A STUDY OF THE PROCESSES OF DIGESTION IN CERTAIN INSECTS

By M. F. DAY* and R. F. POWNING*

[*Manuscript received March* 11, 1949]

Summary

Several aspects of the processes of digestion in the insects *Blattella germanica*, *Periplaneta americana*, and *Tenebrio molitor* are reported. In *Blattella* starved for 2 days, a meal of coloured starch reaches the midgut within 10 minutes and the rectum within 5 hours. The pH of the gut contents on a starch diet is approximately 4.5 in the crop, 6.0 in the midgut, and 8.0 in the hindgut. A protein diet raises the pH of the crop to about 6.0, but does not change that of the other regions. There is a gradient of decreasing redox potential from the crop to the hindgut where the *Eh* approximates − 0.1 V. at pH 8.0.

Concurrent quantitative enzyme estimations and cytological investigations on *Blattella* have proved that the presence of cytoplasmic globules, hitherto generally referred to as cytological evidence of secretory activity, is not associated with an increase in enzyme concentration in the gut contents. The greatest enzyme concentrations are found when the cytoplasm is cytologically uniform. The secretory globules are more probably signs of cell breakdown than an indication of secretory activity.

Digestive enzymes are still present in *Blattella* midgut contents after 3 days' starvation, but the enzymes studied increase in concentration when the insect is fed, irrespective of the diet. A digestive enzyme of *Blattella* decreases in amount when the insect is fed a diet of that particular enzyme substrate for some time. The enzyme concentration is fairly slow to regain its former level.

Evidence is presented that stimulation of epithelial regeneration of the midgut of *Tenebrio* is effected by a factor carried in the blood. There is some evidence, mainly morphological, against the nervous control of midgut secretion.

A study of the localization of various substances shows that different materials may be absorbed in different regions of the gut. Fore-, mid-, and hindguts, and the midgut caeca may all be involved in absorption. The histopathology of a number of insecticides suggests that, except for arsenic compounds, changes produced in the midgut are not sufficient to account for death of the insect.

I. INTRODUCTION

In spite of a considerable number of investigations, there are many problems concerning the digestive processes of insects on which we have inadequate information. This is not surprising, since the same has quite recently been said of vertebrates (Babkin 1944), but the whole process in insects is probably simpler, and a knowledge of it is a necessity for the intelligent formulation of certain types of control measures. We have applied a number of experimental techniques to the study of the alimentary canal, particularly of the German cockroach *Blattella germanica* (L.), although other species have been used when *Blattella* was unsuitable.

* Division of Economic Entomology, C.S.I.R.

Many authors have studied the cytology of intestinal secretion in insects, but there is still considerable doubt about the nature of cell inclusions and their relation to enzyme formation; perfunctory examination of the relation of enzyme production to diet has not yet proved whether digestive enzymes are produced in response to secretogogues or other stimuli or whether they are all produced continuously; while information has been obtained on the site of absorption of some inorganic ions, practically nothing is known of the absorption of organic materials; and the whole problem of intermediary metabolism is almost unexamined. To attempt to elucidate these and related problems the following investigations were undertaken. Details of the methods employed will be found in each section.

The culture of *Blattella* was maintained at a fairly constant temperature of 32°C. and a relative humidity of about 70 per cent. Abundant supplies of cut potato, bran mash, and water were available at all times in the stock cultures. The individuals used in experiments were fed an artificial diet composed of ground whole wheat, dried milk powder, dry yeast, sugar, and fat. Thriving cultures of the larger cockroach, *Periplaneta americana* (L.), and of the mealworm, *Tenebrio molitor* L., the other species used, were maintained at about 27°C. and about 35 per cent. relative humidity.

II. Morphology

The mouthparts, feeding mechanism, and gross morphology of the gut of *Blattella* have been well described by Snodgrass (1944, Figs. 7 and 8) and the morphology and histology of the gut have been studied by Ross (1930). The proventriculus has been described in considerable detail by Judd (1948). Two aspects of the morphology of the *Blattella* gut, which are important from the physiological viewpoint but which have not received attention, are the tracheation and innervation. The tracheal supply of the gut is of Snodgrass's generalized type C (1935, Fig. 223, p. 430), in which visceral tracheae are given off from each abdominal spiracle. In addition to the possibility of ventilation through the lateral trunks, conspicuous anastomoses between relatively large tracheae are found on the haemocoelic surface of the midgut, but not in other regions. The dark field photomicrographs (Plate 5, Figs. 28 and 29) illustrate differences in the form of tracheolar endings in different regions of the gut. In the crop (Plate 5, Fig. 28) fine tracheal trunks send short branches through the muscularis to penetrate the epithelium. Silver nitrate preparations, as used to demonstrate ascorbic acid, show that the tracheoles penetrate some of the epithelial cells. In the midgut (Plate 5, Fig. 29) relatively large tracheae send very short branches with conspicuous end-twigging to and through the muscularis so that every epithelial cell is supplied by tracheoles. This organ is more thoroughly tracheated than any other region of the alimentary tract. In the hindgut the epithelium is composed of patches of cuboidal cells with flattened epithelial cells between the patches. The tracheae run to these patches, where they branch repeatedly to form an intertwining mass of tracheoles. The low epithelial cells are less well tracheated.

The innervation of the gut as seen in methylene blue preparations is described in Section VII below.

III. Time Required for Passage of Food through the Alimentary Tract

Snipes and Tauber (1937) and Snipes (1938) have studied the rate of passage of food through the gut of *Periplaneta*, and the effect of various poisons on it. They were mainly concerned with egestion time, that is, the period from ingestion to the passage of faecal pellets, but Snipes gives some data on the time taken for banana paste to reach various points in the gut. We have obtained similar data for adult *Blattella* starved for 2 days and then fed on starch paste coloured with carmine, trypan blue, or orange G. Typical results are illustrated in Figure 1, in which the position in the gut reached by food after various time intervals is shown by horizontal lines. Broken lines indicate

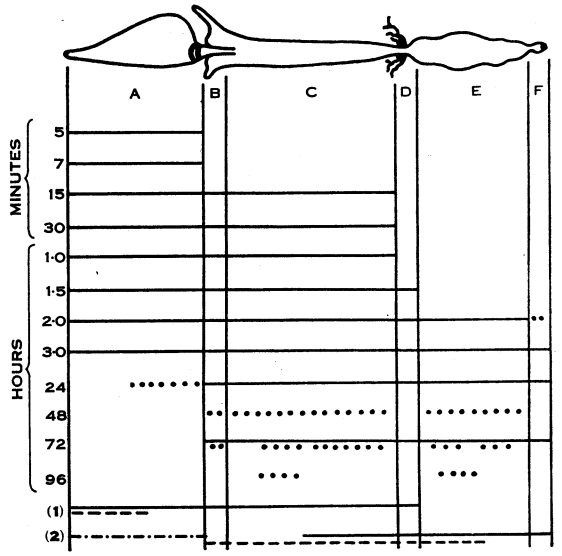

Fig. 1.—Diagram illustrating the passage of food through the *Blattella* alimentary tract. See text for description. *A*, crop; *B*, caeca; *C*, midgut; *D*, ileum; *E*, large intestine; *F*, rectum.

the presence of traces only of colouring matter from the food. Food is held for a short period in the crop by the proventriculus. The next delay is at the entrance of the malpighian tubules into the gut. Passage through the midgut and through the hindgut, once it begins, is relatively rapid. The two examples at the bottom of the figure illustrate the effect of feeding starch paste of one colour and replacing it by another. Thus in (1) after the insect had fed for 20 minutes on carmine paste, it was transferred to trypan blue paste, and examined 20 minutes later. The movement of the blue colour only part way into the crop after 20 minutes indicates the effect of the state of nutrition on the rate of passage. In (2) an adult *Blattella* was fed for 20 minutes on carmine starch

(solid line), then transferred for 2½ hours to trypan blue starch (broken line), and then transferred for 1 hour to orange G starch, after which the midgut was removed and examined. It will be observed that there was some mixing of the first two meals in the hind part of the midgut and the anterior part of the large intestine, but that all the middle meal (trypan blue starch) had passed through the crop into the midgut, its place being taken by the third meal.

The conclusions from these experiments are : (1) In an individual starved for two days, the crop is filled and some food has reached the midgut within 5 minutes. (2) The midgut is filled within 20 minutes, but there is no sign of food in the hindgut until about 2 hours after feeding. (3) The hindgut is slowly filled until after 4 hours it contains material as far as the rectum. (4) If a meal is fed to a replete insect, the second meal has penetrated only as far as the crop after 20 minutes. (5) Some mixing of successive meals can occur in the mid- and hindguts. (6) After a full meal at least three days are required for the crop to empty again. In some insects some dye still remained in granules in the mid- and hindguts 96 hours or even longer after the insects had been removed from food. A readily metabolized material would, of course, have all disappeared from the gut by this time, and insects subsequently fed a normal diet appeared to contain less of the dye than those starved.

From these observations it is clear that: (1) we may consider *Blattella* to be starved when it is removed from all food for 3 days; and (2) on feeding, the cells of the midgut will have an opportunity to react to the presence of food within 10 minutes.

IV. Hydrogen Ion Concentration and Oxidation-Reduction Potential

The pH of the contents of the alimentary canal of insects has been quite extensively studied (see Waterhouse (1940) for review). Wigglesworth (1928) employed a colorimetric comparator method for the study of the *Blattella* gut and found that the pH varied in different regions from a minimum of 4.6 in the crop to a maximum of 6.4 in the midgut but he did not study the pH of the hindgut. He found that the pH of the crop contents was higher after a protein diet than after a carbohydrate one.

Waterhouse (loc. cit.) discussed the methods that are available for the study of the pH of gut contents and concluded that the most satisfactory is to include a series of indicators in the food. We have employed this method in a study of *Blattella* and have extended Wigglesworth's observations in several respects. Cockroaches are not as satisfactory for this investigation as the Diptera which Waterhouse studied, since the contents of the mid- and hindguts are normally brownish and this colour tends to interfere with delicate changes in the colours of indicators. We have succeeded in overcoming this difficulty when necessary by puncturing the gut and comparing the colour of the contents with the colour of the unpunctured gut. Even though the observations cannot be made as accurately as those of Waterhouse, changes in pH are apparent in short sections of the gut, for example, in the vicinity of the malpighian tubules, which would never show up in the method employed by Wigglesworth since it

invariably involves some mixing of the gut contents. The sulphonphthalein indicators were used because of their relatively low salt and protein errors.

Tables 1 and 2 show the results obtained. In general the crop contents are somewhat acid, those of the caeca and midgut almost neutral, while those of the hindgut and in the region of the malpighian tubules are alkaline. A carbohydrate diet reduces the pH of the crop contents, but does not change those of the midgut or hindgut.

TABLE 1

pH OF GUT CONTENTS OF *BLATTELLA* FED STARCH + INDICATORS

Indicator	Crop	Caeca	Midgut	Ileum	Hindgut
Thymol blue	> 2.6 < 8.0	> 2.6 < 8.0	> 2.6 < 8.0	> 2.6 < 8.0	> 2.6 < 8.0
Brom-phenol blue	> 4.5	> 4.5	> 4.5	> 4.5	> 4.5
Brom-cresol green	*c.* 4.6	> 5.2	> 5.2	> 5.2	> 5.2
Brom-thymol blue	< 6.5	< 6.5	< 6.5	> 7.2	> 7.2
Phenol red	< 7.0	< 7.0	< 7.0	< 8.0	< 8.0
Cresol red	< 7.4	< 7.4	*c.* 8.4*		< 7.4*
Range	> 4.5 < 6.5	> 5.2 < 6.5	> 5.2 < 6.5	*c.* 8.0	*c.* 8.0

* Anomalous results.

TABLE 2

pH OF GUT CONTENTS OF *BLATTELLA* FED GELATINE + INDICATORS

Indicator	Crop	Caeca	Midgut	Ileum	Hindgut
Thymol blue	> 2.6 < 8.0	> 2.6 < 8.0	> 2.6 < 8.0	> 2.6 < 8.0	> 2.6 < 8.0
Brom-phenol blue	> 4.5	> 4.5	> 4.5	> 4.5	> 4.5
Brom-cresol green	> 5.2	> 5.2	> 5.2	> 5.2	> 5.2
Brom-thymol blue	< 6.5	< 6.5	< 6.5	> 7.2	> 7.2
Phenol red	< 7.0	< 7.0	< 7.0	> 8.0	> 8.0
Cresol red	< 7.4	< 7.4	< 7.4	*c.* 8.4	< 7.4*
Range	> 5.2 < 6.5	> 5.2 < 6.5	> 5.2 < 6.5	*c.* 8.0	*c.* 8.0

* Anomalous result.

An attempt was made to incorporate the indicators in the artificial diet but its brown colour interfered with the indicator colours.

The figures for the midgut agree closely with those of Wigglesworth, who has discussed them in relation to the pH optima of the digestive enzymes. In some individuals a small region immediately anterior to the point of entry of the malpighian tubules had the pH of the hindgut rather than that of the midgut. When this was noted the peritrophic membrane was coiled in this region.

It is noteworthy that the indicators always penetrated to the ends of the caeca. But they were never found in the haemocoele or in any organs other than the gut. If, however, the same dyes were injected into the body cavity many of them made their way into the alimentary canal.

The oxidation-reduction potential of the insect alimentary tract has not been extensively studied, although Linderström-Lang and Duspiva (1936) have obtained interesting data on the larva of the clothes moth, *Tineola*, indicating very low values in the midgut. We have incorporated redox indicators at a

concentration of 0.1 per cent. in starch fed to *Blattella*. At this concentration the indicators used would probably not alter the poising of the system. Consistent colour changes have demonstrated a decrease in redox potential from the fore to the hind end of the gut; that is, the increase in alkalinity of the gut contents is accompanied by a decrease in redox potential (Table 3). In the regions of low *Eh* the indicators regained their colour on exposure to air. While these data are only approximate, they are interesting in view of the scanty information on the redox systems in the insect gut.

TABLE 3

APPROXIMATE REDOX POTENTIAL (IN VOLTS) OF THE GUT OF *BLATTELLA* FED STARCH + INDICATORS

Indicator		Crop pH 4.5	Midgut pH 6.0	Hindgut pH 8.0
Indigo disulphonate	Colour	Blue	Green	Green
	Eo	$> +0.02$	*c.* -0.07	*c.* -0.15
Indigo trisulphonate	Colour	Blue	Blue	Blue
	Eo	$> +0.06$	> -0.03	> -0.12
Indigo tetrasulphonate	Colour	Blue	Blue	Colourless
	Eo	$> +0.10$	> 0.01	< -0.09
Methylene blue	Colour	Blue	Colourless	Colourless
	Eo	$> +0.13$	$< +0.03$	< -0.02
1-Napthol-2-sodium sulphonate-indo-2, 6-dibromo-phenol	Colour	Colourless	Colourless	Colourless
	Eo	—	$< +0.15$	$< +0.05$
Phenol-indo-2, 6-dibromo-phenol	Colour	Colourless	Colourless	Colourless
	Eo	—	—	$< +0.12$
Range		$> +0.13$	$> +0.01 < +0.03$	$> -0.12 < -0.09$

Some exceptions to the figures given in Table 3 were noted. Thus, methylene blue and potassium indigo tetrasulphonate were observed to be reoxidized in the rectum, indicating a much higher *Eh* in this region than in the hindgut. In one individual fed Janus Green, the indicator was present in the reduced condition in the posterior half of the midgut and in the hindgut, suggesting a much lower *Eh* for these regions than shown by the other indicators.

V. CYTOLOGY OF THE MIDGUT EPITHELIUM

(*a*) *Normal Histology*

In *Blattella* it is apparent, for reasons given in Section VI, that the midgut (with its caeca) is the principal organ concerned with the secretion of digestive enzymes. The midgut is also responsible for some absorption, although the large intestine and the crop also absorb certain substances. The midgut epithelium is, therefore, a tissue of considerable interest and has been extensively studied in insects. Petrunkewitch (1900) and Ross (1930) both figured the

histology of the midgut of *Blattella*, but considerable detail can now be added to their descriptions.

The insect midgut is always composed of a simple epithelium. In some large species it may be folded or convoluted to increase the surface area, but in *Blattella* there is a simple columnar and fairly uniform epithelium. There is, however, a gradual change in the shape of the cells from the anterior to the posterior end. At the anterior end the cells are tall and the nuclei are laterally compressed, while at the posterior end the cells are more cuboidal and the nuclei are almost spherical. Evenly distributed among the epithelial cells are groups of undifferentiated cells, the regenerative nidi (Plate 1, Fig. 2). Mitoses are frequent in these nidi, the cells of which replace the mature epithelial cells as the latter degenerate. There is a conspicuous striated border on the distal end of the cells and this also is shorter in the more posterior cells (compare Plate 1, Fig. 4, with Plate 1, Fig. 1), although it changes in length, perhaps during digestion. The cytoplasm in the anterior cells is denser distally, while in the posterior cells it is more uniform. In the cytoplasm, either basophilic or acidophilic granules are sometimes found, although in the active midgut they are not abundant. Some of these granules give a positive test for acid phosphatase by Gomori's (1941) technique, and some contain glycogen. Very occasionally a cell, always distant from a regenerative nidus, may be seen degenerating (see Plate 2, Fig. 8). The epithelium appears to be homomorphous following the usual histological techniques, but a distinct cell type, confined to the anterior end of the midgut, and containing conspicuous argentophil inclusions, is readily distinguishable by Bodian's silver technique, following alcoholic Bouin's fixative. Figure 5 shows that these inclusions are scattered throughout the epithelium, but that they occur in greater numbers distal to the nucleus. The junction between the region of argentophil cells and the cells lacking these inclusions (shown in Plate 1, Fig. 6) is very sharp. Following fixatives containing osmic acid the epithelium appears to contain two cell types (Plate 5, Fig 30), since the oldest cells (see below) always stain darker. This is especially true after feeding on starch. In a number of cells the nucleus is indented at its proximal pole (Plate 1, Fig. 6) but this is not evident in Fleming-fixed material, suggesting that the indentation may represent a fixation artefact. There is usually a single nucleolus.

An estimate of the number of cells in the midgut epithelium can be made by two methods, either by dividing the average area of a cell (approximately 10 by 10 microns) into the area of the midgut (length x $2\pi r$ = 10 x 2 x 22/7 x 0.5 mm. = 31.4 sq. mm. approx.), or alternatively, by counting the number of cells in a complete longitudinal section (approximately 1000) and multiplying by the number in a 10 micron transverse section (approximately 300). Both methods give a figure in the vicinity of 300,000 cells in the *Blattella* midgut. The number of nidi in the midgut can similarly be estimated to approximate 40,000. No estimate has been made of the number in the caeca.

The epithelium is overlain by a thin connective tissue layer which binds the epithelium to the muscularis. It is usually inconspicuous but is well differen-

tiated by toluidine blue. The epithelial cells separate when placed in an extract of hyaluronidase prepared from hog testes, suggesting that they are bound together by substances similar to those of vertebrates. The muscularis consists of an inner layer of circular muscles and outer longitudinal muscles, which are together responsible for peristalsis. The midgut does not undergo continuous writhing movements as does the crop, but reacts to mechanical stimulus with a slow contracture. The flattened nuclei of the muscle cells may be clearly seen in Plate 1, Figure 6.

(*b*) *Effects of Starvation and Poisons*

Degenerating cells are occasionally observed in the epithelium of normal insects. When midguts of a series of *Blattella* starved for 1 to 5 days are studied, degenerating cells become more abundant with increasing periods of starvation. This degeneration may take the form of the extrusion of many droplets expressed through the striated border (Plate 2, Fig. 7) or of the extrusion of a nucleus and its adherent cytoplasm (Plate 2, Fig. 8). The majority of authors in the past have considered these droplets to represent "merocrine secretion." In a large series of *Blattella* we have both histological preparations and estimations of the concentration of the digestive enzymes, proteinase and amylase. From these data it is clear that the extrusions of cytoplasm are in no way connected with secretion, and they prove, in fact, that the highest enzyme concentrations are associated with epithelia exhibiting a very uniform cytoplasm (as in Plate 2, Fig. 9), hitherto generally referred to as a "resting epithelium." In general, the longer the insect is starved, the more frequent are the extrusions of cytoplasmic fragments, and the lower is the concentration of digestive enzymes.

TABLE 4

RESULTS OF INJECTION OF SODIUM ARSENITE INTO ADULT *PERIPLANETA*

		Controls	One Hour after Injection	Two Hours after Injection
Mitoses per 25 nidi: means of 10 counts		8.2	6.2	5.8
Proteinase (optical density): means of six individuals	Caeca	0.157	0.150	0.174
	Midgut	0.203	0.198	0.240

We have studied this matter further by investigating the so-called "hypersecretion" produced by poisons. Thus Hoskins (1940, p. 355) has reported data obtained by Wilson on "hypersecretion" in the midgut of *Pieris rapae* following injection or ingestion of sodium arsenite. Twelve normal *Periplaneta,* each approximately 1 g. in weight, were injected through the coxo-femoral joint with 1 ml. of 0.1M sodium arsenite. At the end of one hour all roaches showed signs of poisoning and after 2 hours several were moribund (compare Yeager and Munson 1945). It was shown that sodium arsenite in the concentrations employed had no effect on the quantitative estimation of proteinase by the colorimetric method referred to in Section VI below. Counts of mitoses, by the method given in Section VII, and proteinase estimations on the entire midgut and contents

were performed on the same individuals, while histological observations were made on midguts and caeca of insects treated simultaneously in the same way. The histological examination showed the typical "hypersecretion" (Plate 3, Fig. 17). The results of enzyme estimations and mitotic counts are given in Table 4.

None of these differences are significant, but because of the possibility that two hours would not be sufficient time to produce maximum cell breakdown, the experiment was repeated, permitting the poison to act for 2 and 4 hours after injection. In this series, only the peritrophic membrane and its contents were taken for the determinations of proteinase activity. The results are given in Table 5. The differences between treatments are again not significant, indicating

TABLE 5

PROTEINASE ESTIMATIONS IN *PERIPLANETA* POISONED WITH SODIUM ARSENITE

Controls		Two Hours after Injection		Four Hours after Injection	
Weight of Peritrophic Membrane and Contents (g.)	Proteinase (optical density)	Weight (g.)	Proteinase (optical density)	Weight (g.)	Proteinase (optical density)
0.0099	0.162	0.0065	0.184	0.0351	0.254
0.0093	0.201	0.0087	0.212	0.0120	0.070
0.0051	0.157	0.0060	0.119	0.0117	0.088
0.0152	0.123	0.0215	0.219	0.0046	0.311
0.0215	0.254	0.0203	0.349	0.0176	0.211
0.0167	0.327	0.0178	0.327	0.0081	0.116
Mean	0.204		0.235		0.176

that the striking cytological picture of globule formation (hypersecretion) is not accompanied by a comparable rise in either intra- or extracellular proteinase. The results given in Tables 4 and 5 suggest that there may be a slight increase in proteinase 2 hours after injection, and since the cytoplasm of the epithelium must contain proteinases, such an increase would indeed be expected. But it is clear that the tremendous cytological differences are associated with at most only a slight rise in digestive enzyme concentration. Since large amounts of proteinase are found without any visible cytological evidence it is clear that cytoplasmic globule formation is not the normal method of secretion by the *Blattella* or *Periplaneta* midgut epithelium.

A comparison of the data contained in Plate 3, Figures 16 and 17, and Tables 4 and 5 will reveal (i) that there is a more marked breakdown in the epithelium of both caeca and midgut following arsenite poisoning after 2 hours than after 1 hour, though the incipient changes are already clear at the end of 1 hour; (ii) the cells which show the first signs of breakdown are the oldest cells, i.e. the furthest from the regenerative nidi; (iii) the cellular breakdown follows the pattern of the breakdown which may rarely be seen in the normal epithelium, but

which becomes more marked following starvation; (iv) there is no destruction of the peritrophic membrane, as Wilson (see Hoskins 1940) described in *Vanessa;* (v) the number of mitoses does not increase as the epithelium breaks down following arsenite poisoning, but is progressively slightly reduced; and (vi) the proteinase concentration does not increase to the extent expected with increasing cell breakdown if cytoplasmic globule formation represented true secretion.

From these experiments it may be concluded that so-called "secretion droplets" do not indicate the normal mechanism of digestive enzyme secretion, and reports of "hypersecretion" must be accepted with caution unless accompanied by confirmatory quantitative enzyme determinations.

(*c*) *Effects of Feeding*

The effects on the epithelium of feeding are seen in Plate 2, Figures 9 and 10. Whatever the diet, the cytoplasmic fragments described above are no longer visible. Instead, the epithelium presents an appearance which has hitherto been called "resting." Actually it is apparent that this uniform low columnar epithelium is active in both secretion and absorption. When starch is fed, this appearance is most marked since the bulk of the diet ensures a low epithelium and there are no visible signs of absorption by the usual histological techniques. After feeding either water, gelatine (Plate 2, Fig. 10), or fat, conspicuous vacuoles appear in the distal cytoplasm and remain there for some hours. With the usual fixatives it is not possible to differentiate between the epithelia of *Blattella* fed any of these three substances.

The Golgi substance also reacts to the ingestion of foods. Excellent preparations of the Golgi substance in the *Blattella* midgut epithelium are obtained by the Mann-Kopsch technique. It is present as discrete granules in the characteristic pattern of the invertebrates, and shows well-marked changes correlated with the nutritional state and diet of the insects. The normal epithelial cell exhibits a large number of discrete granules mainly located towards the lumen but with a few granules proximal to the nucleus and some against the connective tissue (cf. Gresson 1934). In some cells there are conspicuous masses which presumably result from the clumping of a number of granules. When a cockroach is starved for 3 days the Golgi substance disperses and is found more or less evenly scattered throughout the cell. Aggregations as described in the normal insect are not found. If *Blattella* is starved for 3 days then fed starch, it is found that there is within half an hour a darkening of the whole cell, and conspicuous clumping of the Golgi substance occurs on the lumen side of the nucleus (Plate 5, Fig. 30). Feeding on gelatine also results in clumping of the Golgi substance, but the conspicuous dark staining cells are not present (Plate 5, Fig. 31).

We have presented reasons for the conclusion that the extrusion of cytoplasmic globules and fragments does not represent merocrine secretion. Shinoda (1927) has claimed, with little evidence, that secretion in *Blattella* is usually merocrine, but is holocrine following a period of starvation. This conclusion has been widely quoted (for example, Wigglesworth 1939, p. 264), but

has never been examined critically. Our evidence suggested that the hypothesis was questionable, and it was therefore studied further. If the excised midgut of *Blattella* is placed in acetic-orcein for a few minutes the epithelium can be removed from the connective tissue and muscularis. If the epithelium be then mounted and examined under an oil immersion lens the nuclei are found to be stained and the number of mitoses can be counted. The results, following a variety of treatments, are given in Table 6.

TABLE 6

NUMBER OF MITOSES PER 25 NIDI IN *BLATTELLA* MIDGUT

	Normal Controls	Starved 48 Hours	Starved 48 Hours, then Fed Starch			
			30 min.	60 min.	120 min.	300 min.
Mitoses per 25 nidi: means of 10 counts	4.3	2.6	1.6	3.0	4.6	4.3

There is rarely more than one mitosis per 5 nidi in the *Blattella* midgut, and the number may be as low as 1.6 mitoses per 25 nidi. Thus, taking the estimate of 40,000 nidi, there will be from 2500 to 8000 mitoses per midgut at any one time. If the mitotic process occupies about 60 minutes it would take from 120 hours at the minimum to 40 hours at the maximum replacement rate to regenerate all the epithelial cells in the adult *Blattella* midgut. While this indicates a fairly rapid rate of replacement, the increase is not sufficient to permit of a change to the holocrine mode of secretion. It is apparent that all that takes place is an accelerated rate of the normal sequence of cell division, growth, and regeneration.

(*d*) *Summary of Cytology of Secretion*

A synthesis of these results suggests a concept of the processes of secretion in the cockroach midgut which is probably also applicable to the caeca. The details are summarized in Figure 2. Each nidus is surrounded by a number of epithelial cells (about 10 or 12) which originate from it. The number of pro-epithelial cells in each nidus varies considerably. It is low in starvation, greater in a fed insect. Thus, epithelial replacement can occur either as a result of mitoses in the nidi (Fig. 2, A and B) or by maturation of nidus cells without simultaneous mitosis. The midgut epithelial cell is mature, that is, it functions in secretion and absorption, as soon as it reaches the midgut lumen. As it ages it is pushed farther away from the nidus – although this migration (A^1 to A^4 or B^1 to B^4) need never be greater than 2 or 3 cell widths. During the later stages of the development of the mature cell, granules form in the cytoplasm, and the cell becomes more sensitive to poisoning or to cytolysis caused by starvation or other factors. Next, globules of cytoplasm may be expressed through the striated border (as in cell B^3), a phenomenon hastened by many fixatives, and finally the nucleus and the remainder of the cytoplasm may be extruded into the gut lumen. During starvation, the mature cells continue to break down and are

replaced mainly from the cells already in the nidi. Some mitoses occur, but fewer than during feeding. Following the ingestion of foodstuffs (distilled water produces no effect) the number of mitoses is augmented, increasing the size of the nidi, but not necessarily the number of epithelial cells. Maximum enzyme output occurs from mature, but not from degenerating, cells.

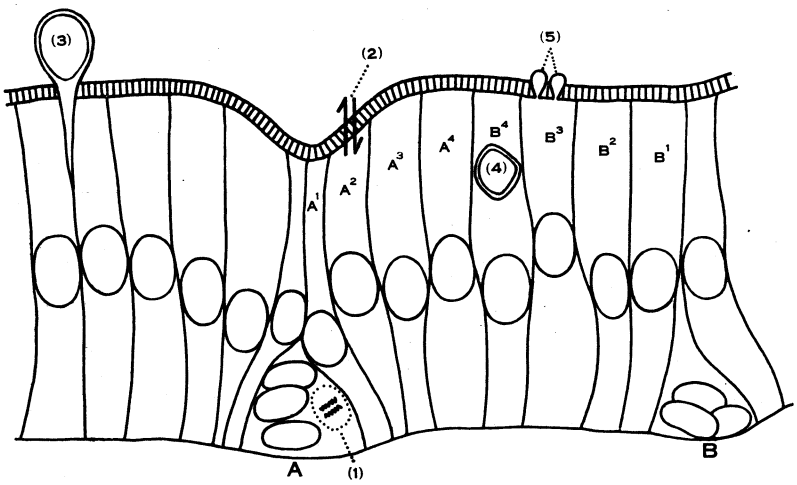

Fig. 2.—Diagram of epithelium of *Blattella* midgut showing sequence of changes occurring from the origin of the cells in the nidi, *A* and *B*, to degeneration (3). $A^1 - A^4$ and $B^1 - B^4$ represent cells of increasing age, which have originated from regenerative nidus A and regenerative nidus B, respectively. (1), a mitotic figure; (2) indicates that secretion of digestive enzymes and absorption occur in the same epithelial cell; (3), the final stage in cell degeneration showing extrusion of nucleus and adherent cytoplasm; (4), a cytoplasmic granule in an old cell: (5), cytoplasmic globules in process of being expelled through the striated border.

VI. Digestive Enzyme Studies

(*a*) *Introduction*

The data presented in the previous section indicated the cytological basis of enzyme secretion in *Blattella*, but a number of questions on enzyme secretion still require elucidation. We have attempted to obtain answers to the following questions:

(i) Where in the gut are the digestive enzymes produced?

(ii) Does the amount of enzyme change with different diets?

(iii) Does the amount of enzyme change during starvation and subsequent feeding?

(iv) Is enzyme production related to the intensity of epithelial regeneration in caeca and midgut?

The last question is considered in Section VII below.

To obtain data on the first three points, quantitative estimations of amylase, invertase, and proteinase have been made, using *Blattella* adults under a variety

of experimental conditions. A few similar experiments were performed on *Periplaneta.* The enzyme systems selected are all of fundamental importance in digestion. Purification of enzyme extracts was not attempted since it was not considered necessary for the purposes in view. The results are a measure of the capacity of the crude extract to break down starch, sucrose, and protein respectively.

(*b*) *Methods*

The tissues to be studied were removed from decapitated insects, freed from adhering tissues, and ground with a little sand. The suspension was diluted with glycerine-phosphate buffer mixture (Linderström-Lang and Duspiva 1936) to a volume depending on the method used, i.e. 1 midgut per ml. for colorimetric proteinase, 2 midguts per ml. for titrimetric proteinase, or 0.4 midgut per ml. for amylase and invertase. After standing with occasional stirring at room temperature for about an hour, the suspension was centrifuged to give a clear extract. When cells only were extracted, double this concentration was used, and in the studies on regional variation of the quantity of enzyme in the gut, the number of insects used was further increased by the number of regions into which they were divided, i.e. three for *Blattella* and four for *Periplaneta.* For the purpose of this experiment, *Periplaneta* was shown in preliminary experiments to have about six times the quantity of enzymes as *Blattella* and the extracts were diluted accordingly. For this reason no comparison of quantity of enzyme between the two species may be made.

Proteinase activity was estimated by two methods, titrimetric and colorimetric. The former was a modification of the Willstätter titration in alcohol, similar to Schlottke's (1937*a*) technique, with the exception that a gelatine substrate was substituted for casein as it was found to be more convenient. Two ml. of 6 per cent. neutral gelatine, 1 ml. of M/15 phosphate buffer pH 8.0, and 0.5 ml. of enzyme extract were mixed, then two 0.5 ml. samples were titrated immediately to a faint blue using thymolphthalein as the indicator with N/20 alcoholic (90 per cent.) potassium hydroxide; 4.5 ml. of absolute alcohol were then added and the mixture titrated to the final faint blue end-point, this titration representing the blank estimation. The enzyme-substrate mixture was held for 24 hours in a water-bath at 37°C., preserved with toluene, and the same titration procedure repeated. The difference between the titrations was transformed as follows. The relative concentration of serial dilutions from a strong enzyme extract gave a curve when plotted against the titration figures (Fig. 3). To permit comparison of results on different parts of the curve the relative enzyme concentration was arbitrarily divided into units and these were used to represent enzyme activity.

The colorimetric method employed was described by Charney and Tomarelli (1947) for the determination of proteolytic activity in duodenal juice. The substrate consisted of a solution of a chromophoric protein derivative (sulphanilamide azocasein), the digestion of which yields coloured components soluble in trichloracetic acid. 0.5 ml. of substrate (pH 8.0) was mixed with 0.5 ml. of enzyme extract and held in a water-bath at 37°C. for 1 hr., after which 4 ml. of

5 per cent. trichloracetic acid solution were added and thoroughly mixed. The unchanged substrate was filtered off and to 3 ml. of the filtrate were added 3 ml. of 0.5N sodium hydroxide to develop the colour. A blank estimation was made using the glycerine-phosphate solution instead of enzyme extract. The optical density (log absorption) was read on a Lange photoelectric colorimeter with a blue filter having a maximum transmission at about 480 mμ and a dilution curve for conversion to units of proteinase was constructed as described above (Fig. 3).

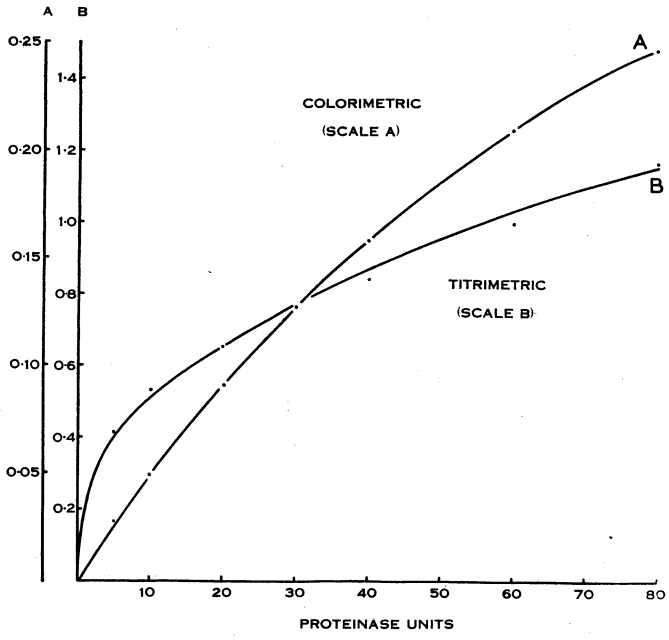

Fig. 3.—Dilution curves of *Blattella* proteinase.
Colorimetric method. Scale *A*. Optical density (log absorption). For details of colorimeter, see text.
Titrimetric method. Scale *B*. Millilitres of N/20 alcoholic (90 per cent.) potassium hydroxide.

The Linderström-Lang and Holter (1933) technique was modified slightly for the estimation of amylase and invertase. A mixture of 5 ml. of 1.5 per cent. soluble starch, 2 ml. of M/15 phosphate buffer pH 6.5, 0.5 ml. of 0.2N sodium chloride, and 0.5 ml. of enzyme extract was used for amylase determination, and a similar mixture containing 5 ml. of 2 per cent. sucrose, 2 ml. of M/15 phosphate buffer pH 6.5, and 1 ml. enzyme solution for invertase. After 1 hr. in the water-bath at 37°C., 3 ml. aliquot samples were added to 2.5 ml. carbonate buffer at pH 10, and 1 ml. of N/10 iodine added. The tubes were stoppered for half an hour, the contents then acidified with 2.5 ml. of 2.4N sulphuric acid and the remaining iodine titrated with N/50 sodium thiosulphate. The differences between these titrations and those of boiled enzyme blanks were transformed by means of a dilution curve (Fig. 4) constructed as described for proteinase and these values were taken as a measure of amylase and invertase activity.

The results of all experiments (except those of Tables 7 and 10) are presented as activity per gut. When expressed as activity per unit weight the results are more variable owing to the large variable fraction of inactive gut contents. The statistical analyses were carried out on the individual titrimetric

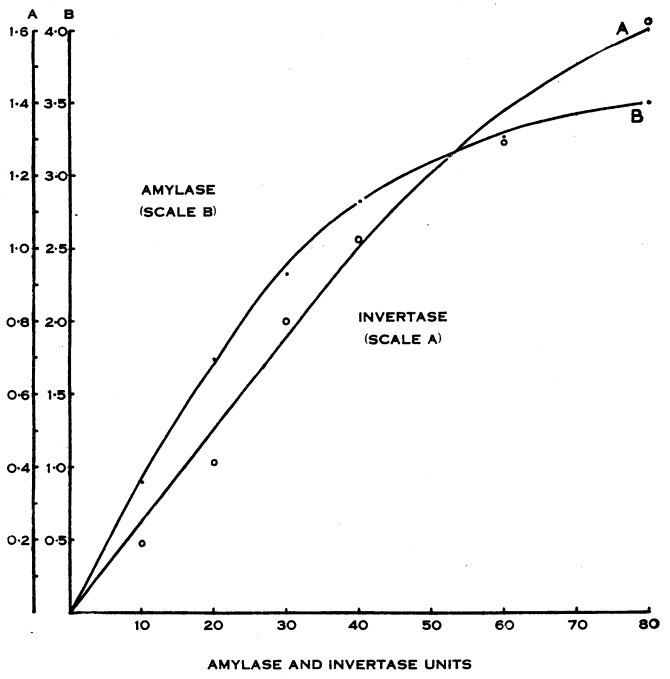

Fig. 4.—Dilution curves of *Blattella* invertase and amylase.
Invertase. Scale *A*. Millilitres of N/50 sodium thiosulphate.
Amylase. Scale *B*. Millilitres of N/50 sodium thiosulphate.

or colorimetric data, but the results are presented for the sake of clarity as average units (determined from the dilution curves).

Proteinase was determined by the colorimetric method in the midguts of ten male and ten female *Blattella* in order to detect any difference in amount of enzyme between the sexes (Table 7).

TABLE 7
PROTEINASE CONCENTRATION IN MALE AND FEMALE *BLATTELLA*

Male		Female	
Midgut Weight (mg.)	Proteinase (units)	Midgut Weight (mg.)	Proteinase (units)
2.09 ± 0.14	0.210 ± 0.022	3.32 ± 0.70	0.247 ± 0.044

The weight of the female midgut was more variable and on the average higher than for males, but the proteinase content was not greatly different. On the basis of this we made no distinction between the males and females in the subsequent experiments.

(*c*) *Enzyme Characteristics*

Of the characteristics of the enzymes, we studied the pH and temperature optima. Wigglesworth (1928) has shown that the pH optimum of *Blattella* amylase and invertase is about pH 6 (Figs. 5A and 5B) and that *Periplaneta* proteinase has an optimum about 7.8 (Fig. 5C) when acting on 5 per cent. gelatine. Two single experiments confirmed these results for amylase and invertase respectively. Another experiment (Fig. 5C) on *Blattella* proteinase showed that this had the same optimum as *Periplaneta* proteinase, i.e. pH 7.8-8.2.

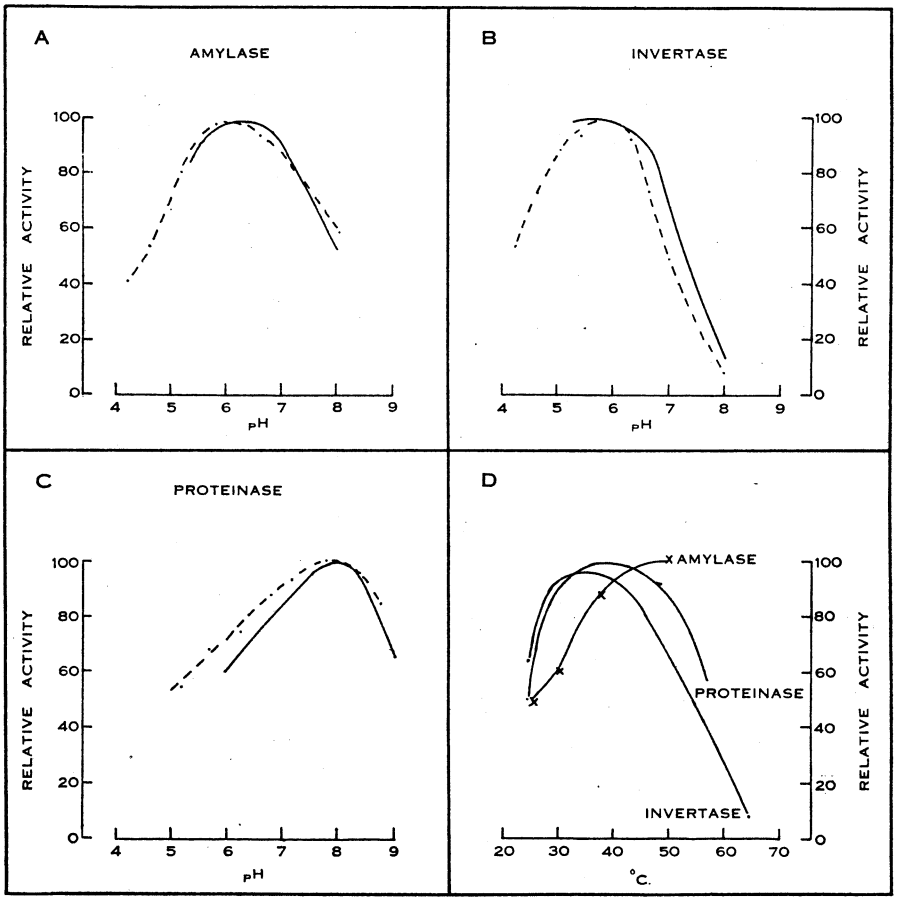

Fig.5.—Characteristics of *Blattella* and *Periplaneta* digestive enzymes. See text for description.

The effect of temperature on the activity of the three enzymes is shown in Figure 5D. Since the temperature optimum is dependent on time of incubation it is not possible to compare proteinase with the other two enzymes, but it is

clear that in a period of 1 hour at 50°C. *Blattella* amylase is not inactivated as rapidly as invertase. When amylase extract in water was heated in a boiling water-bath for varying periods slight activity still remained after 2 minutes, but none after 5 minutes. Proteinase treated similarly had slight activity after 1 minute but none after 2 minutes.

Dialysis of the enzyme extract through a collodion membrane markedly reduced amylase activity, but had little or no effect on proteinase or invertase. Amylase activity was restored on addition of sodium chloride.

It will be observed that the data for both *Blattella* and *Periplaneta* are very similar, and no striking differences in the characteristics examined are apparent between the insect enzymes and those of vertebrates. These preliminary experiments were necessary in order to standardize conditions for the subsequent experiments.

TABLE 8

DISTRIBUTION OF DIGESTIVE ENZYMES IN REGIONS OF THE MIDGUT OF *BLATTELLA*

Gut Region	Amylase (units)	Invertase (units)	Proteinase (units)
Caeca	21.8	63.0	16.0
Anterior midgut	9.0	43.0	21.0
Posterior midgut	13.0	28.0	17.0

TABLE 9

DISTRIBUTION OF DIGESTIVE ENZYMES IN REGIONS OF THE MIDGUT OF *PERIPLANETA*

Gut Region	Amylase (units)	Invertase (units)	Proteinase (units)
Caeca	> 80	> 80	> 80
Anterior midgut	5	> 80	15
Mid midgut	11	67	6
Posterior midgut	5	40	3

(*d*) *Sites of Enzyme Production in Relation to Gut Morphology*

Preliminary experiments indicated that most of the amylase was produced by the salivary glands. Several experiments were designed to give information on the relative amounts of enzyme occurring in the cells in different regions of the midgut. In all experiments on *Blattella* the midgut was divided into caeca, and anterior and posterior parts. The cells alone, free of gut contents, were used for the midgut samples, but whole caeca were taken, since the contents of this part of the gut consist only of substances in solution. Owing to the much larger gut in *Periplaneta* it was possible to divide it into caeca, and anterior, mid, and posterior parts of the midgut.

In all instances the trends are fairly uniform within experiments. Thus in both species there was more amylase in the caeca than in the midgut. The concentration of invertase decreased from anteror to posterior in both species, but the drop was not marked. There is a difference between the two species with regard to the distribution of proteinase activity. Thus in three experiments

the concentration in all parts of the midgut of *Blattella* was similar, but in *Periplaneta* the caeca contained the bulk of the enzyme and the activity was considerably less in the posterior regions of the midgut.

These results cannot be correlated with differences in histological structure or with the absorptive propensities of the cells (see below). In Plate 1, Figures 1 and 4, it will be noted that the epithelial cells in the more posterior part of the *Blattella* midgut are more cuboidal than those in the anterior part, yet both produce equivalent amounts of proteinase. In *Periplaneta* the cytology is not greatly different in the fore and hind parts of the midgut, but the amount of proteinase produced is apparently greatly reduced in the latter region. Summarizing, it has been shown that amylase, invertase, and proteinase are present in extracts from all parts of the midgut, but the amount varies in different regions.

TABLE 10
PROTEINASE CONCENTRATION IN COCKROACH CROPS

	Weight of Sample (mg.)	Proteinase Activity (optical density)
7 full *Blattella* crops	23.6	0.010
10 full *Blattella* crops	40.4	0.007
1 full *Periplaneta* crop	98.6	0.530
1 empty *Periplaneta* crop	59.8	0.011
1 empty *Periplaneta* crop	25.3	0.057

In view of several reports in the literature of the presence of enzymes in the cockroach crop, we were surprised to find very little proteinase in the crop of *Blattella*. In an attempt to find whether there was a difference between *Blattella* and *Periplaneta* in this respect the proteinase activity in one full and two empty *Periplaneta* crops and in two groups of full *Blattella* crops was estimated by the colorimetric method. The results (Table 10) show clearly that proteinase is present in the full *Periplaneta* crop but not found in significant amounts in *Blattella*.

(*e*) *Effect of Diet on Digestive Enzyme Production*

We attempted to obtain data on this point by feeding *Blattella* pure starch, gelatine, or sucrose for varying periods.

(i) In the first series, *Blattella* were fed on pure gelatine, starch, or sucrose for 3 days. These were compared with roaches starved for 3 days and with others fed the normal food which consisted of cut potato and bran mash. After feeding, 4 midguts from each group were extracted, made up to 10 ml., and the invertase and amylase activity determined. A similar series of tests, except that sucrose was omitted, was carried out for proteinase using the titrimetric technique. For these latter, midgut extracts of each group of 4 *Blattella* were made up to 2 ml. The results given in Table 11 demonstrate that amylase activity is high in *Blattella* fed a normal diet, but is considerably reduced by starvation for 3 days. Feeding gelatine or sucrose also reduces it, but to a slightly less degree than starvation. A starch diet for 3 days *decreases* the amylase activity

to less than one-tenth the normal value. The activity of invertase and proteinase is not greatly changed following any of the above treatments.

TABLE 11

CHANGES IN ACTIVITY OF AMYLASE, INVERTASE, AND PROTEINASE IN THE MIDGUT OF *BLATTELLA* ON VARIOUS DIETS

	Amylase (units)	Invertase (units)	Proteinase (units)
Fed normal diet	25.0*	32.4	12
Starved 3 days	4.6	25.4*	14
Fed gelatine 3 days	7.4	49.5	21
Fed starch 3 days	1.7*	43.8	22
Fed sucrose 3 days	6.6	32.3	—

* Indicates the differences are significant.

(ii) In the second series, 3 groups of 4 insects were used for each treatment, which consisted of feeding, after 3 days' starvation, the same types of foodstuff as before for periods ranging from a few minutes up to one hour. Each gut was separated into 3 parts, (*a*) crop, (*b*) cells of midgut, and (*c*) contents of midgut, and amylase and invertase activity determined (Tables 12 and 12A).

TABLE 12

CHANGES IN ACTIVITY OF AMYLASE AND INVERTASE IN DIFFERENT REGIONS OF *BLATTELLA* GUT AFTER FEEDING FOR SHORT PERIODS. ACTIVITY OF AMYLASE AND OF INVERTASE EXPRESSED AS UNITS

Amylase Activity

	Starved 3 Days	Starved then Fed Starch		Starved then Fed Gelatine		Starved then Fed Sucrose	
		10 min.	1 hr.	10 min.	½ hr.	10 min.	1 hr.
Crop	3.3	44.0	21.0	10.0	9.0	4.0	11.0
Midgut cells	1.9	3.0	2.5	3.0	2.0	3.0	1.5
Midgut contents	3.8	3.0	2.5	4.0	3.5	1.5	2.0

Activity in the crop after feeding is significantly higher than that of the starved insects. None of the other differences are significant.

TABLE 12A

Invertase Activity

	Starved 3 Days	Starved then Fed Starch		Starved then Fed Gelatine		Starved then Fed Sucrose	
		10 min.	1 hr.	10 min.	½ hr.	10 min.	1 hr.
Crop	1.2	1.5	1.0	2.3	3.0	1.0	0
Midgut cells	11.0	10.0	7.0	8.5	9.0	12.0	8.0
Midgut contents	19.0	7.0*	15.0	13.0	17.0	5.0*	12.0

* Indicates differences are significant.

Changes in proteinase activity were studied by the colorimetric technique in a separate experiment, using the same food materials except for sucrose.

Proteinase in the entire midgut of five individual insects was estimated for each treatment (Table 13).

Considerable variation can occur between individuals even within a treatment. Thus with the small numbers used only very marked differences between treatments are significant. Amylase increases only in the crop, and this increase is greater after feeding with starch than with either sucrose or gelatine (Table 12). This may be due to the quantity of food ingested (which was greater, as determined by a comparison of weights) or to the consistency of the food, since the greater part of the enzyme is secreted by the salivary glands. There is some amylase in the midgut cells, but whether this is an endoenzyme or due to contamination has not been determined.

Practically no invertase is present in the crop. The treatments cause no change in invertase activity in the midgut cells, but the activity in the contents falls after feeding with starch or sucrose. Both of these diets cause a rise in invertase activity during one hour. From Table 13 it is evident that there is a

TABLE 13

CHANGES IN PROTEINASE ACTIVITY PER WHOLE MIDGUT OF *BLATTELLA* AFTER FEEDING. ENZYME ACTIVITY EXPRESSED AS UNITS

Fed normal diet	36	
Starved 3 days	23	
	Fed Starch	Fed Gelatine
Starved, then fed ½ hour	17	18
Starved, then fed 1½ hours	21	15*
Starved, then fed 3 hours	20	22
Starved, then fed 6 hours	24	26
Starved, then fed 24 hours	27	28

* Indicates the difference is significant.

drop in proteinase activity following starvation and that this is accentuated after feeding gelatine. However, the concentration returns almost to normal within some hours after feeding.

These results suggested that three days' starvation may have an adverse effect on the insects. An experiment was therefore designed to show any reduction in the ability of *Blattella* starved for 3 days to regain their normal enzyme activity. Two groups of 6 control *Blattella* were fed normally for 6 days. Two other groups were starved for 3 days and then fed the normal diet for 3 days. Proteinase activity in midgut extracts was estimated by the colorimetric method. The results (Table 14) indicate that the weights of the midguts from the insects which had undergone 3 days' starvation are lower than the controls, and that, even allowing for the considerable decrease in weight, the amount of proteinase is still lower. This must be kept in mind when drawing conclusions from Tables 12 and 13.

The following general conclusions can be drawn from the data presented in this section.

(1) In general, a decrease in the production of digestive enzymes results from starvation.

(2) All enzymes are secreted irrespective of the nature of the food.

(3) The enzyme activity is reduced following the feeding of its substrate and recovery is comparatively slow. Schlottke (1937*b*) arrived at the same conclusion as a result of his work on *Periplaneta*. The continuous feeding of *Blattella* on starch markedly depletes the salivary amylase.

(4) There are some quite marked differences between *Blattella* and *Periplaneta* as reported by Schlottke (1937*b*), and in part confirmed by us.

TABLE 14

EFFECT OF 3 DAYS' STARVATION ON WEIGHTS AND PROTEINASE ACTIVITY OF *BLATTELLA* MIDGUT

	Weight of Gut Tissue (mg.)	Proteinase Activity (units)
Starved 3 days, then fed 3 days	6.7	36
	6.3	29
Normal controls	8.6	46
	7.8	46

VII. STIMULATION TO SECRETION

It was shown in the previous section that some digestive enzymes increase in concentration in insects which are fed following a period of starvation. There are several possible mechanisms whereby this increased secretion might be stimulated: (*a*) the foodstuff itself or its products may stimulate secretion; (*b*) the stimulus may be nervous, or (*c*) the stimulus may be hormonal. All or any of these factors may be involved as, in fact, they are in vertebrates.

(*a*) It is difficult to prove the action of a secretogogue in insects, but there are several pertinent lines of evidence. For example:

(1) Distilled water alone has no detectable effect on secretion in *Periplaneta* (Table 15).

(2) The caeca of this species may reach considerable lengths (about 14 mm.), and are ill-fitted for the rapid diffusion of materials from the gut lumen to their closed ends.

(3) Schlottke (1937*b*) claimed that the caeca start to secrete before the midgut. This suggests that the caeca receive the stimulus to secrete before the midgut.

(4) However, they are not well innervated (see below) and, if they respond to a stimulus it could only be carried in the caecal contents, by the haemolymph, or through the tracheae. The latter route seems very unlikely, not only because no such mechanism seems to have been employed by insects, but also because of the anatomical relations of the tracheal trunks.

The above evidence is insufficient to decide whether stimulation of the caeca and midgut is mediated by secretogogues.

(*b*) The vital methylene blue technique, modified from Kuwana (1935), was found to demonstrate the innervation of the *Periplaneta* and *Blattella* alimentary canal. The stock rongalit solution, made up in distilled water and used concentrated, was injected into both species until they were distended. Optimum staining was obtained after 3 hours at room temperature (about 18°C.). The tissues were dissected, immersed in a saturated aqueous solution of ammonium molybdate, and the spreads studied under high dry and oil immersion objectives. The same insects which showed excellent staining of the nerves, nerve endings, and nerve cell bodies in the crop showed no trace of nerves in the caeca. The epithelial cells of both caeca and midgut were, however, outlined in surface view by fine, blue granules which, under high magnification, could be seen to be confined to the region of the striated border. No trace of methylene blue stained bodies could be seen towards the proximal end of the cells. If the caeca are innervated, their nerves must be refractory to methylene blue. An occasional cell body and a few extremely fine nerves were found in the best preparations of the *Periplaneta* midgut. These nerves were entirely superficial and almost certainly innervated only the muscularis. The salivary glands, on the other hand, were extremely well innervated, being completely enveloped by a fine mesh of nerve fibres. While nerve cell bodies could be seen on the adjacent pharynx in this region none was found on the salivary glands. Only an occasional very fine nerve could be found innervating the salivary reservoirs.

In *Blattella* the caeca were devoid of visible nerves, but the midgut was quite conspicuously innervated over its entire length. In contrast, the salivary glands were comparatively poorly supplied with nerves. Thus *Blattella* and *Periplaneta* are quite different in regard to the innervation of their respective midguts and salivary glands. In both cases, however, it was concluded that the nerves to the midgut innervated the muscularis, and could not be concerned with the stimulation of the epithelium. This conclusion was strengthened by the results of the use of the classical silver staining techniques for nerve endings. We obtained successful impregnation of nerves in the crop by the Hortega method, but better results were obtained by Boeke's technique (see Lee, p. 578). Plate 3, Figures 14 and 15, shows sections from the same slide of caecum and crop. Nerves are visible running between and into the epithelial cells of the latter, but there is no trace of them in the caeca. Bodian's protargol method did not demonstrate nerves in *Blattella* or *Periplaneta* although it gave excellent preparations of some other insects.

The above data indicate that the stimulus to secretion of the caecal and probably of the midgut epithelia is not mediated by nervous impulses, in spite of the importance of this type of stimulus in vertebrates. The time relations of the responses support this conclusion.

(*c*) There remains the possibility that a factor, liberated into the haemocoele and carried by the haemolymph, influences the secretion of the midgut epithelium.

We have found in the literature no previous attempt to examine this hypothesis, and we were not able to test it for *Blattella* because of the small size of this species. The following experiments were, therefore, performed on *Periplaneta*.

We considered that the rate of regeneration of midgut cells, as indicated by the mitoses in the nidi, would provide data on the physiological state of the midgut. If the whole midgut of *Periplaneta* is dropped into acetic orcein for ten minutes, and the caeca cut off, their contents and the epithelium can be squeezed on to a slide. The epithelium from each caecum is then covered with a 1 in. by 1 in. coverslip which is pressed down firmly with blotting paper. The number of mitoses in the regenerative nidi can then be counted under a high dry objective and gives a measure of the regenerative activity of the caeca. Plate 5, Figure 26, shows a nidus and mitotic figure. The midgut itself is more difficult to study in

TABLE 15

NUMBER OF MITOSES IN 25 NIDI IN CAECA OF *P. AMERICANA*. EACH FIGURE IS THE MEAN OF 10 COUNTS

Starved 7 days	1 Hour	2 Hours	4 Hours
		Period after feeding artificial food	
2.5	3.7	7.0	4.6
		Period after feeding distilled water	
	5.6	2.9	3.9
		Period after feeding cut potato	
6.5	5.4	9.5	6.3

this way since the regenerative cells are arranged in long anastomosing rows, but the number of mitoses per oil immersion field can be estimated. In each caecum 10 counts are made of the number of mitoses per 25 nidi. In the normal feeding cockroach the number is approximately 8. In an experiment to determine the relation between mitotic index and enzyme production in a series of insects three caeca were used for the estimation of proteinase and three from the same insects for mitotic counts. The results suggested that there is probably a weak inverse correlation between cell replacement by mitotic activity in the nidi and enzyme concentration, although the relationship is not precise. This is rather to be expected, in view of the fact that the enzymes are probably reduced from cells some time after they have undergone mitosis (see previous section).

A lag between stimulus and response was demonstrated in Schlottke's (1937*b*) data on the effects of feeding on enzyme production. Such a lag could not be expected on the basis of a neural mechanism, so an attempt was made to determine whether there was any mitotic response within a few hours to ingested material. The results (Table 15) were variable and showed no significant difference between treatments, indicating that there is probably no detectable stimulation in *Periplaneta* within 4 hours.

These results were, however, suggestive, so experiments were designed to provide evidence for the possibility of the existence of a factor in the blood of a feeding *Periplaneta* which would affect the mitoses in the caeca. Blood from

feeding *Periplaneta* was removed from a neck puncture, sucked into a glass needle, and injected into the coxo-femoral joint of a starved individual. The technique employed by Yeager and Munson (1945) was found satisfactory, but unless the operation was performed rapidly the blood coagulated and clogged the needles before the injection could be completed. Clean needles were employed for each injection. Insufficient blood could be obtained from starved *Periplaneta* for a control to be employed to demonstrate that the effect was not due to a factor in the blood itself unconnected with feeding. The results (Table 16) gave some indication that there may be an effect of the injected blood. But the data are variable and the technique is not easy.

TABLE 16

NUMBER OF MITOSES IN 25 NIDI IN THE CAECA OF *P. AMERICANA*. EACH FIGURE IS THE MEAN OF 10 COUNTS

Normal Fed (*A*) Donors of Blood	Starved 7 Days (*B*)	One Hour after Blood from (*A*) Injected into (*B*)	Two Hours after Blood from (*A*) Injected into (*B*)
7.1	3.7	4.8	4.3

Difference significant at 5 per cent. level, 1.6.
Difference significant at 1 per cent. level, 2.9.

The difficulty of obtaining statistically significant results with *Periplaneta* led to the search for more satisfactory material, and attention was directed to the Coleoptera, in some groups of which the regenerative cells are contained in crypts which extend into the haemocoele. (For a description of the crypts of *Dytiscus*, see Duspiva 1939.) It was found that mitoses could be readily counted in the crypts of *Tenebrio molitor* if small segments of the orcein stained midgut were examined under the oil immersion objective. In the adult *Tenebrio* there are about 3000 crypts. Those of the anterior and mid parts of the midgut are larger than those of the posterior region. A section of crypt from the *Tenebrio* midgut is illustrated in Plate 5, Figure 27.

Tenebrio molitor has the additional advantage over *Periplaneta* that the food passes almost immediately into the midgut, instead of being retained for considerable periods in a storage crop, as it is in the Blattidae.

In all experiments with *Tenebrio,* counts of the number of mitoses in 10 crypts from sections of the gut at the anterior end, the mid part, and the posterior region of the midgut were made. The number of individuals per treatment varied from 2 to 6 in different experiments, but was usually 4.

Firstly, to determine whether the results might be affected by different periods of the day at which counts would be made, *Tenebrio* were fixed every 2 hours from 8 a.m. to 4 p.m. The results (Table 17) indicated the absence of a detectable diurnal rhythm.

Some idea of the frequency of mitoses and the time required for the mitotic cycle was obtained from the study of colchicine injected *Tenebrio.* Normal insects were injected with about 0.1 ml. of 0.5 per cent. colchicine in 0.7 per cent. saline solution. This produced toxic symptoms within one hour and the mitoses in

many of the caeca were difficult to count. After 2 hours many of the crypts were very reduced in size. The injection of saline alone produced no discernible effects.

TABLE 17

MEAN NUMBER OF MITOSES IN EACH OF 30 CRYPTS FROM THE MIDGUT OF *TENEBRIO MOLITOR*. ALL INSECTS FED IN SEPARATE CONTAINERS

8 a.m.	10 a.m.	Noon	2 p.m.	4 p.m.
3.9	4.2	4.0	4.5	4.0

TABLE 18

MEAN NUMBER OF MITOSES IN EACH OF 30 CRYPTS FROM THE MIDGUT OF *TENEBRIO*

Normal Fed	Time after Injection of Colchicine				
Controls	15 min.	30 min.	60 min.	120 min.	240 min.
4.5	3.3	5.6	4.2	7.2	8.6

From these data it will be seen that, under the conditions of the experiment, mitoses occurred at the rate of about 1 per hour. Further, it can be calculated by the method employed by Leblond and Stevens (1948) that the duration of mitosis is about 2 hours. This is slow compared with the mitotic rate in *Drosophila*, for example (cf. Sonneblick 1948), but is of the correct order of magnitude. (It should be mentioned that attempts to use the colchicine method on *Blattella* or *Periplaneta* were not successful. In these insects colchicine decreases rather than increases the number of mitoses, indicating that it does not inhibit the functioning of the spindle, but merely appears to poison the mitotic process. This is unexpected in view of the success of a number of authors to inhibit spindle formation in orthopteran gametogenesis.)

The results of various periods of starving and subsequent feeding on *Tenebrio* are given in Table 19.

TABLE 19

MEAN NUMBER OF MITOSES IN EACH OF 30 CRYPTS FROM THE MIDGUT OF *TENEBRIO*

Normal Fed	Period of Starvation			
	24 hr.	48 hr.	72 hr.	96 hr.
3.8	3.5	3.3	2.6	2.5

Starved 96 Hours, then Fed Potato

½ hr.	1 hr.	1½ hr.	2 hr.	2½ hr.	3 hr.	4 hr.
2.7	2.6	2.9	3.1	3.5	3.4	4.1

Difference significant at 5 per cent. level, 0.71.

It is evident that the effects of starvation are marked after 3 days, and that the effect of feeding increases up to 4 hours. If the beetles are allowed to feed for 4 hours only and are then examined daily, it is found that the effect of feeding persists for 24 hours but is not present after 48 hours (Table 20).

TABLE 20

MEAN NUMBER OF MITOSES IN EACH OF 30 CRYPTS FROM THE MIDGUT OF *TENEBRIO MOLITOR*

Normal Fed	Starved 3 Days	Starved, then Fed Potato			
		4 hr.	24 hr.	48 hr.	72 hr.
3.3	2.4	4.1	4.4	3.3	3.3

Difference significant at 5 per cent. level, 0.81.

The effects of feeding gelatine or starch, rather than potato, showed that both diets had a similar effect on the number of mitoses (Table 21). While stimulation is induced by either diet the histological effects of these two diets are dissimilar. The intercryptal epithelium is normal following starch, but greatly reduced following gelatine feeding.

TABLE 21

MEAN NUMBER OF MITOSES IN EACH OF 30 CRYPTS FROM THE MIDGUT OF *TENEBRIO MOLITOR*

Controls Normal Fed	Starved 48 Hours	Starved 48 Hours, then Fed Gelatine			Starved 48 Hours then Fed Starch		
		½ hr.	2 hr.	6 hr.	½ hr.	2 hr.	6 hr.
4.6	2.9	3.5	2.1	3.7	3.4	2.1	3.5

Difference significant at 5 per cent. level, 0.84.

It will be observed that the period of maximum mitotic activity follows some hours after feeding. However, this is the normal *response* which is a slow one, and the stimulus may be expected to have exerted its effect some time previously. The critical experiment to determine the presence in the blood of a factor affecting midgut mitoses, was made by comparing the effect of blood from starved or from fed *Tenebrio* when injected into starved individuals.

After trying several methods the injections were most satisfactorily performed by allowing blood, obtained from an adult *Tenebrio* by puncture of the intersegmental membrane in the cervical region, to form a drop on a clean microscope slide. This drop, of approximately 50λ, was immediately drawn into a fine glass needle into which the shortened shank of a No. 20 hypodermic needle was fixed by shellac. The advantages of a glass needle are: (1) that the amount of blood can be regulated visually, and (2) that there is no question of the completeness of the injection. Injection was made into the haemocoele through the soft abdominal tergum normally concealed beneath the elytra.

Blood from a fed insect causes a slight rise in the number of mitoses in the midgut crypts after 30 minutes. Blood from a starved insect causes no such rise (Table 22). This experiment was repeated with the same results, and together they strongly suggest the presence of a factor in the blood of fed, but not of starved, *Tenebrio*, which affects the number of mitoses in the midgut crypts.

After normal feeding, the response does not become evident for about 1½ hours and increases up to 2½ hours (see Table 19 above). After the injection

of blood from a fed individual, however, the response is more rapid, and, in addition, is transient as is shown in two separate experiments (Table 23).

TABLE 22

MEAN NUMBER OF MITOSES IN EACH OF 30 CRYPTS FROM THE MIDGUT OF *TENEBRIO MOLITOR*

Normal Fed Controls (*A*)	Starved 3 Days (*B*)	Blood from (*B*) Injected into (*B*) after ½ hour	Blood from (*A*) Injected into (*B*) after ½ hour
3.7	2.3	2.2	2.9

Difference significant at 1 per cent. level, 0.5.
Difference significant at 0.1 per cent. level, 0.7.

TABLE 23

MEAN NUMBER OF MITOSES IN EACH OF 30 CRYPTS FROM THE MIDGUT OF *TENEBRIO MOLITOR*

Fed Donors	Starved 2 Days	Time after Injection of Blood from Fed Donors into Starved Individuals			
		½ hr.	1 hr.	2 hr.	4 hr.
3.3	2.3	3.6	2.6	1.6	2.8

Difference significant at 5 per cent. level, 1.0.

Controls Starved 4 Days (*A*)	Half an hour after Injection of Blood from Fed Insects into (*A*)	One hour after Injection of Blood from Fed Insects into (*A*)
2.9	3.9	2.3

Difference significant at 5 per cent. level, 0.4.

There is no question, therefore, that the response following injection differs from that of the normal insect fed after a period of starvation. In spite of this there is strong evidence that a factor in the blood of a normal feeding *Tenebrio* is able to affect the rate of mitosis in the midgut crypts. We shall refer to the factor as the "midgut regeneration stimulating factor" (M.R.S.F.).

It is clear that the injection type of experiment is not as well suited to an investigation of this kind as parabiotic experiments, but neither *Periplaneta* nor *Tenebrio* is amenable to such operative techniques.

(*d*) *Source of the M.R.S.F.*—A number of sources for the production of the M.R.S.F. may be considered: (i) the corpora allata and cardiaca, (ii) the salivary glands, and (iii) the epithelial cells of the midgut.

(i) The innervation of the corpora allata and cardiaca differs from that of most organs in that nerves are received from the supraoesophageal ganglion and also from the hypocerebral ganglion which is part of the stomodeal nervous system, and is in neural connection with the frontal ganglion. This latter ganglion innervates the pharynx and buccal cavity, and is in a position to receive sensory

impulses arising as a result of feeding. It was thought, therefore, that the corpora allata or cardiaca, which are obviously endocrine organs, may be involved in the production of a M.R.S.F. This hypothesis gains credence from the suggestion that the corpora allata are involved in moulting and metamorphosis (Wigglesworth 1939), and both these processes are usually accompanied by great increases in mitotic activity in the midgut (Henson 1946). Plate 3, Figure 18, illustrates the results of this mitotic activity in a moulting nymph of *Blattella*.

It is known that fuchsinophilic secretory granules occur at times in the corpora cardiaca. But no correlation could be found between their presence in *Periplaneta* and the nutritional state of the insect. It would appear that the corpora cardiaca have other functions, but no evidence has been brought forward that they are active in the production of the M.R.S.F. Wigglesworth's (1948) indication that food is digested more rapidly in *Rhodnius* females under the influence of the hormone affecting egg maturation may be significant in this connection.

(ii) The salivary glands are, like the corpora allata and cardiaca, innervated from the stomatogastric nervous system. The glands contain two cell types, but it is not known whether they are both secretory and whether they both produce constituents of the saliva.

(iii) The cells of the alimentary tract itself may produce the M.R.S.F., since in vertebrates they produce gastrin, etc., but data on the origin of the M.R.S.F. in insects await further research.

Summary.—It will be clear from the above experiments that secretion in the insect midgut may be initiated either by the action of secretogogues or by a hormone. Evidence for the former is not yet available, but suggestive evidence is presented in favour of the latter hypothesis. A neural stimulus does not seem to be involved.

VIII. The Role of the Alimentary Canal in Absorption

Ingested food may be either in a condition for direct absorption through the gut wall, or may be digested to such a state by the enzymatic processes discussed in the previous sections. The permeability of various parts of the alimentary tract of any insect has never been fully investigated and it is not known, for example, whether proteins may be absorbed directly, or whether they must be completely broken down to amino acids before absorption.

Many authors have claimed that fat is absorbed directly through the cockroach crop (Abbott 1926; Petrunkewitch 1900; Scharrer 1947), though how this occurs is still obscure. The tracheae were at one time considered to play an active part but this theory has been discounted (Schluter 1912). Absorption of fat also occurs in the midgut and caeca (Scharrer 1947).

The absorption of inorganic ions, for example, those of iron, has likewise been widely studied. In fact, a number of conclusions regarding the absorptive cells in insects have relied on the localization of iron absorption as their sole evidence.

Our studies have indicated, not only that different substances may be absorbed in different regions, but also that considerable differences may be found even between insects belonging to a single family. Data will be presented on absorption of the following substances:

(*a*) Cations, Fe^{++}, Fe^{+++}, Ba^{++}, Sr^{++}, Cu^{++}.

(*b*) Anions, $H_2PO_4^+$.

(*c*) Ascorbic acid.

(*a*) *Cations.*—The distribution of a number of cations in insect tissues has been studied in this laboratory by D. F. Waterhouse, who has kindly permitted the quotation of his unpublished results (for methods, see Waterhouse 1940*b*). The distribution of these ions in the alimentary tracts of *Blattella* and *Periplaneta* is given in Table 24.

TABLE 24

DISTRIBUTION OF ANIONS IN ALIMENTARY CANAL OF *PERIPLANETA* AND *BLATTELLA*

	Crop	Caeca	Midgut	Anterior Hindgut	Mid Hindgut	Rectum
Periplaneta						
Normal food	–	Fe^{+++}	–	–	Fe^{+++}	–
Metal enriched food	–	$Fe^{+++}Fe^{++}$	Fe^{+++}	–	$Fe^{+++}Fe^{++}$ Ba^{++}	–
Blattella						
Normal food	–	–	–	–	$Fe^{+++}Fe^{++}$	–
Metal enriched food	–	Fe^{+++}	Fe^{+++}	–	$Fe^{+++}Fe^{++}$ Ba^{++}	–

It is to be especially noted that absorption in the hindgut is confined to a band of cells, not as clearly defined as the iron cells of *Lucilia* (Waterhouse 1940*b*), but nevertheless to a quite well-defined zone. Ferric iron is invariably found in high concentration in this region of both *Periplaneta* and *Blattella*. This ion is usually found also in the caeca in *Periplaneta*, but not in *Blattella*.

Absorption of copper could not be demonstrated either in *Periplaneta* or *Blattella*. When very large amounts are ingested from enriched foods barium may be detected in the caeca of the midgut of *Periplaneta* and of *Blattella* and strontium in the caeca of *Periplaneta* and in the hindgut of *Blattella*.

These data are more complete than those of previous authors who have studied the absorption of cations in the insect gut. It is clear that there are marked differences even between insects of a single family, but the reasons for these differences are still quite unknown.

(*b*) *Anions.*—We attempted to study the absorption of phosphate by feeding the dibasic sodium salt. When the monobasic salt was added to the diet the roaches died, although Lindsay and Craig (1942) used it without these effects in their studies of the absorption of radiophosphorus. Equal quantities of the sodium dihydrogen phosphate and the artificial diet were mixed thoroughly

and moistened with a small amount of water. Sections were cut and treated in the usual manner for controls for the Gomori alkaline phosphatase technique. In normal *Blattella* no phosphate is demonstrated in the midgut by this method and only a small amount in the hindgut. After feeding on the above diet for four days there are small accumulations in the distal regions in the caecal epithelium, a smaller, but still detectable amount in the same regions of some midgut epithelial cells (mainly in the anterior half of the midgut), and very considerable quantities in the epithelium of some regions of the hindgut. Since, however, there is frequently phosphate in these hindgut cells it is difficult to determine whether it is absorbed in this region. Lindsay and Craig's (1942) data suggest that it is not.

If *Blattella* is fed *d*-glucoascorbic acid, the amount of phosphate in the hindgut is greater than in normal insects (cf. Plate 3, Fig. 19, and Day 1949*a*).

(*c*) *Ascorbic Acid.*—Prior to the recent investigation by Day (1949*b*) no studies had been reported on the histological localization of ascorbic acid in insect tissues. It is now known to occur in a wide variety of tissues and in some tissues of practically all insects studied. In the *Blattella* gut ascorbic acid occurs normally only when the insects are fed on a diet containing it. If the culture is maintained on an ascorbic acid free diet and then transferred to a diet rich in it, differences in its absorption in different regions of the midgut become apparent. Since details have been published recently (Day 1949*b*) it is sufficient to mention that both midgut and caeca absorb ascorbic acid in *Periplaneta* and *Blattella*. The hindgut does not appear to be involved in the absorption of this material.

It has been indicated that the caeca of *Blattella* would seem to be better suited to the function of secretion than to that of absorption. However, they absorb ascorbic acid, and there is other evidence that they may be more efficient in absorption than might appear: (i) small quantities of phosphate and iron may also be absorbed by the caecal epithelium, (ii) when dyes are introduced into the alimentary canal, as in the hydrogen ion experiments previously considered, the dyes always fill the caecum lumen, and (iii) the absorption of the products of starch digestion as evidenced by the dark cells following the Mann-Kopsch Golgi technique is as conspicuous in the caeca as in the midgut (Plate 5, Fig. 30).

The two general conclusions from these studies on absorption are: (1) that different substances are absorbed in different regions of the gut of a single species of insect, and (2) that the same substance may be absorbed in two different regions of the gut of different species even though they be closely related. Gresson's (1934) conclusion (based on the use of iron saccharate) that the forepart of the midgut of *Periplaneta orientalis* is mainly secretory while the posterior part is mainly absorptive, is not substantiated with *P. americana* or *B. germanica.*

IX. The Function of the Alimentary Canal in Intermediary Metabolism

The examination of some of the substances concerned in intermediary metabolism cannot provide an indication of the multitudinous processes occurring following the absorption of foodstuffs. We have studied the distribution of glycogen, alkaline phosphatase, and lipase in the *Blattella* gut, as a preliminary essay in this field.

(*a*) *Glycogen.*—No study of the distribution of glycogen in the cockroach gut is known to us, and there are in fact a number of disagreements in the literature regarding its presence in the insect alimentary canal (see Yeager and Munson 1941; Babers 1941, for reviews of the literature). Difficulties with methods are undoubtedly the cause of these differences of earlier workers, and indeed the Bauer, Best's carmine, and iodine methods do give capricious results even in the hands of experienced investigators. A recent technique (Gomori 1946) in which tissues are incubated in silver methenamine solution, has given uniformly good results with *Blattella* tissues, and may be considered specific if studied in conjunction with controls treated with salivary ptyalin. (The only structure in the insects we have studied which still gives a positive reaction in saliva treated controls is the peritrophic membrane of *Lucilia cuprina* larvae.)

An unexpected distribution of glycogen in the *Blattella* midgut has been found. While a small amount is present throughout the midgut (Plate 2, Fig. 12), a ring of cells near the anterior end in the region of the proventriculus is particularly rich in glycogen (Plate 2, Fig. 11). The distribution in the cells is characteristic. The greater part of the glycogen is just above the nuclei. There is an area of lower concentration below the striated border and the latter gives an intensely positive reaction. Some of the cell inclusions apparently contain some glycogen. It is particularly interesting that the cells rich in glycogen are in the same region as those showing the argentophil granules described above. However, the distribution in the cells is different and the two substances are obviously not the same. Wigglesworth (1942) has observed glycogen (iodine method) in some clearly defined regions of the *Aedes* midgut, but most of it was in the posterior half.

From a study of the midguts of *Blattella* which have been either fed a normal diet, starved for 3 days, fed starch 3 days, or fed gelatine 3 days, it is clear that the glycogen in the midgut is fairly constant in quantity. Yeager and Munson (1941) conclude that "the gut-cell glycogen varies with the intake of carbohydrate," but our data do not substantiate this hypothesis. In fact the starch fed insects had conspicuously less glycogen in the midgut than the other treatments (Plate 2, Fig. 13). Starving for 3 days does not appear to deplete the stainable glycogen of the midgut to any observable extent.

(*b*) *Alkaline Phosphatase.*—The occurrence in the gut of phosphatase with an optimum at pH 5.0 has been mentioned on p. 181. Drilhon and Busnel (1945) have reported that phosphatase active at pH 9.5 is present in the alimentary tract of certain insects, and Bradfield (1946) has located this enzyme histochemically in the midgut of *Cossus*, using the technique of Gomori (1939). The results of

the application of this method to the tissues of a number of insects have recently been reported (Day 1949*a*). In *Blattella* the enzyme is present in the foregut, hindgut, and rectum, but it is particularly concentrated in the circular muscles at the anterior end of the midgut. The functional significance of these results is by no means clear. A variety of diets and experimental treatments failed to influence the distribution and this fact validates the study of the sites of absorption of phosphate as described in the previous section.

(*c*) *Lipase.*—Of the digestive enzymes, a method has been described only for the detection of lipase (Gomori 1946). This method has been used on *Blattella* with success. The enzyme appears to be labile, although satisfactory preparations have been obtained when all operations were performed with a minimum of delay.

In normal *Blattella,* lipase is found to be fairly uniformly distributed through all midgut epithelial cells, but it is perhaps slightly more abundant in those of the regenerative nidi than in the mature cells. It was not found in the epithelium of the crop, which is perhaps surprising in view of the evidence that the crop can absorb fat (see Section VIII). It is more abundant in the hindgut. In this location, but not in the midgut, the intensity of the reaction appears to be somewhat lower following a prolonged fat diet.

X. Histopathology of Insecticides

It may be expected that the study of the effect of appropriate substances on the alimentary canal will elucidate certain details of the physiology of this organ. We have attempted to investigate the histopathology of insecticides with particular attention to the incipient changes in the midgut epithelium. First it was necessary to differentiate between necrosis and effects which are attributable to the specific action of the insecticides. We therefore studied a series of *Blattella* killed by (*a*) decapitation, (*b*) heat, (*c*) carbon dioxide, and (*d*) cyanide.

(*a*) Twenty-four hours after decapitation the insects may still move their appendages violently when stimulated, but conspicuous changes are found in the midgut. These probably represent incipient necrotic changes and are characterized by increasing numbers of cytoplasmic inclusions, increasing nuclear acidophily, and finally destruction of the striated border with cytolysis (Plate 4, Fig. 20).

(*b*) After killing by heat (56°C. for 15 min.) the cells of the midgut epithelium become separated and present a characteristic form of granular disintegration of the cytoplasm with clumping of the chromatin leading to karyorrhexis (Plate 4, Fig. 21). After 60 minutes at the same temperature the striated border is still conspicuous, but the nuclei have lost their ability to stain differentially.

(*c*) Carbon dioxide acts very rapidly as an anaesthetic to *Blattella,* but they recover quickly even after a 3 hour exposure to it. After a 6 hour exposure only a small percentage recovers. Insects fixed after this treatment show only slight cytoplasmic vacuolization in the anterior part of the midgut. However, in

the posterior part of the midgut cytoplasmic globules, expressed through the striated border, increase in number and may become very numerous (Plate 4, Fig. 23). The globules frequently are vacuolated. Cell breakdown may become so extensive as to produce a crenulated appearance in the epithelium like that of *Periplaneta.*

(*d*) *Blattella* is rapidly killed by exposure to potassium cyanide. After a 3 hour exposure recovery never occurs. The midgut of such an insect is considerably damaged. Cytoplasmic globule formation is frequent in the anterior and posterior parts of the epithelium and whole cells may be discharged into the lumen (Plate 4, Figs 24 and 25). The globules are more deeply staining than those produced by the effects of carbon dioxide.

With these preliminary data the histology of the epithelium of *Blattella* treated with several insecticides was examined. The insecticides were incorporated in the artificial diet, and although some food was ingested they could have been absorbed through the cuticle. Three types of effects were observed: (i) marked epithelial breakdown by the arsenic compounds, (ii) slight epithelial changes by chlordane, BHC, and "Ryanex,"* and (iii) almost no effect by DDT.

(i) Sodium arsenite was studied in *Periplaneta* in relation to so-called "hypersecretion" (see Section V). The effects in *Blattella* are essentially the same. One hour after injection a number of the oldest epithelial cells show the early signs of cytoplasmic globule formation (Plate 3, Fig. 17), while after 2 hours many cells have discharged their cytoplasm, resulting in some places in the complete disorganization of the epithelium. It should be noted especially that it is the oldest cells farthest from the nidi which degenerate first.

(ii) Poisoning by chlordane results in the loss of the striated border, separation of some epithelial cells, and incipient cytolysis. Conspicuous dark brownish deposits are found in the distal region of the epithelial cells and acidophilic inclusions are frequent in the cytoplasm proximal to the nucleus. BHC and "Ryanex" produce vacuoles in the cytoplasm, the circular muscles contract and become conspicuous but otherwise the midgut is unaffected (Plate 4, Fig. 22).

(iii) DDT produces a tendency towards cytoplasmic globule formation but has remarkably little effect on the midgut.

All insecticides examined, other than sodium arsenite, appear to exert their lethal action with little visible change in the midgut, even though this organ of *Blattella* undergoes striking histological changes. This fact gives a special interest to those substances which do produce distinctive changes in the midgut. Sodium arsenite, for example, certainly seems to hasten cell breakdown in *Blattella* as it does in *Vanessa* and *Locusta* (Pilat 1935) and *Prodenia* (Woke 1940). Further work in this field of insect pathology is most desirable.

* "Ryanex" described as "100% production grind for dusts" kindly supplied by Merck & Co. Inc.

XI. Discussion

Some of the data presented in the previous sections are at variance with current concepts and others represent new aspects of the processes of digestion in insects. These will be discussed in the following paragraphs.

(*a*) It is sometimes considered that *Blattella* and similar insects are discontinuous feeders, in contrast to many other insects, particularly larvae, which are able to ingest almost continuously. Crowell (1943) has shown that even in the larva of *Prodenia* there are several periods of non-feeding per hour which almost equal the actual feeding time. But the alimentary tract is certainly always kept full of food. Our observations on the rate of passage of foodstuffs through the gut of *Blattella* indicate that in this species also the midgut is normally kept full of food and a mechanism for the stimulation of secretion at the time of feeding may therefore not be required. The continued production of digestive enzymes during starvation and the damaging effect of 3 days' starvation on *Blattella* provide further evidence for the fact that this species, while it may ingest food in a discontinuous manner, is not a discontinuous feeder in the usual sense. Food passes through the alimentary tract of *Blattella* at a considerably greater rate than it does through that of *Periplaneta* (Snipes 1938).

(*b*) The reducing conditions demonstrated in the hindgut of *Blattella* would be presumably more difficult to maintain in the presence of an abundant supply of oxygen. It is, therefore, interesting to note the poorer tracheation of the large intestine in comparison with that of the midgut (Plate 5, Fig. 29) and of the rectum, in the contents of which the reducing conditions are not maintained. The information is not yet available for a more detailed comparison between redox conditions and tracheation in the insect gut, but the problem would be worthy of future study.

(*c*) The correlation of histological structure with secretory activity is not the simple situation which has frequently been described in the insect midgut. The precise nature of many cell inclusions is still unknown, but there is no justification for describing "secretion" or "absorptive" inclusions without more information than can be obtained by histological or histochemical techniques. The hypothesis of Shinoda (1927) that the midgut epithelium of *Blattella* produces enzymes normally by merocrine secretion, but by holocrine secretion after starvation, has been shown to be untenable. In its place we have a concept of the replacement of worn out cells by regeneration from the nidi, while the main burden of both secretion and absorption is carried on by the epithelial cells previously referred to as "resting." This hypothesis is strengthened by the critical study of the cytology of the midgut epithelium and the observation that the cells which degenerate are always the old cells far from the regenerative nidi. Henson (1929) and certain earlier authors, on morphological evidence, arrived at similar conclusions.

Steudel (1912) concluded from his studies that the absorption of iron and fat occurred in the epithelial cells previously described as resting. We concur with this opinion and from our enzyme studies we are able to go one step further,

for we have proved that cells in this same condition are also active in secretion. The conclusion follows that in *Blattella,* secretion and absorption go on simultaneously from and into the same cell. This is in distinction to a widely accepted hypothesis that epithelial cells go through a cycle, being first absorptive and later secretory in function. Gresson (1934) concludes that both secretory and absorptive cells occur in the caeca and in the anterior part of the midgut, but maintains that secretion and absorption never take place in the same cell. Steudel (1912), in common with the majority of authors, described secretory stages which are probably degenerating cells.

The rapidity of the renewal of the insect midgut epithelium indicates the intense activity characteristic of this tissue. Few other animal tissues are provided with such well-defined nidi of embryonic cells. But this short life of the midgut epithelium is not confined to insects, for Leblond and Stevens (1948) have calculated that the duodenal epithelial cell of the albino rat lives only about 36 hours.

The general conclusions from these data are that both secretion and absorption can go on without visible cytological changes, and that both processes go on in epithelial cells as soon as they reach the lumen of the gut. We have demonstrated an approximate inverse relationship between the mitoses in the regenerative nidi and proteinase activity. We would not expect this relationship to be close since we have produced strong evidence that the most actively secreting cells are considerably older than those which undergo mitosis. There are very few data in the literature on the rate of regeneration of the midgut. Hodge (1937) reported that the number of mitoses per section almost doubled in *Melanoplus* (6.4 to 11.6) when the insects were fed on an incomplete diet as compared with a satisfactory one. These figures are difficult to evaluate since Hodge reported that the number of nidi increased on the unsatisfactory diet. However, they suggest compensatory hypertrophy of the epithelium attempting to cope with the unsatisfactory diet.

(*d*) The demonstration of digestive enzymes in the crop by both Abbott (1926) and Schlottke (1937), and confirmed in our experiments, raises the question of their origin. Our study of the time relationship between the appearance of proteinase in the crop in relation to its secretion into the caeca and midgut confirms the theory that the enzymes are regurgitated. A mechanism for this is obvious in the striking movements of the oesophageal invagination which appear to result in a sucking action in the isolated alimentary canal in saline. Wigglesworth (1930) has stressed the hypothesis that the function of this invagination is to permit the peritrophic membrane to arise anteriorly to the point of entry of the food into the gut. Moreover, he maintained that the peritrophic membrane is "pressed" between the invagination and the midgut wall. This may well be true in, for example, Diptera and in Dermaptera where there is a conspicuous annular ring on the posterior edge of the invagination which might well act as a press. But it certainly does not appear to be the case in *Blattella* or in *Periplaneta.* In these Orthoptera the distance between the

oesophageal invagination and the midgut epithelium is very many times the thickness of the peritrophic membrane. Moreover, the invaginated organ undergoes normal writhing movements which would render it unsuitable as a press. Consideration of these two points, together with the laminated structure of the peritrophic membrane, suggests that the complex structure of the invagination serves other purposes, and one of these is undoubtedly to regurgitate digestive enzymes from the midgut into the crop. However, the morphology of the organ is not dissimilar in *Periplaneta* and in *Blattella*. Yet we have shown that the *Periplaneta* crop may contain proteinase while it does not appear to be regurgitated into the *Blattella* crop. Certainly some additional function must be ascribed to the oesophageal invagination. A function, and an important one, of the crop is to become dilated with air during moulting to enlarge the cuticle, before hardening occurs. A study of the crop in moulting insects reveals that it is the main organ distended with air and that there must be an effective mechanism preventing the air passing into the midgut. The oesophageal invagination must also serve this purpose.

(*e*) While we have presented evidence for the presence in the blood of a fed *Tenebrio* of a factor resulting in increased mitoses in the midgut crypts, we have no data on the relationship between mitoses and enzyme formation in this species.

The evidence for the lack of a neural mechanism for the stimulation of secretion is anatomical. But it should be stressed that the innervation of the gut varies considerably, even within the Orthoptera. Nesbit (1941) has shown that the stomodeal nervous system may innervate the caeca in some species and even the midgut in, for example, *Rhomalea*. The study of species sufficiently large to permit artificial stimulation of these nerves will be necessary to decide whether they are simply motor in function.

It is not expected that all insects (continuous feeders, for example), would have a mechanism for stimulating midgut activity. A discontinuous feeder amenable to parabiotic experiments should provide suitable material for the continuation of the study of the problems associated with the initiation of secretion.

(*f*) During the course of these investigations it has become apparent that there are marked differences between the details of the digestive physiology of the two cockroaches, *Blattella* and *Periplaneta*. These differences are as follows: (i) the innervation of the midgut and salivary glands; (ii) the arrangement of the regenerative nidi in the midgut; (iii) the distribution of glycogen; (iv) the distribution of invertase and proteinase production in the midgut; (v) the presence of proteinase in the crop; (vi) the period during which food remains in the crop (compare our results with those of Snipes 1938); (vii) the extent of enzyme concentration changes during starvation (compare our results with those of Schlottke 1937*b*); and (viii) the presence of invertase in the *Blattella* salivary glands and its absence in those of *Periplaneta* (Wigglesworth 1927).

The few data on each of the aspects of the physiology of digestion which we have presented above, when integrated with previous work, do not yet outline a comprehensive theory of the digestive processes of *Blattella*. They serve rather to indicate the lacunae in our knowledge, but advances are difficult and can only be expected as specialized microtechniques become available.

XII. Acknowledgments

The work described in this paper was carried out as part of the research programme of the Division of Economic Entomology, C.S.I.R. The authors are greatly indebted to many of their colleagues for assistance in the preparation of this paper, and especially to A. T. James, Section of Mathematical Statistics, C.S.I.R., for performing the statistical analyses.

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EXPLANATION OF PLATES 1-5

PLATE 1

Midgut of *Blattella germanica.*

All photomicrographs were taken with Zeiss Ibso attachment under oil immersion x 96 objective, x 8 ocular, magnification x 580. Sections 10 microns. Alcoholic Bouin fixation and Mallory's Triple stain unless otherwise specified.

Figs. 1-4.—Longitudinal sections of one midgut, showing changes in cell size and shape in different regions of the gut. Figure 1 is in the region of the stomodaeal invagination. Note cytoplasmic granules. Figure 2 slightly posterior to the stomodaeal invagination. Note the characteristic arrangement of the cells in the regenerative nidus. Figure 3 is about two-thirds the length of the midgut from the oral end; and Figure 4 is just anterior to the point of entry of the malpighian tubules. Note shorter striated border and laminated peritrophic membrane in Figures 3 and 4.

Figs. 5, 6.—L.S. midgut; Bodian technique. Figure 6 shows conspicuous argentophil inclusions, mainly distal to the nucleus, but some proximal to it. Figure 7 is slightly caudad to Figure 6 and shows the complete absence of argentophil inclusions, and of the dense band just beneath the striated border. The indentations at the proximal side of the nuclei, characteristic of alcoholic Bouin fixation, are conspicuous.

PLATE 2

Details as for Plate 1.

Fig. 7.—Midgut of individual starved 3 days; showing cytoplasmic globules expressed through striated border. The high columnar epithelium is characteristic of individuals lacking gut contents.

Fig. 8.—Midgut of individual starved 2 days. A nucleus and surrounding cytoplasm are extruded into the midgut lumen. This is rarely found in fed insects.

Figs. 9, 10.—Effects of feeding. Fig. 9.—The active epithelium associated with absorption and high enzyme output, following starch ingestion for 3 hours. Note the relatively large number of cells in the regenerative nidi. The epithelium is stretched owing to the bulk of the diet. Fig. 10.—After feeding gelatine for 3 hours. The vacuoles distal to the nucleus are similar to those following ingestion of distilled water. Since gelatine is less bulky than starch the gut is less distended and the epithelial cells are more columnar.

Figs. 11-13.—Glycogen (Gomori technique) in *Blattella* midgut. Striated border is intensely stained. Fig. 11.—Normal individual – glycogen rich region. Fig. 12.—Normal individual – glycogen poor region. Note positive cytoplasmic granule. Fig. 13.—Starch fed individual – glycogen rich regions. Note less glycogen than in Figure 16.

Plate 3

Alimentary canal of *Blattella* and *Periplaneta* – various techniques.

Fig. 14.—T.S. *Blattella* caeca, Boeke technique. No trace of nerves innervating the epithelial cells. x 580.

Fig. 15.—L.S. crop on the same slide, showing nerves (at *A*) running between epithelial cells. x 580.

Fig. 16.—T.S. *Periplaneta* caecum, one hour after injection of sodium arsenite. Incipient cell breakdown visible at (*A*). x 355.

Fig. 17.—The same after 2 hours – "hypersecretion" evident at the apices of most groups of cells. x 205.

Fig. 18.—L.S. *Blattella* midgut. At time of last ecdysis, but before there are exernally visible signs of moulting. The nymphal epithelium is greatly vacuolated. Beneath it there is a layer of horizontally disposed cells, and beneath that is the adult midgut epithelium of which the cells are not yet mature, and many of them are in mitosis. x 355.

Fig. 19.—L.S. small intestine of adult *Blattella* fed *d*-glucoascorbic acid. Gomori phosphate stain, showing considerable deposit of inorganic phosphate in these cells. x 580.

Plate 4

Histopathology of *Blattella* midgut. Alcoholic Bouin fixation and Mallory's Triple stain. All x 580.

Fig. 20.—Twenty hours after decapitation. Note incipient necrosis increased cytoplasmic inclusions. Also apparent in the original is nuclear acidophily and incipient destruction of the striated border.

Fig. 21.—The effect of heat (15 minutes at 57°C.). Note separation of the epithelial cells, marked basophilia, and granular cytoplasm.

Fig. 22.—The effect of the insecticide, "Ryanex"– the production of conspicuous vacuoles in the cytoplasm.

Fig. 23.—The effect of 6 hours exposure to carbon dioxide. Posterior part of the midgut showing incipient cytolysis and many cytoplasmic granules expressed through the striated border. Note crenulation of epithelium.

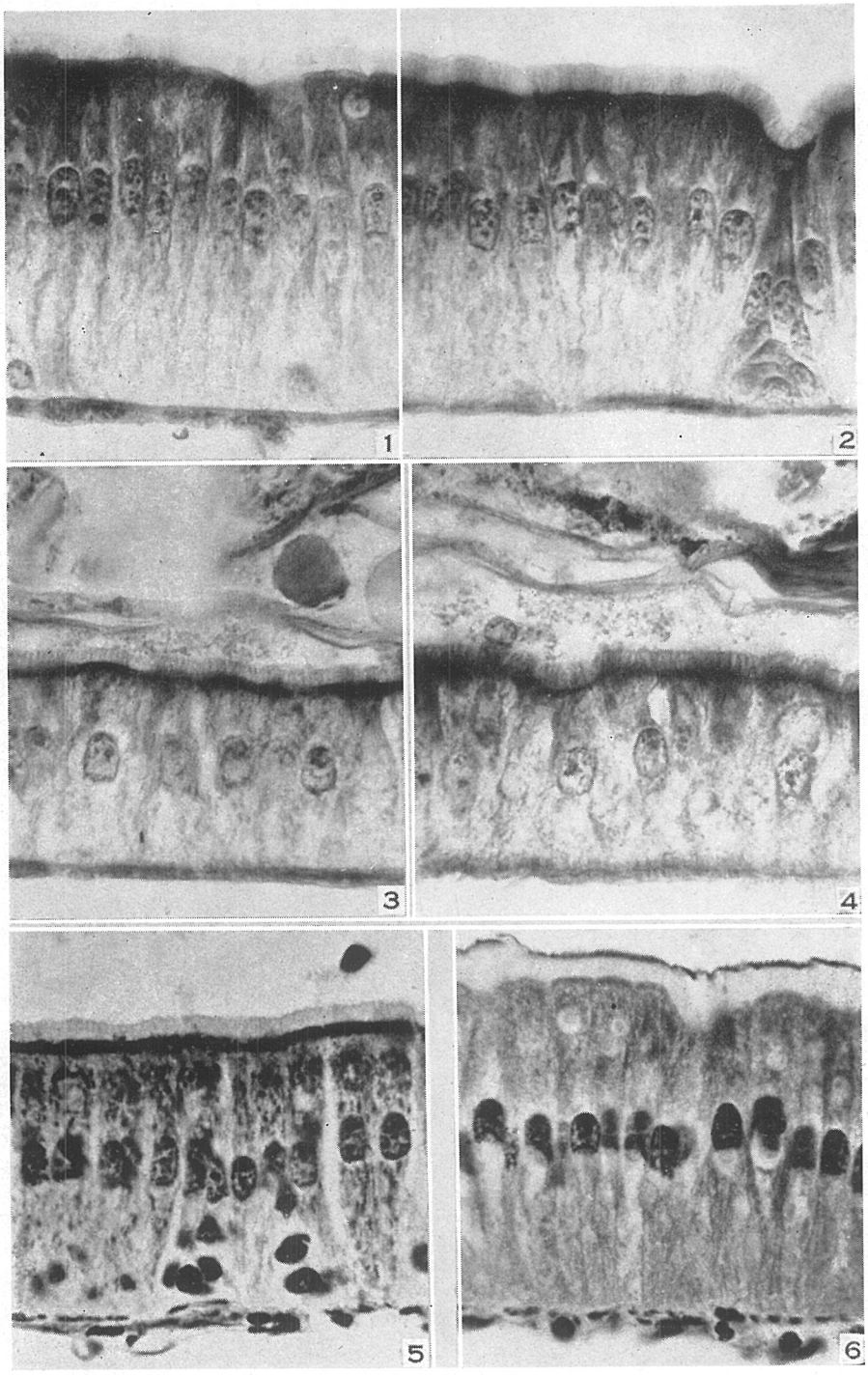
Fig. 24.—The effect of 3 hours exposure to potassium cyanide fumes. Conspicuous cytoplasmic globule formation and chromatic granules in the cytoplasm.

Fig. 25.—L.S. of a caecum of the same insect. Epithelial cells distorted towards midgut attachment of caecum. Cytoplasmic fragments, some containing nuclei, abundant in lumen.

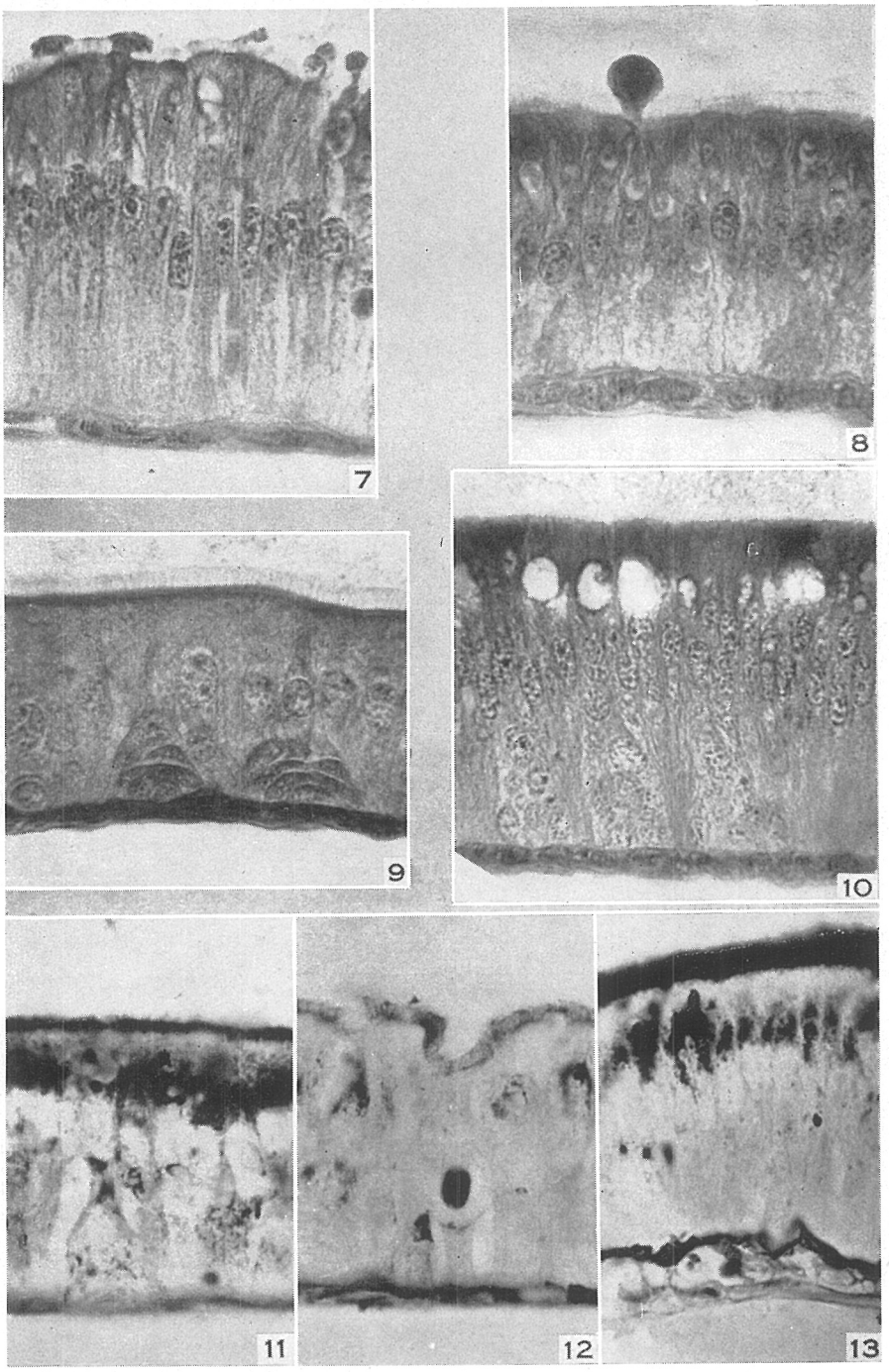
Plate 5

Alimentary tracts of *Periplaneta, Blattella,* and *Tenebrio* – various techniques.

Fig. 26.—Regenerative nidus from spread of *Periplaneta* caecum, stained in acetic orcein, and showing mitotic figure.



Day and Powning.— A Study of the Processes of Digestion in Certain Insects



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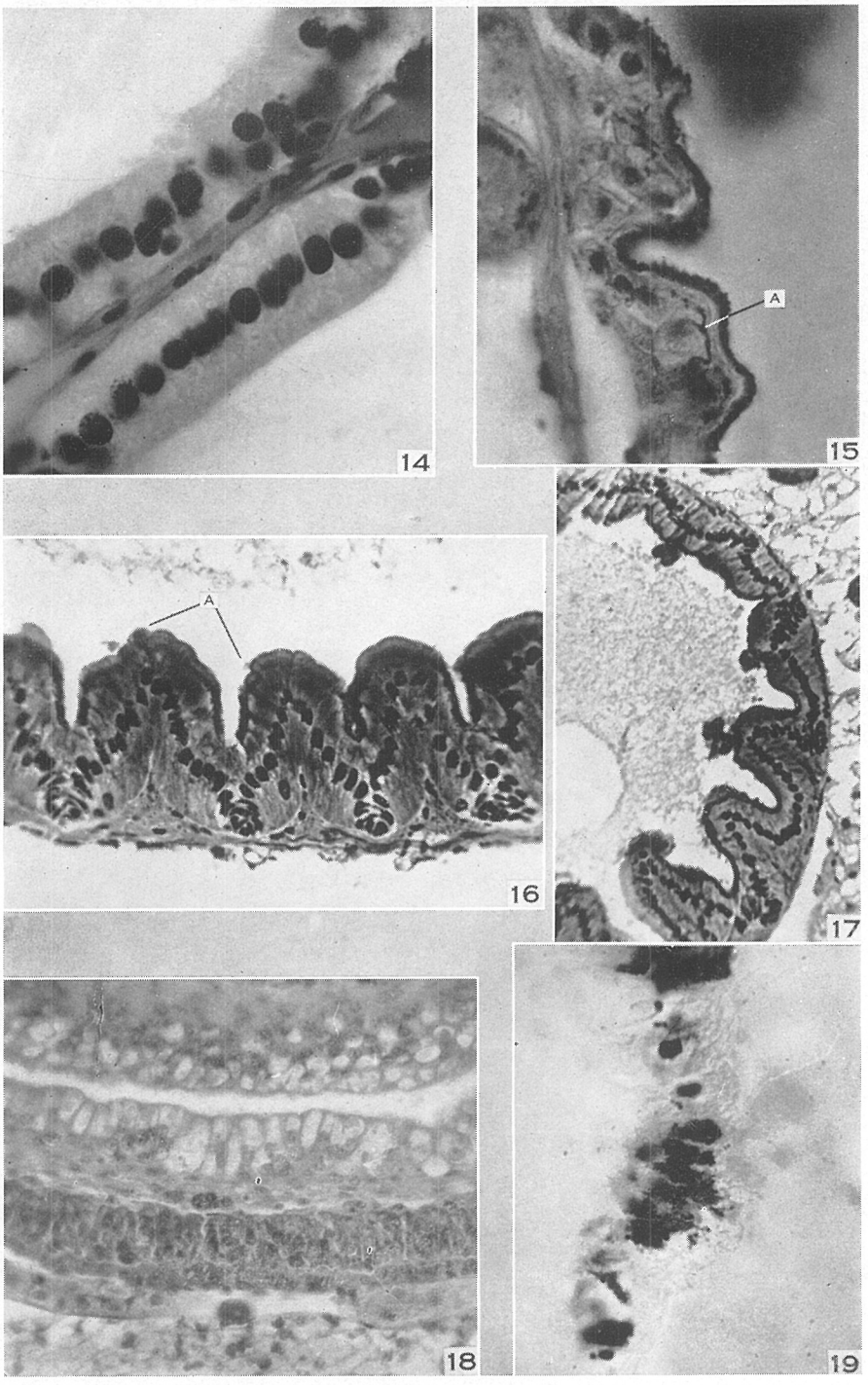

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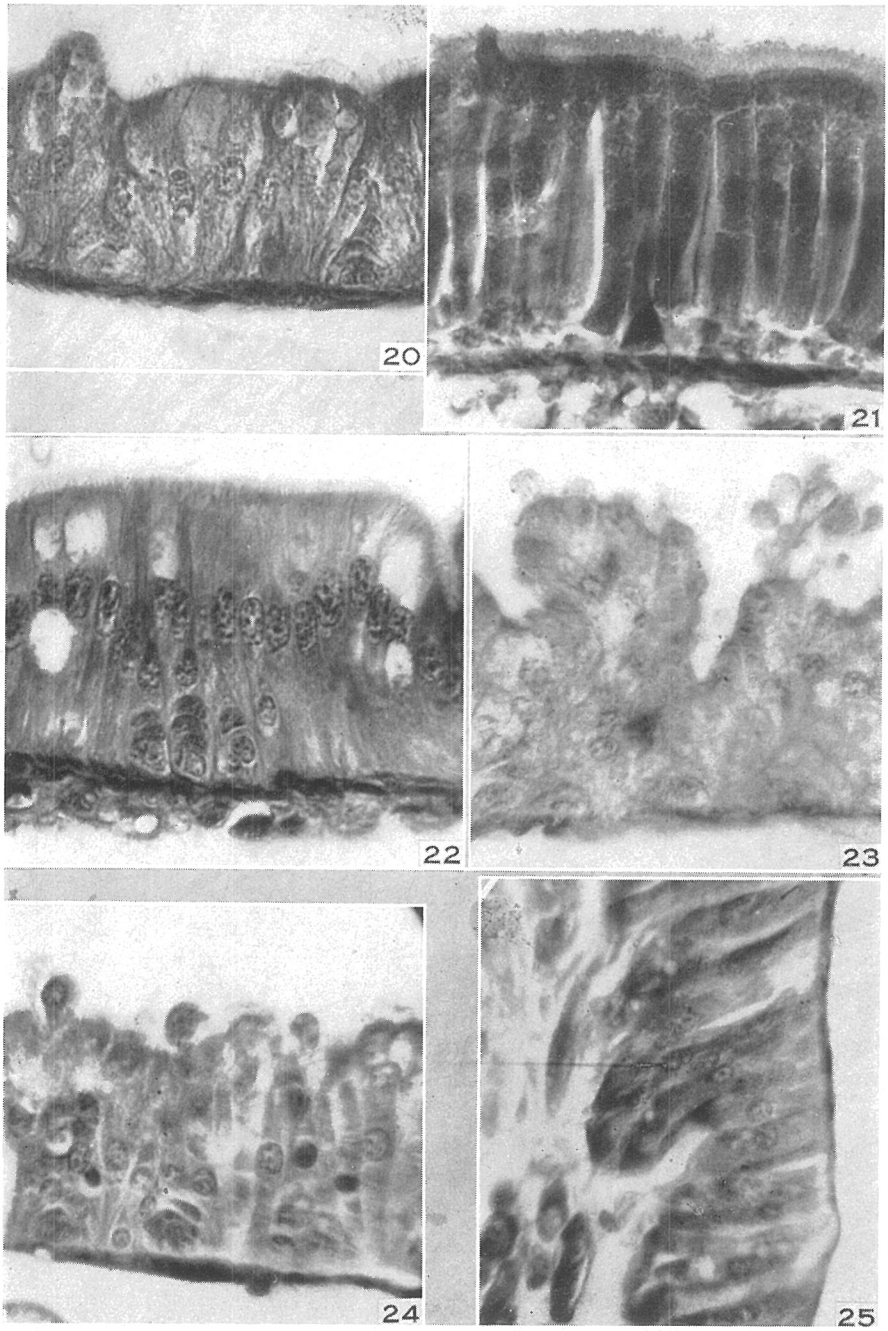

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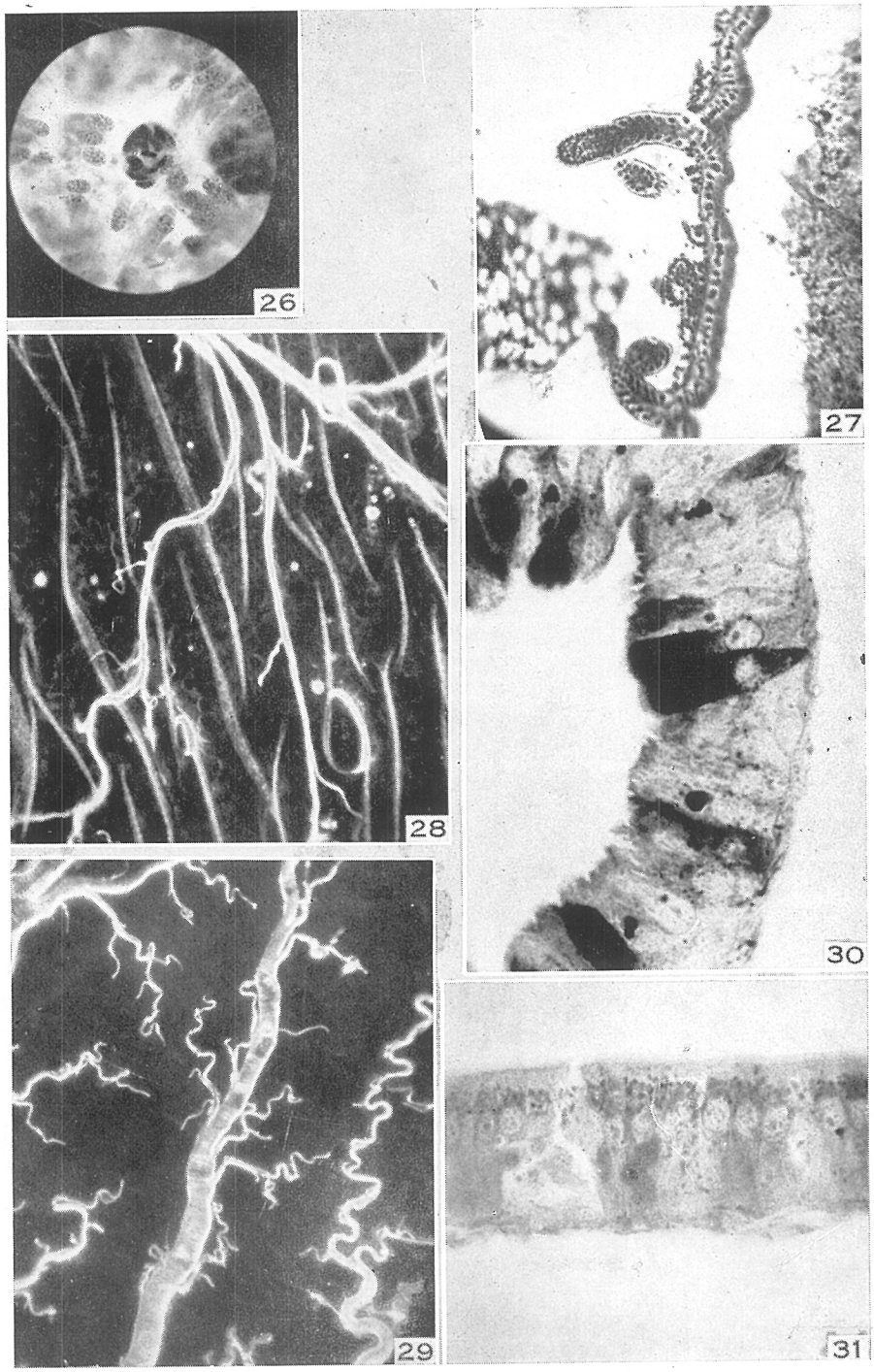

DAY AND POWNING.— A STUDY OF THE PROCESSES OF DIGESTION IN CERTAIN INSECTS

Fig. 27.—L.S. *Tenebrio* midgut showing midgut contents on the right, cuboidal intercryptal epithelium and section of regenerative crypt. The regenerative cells are at the blind end of the crypt. Fat body at extreme left. Bodian technique. x 130.

Fig. 28.—Spread of *Blattella* crop under dark field showing distribution of tracheae and some tracheoles. x 355.

Fig. 29.—The same of midgut showing large tracheal trunks and characteristic end twigging. Every epithelial cell is tracheolated. x 355.

Fig. 30.—T.S. Caecum of *Blattella* fed starch 3 days, Mann-Kopsch technique. Golgi substance clumped towards lumen side of nucleus. Note dark-staining cells found after feeding on starch. x 355.

Fig. 31.—L.S. Midgut *Blattella* fed gelatine 3 days, Mann-Kopsch technique, showing characteristic position of Golgi substance in feeding individuals. x 355.