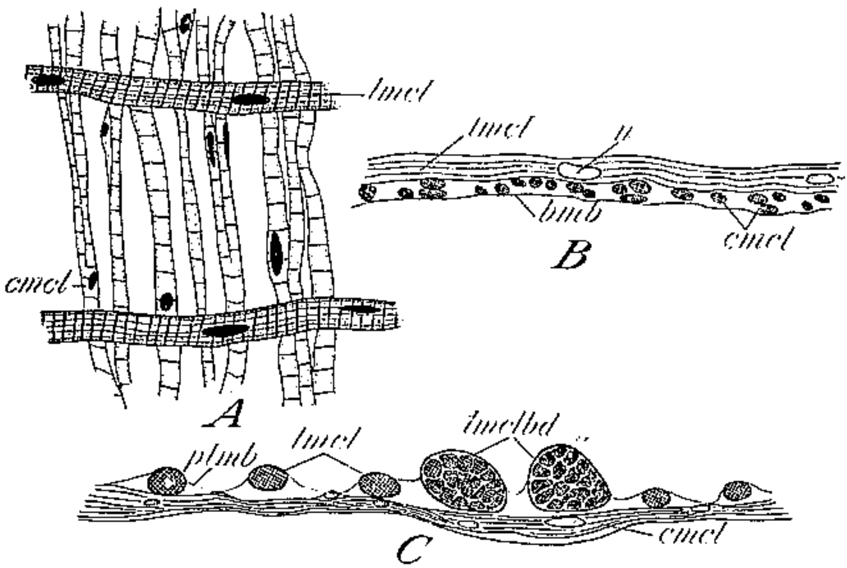

FIGURE 7.—Musculature of midgut of a late sixth-instar southern armyworm. *A*, Circular and longitudinal fibers drawn from whole mount, ×290. *B*, Longitudinal section from anterior third of midgut, ×400. *C*, Transverse section through wall of midgut, ×180.

The regular longitudinal fibers (fig. 7, *A*, *B*, *C*, *lmcl*) are widely spaced and occur at fairly regular intervals around the tube. In the late sixth instar there are usually 17 to 22 fibers per millimeter. As many as 228 per millimeter have been found in the late first instar. In fresh preparations they measure approximately 3 to 5μ in width in the first instar, and up to 15 to 25μ in the late sixth instar. The distance between the fibers varies according to the degree of relaxation or distention of the wall. The fibers occasionally lie adjacent to one another in preparations. In apparently normal distention they are usually separated by a space of about 6μ (3 to 9μ) in the first instar and 70μ (64 to 106μ) in the late sixth instar. The number of fibers around the tube is probably constant throughout development. With increasing circumference of the tube the fibers simply become spaced farther apart.

The muscle-fiber nuclei are distinct, elongate, centrally located, and regularly spaced.

Two large bundles of muscle fibers (fig. 7, *C*, *lmclbd*) extend the full length of the midgut on the dorsal and two others on the ventral side. In unfixed preparations of late sixth instars these may measure 60 to 90μ in width. Those in fixed and stained preparations have usually contracted somewhat. The two muscles in each pair lie close together. A single bundle of fibers occurs on each side of the alimentary canal. This muscle is much branched, especially near its terminations at the end of the midgut. The longitudinal layer of muscle fibers is covered by a very thin peritoneal membrane (*C*, *ptmb*) and lies over a layer of circular fibers.

The circular fibers (fig. 7, *A*, *B*, *C*, *cmcl*) are much smaller than the longitudinal fibers and are eight or more times as numerous. They lie close together or separated by narrow spaces.

Peritrophic Membrane

The peritrophic membrane occurs as a detached sheath closely surrounding the food mass in the midgut, and extending into the hind-gut. In histological sections the membrane is usually separated some distance from the distal ends of the epithelial cells. The space between the epithelium and the peritrophic membrane is clear, with scattered granular material. The peritrophic membrane may be removed from the midgut entire with the contained food material by pulling from the anterior end. It is apparently produced by the enteric epithelium as a whole (*11*, *26*, *30*) and serves to protect the delicate epithelium against the coarse material of the food.

Epithelium

The epithelium consists of a single layer of highly developed cells of three principal kinds—columnar or cylindrical cells, calyciform or goblet cells, and interstitial or regenerative cells. Histological evidence indicates that the interstitial cells become columnar and calcyiform cells by differentiation and development. The columnar and calcyiform cells are morphologically distinct. All these cells occur throughout the epithelial layer in all instars. Representative cells from the different instars as they appear in fixed and stained preparations are illustrated in figure 8.

The marked development of these cells is related to their function in the elaboration and discharge of the secretions required for digestion, and in the absorption of selected products of the digestion and transference of the products to the hemolymph. The precise manner in which secretion, absorption, and transference of materials takes place in the cell and the effects of these processes upon the cell are of much interest and importance; yet surprisingly little is known on the subject. Further knowledge of the activities of the cells under normal conditions should aid in an understanding of the absorption and disposition of ingested insecticides.

INTERSTITIAL CELLS

Interstitial cells of the insect midgut are commonly regarded as regenerative in function (*25*, *30*). The derivation of columnar and calyciform cells from interstitial cells in lepidopterous larvae has been reported by Henson (*16*) for *Vanessa urticae* and by Tchang (*27*) for

Galleria mellonella (L.). The following account and discussion is on original observations in the southern armyworm.

Interstitial cells occur singly and in groups throughout the midgut epithelium in all instars. Multiplication by mitosis was not definitely established. The cells vary in form and appearance in such a manner that, when the forms are arranged serially, development of the principal epithelial cells from small basal cells is strongly suggested. Further evidence on this point will be given below.

Nidi (fig. 8, *O*, *ni*) rest on the basement membrane between the bases of the principal cells. Each nidus is roughly triangular. Numerous epithelial nuclei occur in the basal half of the nidus. The chromatin material is coarsely granular. The cytoplasm is homogeneous and stains more intensely with the hematoxylin stain than does that of the older cells. The nuclei are surrounded by a very little cytoplasm. The greater quantity is found toward the distal part of the nidus. In many nidi there are faintly perceptible dividing lines extending upward toward the distal part. These probably indicate boundaries of forming cells.

Very small isolated cells are scattered throughout the epithelial layer, resting on the basement membrane between the bases of the principal cells. They occur singly, or several may occur together (fig. 8, *F*, *H*). These cells are rounded basally, slightly elongated, and narrowed distally. The nucleus occupies almost the entire basal part, and is surrounded by deeply staining cytoplasm.

The interstitial cells exist in all gradations in size up to fully mature cells. The small and intermediate cells are arranged singly or in groups according to their probable origin from isolated basal cells or from nidi. Those shorter than fully mature cells are also very slender (fig. 8, *G*, *I*, *J*, *inc*). The cell wall is usually bulged about the proportionately large nucleus, which is elongated in the direction of the longitudinal axis of the cell. The cytoplasm stains deeply with hematoxylin. Interstitial cells of the height of the principal cells (*R*) take the hematoxylin stain in the same intensity as do the mature cells.

The sequence of development and differentiation by which the principal cells of the epithelium (fig. 8) appear to be derived from the small basal cells is as follows: Embryonic rudiments on the basement membrane add new cells, which grow upwards between the main cells and on reaching the height of the main cells differentiate into columnar (*R*) and calyciform (*Q*) cells. During a period of further growth the newly differentiated cells reach the full size and appearance of the principal cells. Those destined to become columnar cells increase in diameter and develop a striated border (*R*). Those destined to become calyciform cells develop a small goblet in the distal part of the cell, which increases in depth (*M*, *Q*) until it occupies the entire cell (*I*, *N*, *P*, *V*) except a small basal part containing the nucleus. As the goblet increases in depth, the nucleus descends before it and becomes reorientated to lie with its longitudinal axis at right angles to that of the cell. While the goblet deepens the cell increases in diameter also.

This suggested role for the interstitial cell appears to be established by observations of successive periods during the first and second

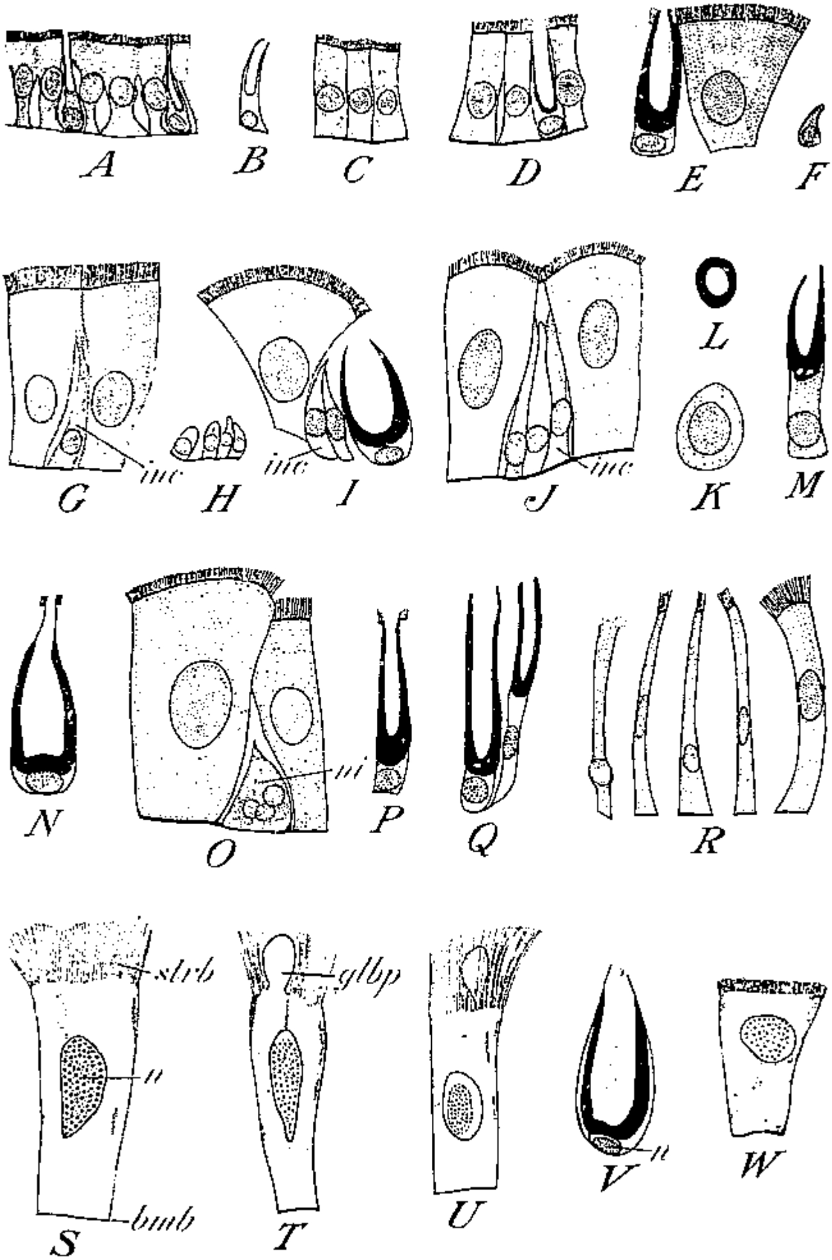

FIGURE 8.—Epithelial cells from the midgut of the southern armyworm. *A*, Primordial columnar and calyciform epithelial cells from first instar 10 minutes after hatching. *B*, Primordial calyciform epithelial cell from first instar 10 minutes after hatching. *C*, Primordial columnar epithelial cells from first instar 4 hours after hatching, showing clear spaces in cytoplasm. *D*, Primordial columnar and calyciform epithelial cells from first instar 12

Continued on next page.

stadia. Here the progressive stages of development can be easily followed.

No form of interstitial cell was found during the first 24 hours of larval life. At 39 hours there were a few very small cells and a few nidi in the anterior part of the midgut, and a few slightly more elongated ones in the posterior part. Just before the first molt the cells of the anterior part were much elongated, and those of the posterior part were one-third to two-thirds the height of the columnar cells, and numerous. At age 60 hours, 3 hours after the molt to the second instar, there were already numbers of interstitial cells as tall as the columnar cells. The cytoplasm had assumed the same staining qualities as the mature cells. These partially developed cells were narrow, and the nuclei were so large that the cells were bulged about them. At age 69 hours, 12 hours past the first molt, some of these tall, narrow cells contained small goblets. Among the developing interstitial cells no distinctive character was observed to separate those that were to become columnar from those that were to become calyciform cells. Not until the goblets appeared were the two distinguishable, and then for several hours development was toward increasing diameter of both the columnar and the calyciform cells, and deepening of the goblet in the calyciform cells.

Numbers of nidi and small interstitial cells, isolated or in groups, appeared just after the molt to the second instar. There were also partly developed cells which may have come over from the previous instar. Soon numerous nidi and all sizes of interstitial cells were present, and this condition continued throughout the rest of the

FIGURE 8—Continued.

hours after hatching. *E*, Primordial columnar and calyciform cells from first instar 50 hours after hatching. *F*, A basal interstitial cell from first instar 50 hours after hatching. *G*, A developing interstitial cell pushing upwards between two primordial columnar epithelial cells, from a first instar 56 hours after hatching and near the molt to second instar. *H*, A group of basal interstitial cells from a second instar 24 hours after the molt. *I*, Two developing interstitial cells between a columnar and a calyciform epithelial cell, from a second instar 48 hours after the molt. *J*, Three developing interstitial cells pushing upwards between two columnar epithelial cells, from a second instar 48 hours after the molt. *K*, Transverse section of a columnar epithelial cell through the nucleus, from a second instar immediately before the molt to third instar. *L*, Transverse section of a calyciform epithelial cell through the lower part of the goblet, from a second instar immediately before the molt to third instar. *M*, A calyciform epithelial cell in which the goblet extends over half way toward the base of the cell, from a second instar immediately before the molt to third instar. *N*, A fully developed calyciform epithelial cell from a third instar 5 hours after the molt. *O*, A nidus between two columnar epithelial cells from a third instar 56 hours after the molt. *P*, A calyciform epithelial cell in which the goblet reaches nearly to the bottom of the cell, from a fourth instar 3 hours after the molt. *Q*, Two calyciform epithelial cells from a fourth instar 24 hours after the molt. *R*, Five developing interstitial cells of the height of the principal epithelial cells, from a fifth instar 2 hours after the molt. *S*, A typical columnar epithelial cell from the anterior third of the midgut of a late sixth instar. *T*, A columnar epithelial cell from the anterior part of the midgut of a late sixth instar, bearing a globular protrusion. *U*, A typical columnar epithelial cell from the middle third of the midgut of a late sixth instar *V*, A typical calyciform epithelial cell from the middle third of the midgut of a late sixth instar. *W*, A typical columnar epithelial cell from the posterior third of the midgut of a late sixth instar. All ×540.

larval development. None were found, however, in the sixth instar 60 hours or more after the molt. New columnar and calyciform cells therefore appear to be added continuously throughout larval development, beginning early in the second stadium and ending some time in the last stadium.

COLUMNAR CELLS

Of the different kinds of cells, those designated as columnar cells are the most conspicuous by reason of their numbers and appearance. The term "columnar" or "cylindrical" generally applied to cells of this kind is scarcely descriptive. In shape and appearance they are very variable, depending on the instar, on the stage of development, on their location and physiological state, and on the degree of contraction or relaxation of the tube wall. When the gut wall is shrunken, the cells become laterally compressed, tall, and narrow. The cells frequently approach the truly cylindrical form (fig. 8, *C, K, S, U*), but are usually enlarged distally with more slender basal ends (*E, G, I, J, W*). They are tall and elongated in the direction of the diameter of the gut, never along the anteroposterior axis, as occurs in many kinds of insects. Henson (*16*) found that the latter condition sometimes exists in larvae of *Vanessa urticae*.

The columnar cells of the posterior part of the midgut in all instars are smaller and shorter than those of the anterior part. The decrease in size from the anterior to the posterior end is gradual. The three similar forms from the anterior, middle, and posterior thirds of the midgut of the sixth instar, shown in figure 8, *S, U*, and *W*, are of about average size and appearance for their respective regions.

The individual cells possess distinct lateral boundaries and exhibit marked polarity. They usually appear to cohere by their lateral surfaces. Their slender basal ends rest on the basement membrane, while the surfaces of the enlarged distal ends are exposed to the gut lumen.

The cytoplasm is finely granular. Clear spaces sometimes occur, especially near the distal end. Many of the cells are longitudinally striated in the basal part.

The nucleus occupies a central position, usually slightly toward the distal part of the cell. It is elongated in the direction of the longitudinal axis of the cell.

A striated border is present on the free surface of the cells in all instars. It is present as a low, brushlike border on the primordial columnar epithelial cells at hatching, and increases in height with the subsequent development of the cells. In all instars the border on the cells of the anterior part of the midgut is taller than that on the smaller cells of the posterior part. The striated border is a delicate structure of apparently cytoplasmic origin. In fixed and stained preparations it is marked with parallel channels or striae arranged in the longitudinal axis of the cell. Since it is probable that secreted and absorbed materials pass through this structure, its purpose may be to enlarge the functional cell surface. One function of these cells must be to select the kinds of materials to be absorbed. No morphological part of the cell seems better suited by position and structure to perform this act than the striated border. The structure and function of the border in other arthropods has been the subject of much discussion in the literature. The interested reader is referred to the recent papers by Newell and Baxter (*20*) and Zilch (*32*) for detailed discussions of the views expressed by different investigators.

The primordial columnar epithelial cells that are present at the time the larva leaves the egg have the general features of the full-size cells that they later become. They differ in many ways from the developing interstitial cells, which are found late in the first stadium and thereafter throughout the remainder of the growth period. They develop rapidly during the first stadium. Each cell approximately doubles its height and its diameter, thereby increasing its size several times. Immediately after the larva leaves the egg, the columnar epithelial cells of the anterior part of the midgut average in height approximately 22 mm., in basal diameter approximately 4.8 mm., and in diameter of apex approximately 5.4 mm. The nuclei are ovoidal in section and average about 8.3 mm. in length by 5.6 mm. in greatest diameter. The cells of the posterior part of the midgut are smaller.

In sections prepared from larvae 10 minutes out of the egg (fig. 8, *A*) the columnar cells are typically slender basally, expand slightly distally, and merge with adjacent cells in the distal part. A clear space of variable size is thus left between the basal parts of adjacent cells. This space extends upwards to the point where the adjacent cells merge, which may be about half way up the cell, as shown in figure 8, *A*, or much nearer the distal end. In some preparations no space appeared between the cells, and in such instances the cell boundaries were often indistinct or impossible to discern.

The cytoplasm is usually homogeneous. It is sometimes broken up by clear spaces, as seen in figure 8. *C*. The nucleus usually is so large that the cell cytoplasm is bulged about it.

Later development (fig. 8, *D*, *E*, *G*) is accompanied by rapid increase in the quantity of cytoplasm and subsequent enlargement of the cells. The cells may be almost perfectly cylindrical (*C*) or expanded distally (*E*). Greater variance in shape exists toward the end of the stadium (*G*). The cells found near the end of the stadium approach closely in size and appearance this kind of cell found in all subsequent instars. The spaces between the bases of the cells become smaller or obliterated entirely, permitting the lateral surfaces of adjacent cells to meet. To a limited extent, however, spaces between the cells do persist throughout the first stadium and are found even in the second and third stadia. In the late part of the first stadium and in all the other stadia the spaces are in part occupied by interstitial cells. The lateral attachment of the cells is apparently not firm, since the cells usually occur singly in tissue smears of epithelium. The cytoplasm of cells in the early part of the stadium more frequently contains large irregular clear spaces. Later the cytoplasm is most likely to be finely granular and homogeneous. The nucleus increases in size and at the end of the stadium is surrounded by considerable cytoplasm. The striated border increases in height during the stadium from 2.7μ in the beginning to 5.3μ near the end (measured in the anterior region of the gut).

At the beginning of the second stadium the columnar epithelial cells of the anterior part of the midgut average approximately 46 mm. in height, in basal diameter 10 mm.. and in diameter of apex 20 mm. The nuclei average about 16.9 mm. in length by 9.7 mm. in greatest diameter. It is evident that during the first stadium the size of the cells has increased more than 10 times. Full size is reached early in the second stadium. The new cells that develop from this point on do not exceed, on an average. the size attained by the first cells to mature.

Mature cells of the anterior part of the midgut of the late second instar, and of the third, fourth, fifth, and sixth instars, measure on an average approximately 50.0 mm. in height, 12.5 mm. in basal diameter, and 27.1 mm. in diameter of apex. The nuclei average about 20.5 mm. in length by 12.6 mm. in greatest diameter. There is a tendency for greater deviation from the mean average size with succeeding instars. The cells of sixth instars may vary between 35 and 70μ in height.

The cells of the later instars usually are closely appressed laterally and apparently cohere. However, it has been observed in histological preparations, even of the sixth instar, that cells in the posterior part of the midgut are often distinctly separated from neighboring cells by a clear space.

The development of the cells during the early part of larval life is regarded as a continuation of embryonic processes.

There is a question regarding the significance of the globular protrusions that are observed in histological preparations on the distal ends of many of the columnar epithelial cells of late sixth instars (fig. 8, *T*). Such formations were never found in earlier instars, or in the early part of the sixth stadium. A series can be arranged in which the globule is seen first in a period of apparent formation in the distal end of the cell, then pushing through the striated border, later as a bubble attached by a narrow stalk extending through the striated border to the cell, and finally completely detached and free in the area between the epithelium and the peritrophic membrane. The globule is often clear, but as often it is partially or completely filled with granules that stain like those of the cytoplasm of the cell. Such formations in other species of insects, including lepidopterous larvae, frequently have been described as probably secretion vesicles. Suggestions have been made that the formations may occur as a process of cell disintegration or as artifact. Among the many investigators who have studied and discussed this question are Boardas (*4, 5*), Buchmann (*6*), Gehuchten (*12*), Haseman (*15*), Henson (*16*), Newcomer (*19*), Pavlosky and Zarin (*21*), Shinoda (*22, 23*), Snodgrass (*25*), Tchang (*27*), and Wigglesworth (*30*). In the southern armyworm it appears unlikely that these formations are related to the secretory process, whereby digestive enzymes are produced throughout larval life. Feeding is continuous, and the kind of food ingested is unchanged throughout the larval period. It follows that secretion of digestive enzymes should likewise be continuous and probably unchanged in kind or manner. The fact that these formations are found only in late sixth instars suggests that the usual mode of secretion must be of a different nature. The writer prefers to interpret the globular protrusions as evidence of beginning disintegration of the cells preceding the metamorphosis.

CALYCIFORM CELLS

Calyciform cells occur throughout the midgut epithelium in all instars. The ratio of calyciform to columnar cells is generally about 1:3 or 1:2; occasionally it is 1:1. The calyciform cells have never been observed to exceed the columnar cells in number.

Calyciform cells are present in the epithelium at the time the larva hatches (fig. 8, *A*, *B*). They increase in size during the first and second stadia at about the same rate as the columnar cells.

The fully developed calyciform cells (fig. 8, *N*, *V*) are usually expanded in their middle part, slightly narrowed basally, and distally tapered to a narrow neck which communicates with the gut lumen. They are equal in height to the columnar cells, but are generally narrower even in the widest part. They are therefore smaller than the associated columnar cells. The basal part of the cell rests on the basement membrane and fills the spaces between the more slender basal parts of the columnar cells. The lateral boundaries are always distinct.

The large goblet or calyxlike part occupies most of the cell. The cavity appeared clear in the histological sections prepared for this work. The inner wall of the goblet is lined by a structure (fig. 8, *L*) that has the same affinity for erythrosin counterstain as the striated border of the columnar cells. Its position in relation to the rest of the cell suggests that it may be an invagination of the distal cell surface. Another basis for this conception is the probable sequence of its development from interstitial cells, as outlined in the discussion of these cells. It is therefore thought to be homologous to the striated border of columnar cells. The invagination of the cell surface would thus give a far greater surface exposure than is had by the columnar cells. While striae and other structural details characteristic of the striated border of columnar cells were not perceived, this might be due to greater compactness of the structural elements.

The cytoplasm and nucleus of the cell are crowded by the goblet into the extreme basal part. The cytoplasm is like that of the columnar cells. The coarsely granular nucleus occupies most of the basal part. It is ovoidal and is orientated with its longitudinal axis at right angles to the longitudinal axis of the cell.

INTERRELATIONSHIP OF THE THREE KINDS OF CELLS

The presence of three distinct kinds of cells in the midgut epithelium brings up the question of their morphological relation to one another. As to the interstitial cells, their position as young columnar and calyciform cells appears well established by the morphological evidence. The great numbers in which they are produced suggest that their primary role is to increase the number of regular epithelial cells progressively during larval development. Their regenerative function in certain lepidopterous larvae is discussed by Henson (*16*) and by Tchang (*27*).

The morphological relationship of the columnar and the calyciform cells of lepidopterous larvae has received much attention. Some writers, notably Shinoda (*22*, *23*) and Buchmann (*6*), regard the two forms as homomorphous, whereas Henson (*16*) and Tchang (*26*, *27*) believe them to be dimorphous. Shinoda regards the different forms as functional variations of a single cell type. He associates the functions of secretion and absorption with the columnar phase, and states of senescence and rejuvenescence with the calyciform phase. The transition from one phase to the other is shown by the presence of cell forms that are intermediate in structure and appearance. Tchang and Henson regard the columnar and calyciform cells as distinctly different kinds of functional epithelial cells and find that both are derived directly from the interstitial cells.

The following observations indicate that in southern armyworm larvae the forms are dimorphous: (1) No intermediate forms were found in the first stadium or early in the second stadium, even though both columnar and calyciform cells were present at the time the larva left the egg and both these forms underwent development with increase in size during this period. The presence of calyciform cells at hatching would not in itself invalidate their status as senescent cells, for digestion of yolk and egg chorion might already have begun. But the complete lack of any cells remotely suggesting transition forms seems to rule out changes from the one form to the other during this period. (2) The forms present in the second and later instars that might be taken for transition forms are more likely calyciform cells newly differentiated from interstitial cells. (3) The evidence indicates that both forms in the later instars are derived from interstitial cells.

An assumption that the calyciform and columnar forms are different kinds of cells leads to the question of their respective functions. The single kind of functional epithelial cell present in insects lacking calyciform cells resembles the columnar cell of lepidopterous larvae in cytoplasmic characteristics although it may be different in shape. Since both functions are necessary, this cell must be both secretory and absorptive. From morphological evidence there appears to be no reason why the columnar cells of lepidopterous larvae might not also perform both functions. The goblet cell is not known to be the only, or even the main, type of cell in any insect. It appears less adapted to the function of absorption because of the position of the cell surface within the goblet, where it is not directly exposed to the fluids of the gut lumen. The cell may be secretory. No evidence is available to reveal the nature of the contents, if any, of the goblet. The cavity would appear to be a satisfactory reservoir for secretions. They might readily reach the gut lumen continuously or as needed through the narrow neck of the goblet.

GROWTH OF THE MIDGUT EPITHELIUM IN RELATION TO CELL SIZE AND CELL NUMBERS

The growth of the midgut epithelium between hatching and maturity of the insect is due to the increase in the size of the epithelial cells during the first stadium and the early part of the second stadium, and to the increase in numbers of cells throughout the remaining larval stadia.

The primordial epithelial cells, both columnar and calyciform, have been shown to increase to several times their original size during the first stadium and reach full size in the early part of the second stadium. No increase in numbers takes place during this time, although by the beginning of the second stadium many of the interstitial cells have attained the height of mature cells. The increase in size of the midgut epithelium during this period must therefore be due almost entirely to the increase in the size of the constituent primordial cells.

The continued growth from the second instar to the mature larva must be attributed entirely to the increase in numbers of cells derived from the large numbers of regenerative cells, since the newly formed cells do not exceed the primordial cells in size. There are approximately 55 cells in a transverse section of the midgut of an early second instar and 700 to 725 in the sixth instar. The increase in numbers is in approximately the same ratio as the increase in the circumference of the midgut. No evidence of division of the functional epithelial cells was found. Their development in the larva is evidently a continuation of embryonic processes.

Henson (*16*) found that the midgut epithelium of the larva of *Vanessa urticae* (Lepidoptera) likewise grows both by the increase in size of the original cells and by the addition of new cells. Trager (*28*) found that the midgut cells of *Bombyx mori* (Lepidoptera) increase in length during the first and second stadia in proportion to the length of the body, but during the third and fourth stadia they remain of constant size. No measurements were made to determine the condition in the fifth (last) stadium. The situation is different for some other insects. Trager (*28*, *29*) has shown that the increase in size of the midgut epithelium of *Lucilia sericata* and *Aedes aegypti* (Diptera) throughout larval life is a result of a proportionate increase in size, not in number, of the midgut epithelial cells. Berger (*3*) found that in *Culex pipiens* (Diptera) the functional midgut epithelial cells increase in size throughout larval life but do not divide. Regenerative cells replace those shed into the lumen and also increase the number of functional cells. Abercrombie (*2*) found, in the Japanese beetle (*Popillia japonica* Newm.), that increase in cell volume of the midgut columnar cells is proportional to the increase in size (weight) of the entire larva.

HIND-GUT (PROCTODAEUM)

The hind-gut, or proctodaeum, increases greatly in size during larval development. The total length increases from approximately 0.3 mm. in newly hatched larvae to 8 mm. in late sixth instars. The general structural characteristics are constant. Except where specifically stated otherwise, the description that follows is of the late sixth instar.

The hind-gut (fig. 9, *A*) extends directly from the posterior opening of the midgut to the anus. The pylorus, the anterior intestine, and the posterior intestine are distinctly differentiated, and their points of separation are marked by strong sphincter muscles. The appearance of the parts varies greatly according to the state of contraction and the quantity and distribution of material in the lumen. In the condition of moderate distention the three regions are approximately equal in length. When strongly contracted longitudinally, the pyloric region may be very short. When fully distended, both the anterior and the posterior intestine are globular and separated by a definite stricture. The structure between the pylorus and the anterior intestine is known as the pyloric valve, and that between the anterior intestine and the posterior intestine as the rectal valve. It should be pointed out that in lepidopterous larvae the proctodaeal or pyloric valve is formed, not between the midgut and the hind-gut, but between the pylorus and the anterior intestine.

The epithelial cells gradually increase in size during larval development. They apparently do not increase in numbers either through

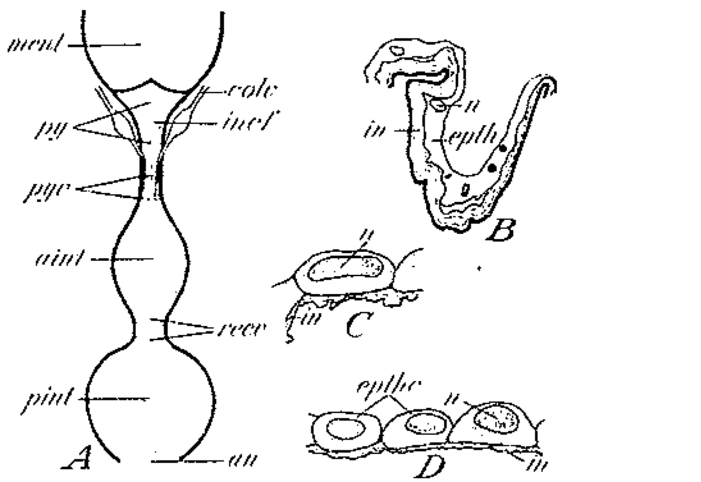

FIGURE 9.—*A*, Ventral view of hind-gut (proctodaeum) of late sixth-instar southern armyworm, showing entrance of collecting vessels of the Malpighian tubules, ×4. *B*, Longitudinal section through wall of pylorus of late sixth instar, ×200. *C* and *D*, Longitudinal section through wall of anterior (*C*) and posterior (*D*) intestine of third instar 30 hours after the molt, showing an epithelial cell and the intima, ×400.

division or through development from regenerative cells. Henson (*17*) found that in *Vanessa urticae* the hind-gut grows by increase in size of the cells and not by increase in cell numbers. Trager (*28*) found that the hind-gut cells of *Lucilia sericata* continued to increase in size throughout larval life in the same proportion as the body length of the larva.

PYLORUS

The pylorus (fig. 9, *A*, *py*) is a well-developed region of the hind-gut and forms a connecting link between the midgut and the intestine. The anterior part is enlarged and calyxlike, and the posterior end is much constricted.

To the unaided eye the enlarged anterior part may appear as a part of the midgut. It may readily be distinguished, however, by its transparent wall and by an abrupt change in the musculature.

An internal circular fold of the wall of the pylorus (fig. 9, *A*, *incf*) occurs at approximately the middle of the enlarged part. Invaginations of the wall in the fold project anteriorly and posteriorly. The anteriorly directed invaginated structures in late sixth instars are about 40 in number and appear as oval or elongate padlike thickenings approximately 100μ long. Each bears numerous teeth about 7μ long. The epithelial cells are large with distinct nuclei, and the intima is much thickened on the distal part.

The much constricted extreme posterior part of the pylorus is encircled by broad (about 80μ) circular muscle bands, which lie close together and form a strong sphincter valve, the pyloric valve (fig. 9, *A*, *pyv*). The function of this valve is probably to regulate the passage of material into the intestine.

The excretory diverticula (fig. 1, *A*, *malt*) consist of two groups of three Malpighian tubules. A short collecting vessel (fig. 9, *A*, *colv*) for each group of tubules opens ventrolaterally into the lumen of the posterior part of the pylorus after having entered from beneath the pyloric sphincter. Each collecting vessel receives a Malpighian tubule and another short vessel, which in turn receives two more Malpighian tubules. Each tubule is long, slender, and sinuous. The walls are thin, and ovoidal dilations are frequent. All the tubules extend forward, adhering closely to the wall of the midgut to about the region of the third or second abdominal segment, turn abruptly, and return along the wall to the hind-gut. After many convolutions in the region of the pylorus, each tubule finally enters the posterior intestine near its anterior end. The lumen of the tubules contains numerous white crystals, and on this account the tubules are conspicuously visible through the ventral body wall of the larva.

The musculature of the pylorus consists of an underlying layer of circular muscle fibers and an overlying layer of widely spaced longitudinal muscle fibers. The region of the pylorus anterior to the internal circular fold is surrounded by smaller circular fibers about 26μ broad in late sixth instars, which lie close together or separated by a space equaling or less than the width of the fiber. They are interconnected by strands of muscle tissue. The circular fibers lying between the invaginated structures and the sphincter muscles are broader (about 43μ). Widely spaced and branching muscle fibers extend longitudinally over the circular muscle fibers. They are inserted beneath the sphincter muscle of the pyloric valve.

The epithelium of the pylorus (fig. 9, *B*) is flat and averages about 5.8μ in thickness, with considerable variation. The cell boundaries are indistinct or absent. The nuclei are large and distinct. The cells of the extreme anterior end of the pylorus are much larger (up to 28μ in length) and have distinct boundaries. They may function (*17*) to regenerate the epithelium of the hind-gut during metamorphosis. The structure of the intima appears to be similar to that of the foregut. It averages about the same in thickness as the pyloric epithelium (5.8μ) and also varies considerably.

Anterior Intestine

The anterior intestine (fig. 9, *A*, *aint*) is an undifferentiated saclike chamber. The wall is thrown into numerous internal longitudinal folds. The musculature consists of branching and uniting circular fibers arising as branches of widely separated (250 to 450μ)

and irregular longitudinal fibers. The circular fibers in late sixth instars are about 37μ broad and lie about 26μ apart. In early second instars they are about 2.5 to 4μ broad and lie 5.3 to 7.4μ apart. They are interconnected by strands of muscle tissue. The epithelial cells (fig. 9, *C*) are comparatively large (15 to 30μ in diameter) in mature larvae and possess distinct lateral walls. The nuclei are distinct. The intima is thin.

The rectal valve (fig. 9, *A*, *recv*) appears as a stricture between the anterior intestine and the posterior intestine or rectum. Internal longitudinal folds of the anterior intestine are prolonged into the region of the rectal valve. The region is surrounded by broad muscle bands (about 65 to 80μ broad), which lie adjacent to one another or are separated by narrow spaces (up to 21μ).

POSTERIOR INTESTINE

The posterior intestine, or rectum, (fig. 9, *A*. *pint*) is a second undifferentiated saclike chamber. Two thin membranes lie between the basement membrane of the epithelium and the muscle layer. The Malpighian vessels are disposed in convolutions in the space between the epithelium and the membranes, and between the two membranes. The circular fibers are small (about 10μ broad) and lie close together, forming a practically solid layer over the rectum. A large sphincter muscle surrounds the anus. Dilator muscles that have their attachment in the body wall of the posterior segment extend over the rectum and are inserted on the wall of the rectal valve and the anterior intestine. The epithelium and intima of the posterior intestine are similar to those of the anterior intestine (fig. 9, *D*).

Structures corresponding to those described as rectal glands in various insects were not found. Rectal glands are reported to be present in adult Lepidoptera, but absent in their larvae (*1*, *p. 243*).

SUMMARY

The structure, microscopic anatomy, and postembryonic development of the alimentary canal of the larva of the southern armyworm (*Prodenia eridania* (Cram.)) are described and figured in preparation for physiological and toxicological investigations on this insect.

The alimentary canal is similar to the alimentary canals of other lepidopterous larvae that have been described. The general information gained from the study is already well known in the field of insect anatomy. Specific information on the conditions found in this particular species was required for practical reasons. No previous work has been reported. The structure and development of each part of the canal is described.

The foregut and hind-gut increase in size by increase in size of the constituent cells, and the midgut increases partly in this manner but primarily by increase in numbers of cells. The columnar and calyciform cells are already differentiated at hatching. They reach full size during the second stadium. Interstitial cells arise during the first stadium, develop during the first stadium and the early part of the second stadium, differentiate into calyciform and columnar cells, and complete development. These cells are of the same size and

appearance as those that develop from the primordial cells. New epithelial cells are continuously produced throughout larval life by development and differentiation from interstitial cells.

ABBREVIATIONS USED ON ILLUSTRATIONS

aint, anterior intestine.
an, anus.
ant, antenna.
aph, anterior pharynx.
bmb, basement membrane.
br, brain.
brdm, branch of dilator muscle.
clp, clypeus.
cmcl, circular muscle fiber.
colv, collecting vessel of Malpighian tubules.
cr, crop.
dmcl, dilator muscle.
epis, epistomal suture.
epth, epithelium.
epthc, epithelial cells.
glbp, globular protrusion.
in, intima.
inc, interstitial cell.
incf, internal circular fold.
labgl, labial gland.
labhphy, labial hypopharynx.
lm, labrum.
lmcl, longtitudinal muscle fibers.
lmclbd, longtitudinal muscle band.
malt, Malpighian tubules.
mcl, muscle.
md, mandibles.
ment, mesenteron (midgut).
mth, mouth.
mx, maxilla.
n, nucleus.
ni, nidus.
oe, oesophagus.
p, parietal.
pint, posterior intestine.
pinvst, postpharyngeal invaginated structures.
pph, posterior pharynx.
prc, preoral cavity.
proc, proctodaeum (hind-gut).
prov, proventriculus.
ptmb, peritoneal membrane.
py, pylorus.
pyv, pyloric valve.
recv, rectal valve.
scl, sarcolemma.
soegng, suboesophageal ganglion.
sp, spinneret.
stom, stomodeaum (foregut).
stomv, stomodaeal valve.
strb, striated border.
t, teeth.

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