FUNCTIONAL DIFFERENTIATION OF THE HINDGUT EPITHELIUM OF THE BLOWFLY LARVA INTO LONGITUDINAL BANDS

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Summary

The hindgut of blowfly larvae commences a short distance posterior to the entry of the malpighian tubules into the alimentary canal. It is divided into short anterior and posterior regions, which function principally as sphincters, and a long central region. The central hindgut is composed of three histologically distinguishable cell types, each forming a longitudinal band. The bands produced by cell types *A* and *B* each form about one-half of the circumference of the hindgut, whereas the cells of type *C* form two narrow longitudinal strips, each one cell wide, situated at the junctions of the bands of *A* and *B* cells.

The *A* cells are rich in potassium, acid phosphatase, dehydrogenases, and acetyl esterase. On the other hand, the *B* cells react strongly for ammonia and stain diffusely for barium in larvae fed on a barium-enriched diet. The *C* cells react very strongly to the test for ammonia and accumulate barium-rich granules.

The hindgut epithelium rapidly takes up ammonia directly from the haemolymph and the *B* and *C* cells are particularly active in this process. Much of the ammonia appears to be eliminated as bicarbonate.

Evidence is steadily accumulating that the hindgut epithelium is an active tissue which plays an important role both in metabolism and in regulating the products of excretion.

I. INTRODUCTION

The hindgut of insects is no longer regarded merely as a passage for the discharge of food residues from the midgut to the exterior. Except for sphincters at the anterior and posterior ends it appears, in a few species, to be of uniform structure throughout. In the majority of species, however, there is some degree of morphological differentiation, which most commonly takes the form of differences in diameter of various regions. Specialized rectal pads are also present in many insects. These play an important part in the process of absorbing water from the food residues before discharge and may also be concerned in recovering other valuable constituents (Wigglesworth 1953). Ramsay (1950) has shown in *Aedes detritus* that the anterior part of the rectum has a different epithelium from the remainder (a distinction not found in *A. aegypti*) and that this is correlated with the ability of the former but not the latter species to produce a hypertonic fluid in the rectum. A few papers also describe restricted distributions in the insect hindgut for alkaline phosphatase, iron, strontium, and barium (Waterhouse and Day 1953).

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Following the demonstration of a striking functional differentiation of various groups of cells in the midgut of blowfly larvae (Waterhouse and Stay 1955), similar techniques were applied to the larval hindgut about which almost nothing was known. It is shown here that, although the hindgut is not as involved as the midgut in absorption and intermediary metabolism, nevertheless, it is a very active organ. Furthermore, for the greater part of its length it is highly differentiated both histologically and functionally into longitudinal bands, each formed from one of three distinct cell types.

II. Methods

Almost mature third instar larvae of the Australian sheep blowfly *Lucilia cuprina* (Wied.) reared at 30°C on a standard artificial medium (Waterhouse and Stay 1955) or on liver were used in all experiments.

Histological observations were made on material fixed in alcoholic Bouin's fluid and stained with either Mallory's triple connective-tissue stain, Delafield's haematoxylin and eosin, or Bodian's protargol silver method. Regaud's method was used for staining mitochondria.

Histochemical tests for the following were performed in a manner already described (Waterhouse 1951; Waterhouse and Stay 1955): lipoid, glycogen, iron, copper, barium, and strontium, potassium, ascorbic acid, dehydrogenases, and cytochrome oxidase. Tests were also performed for zinc (dithizone—Mager, McNarry, and Lionetti 1953), uric acid (methenamine-silver nitrate—Gomori 1952), amine oxidase (Blaschko and Hellmann 1953), phenol sulphatase (Rutenburg, Cohen, and Seligman 1952), and lipase (the "Tween" 20, 60, and 80 substrates of Gomori (1952) after fixation in cold formalin). In addition, the following tests were carried out:

(*a*) *Ammonia*

Larvae were dissected rapidly under saline and then immersed either in Nessler's or Riegler's reagents (Lennox 1941*a*; Waterhouse 1950). The characteristic orange-brown reaction product of Nessler's reagent with ammonia is sufficiently stable when tissues are passed rapidly through the higher alcohols and xylene for its distribution to be examined in cleared whole mounts or after paraffin sectioning. Fading occurred fairly rapidly in xylene, tetrachloroethylene, and balsam prepared with these solvents, but there was sufficient time to obtain a photographic record if desired.

Other tissues were transferred after several minutes in Riegler's reagent to saturated aqueous calcium hydroxide, whereupon regions containing ammonia turned red. Riegler's reagent, as in earlier tests, appeared to be considerably less specific than Nessler's reagent.

(*b*) *Carbonate or Bicarbonate*

The evolution of carbon dioxide on addition of 5N sulphuric acid was detected by absorbing it, in the apparatus described by Feigl (1947), in a dilute (about 0·005N) sodium carbonate solution coloured red with phenol-

phthalein. The formation of bicarbonate was indicated by decolorization of the indicator. After the macerated tissue and acid had been introduced into the apparatus they were mixed by means of a magnet and stirring bead. Gas evolution was assisted by gentle warming in a light beam.

(*c*) *Phosphatases*

In addition to the Gomori techniques (Gomori 1952) using sodium glycerophosphate, the α-naphthyl phosphate-azo dye methods of Gomori (1952) for alkaline phosphatase and of Burton (1954) for acid phosphatase were employed. Initial fixation for 2-6 hr in ice-cold, buffered, 10 per cent. formaline preserved the tissues better than alcohol or acetone, without otherwise influencing the results. However, even with formalin fixation, considerable disintegration occurred in the ammonium sulphide step of the glycerophosphate method. Brief (5-10 min) fixation in alcoholic Bouin immediately before this step prevented disintegration, apparently without producing any artefacts.

(*d*) *Acetyl Esterase*

The α- and β-naphthyl acetate techniques for aliesterase activity, using either Diazo Fast Blue RR or Diazo Blue B were employed (Gomori 1952; Ravin, Zacks, and Seligman 1953). The substrate-dye mixtures were chilled with ice during incubation and replaced every 15-20 min for 1-2 hr. Tissues were fixed in neutral buffered formalin and either mounted in glycerogel or, alternatively frozen sections were prepared from tisses embedded in gelatin.

III. Results

(*a*) *Morphology and Histology of the Hindgut*

In the *L. cuprina* larva the hindgut and the midgut (relative lengths 2 : 3) are together some five to six times the length of the body, a figure which is unusually high compared with that for most other insects. This may be related to the high efficiency demanded of the blowfly alimentary canal during the very short larval growth period.

Although it is a convenient, broad generalization in insects to regard the hindgut as commencing at the point of discharge of the malpighian tubules into the digestive tract, this is not always so. In *L. cuprina* larvae, sections show that midgut cells extend for a short distance posterior to this point (Fig. 1, *X*). The hindgut first narrows for a short sphincter region and then expands slightly to maintain a more or less constant diameter (varying slightly with distension by food) until it terminates with a narrower sphincter region at the anus. It first pursues a winding course forwards from near the posterior end of the larva to the vicinity of the proventriculus and then backwards to the anus, receiving its tracheation from the same main trunks which supply the adjoining loops of the midgut. Numerous intracellular tracheae and tracheoles, which run both circularly and longitudinally through the epithelium, are a constant feature of the hindgut and are very clearly seen in Bodian preparations (Plate

1, Fig. 2). The peritrophic membrane extends continuously throughout the hindgut and is discharged undamaged at the anus, extruded lengths being detached mechanically from time to time (Waterhouse 1954). The pH of the hindgut contents is uniform throughout and lies in the range 7·8-8·0 (Waterhouse 1940).

The characteristic histology of the three regions of the hindgut epithelium is indicated in the inserts in Figure 1.

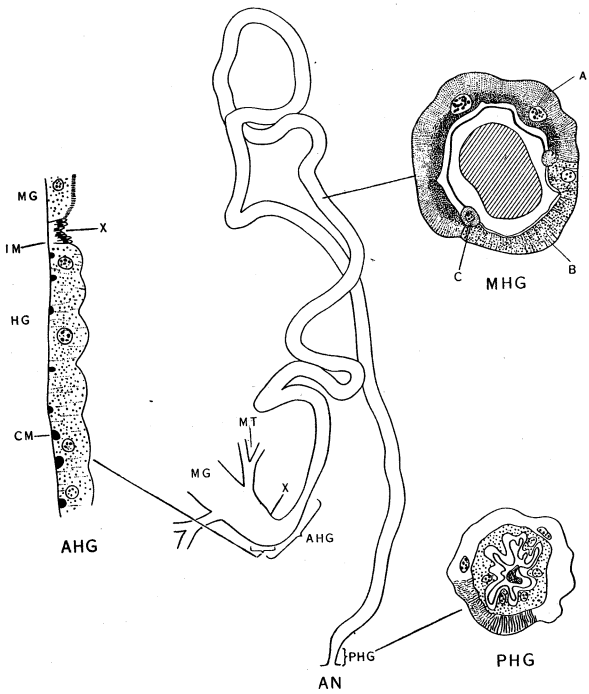


Fig. 1.—Diagram of the hindgut of a *L. cuprina* larva with inserts indicating the typical histology of the various regions. *AHG*, anterior hindgut (longitudinal section); *MHG*, central hindgut; *PHG*, posterior hindgut; *A*, *B*, *C*, cell types in central hindgut; *CM*, circular muscles; *HG*, hindgut; *IM*, imaginal cells; *MG*, midgut; *MT*, malpighian tubules; *X*, junction of mid and hindgut.

(i) *Anterior Hindgut* (*AHG*).—The hindgut commences where the alimentary canal diminishes sharply in diameter (Fig. 1, *X*); it is separated from the midgut by a ring of small imaginal cells which later form portion of the adult hindgut. The first true hindgut cells contain many intracellular tracheoles (Plate 1, Fig. 2) in contrast with the midgut cells where these are comparatively rare. The hindgut cells in this region all have a similar appearance, the nucleus is basal or median, the cytoplasm is uniform, and there is rather narrow cuticular lining. With Mallory's stain the basal cytoplasm is somewhat fuchsinophilic, the apical half of the cell staining predominantly with aniline blue. The

first short zone of the hindgut is richly supplied with muscles, particularly with circular muscles. These form both a sphincter and a means for marked peristaltic activity when food residue is passed on from the midgut. The circular muscles are invaginated into the base of the epithelial cells at the rate of two or three groups of muscles per cell (Fig. 1, *CM*; Plate 1, Fig. 2). This arrangement gives the impression, in 10 μ transverse sections, that the muscles are intracellular.

(ii) *Central Hindgut.*—The histology of the hindgut is apparently uniform from a short distance posterior to the commencement of the hindgut proper to the anal sphincter. Throughout this region the epithelium is differentiated into three cell types (Table 1; Fig. 1, *MHG*). These types can also be distinguished in the freshly-hatched larva.

Cell type *A*.—These cells usually form rather more than half of the circumference of the hindgut, although they may constitute as little as one-third (Plate 1, Fig. 1). They have an apical nucleus, are typically higher, and possess a wider cuticular lining than the two other cell types present. The apical cytoplasm contains both basophilic and fuchsinophilic material and is granular, whereas the basal cytoplasm is vertically striated and stains relatively lightly, except with Bodian. Numerous rod-like and filamentous mitochondria are present in the basal cytoplasm, whereas they are scarce in the apical portion of the cell.

Cell type *B*.—These cells generally occupy less than half (sometimes up to two-thirds) of the hindgut circumference (Plate 1, Fig. 1). They have a basal or median nucleus and a relatively narrow cuticular lining. The cytoplasm is finely granular and is not differentiated into apical and basal zones by the staining methods employed. Mitochondria are fairly abundant and are distributed evenly throughout the cytoplasm.

Cell type *C*.—These cells, which are much smaller than the other types, are situated at the junction of the bands of *A* and *B* cells (Plate 1, Fig. 3), and form two narrow longitudinal strips each one cell wide. Each *C* cell is longitudinally elongated, tapers at both ends, and has a length equivalent to that of several *A* or *B* cells (Plate 1, Fig. 6). Type *C* cells do not always touch each other, so that the two strips of *C* cells are not represented on either side of all sections. Occasional mitochondria are distributed throughout the cytoplasm.

(iii) *Posterior Hindgut.*—Shortly before its termination at the anus the epithelium becomes uniform again (Fig. 1, *PHG*). The cuticular lining becomes relatively thick and the circular musculature is well developed to form an anal sphincter.

No spines have been observed in the cuticular lining of any region of the larval hindgut. These are a feature of the adult hindgut in many higher Diptera.

(*b*) *Histochemical Tests*

(i) *Lipoid.*—No osmium tetroxide-reducing materials were detected in the hindgut epithelium, irrespective of the larval diet. After formalin fixation and Sudan black B treatment the hindgut cells were lightly and uniformly stained,

this being due to a general ground coloration of the cytoplasm. When such preparations were slit longitudinally and mounted flat, the *C* cells appeared to be slightly more heavily stained than the *A* or *B* cells. With Nile blue, a rather generalized, non-specific staining resulted. Thus, there was no indication that deposits of lipoid were accumulated by the hindgut epithelium.

TABLE 1

COMPARISON OF THE THREE CELL TYPES FORMING MOST OF THE HINDGUT OF *L. CUPRINA*

Cell Part and Staining Reactions	Cell Type		
	A	*B*	*C*
Proportion of circumference	⅔ to ½	⅓ to ½	Nil to very small
Height	Relatively high	Generally lower than *A*	Generally ⅓ to ¼ of *A*
Nucleus	Apical	Basal or median	Apical, smaller than *A* or *B*
Cuticular lining	Wide	Narrow	Very narrow
Cytoplasm:			
Apical	Finely granular	Weak vertical striations	Finely granular
Basal	Vertical strong striations	Finely granular	Finely granular
Mitochondria	Mainly basal	Evenly distributed	Sparse, even
Mallory:			
Apical	Fuchsinophilic	Uniformly stained with all components	Weakly fuchsinophilic
Basal	Aniline blue predominates		
Bodian:			
Apical	Lightly stained	Uniform light, with few scattered black granules	Uniform light
Basal	Sometimes more heavily stained; median zone of black granules at times		
Haematoxylin-eosin:			
Apical	Basophilic; cell processes extend into cuticular lining	Staining intermediate between the two regions of *A*	As for *B* or slightly more basophilic
Basal	Light staining		

(ii) *Glycogen.*—After freeze-drying, the cytoplasm of all cell types stained a little more deeply with the periodic acid—Schiff (PAS) test before than after digestion by saliva, although no difference was observed after ordinary fixation. PAS-positive granules were seldom seen and it is evident that little or no glycogen is stored in the hindgut epithelium. The circular muscles forming

the sphincter at the anterior end of the hindgut were found to contain some glycogen.

Staining, which proved resistant to digestion by saliva, occurred in the cuticular lining and the basement membrane of the hindgut epithelium and also in the peritrophic membrane. This staining was virtually abolished by acetylation (McManus and Cason 1950), indicating that these structures contain carbohydrate other than glycogen.

(iii) *Potassium.*—When fresh hindguts were tested for potassium one side of the middle region stained much more heavily than the other. The anterior and posterior regions only stained lightly. The deeply staining side of the middle region consisted of *A* cells. The basal, vertically-striated cytoplasm of these cells stained heavily although the apical cytoplasm also gave a positive reaction (Plate 1, Fig. 5). Numerous large granules, some black or brown, some yellow, occurred in the *A* cells, being concentrated in the median zone of the cell. The *B* cells stained very lightly and contained relatively few, scattered granules similar to those in the *A* cells. These granules tended to be concentrated at the base of the *B* cells. The *C* cells reacted very weakly, staining about as intensely as the apical cytoplasm of the *A* cells or failing to stain.

The addition of 2·5 mg KCl per g medium slightly intensified the staining reaction without altering its character or distribution.

(iv) *Barium and Strontium.*—Neither barium nor strontium could be detected in the hindgut epithelium of larvae fed on the standard medium.

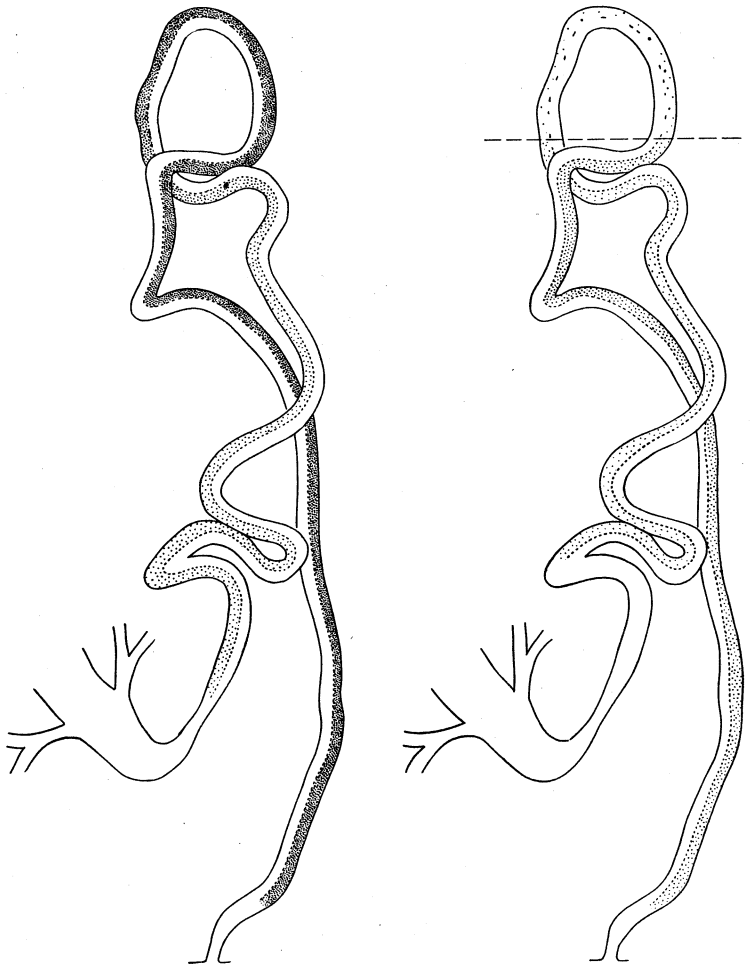
However, after the addition of 0·25 mg $BaCl_2$ per g medium, but not after the addition of a similar amount of $SrCl_2$, one side of hindgut epithelium often stained pink with rhodizonate. Furthermore, portions here and there of the bands of *C* cells stained very heavily (Plate 1, Fig. 4).

Sections demonstrated that the cytoplasm of the *B* cells was staining uniformly and lightly for barium, but that the *A* cells were not reacting. The *C* cells at times contained many small, dark red granules (Plate 1, Fig. 6) and sometimes also clusters of large, brownish red, rod-shaped crystals concentrated near the gut lumen. At other times the *C* cells stained lightly or failed to react.

A characteristic feature of larvae fed on barium-enriched food was the presence of large, light brown crystals attached to the hindgut epithelium and protruding into the gut lumen. These crystals, which were often but not always associated with the *C* cells, generally failed to react with rhodizonate.

(v) *Ammonia.*—Nessler's reagent rapidly produced a conspicuous red-brown coloration for ammonia along most of the central hindgut of feeding larvae, whereas the anterior and posterior hindguts were unstained (Fig. 2(*a*)). In the central hindgut a striking reaction often occurred along one side only, whereas the other side was unstained or comparatively lightly stained. The intensity of staining increased progressively along the first half of the central hindgut to remain at a fairly uniform high level throughout the second half.

When the staining was weaker than usual it appeared to be largely restricted to a narrow, not necessarily continuous, band on either side of the central hindgut. On the other hand, when the staining was particularly intense, the reddish brown precipitate occurred not only throughout the entire epithe-



(a) (b)

Fig. 2.—The distribution of ammonia (intensity indicated by stippling) in the hindgut of *L. cuprina*. (*a*) Actively feeding larva; (*b*) ligatured larva, injected posterior to dotted line (see text).

lium, but also on the outside of the gut. This suggests that there is a tendency for ammonia to diffuse out of the gut during manipulation. Lennox (1941*a*) recorded changes in intensity of staining along the hindgut but not any tendency for the main staining to occur along one side.

Riegler's reagent at first produced a deeper stain along one side of the central hindgut than along the other, but this distribution was soon lost with the development of a more generalized, apparently non-specific staining throughout the larva.

In typical sections of Nessler-stained hindgut there was an intensely coloured band formed on either side of the gut by the *C* cells, together with the immediately adjoining portions of the bands of *A* and *B* cells (Plate 2, Fig. 1). The cytoplasm of this region was yellowish brown and contained many small, dark brown granules. Elsewhere the cytoplasm of the *A* cells was colourless, although large brown granules sometimes occurred in the cuticular lining. By contrast, in the *B* cells a positive reaction almost always occurred which involved all apical and basal structures. The *C* cells generally stained heavily to very heavily, although at times they gave a very weak or negative reaction. Nuclei of all cells usually failed to react.

In sections of very heavily stained hindguts the entire cytoplasm of the *B* cells was brown and contained numerous dark brown granules (Plate 2, Fig. 2). The *A* cells also stained similarly, but usually less intensely.

These results raised the question of the route of ammonia excretion from the body, i.e. whether the hindgut ammonia came either via the malpighian tubules, as claimed by Lennox (1941*a*), or by direct uptake by the hindgut from the haemolymph as suggested by Hurst (1941). When larvae were tested at varying intervals after transfer from their food to individual containers with 1·5 per cent. agar, the ammonia reaction diminished progressively as their guts emptied of food. Under these conditions the areas in the vicinity of the *C* cells retained their strong positive reaction for longer than elsewhere. After several hours the hindgut generally reacted only weakly.

When larvae in the latter condition were ligatured so that a short loop of the hindgut was excluded from the posterior portion (Fig. 2(*b*)) and either blood from actively feeding larvae or 1 per cent. ammonium chloride in saline injected into the posterior portion, the strong positive reaction was re-established after a few minutes in the posterior portion only. This is clear evidence that ammonia is taken up directly from the haemolymph, at least by the posterior segment of the central hindgut.

No ammonia was detected in the malpighian tubules in this test, or for that matter with Nessler's reagent in actively feeding larvae, although it is known from other tests to be present in the tubules (Lennox 1941*a*; Waterhouse 1950). It is, however, not possible to say whether the excretion from the malpighian tubules carries with it any important quantity of ammonia.

(vi) *Carbonate or Bicarbonate.*—When a dissected alimentary canal was flooded with dilute sulphuric acid and pricked at intervals along its length with a fine pin, bubbles of gas were produced from the central hindgut and particularly from the posterior half of this region. The hindguts of larvae from batches which gave a particularly intense reaction for ammonia also produced many bubbles of gas, whereas those from batches giving a weak Nessler reaction evolved relatively little gas. Medium on which larvae had fed for several

days, and also the crop contents of these larvae, gave a strong Nessler reaction and evolved gas on acid treatment. Elsewhere the alimentary canal produced no gas.

The sodium carbonate-phenolphthalein test demonstrated that the gas evolved was carbon dioxide. Cyanides, azides, sulphides, sulphites, and thiosulphates were prevented from interfering by the methods of Feigl (1947). Control tests using all larval tissues except crop, malpighian tubules, and hindgut indicated that little or no carbon dioxide was evolved under these conditions. Brown (1938) recorded the presence of ammonium bicarbonate in the food on which larvae had grown for several days and it can probably be assumed that the same compound is present in the larval body.

(vii) *Phosphatases.*—Alkaline phosphatase activity was very weak or absent, regardless of whether the entire hindgut or sections of it were incubated, and of whether the substrate used was glycerophosphate or β-naphthyl phosphate. On those occasions when very weak activity was observed, staining appeared to be a little more distinct in the *C* than in the *A* or *B* cells. The peritrophic membrane and its contents sometimes stained fairly heavily.

Acid phosphatase was only demonstrated when the whole hindgut was incubated and, even under these conditions, staining was far less pronounced than in the midgut. Uniform, moderate activity was demonstrated in the anterior hindgut and along one side of the central hindgut. The short posterior hindgut showed comparatively little activity. In the central hindgut moderate activity was observed in the strip of *A* cells (Plate 2, Fig. 4). The cuticular lining and the basement membrane of the *A* cells together with the investing muscles, all stained quite distinctly. At times weak cytoplasmic staining and scattered black or brown granules were observed adjacent to the cuticular lining. Nuclei sometimes gave a positive reaction. On the other hand, the strip of *B* cells typically failed to react, although there was sometimes some staining of the investing muscles, and particularly those adjacent to the *A* cells.

(viii) *Dehydrogenases.*—The neotetrazolium chloride (NTC) test for dehydrogenase enzymes resulted in one side of the central hindgut staining strongly with deep purple formazan (reduced NTC), whereas the other side only attained a light pink coloration.

Frozen sections demonstrated that the *A* cells contained numerous purple granules, generally evenly distributed throughout the cytoplasm, but occasionally concentrated in the apical region (Plate 2, Fig. 6). On the other hand, the *B* and *C* cells were colourless or stained a light pink and contained very few scattered granules.

Fresh hindguts reduced NTC without added substrate. However, when the hindguts were frozen in saline and thawed, endogenous substrate was lost from the tissues and colour was formed only in the presence of added succinate (0·05M). Lactate, glycerophosphate, malate, fumarate, and citrate at 0·05M were not effective substrates. Deposition of formazan in the presence of succinate was partially inhibited by malonate (0·1M) and completely inhibited by monoiodoacetate (0·025M).

(ix) *Acetyl Esterase.*—A strong positive reaction for acetyl esterase was obtained on one side only of the hindgut, the other side reacting very weakly or not at all. As with the test for dehydrogenases, the heavy staining occurred in the band of *A* cells. In frozen sections it could be seen that the basal cytoplasm of these *A* cells stained a deep pink and contained numerous small red granules, whereas the apical half of the cells stained rather less heavily but, at times, contained numerous, rather larger deep red granules (Plate 2, Fig. 3). The *B* and *C* cells were generally unstained and only occasionally became light pink (Plate 2, Figs. 3 and 5). These cells rarely contained a few scattered red granules.

(x) *Phenolsulphatase.*—Very weak phenolsulphatase activity (in the form of a diffuse light pink coloration) was observed in the hindgut after long incubation periods, but there was no indication of restriction to any particular cell type.

(xi) *Cytochrome Oxidase.*—The entire hindgut stained rapidly and apparently uniformly with the Nadi reagent for cytochrome oxidase although it is possible that the strong reaction in the musculature might have obscured a difference in the *A* and *B* bands. In mounted preparations the staining was seen to be caused to a large extent by small blue granules scattered throughout the cells.

(xii) *Hydrogen Ion Concentration.*—Saturated solutions of sulphonphthalein indicators* in saline were injected into the posterior segment of larvae ligatured so that a small loop of the hindgut was enclosed in the anterior segment. This enabled indicator taken up by the hindgut epithelium posterior to the ligature to be observed without the complication of colour in the lumen caused by indicator which had come via the midgut or malpighian tubules. One side of the hindgut epithelium was found to absorb and accumulate the various indicators, whereas the other side only contained traces or none at all. The side which accumulated indicators strongly had a pH of about 7·4 (very faint pink with phenol red and showing the alkaline colours of bromthymol blue, bromcresol purple, and bromcresol green). The other side appeared on the basis of the very weak colours observed to be rather more acid, perhaps a pH of about 6·4 (a light yellowish green with bromthymol blue, turning light blue on addition of alkali). When indicator-stained guts were tested with the Nessler reagent the principal reaction for ammonia appeared in the lightly stained cells. The alkaline cells are, therefore, presumably the *A* cells.

(xiii) *Negative Tests.*—No ionizable iron, copper, or zinc was detected in the hindgut epithelium. Tests were also negative for inorganic phosphate, uric acid, ascorbic acid (even after feeding on ascorbic acid-enriched food), lipase, and amine oxidase (which occurs in Crustacea (Blaschko and Himms 1954)).

(*c*) *Comparison with Other Dipterous Larvae*

A brief examination of the larvae of several other blowfly species (*Lucilia sericata* (Meig.), *Calliphora stygia* (F.), *C. augur* (F.)), of the housefly (*Musca*

* BDH water soluble indicators.

domestica L.) and of *Drosophila melanogaster* Meig. indicated that, in these species also, the hindgut epithelium is histologically and functionally differentiated into longitudinal bands of cells. *L. cuprina,* therefore, is not unique in this respect.

IV. Discussion

A striking feature of the present investigation was the finding that, for the greater part of its length, the hindgut of blowfly larvae is differentiated into longitudinal bands, each formed by a single cell type. This kind of differentiation does not appear to have been recorded previously and is quite different from the typical successive zones of different morphology, histology, and function into which the insect gut can normally be divided.

Table 2

TYPICAL REACTION OF *L. CUPRINA* HINDGUT TO VARIOUS HISTOCHEMICAL TESTS

A blank or − indicates a negative reaction, ± indicates a very weak reaction, and + to +++ indicates increasing intensity of reaction

Histochemical Test	Anterior Hindgut	Central Hindgut			Posterior Hindgut
		A Cells	*B* Cells	*C* Cells	
Potassium		++	±	±	
Barium			+	± to ++	
Ammonia		− to +	+++	+++	
Acid phosphatase	+	++	±	±	±
Dehydrogenases	− to ±	+++	− to ±	− to ±	− to ±
Acetyl esterase		+++	− to ±	− or ±	
pH injections		about 7·4	? about 6·4		

No Reaction in Hindgut for:	Weak Uniform Reaction in Hindgut for:
Iron	"Ground" lipoid
Copper	Glycogen
Zinc	Alkaline phosphatase
Strontium	Phenol sulphatase
Phosphate	
Uric acid	Strong Uniform Reaction in Hindgut for:
Ascorbic acid	
Lipoid spheres	Cytochrome oxidase
Amine oxidase	
Lipase	

Table 2 summarizes the results of the various histochemical tests performed. There is no doubt that the three types of cell producing the longitudinal bands each behave in a distinct fashion, although it is not yet possible to define their differing functions in any detail.

The observations in the present paper enable a clearer picture to be obtained of the route of ammonia excretion in blowfly larvae. When larvae are fed on fresh food, proteolytic enzymes in the digestive tract lumen carry out their degradation without appreciable ammonia production. Ammonia is, however, produced from the partially split products, but only after these have been absorbed by the midgut epithelium (Brown 1938; Lennox 1941*b*). The mid midgut gives a weak Nessler reaction, possibly because the acidity of its contents results in a greater ammonia capacity than that of the remaining, alkaline regions of the midgut. However, the posterior midgut cells appear to possess the greatest deaminase activity (Lennox 1941*b*).

The ammonia produced appears not to pass into the midgut lumen but out into the haemolymph which contains the surprisingly high concentration of 12 mg ammonia-N per 100 ml (Lennox 1941*a*). Some of this ammonia is absorbed by the malpighian tubules and is presumably discharged into the hindgut or bound as magnesium ammonium phosphate (Waterhouse 1950). However, the bulk of the ammonia is probably taken up directly from the haemolymph by the central hindgut. Two of the three types of epithelial cell present appear to be specially involved in this process.

Carbon dioxide elimination is linked, in part, with ammonia excretion. Tests indicated that regions in the gut of high ammonia and high carbonate coincided. Ammonia liberated by deaminase action may be pictured as passing from the haemolymph to the hindgut lumen where it is associated with bicarbonate. The presence in the excreta of the relatively unstable bicarbonate (rather than of other more stable ammonium salts) would account for the strong ammoniacal odour characteristic of growing blowfly larvae. The binding of carbon dioxide in the excreta would also explain the extremely low respiratory quotient of *Lucilia* larvae (Brown 1938). It may be that the uptake of respiratory carbon dioxide by the hindgut is largely pH-controlled so that, as the pH of the cell and gut contents increases, the rate of carbon dioxide uptake (or retention) is increased due to a change in the carbon dioxide concentration gradient to the tracheal system.

It should be noted that the binding of carbon dioxide in the excreta and a low respiratory quotient from this source is only possible when the deaminated amino acid is either completely oxidized to carbon dioxide and water or, and rather unlikely, converted to a neutral substance such as an alcohol or an aldehyde. This follows from the fact that at a pH of 7·0-7·5 deamination of an amino acid would have little effect on the pH, but that complete oxidation of the fatty acid (which results in the production of several equivalents of carbon dioxide for each equivalent of ammonia produced by deamination) would cause an increase in pH if an appreciable amount of the carbon dioxide produced was lost, for example, by diffusion into the tracheal system.

When, instead of fresh food, food contaminated by excreta is ingested, the crop many contain a high concentration of ammonium bicarbonate, but this is apparently transferred into the haemolymph when the food reaches the anterior midgut. It has been claimed that blowfly larvae are able to excrete their

nitrogen as ammonia, because, like fish, they are provided with an aqueous environment, and because a copious supply of water is necessary for any organism which excretes ammonia (Lennox 1941*a*; Baldwin 1949). However, this is certainly not the whole story, since blowfly larvae live perfectly well in culture medium into which a great deal of ammonium bicarbonate has been excreted. It appears rather that they are able not only to tolerate relatively high concentrations of ammonia in their haemolymph and food, but also to eliminate it effectively by the activity of portion of their hindgut epithelium. Many aquatic insects excrete ammonia, but evidence so far available suggests that blowfly larvae may be unusual in the concentration that they can tolerate in their haemolymph (Staddon 1955).

No functional relationship can be suggested for the virtual restriction of the reactions for potassium, acid phosphatase, dehydrogenases, and acetyl esterase to the band of *A* cells, unless it is that the function of ammonia excretion by the *B* and *C* cells precludes these from many other activities. If, as appears probable from injection studies, the general cytoplasm of the *A* cells has a pH of about 7·4, it is perhaps not surprising that the acid phosphatase reaction is confined to the wide inner cuticular lining and to the base of the cells. In the mid midgut, acid phosphatase and dehydrogenases occur in different cell types (Waterhouse and Stay 1955). On the other hand, acetyl esterase and dehydrogenase activity is associated in the same cell type in both regions (Waterhouse, unpublished data).

The significance of the presence of acetyl esterase in the hindgut is not clear, although it occurs widely in many animal tissues and has been recorded in extracts of a number of insects (Lord and Potter 1953, 1954).

No evidence was obtained that the hindgut of blowfly larvae plays the important part in water economy that it does in many other insects. It is interesting to note, too, that a good deal of the protease secreted into the midgut lumen passes out to the exterior through the hindgut, apparently without appreciable destruction or absorption (Hobson 1931; Brown and Farber 1936). However, too little is known of the enzyme content of insect excreta to indicate clearly how unusual this may be.

There is little doubt from the evidence presented that the hindgut epithelium of blowfly larvae is an active and highly differentiated tissue which plays an important role both in intermediary metabolism and in regulating the products of excretion.

V. Acknowledgment

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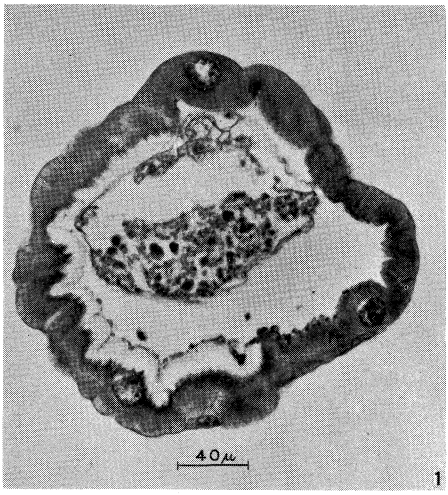
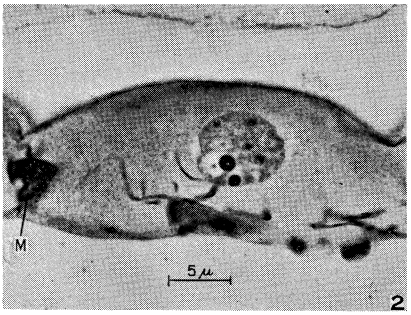
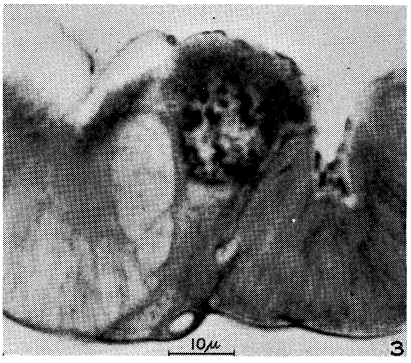
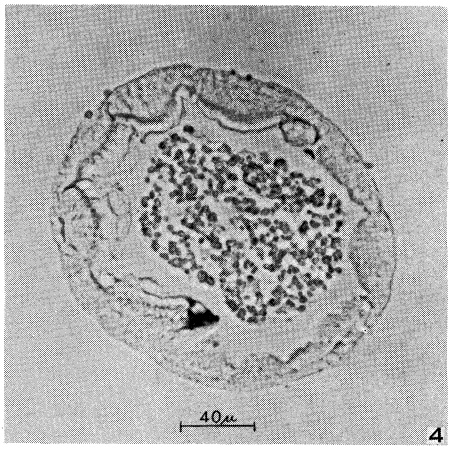
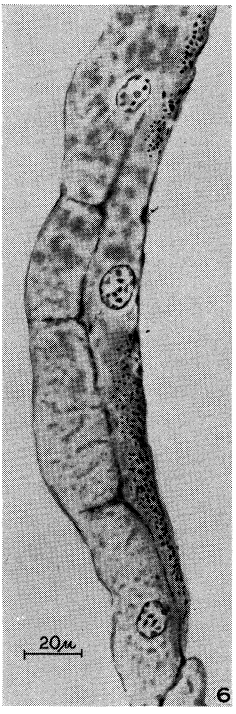
FUNCTIONAL DIFFERENTIATION OF BLOWFLY HINDGUT

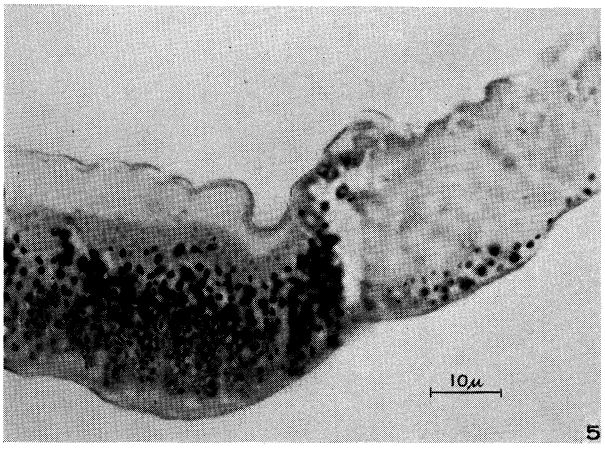












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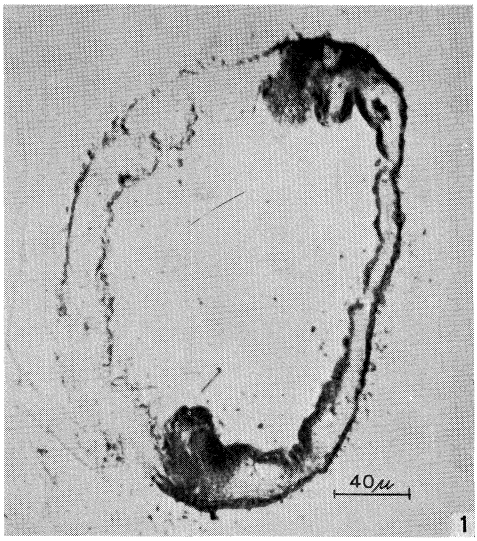

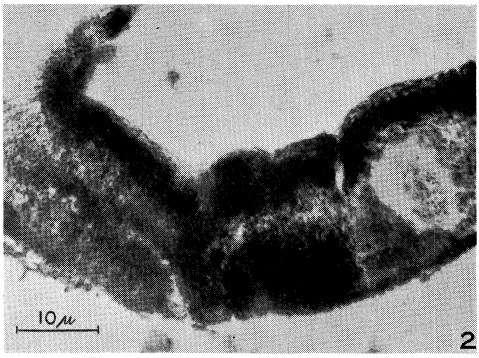

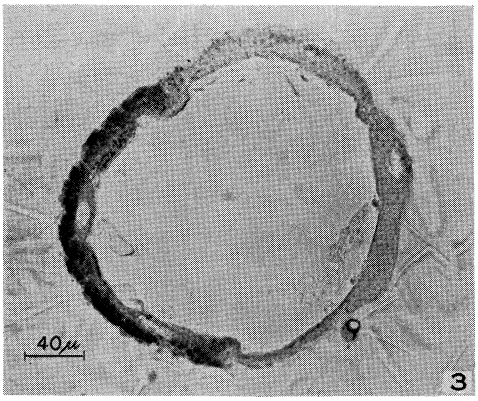

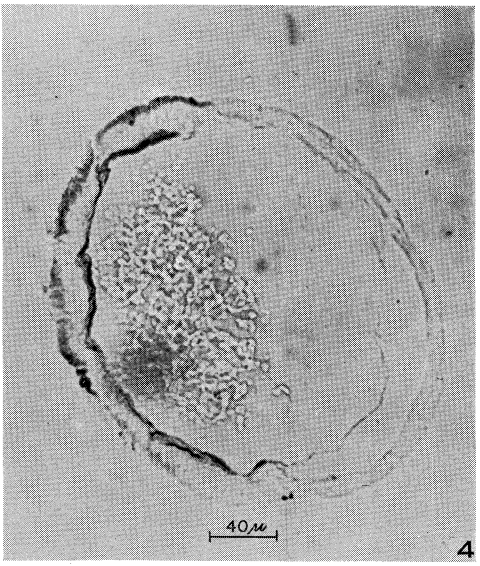

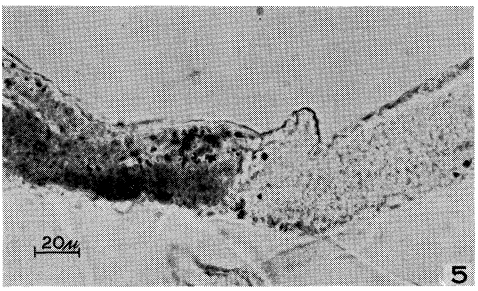

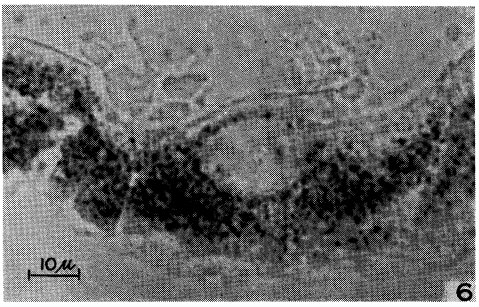

Explanation of Plates 1 and 2

Plate 1

Histology, Potassium, Barium

Fig. 1.—Delafield's haematoxylin and eosin after alcoholic Bouin's fixation. 10 μ cross section of central hindgut showing *A* cells with apical nucleus and wide cuticular lining and *B* cells with median nuclei and narrow cuticular lining.

Fig. 2.—Bodian's silver protargol after alcoholic Bouin's fixation. 10 μ longitudinal section of anterior hindgut showing intracellular tracheae and circular muscles (*M*) invaginated into cells.

Fig. 3.—As for Figure 1, showing *C* cell with nucleus lying between *A* cell (left) and *B* cell (right).

Fig. 4.—Neutral formalin fixation, rhodizonate test for barium. 10 μ cross section of central hindgut showing positive reaction (black) in *C* cell at 7 o'clock and negative reaction elsewhere, including the *C* cell at 1 o'clock.

Fig. 5.—Retouched photograph of Macallum test for potassium in central hindgut, indicating intense reaction (black) in the *A* cell on left and weaker reaction in *C* and *B* cells (centre and right).

Fig. 6.—As for Figure 4. Retouched photograph of longitudinal section of central hindgut, showing *C* cell (right) containing granules stained for barium and *A* cells (left).

Plate 2

All photographs are cross sections of the central hindgut.

Reactions for Ammonia, Acetyl Esterase, Acid Phosphatase, and Dehydrogenases.

Fig. 1.—Nessler test for ammonia. 10 μ paraffin section showing the main reaction in the vicinity of the *C* cells and positive, but weaker, reaction apically and basally in the band of *B* cells.

Fig. 2.—As for Figure 1 showing the distribution of the Nessler reaction for ammonia in two heavily stained *B* cells.

Fig. 3.—Esterase demonstrated with β-naphthyl acetate and Fast Blue RR. Formalin fixation. 10 μ gelatin section showing intense esterase activity restricted to the band of *A* cells.

Fig. 4.—Gomori's acid phosphatase (modified, see text). Note restriction of reaction to apex and base of *A* cells.

Fig. 5.—As for Figure 3 showing esterase reaction in *A* cell (left) and absence of reaction in *B* and *C* cell (right and centre). Note large granules in apical half and uniform positive ground coloration in basal half.

Fig. 6.—Dehydrogenases demonstrated with NTC. Formalin fixation. 10 μ gelatin section showing *A* cells only. Formazan (reduced NTC) is deposited in large granules in the apex of left cell and generally throughout the cytoplasm of both cells.