PROTEOLYTIC ACTIVITY OF THE MIDGUT IN RELATION TO FEEDING IN THE BEETLES *TENEBRIO MOLITOR* L. AND *DYTISCUS MARGINALIS* L.

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Where quantitative methods have been applied to the study of insect digestion, enzyme activity has generally been shown to increase after feeding, often with change in location of maximum enzyme concentration (Schlöttke, 1937*a–c*; Duspiva, 1939; Day & Powning, 1949; Fisk & Shambaugh, 1952). The most marked responses described by these authors occur in carnivorous and blood-sucking insects, and to some extent this supports the view put forward on histological grounds by Haseman (1910), that discontinuous secretion is associated with the carnivorous habit of taking discrete meals.

The work described here indicates the extent to which food regulates digestive secretion in *Tenebrio molitor* L. and *Dytiscus marginalis* L., two beetles of widely different feeding habit. *Tenebrio* passes its entire life cycle in its food (flour and like substances) and feeding appears superficially continuous during its larval stages, whereas *Dytiscus* is an aquatic carnivore whose meals depend on the fortunes of predation. Duspiva (1939) found protease discharge in *Dytiscus* to depend on taking food; this relationship is briefly re-examined for comparison with the situation in *Tenebrio*.

Hitherto the possibility of an endogenous rhythm in secretion related to age or development has been neglected, although such periodicities have been suggested on histological grounds in various beetles (Wigglesworth, 1953). As such a rhythm might mask effects due to feeding, secretory changes connected with development were examined in *Tenebrio* before testing the effect of food.

METHODS

(*a*) *General procedure*

Enzyme concentration in insect digestive tissues has usually been measured on extracts prepared from groups of similarly treated insects assumed to be physiologically homogenous. By recording enzyme values for individual insects, it was hoped that variation within experimental groups would be more readily appreciated.

Preliminary work with *Tenebrio* showed the enzyme content of the midgut to depend on age, stage of development and feeding history. Within any group of insects defined by these factors the range of enzyme values was wide, necessitating the use of large numbers of individuals before differences between groups became apparent. As the techniques involved precluded large numbers of concurrent

determinations, data were accumulated over a period of months, using sets of six to twelve insects from different experimental groups at a time. When further replication failed to alter the mean value of a group appreciably, it was considered adequately sampled. The definition of experimental groups is discussed later.

Examination of *Dytiscus* was limited to changes in secretion associated with feeding. These were sufficiently pronounced to be apparent from a comparison of individual beetles.

(*b*) *Preparation of standard enzyme extracts*

Similar techniques were followed in the preparation of gut extracts for both species, and in the subsequent evaluation of their protease activity.

Whole midguts were dissected from live *Tenebrio*, freed from adhering fat-body, and weighed on a torsion balance. For the measurement of tissue enzyme the midgut was slit open and washed free of contents in insect saline; after drying for a few seconds on filter-paper the washed tissue was weighed. The whole midgut or washed tissue was ground with a small quantity of kieselguhr in a thick-walled ignition tube, and 2 ml. of phosphate-buffered glycerin extraction fluid (Linderström-Lang & Duspiva, 1936) added with thorough mixing. After standing, with periodic agitation, for 1 hr., the tube was centrifuged at 3000 r.p.m. for 15 min., when the kieselguhr and tissue solids formed a compact pad from which the clear extract was easily decanted.

Separate extracts of the crop contents, midgut contents, and midgut tissue were prepared from each *Dytiscus*. Crop and midgut were dissected from the live beetle and removed to a watch-glass, where the former was transected at the cardiac valve, slit open, and its contents washed out in 2 ml. of extraction fluid. After thorough mixing this was removed to an ignition tube for centrifugation. The midgut contents were likewise dealt with. The washed midgut tissue was dried, weighed, and extracted as described for *Tenebrio*.

(*c*) *Protease determination*

The titimetric method of Day & Powning (1949) was followed with slight modification. A gelatin substrate was mixed in bulk, using 1 part of M/15 phosphate buffer of pH 8·0 to 2 parts of 6% bacteriological gelatin. 10 ml. of toluene were added to each litre of substrate mixture which could then be stored in a refrigerator without deterioration. For each experimental run 7·5 ml. of this substrate were mixed with 0·5 ml. of enzyme extract and incubated at 39° C. for 18 hr. 7·5 ml. of substrate plus 0·5 ml. of extraction fluid were used for blank runs. A drop of toluene was added to each run before incubation to prevent bacterial action. After incubation 0·5 ml. samples were titrated with N/40 alcoholic (90%) KOH as described by Day & Powning. The difference between titres for experimental and blank runs gave a measure of the protease activity of the extract. Transformation to arbitrary comparative units was effected by reference to a dilution curve prepared by plotting the titres for serial dilutions from a concentrated extract. Curves for both larval and adult *Tenebrio* extracts were similar, and one was selected for deriving all *Tenebrio* protease values. A separate dilution curve was prepared in a similar manner for use with *Dytiscus*.

(*d*) *Observations on the development of* Tenebrio *and their application in defining experimental groups*

A stock culture of *T. molitor* L. was obtained from the Imperial College insectary at Silwood Park, Berks, and maintained at a constant temperature of $27 \pm \frac{1}{2}$° C. and a R.H. of 50 ± 15 %. The insects lived in a mixture of wholemeal flour and middlings in 7 lb. glass jars containing up to 500 larvae per jar. Individuals at appropriate stages of development were confined singly in $3 \times 1\frac{1}{2}$ in. glass specimen tubes, where their pre-experimental history could be accurately noted and adjusted. Thus isolated, times of moult, pupation and emergence were known, and growth curves were obtained by periodic weighing.

Classification into experimental groups was based on age in relation to a moult or emergence, and the occurrence or non-occurrence of feeding. Feeding was presumed to have occurred during any specified period if a weight increment was recorded. It could not be assumed that weight loss implied non-feeding, as this might result if defaecation exceeded food ingestion. Those designated 'unfed' were therefore deprived of food from moult or emergence. As the midgut is largely emptied at these times, the effect of variable storage of food was avoided.

In some larvae removed from food at moult, especially those in a late instar, further moulting could occur (Buxton, 1930; Leclercq, 1949). Thus it would have been possible for those classified as 'unfed' to be in a premoult or prepupal condition. However, a study of larval growth curves showed this to be unlikely in larvae less than 90 mg. in weight. If these had moulted once after removal from food, a state of starvation uncomplicated by imminent metamorphosis was reasonably assured.

From growth-curve data it was possible to determine approximately when a moult or pupation was about to occur in larvae developing on a normal diet. Weight increases regularly during the early part of each instar; feeding ceases shortly before a moult, which is preceded by a period of 5 to 6 days during which weight is lost (Murray, 1956). Dissection of the midgut at this time revealed greatly reduced contents. As freshly moulted larvae always had a small residue in the posterior part of the gas-distended midgut, it appears that complete evacuation only occurs before pupation. In prepupae, which may be recognized by their characteristic appearance (Stallwaag-Kittler, 1954), the midgut is completely empty.

Using the foregoing information it was possible to relate the age of any insect approximately to a moult, pupation, or emergence, and to classify it as 'fed' or 'unfed' in the sense defined above.

VARIATION IN MIDGUT PROTEASE IN ADULT *TENEBRIO*

The midgut protease of unfed adults was measured at various times after emergence. Separate insects were used to provide data for the total midgut (midgut tissue plus contents) and midgut tissue only. Protease values for the total midgut are recorded in Table 1, together with the means for each age group, and those for tissue only in Table 2.

The data of Table 1 were examined after separation into male and female sets, and group means for each sex are included. Though indicating slightly greater enzyme content in the female, the similarity between the sexes was considered sufficient to justify combining all data.

Table 1. *Total midgut protease in adult* Tenebrio *isolated without food from emergence*

Days after emergence ...	1	2	3	4	5	6	9	14	21
Individual protease values	2	7	11	25	30	41	19	5	11
	1	3	10	14	43	14	23	14	13
	1	6	6	36	40	50	27	19	6
	1	3	7	23	38	37	6	21	18
	0	1	6	31	34	38	34	20	16
	0	1	2	40	29	44	23	9	10
	0	0	8	20	34	39	23	20	4
	0	1	6	19	28	26	23	5	25
	1	3	8	19	43	25	11	17	3
	—	3	5	23	20	23	32	19	7
	—	—	9	—	—	—	46	30	—
	—	—	1	—	—	—	23	13	—
	—	—	—	—	—	—	41	15	—
	—	—	—	—	—	—	34	15	—
	—	—	—	—	—	—	22	18	—
	—	—	—	—	—	—	27	26	—
	—	—	—	—	—	—	—	17	—
Means (both sexes)	1	3	7	25	34	34	26	17	12
Means (males only)	1	3	6	20	33	31	25	13	15
Means (females only)	1	3	7	28	35	36	27	20	10

Table 2. *Midgut tissue protease in adult* Tenebrio

	Unfed from emergence								Flour taken within 24 hr. of dissection	
Days after emergence...	1	2	3	4	5	6	9	14	9	14
Individual protease values	0·0	0·0	1·5	3·0	1·0	4·5	3·0	1·5	2·0	3·5
	0·0	0·0	0·5	1·5	2·0	12·0	2·0	0·0	4·0	3·5
	0·0	1·0	1·0	4·0	1·0	2·0	0·0	0·0	2·0	2·0
	0·0	0·0	3·0	4·5	4·0	3·0	0·0	0·0	—	1·5
	—	1·0	1·0	12·5	2·5	5·5	1·0	0·5	—	—
	—	2·0	4·0	9·5	3·0	2·5	0·5	0·5	—	—
	—	—	2·5	1·0	2·5	2·0	0·0	0·0	—	—
	—	—	0·0	1·5	2·5	4·0	0·0	0·0	—	—
	—	—	1·5	11·0	—	—	1·5	0·5	—	—
	—	—	0·0	—	—	—	1·5	—	—	—
	—	—	—	—	—	—	2·0	—	—	—
Means	0·0	0·5	1·5	5·5	2·5	4·5	1·0	0·5	2·5	2·5

To assess the effect of feeding, unfed adults were allowed access to wholemeal flour, damp cellulose powder or water at various times after emergence, and periodic weighing so arranged that feeding was known to occur during particular periods preceding dissection. Values for total midgut protease of adults thus fed are listed in Table 3. Data for tissue protease are included in Table 2.

Table 3. *Total midgut protease in adult* Tenebrio *allowed to feed after various periods of starvation from emergence*

	Food withheld 13 days after emergence: ingestion occurred during 14th day					
	Flour				Cellulose powder	Water
Days after emergence ...	6	9	14	17	14	14
Individual protease values	32	41	42	32	54	48
	26	21	48	32	24	31
	45	39	41	—	24	34
	35	31	53	—	18	22
	32	58	48	—	12	17
	—	—	33	—	14	20
	—	—	23	—	28*	42*
	—	—	28	—	42*	45*
	—	—	19*	—	42*	45*
	—	—	22*	—	76*	22*
	—	—	—	—	16*	24*
	—	—	—	—	37*	29*
	—	—	—	—	15*	—
Means	34	38	36	32	31	32

* Ingestion occurred within 4 hr. of dissection.

In Fig. 1 group means for tissue protease and total protease of unfed adults are plotted against time after emergence. The two curves fitted visually to the points so obtained are taken to represent the fluctuation of protease in the midgut as a whole, and in the midgut epithelium. At emergence proteolytic enzymes are lacking. They build up from the 1st day after emergence until total midgut protease reaches a maximum between the 5th and 6th post-emergence days, with the greatest rate of increase occurring on the 3rd and 4th days. Thereafter, it gradually decreases on continued starvation until death ensues. Tissue protease, which becomes comparatively high between the 4th and 6th days, falls to a negligible residual value by the 9th day, and remains so until death.

Comparison of the means for total protease in fed adults (Table 3) with those unfed of the same age (Table 1) indicates the effect of feeding. Between the 5th and 6th days after emergence, when in unfed adults the spontaneous increase is maximal, the amount of protease accumulated is unchanged. At later times, when in the absence of feeding total protease is reduced, the ingestion of flour causes an increase to a value approximating that attained initially. Similar events follow the ingestion of damp cellulose or water, and are apparent within 4 hr. In adults fed flour at these later times tissue protease rises from the residual starvation level to values comparable with those reached spontaneously after emergence (Table 2).

These results show that the secretion of protease is stimulated by endogenous events at emergence, and after the ingestion of food (or nutritionally valueless material) in the mature adult. From a comparison of the values for tissue protease and total protease it is clear that the latter is largely a measure of discharged enzyme in the midgut lumen, epithelial accumulation being negligible. This being so, tissue

protease may reasonably be considered an index of the rate of synthesis of enzyme in the secretory cells. As large increases in total (i.e. discharged) enzyme occur when tissue enzyme is comparatively high, it appears that synthesis and discharge form an integrated process.

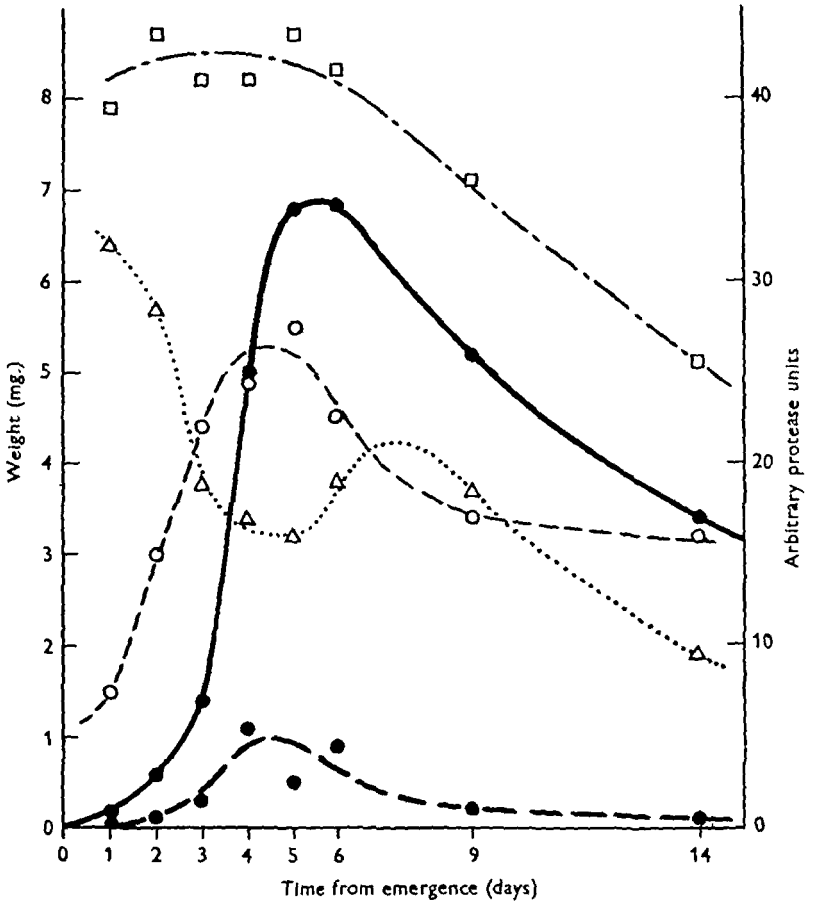

Fig. 1. Changes in midgut weight and protease in adult *Tenebrio*. —●—●—●—, total midgut protease; —●—●—●—, midgut tissue protease; —○—○—○—, weight of midgut tissue; —□—·—□—·—□—, weight of total midgut; ···△···△···△···, weight of midgut contents.

VARIATION OF MIDGUT WEIGHT IN ADULT *TENEBRIO*

When tissue protease was measured weights of total midgut and washed midgut tissue were recorded. These are listed in Table 4 and group means plotted against time after emergence in Fig. 1, together with the values obtained by subtracting tissue weight means from corresponding total weight means. The curves fitted visually to these points represent the fluctuation of tissue weight, total weight and weight of midgut contents with time, and may be compared with the curves showing variation in tissue and total protease.

Corresponding changes in the weight and protease of the tissue are evident.

Tissue weight increases as tissue protease rises from zero at emergence, and falls to a more or less stationary value as protease decreases to a low residual level; increase in weight marks the increase in tissue enzyme which follows feeding. The initial weight increment is unlikely to represent formed enzyme. This has been shown to accumulate mainly in the midgut contents, and no corresponding weight increase occurs for the total midgut. It follows that epithelial growth occurs during active secretion. This conclusion accords with the observation of accelerated mitosis in the midgut regenerative cells of *Tenebrio* during secretion (Day, 1949), and supports the view that epithelial regeneration is an important feature in the secretory process of some insects.

Table 4. *Weights of total midguts and midgut tissues in adult* Tenebrio

	Unfed from emergence								Flour taken during previous 24 hr.	
Days after emergence ...	1	2	3	4	5	6	9	14	9	14
Individual weights of midgut tissue (mg.)	1·3	2·6	3·2	5·0	6·6	3·4	3·2	4·2	4·5	3·8
	1·2	2·8	3·4	5·2	5·8	3·5	3·2	2·4	4·0	4·9
	1·6	3·6	3·6	3·9	4·7	4·2	2·4	3·5	5·0	2·8
	1·7	3·7	4·3	4·6	4·9	4·3	4·1	3·1	—	3·5
	—	2·3	3·5	4·2	4·7	5·6	2·4	3·5	—	—
	—	2·8	3·7	5·2	6·8	5·3	5·3	2·6	—	—
	—	—	3·5	5·2	5·4	3·1	3·0	1·8	—	—
	—	—	6·5	4·9	4·7	6·0	3·7	2·9	—	—
	—	—	5·4	4·9	—	—	3·5	5·0	—	—
	—	—	7·1	—	—	—	3·9	—	—	—
	—	—	—	—	—	—	2·8	—	—	—
Tissue weight means	1·5	3·0	4·4	4·8	5·5	4·5	3·4	3·2	4·5	3·8
Weights of individual total midguts (mg.)	8·0	6·1	6·0	6·6	10·8	8·1	5·1	9·1	8·8	8·5
	7·6	9·1	5·1	8·8	8·5	9·1	5·0	4·2	9·3	10·0
	8·2	8·8	7·7	8·3	6·8	8·6	4·4	4·7	8·2	7·6
	7·9	11·0	9·3	8·1	7·3	7·7	6·5	4·2	—	8·8
	—	9·0	7·9	5·3	8·7	8·1	6·5	5·0	—	—
	—	8·4	6·4	11·0	10·7	10·5	8·4	3·7	—	—
	—	—	8·8	8·5	8·1	6·5	5·1	3·2	—	—
	—	—	11·2	9·2	8·7	8·9	9·0	5·0	—	—
	—	—	9·9	8·7	—	—	7·6	8·6	—	—
	—	—	10·0	—	—	—	6·0	—	—	—
	—	—	—	—	—	—	7·6	—	—	—
Total weight means	7·9	8·7	8·2	8·2	8·7	8·3	7·1	5·1	8·8	8·7
Difference between means (weight of midgut contents)	6·4	5·7	3·8	3·4	3·2	3·8	3·7	1·9	4·3	4·9

As total midgut weight remains constant during tissue growth, midgut contents must diminish. At first sight growth appears to be at the expense of midgut contents, but these weight relationships would hold if tissue growth at the expense of body fluid were accompanied by a loss of contents from the midgut. The available data provide no means of distinguishing between these possibilities.

In the unfed adult total midgut weight falls steadily from the 6th to the 14th day. As tissue weight remains nearly constant after the 9th day this decrease must be due to the evacuation of midgut contents, and largely accounts for the decline in

total midgut protease over this period. From the 5th to 9th days tissue weight decreases more than total weight; it may be supposed that during this period gut contents are augmented from tissue material, perhaps by the disruption of senescent cells as secretion declines.

VARIATION IN MIDGUT PROTEASE IN THE *TENEBRIO* LARVA

On cursory examination secretory events in the larva proved similar in many respects to those of the adult, so fewer individuals were used for each experimental group In order to limit variation due to size, only larvae weighing between 75 and 90 mg. at the time of dissection were used; it was hoped that this might incidentally result in the majority being of the same instar. (Instar number is not easily determined in *Tenebrio*.) Furthermore, larvae of this weight were unlikely to be entering the physiologically complicated prepupal state. Protease values for the total midguts of larvae, grouped according to the criteria described earlier, are listed in Table 5.

Table 5. *Total midgut protease in* Tenebrio *larvae*

Time from moult (in days) ...	Without food from emergence											Premoulting larvae		Pre-pupae
	½	1½	1	2	3	4	5	6	7	14	42	−3	−2	
Individual protease values	1	12	21	19	26	16	15	23	42	23	11	16	4	0
	18	12	13	19	16	16	9	30	30	12	9	23	3	0
	13	14	11	11	10	16	12	18	11	26	13	16	—	—
	0	6	16	22	15	15	15	19	15	25	15	10	—	—
	14	18	14	17	22	16	23	17	20	29	17	—	—	—
	5	7	19	16	—	18	20	16	17	15	—	—	—	—
	—	10	—	14	—	27	17	26	—	12	—	—	—	—
	—	—	—	—	—	15	—	—	—	—	—	—	—	—
Means	9	11	16	17	18	17	16	21	23	20	13	16	4	0

Flour taken during the 24 hr. preceding dissection

	Flour available from moult									Flour withheld for 21 days after moult		
Days after moult ...	4	5	6	7	8	10	11	12	16	22	23	26
Individual protease values	32	43	37	42	28	30	40	27	27	35	44	33
	—	49	57	—	—	36	—	27	23	29	31	—

In contrast to the newly emerged adult, the larva has considerable protease immediately after moulting. Part of this may be ascribed to the carry-over of residual gut contents from the previous instar. Comparison with the build up of protease in the young adult suggests that secretion may commence before moulting is externally apparent. During the day after moulting protease rises to a stationary level; a further slight increase may occur between the 5th and 7th post-moult days, but the data are insufficient to decide this conclusively. Thereafter, total protease

decreases very gradually, contrasting in this respect with the adult, where enzyme activity in the midgut is halved after a week's starvation. The high level of protease conserved over long periods suggests both an efficient retention of gut contents (of importance to the larva in its ability to withstand months of starvation and desiccation) and continuous secretion at a low rate.

In larvae that have fed within the preceding 24 hr. protease values are higher than in those unfed from moult. This difference is very pronounced at the start of a feeding period, whether this follows normally from moulting, or after starvation. As feeding proceeds, the difference from the starvation level of enzyme decreases, and may indicate a diminished secretory response to feeding or an increase in the rate of evacuation of gut contents.

It may be noted that the second spontaneous increase in enzyme occurs 5 days after moulting, when secretion is maximal if food is available. This suggests that a tendency to continuous secretion has developed in association with regular feeding. If so, secretion may here be supposed an endogenously initiated function of the digestive epithelium subject to a limited regulation in response to stimuli arising during feeding.

Shortly before moulting the larva loses weight as feeding ceases, and the low enzyme values then recorded may be ascribed to the evacuation of most of the midgut contents at this time. Complete evacuation precedes pupation; in two prepupae examined the empty gut lacked proteolytic activity.

A systematic study of midgut tissue protease in the larva was not made. However, while working on enzyme distribution in the midgut epithelium, tissue protease was found to be of the same order of magnitude in larvae and adults. It may be concluded therefore that, as in the adults, little protease is retained in the epithelium.

PROTEASE SECRETION IN *DYTISCUS*

Adult *D. marginalis* L. were obtained from a dealer and isolated in 7 lb. glass jars containing about 2 in. of tap water at room temperature. (This varied between 15 and 20° C. during the course of the experiments.) On reception they were allowed to feed to repletion on whalemeat, removed to clean water devoid of ingestible material, and starved for 1 month. Preliminary work had shown that after this period the meat was completely digested and the crop empty. Beetles thus starved were offered meat, and, at various times after commencing to feed, were dissected for the measurement of gut protease. The data for one set of insects are listed in Table 6 and represented graphically in Fig. 2. Two other sets, though less detailed, gave essentially similar results.

Clearly, tissue protease is high during starvation and very low subsequent to feeding, whereas the converse applies to protease in the crop. Within 15 min. of feeding tissue enzyme is much reduced, and after an hour protease in the midgut tissue and lumen is virtually zero. Enzyme lost from the midgut appears in the crop; this organ contains over 90% of the total protease an hour after taking food. Protease recurs in the midgut tissue 3 hr. after feeding; by this time the synthesis

of additional enzyme must have started in the epithelial cells. Protease steadily increases in the crop and midgut contents during the 12 hr. after feeding. As midgut tissue remains low during this period, it may be inferred that the synthesis of fresh enzyme is accompanied by its continual discharge and translocation forward to the crop.

Table 6. *Protease in the crop and midgut of* Dytiscus

Sex	Wt. of beetle (g.)	Wt. of midgut tissue (mg.)	Time from commencement of feeding	Protease values			
				Crop contents	Midgut contents	Midgut tissue	Total
Female	1·7	11	15 min.	35	24	2	61
Female	1·8	17	1 hr.	54	4	0	58
Male	2·3	16	3 hr.	74	7	1	82
Female	1·9	29	12 hr.	*c.* 150	30	6	180
Female	2·0	27	24 hr.	45	6	4	55
Male	1·9	22	24 hr.	31	53	11	95
			Mean, 24 hr.	38	30	8	75
Female	1·8	32	2 days	54	3	8	65
Female	1·7	14	5 days	90	24	11	125
Female	1·7	31	2 weeks	0	46	36	79
Female	2·0	29	4 weeks	12	21	26	59
Male	1·6	14	4 weeks	1	20	13	34
			Mean, 4 weeks	7	21	20	47
Female	1·9	25	5 weeks	3	35	24	62
Female	1·9	17	7 weeks	0	4	7	11

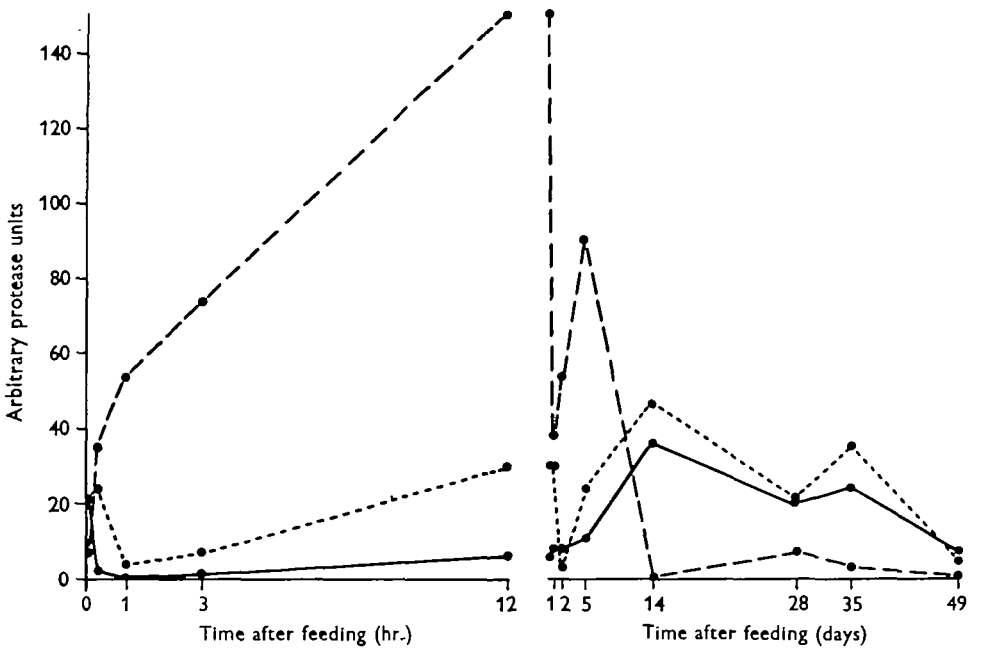

Fig. 2. Distribution of protease in the gut of *Dytiscus* during digestion and subsequent starvation. ————, midgut tissue; ------, midgut contents; — — — —, crop contents.

For some days after feeding tissue protease remains low. During this period the variable distribution of enzyme between crop and midgut lumen is best accounted for by the periodic evacuation of the products of digestion in the crop backwards through the midgut. By the second week tissue protease has built up to a high level, and thereafter it slowly decreases if starvation is prolonged. When tissue protease is maximal, crop protease is virtually zero, and it remains so on starvation; when this condition obtains the crop is found to be empty.

These results suggest that while the crop contains undigested material, continual synthesis and discharge of protease occur from the midgut epithelium. On complete evacuation of the crop discharge ceases, allowing the accumulation of enzyme in the epithelium. This view differs somewhat from that of Duspiva (1939), who on histological grounds considered the epithelial cells in a 'resting condition' a few hours after the initial discharge. Though the foregoing results might be accounted for in terms of a secretion cycle of a few hours' duration, histological examination always revealed the characteristic condition associated with enzyme discharge if the crop contained undigested contents. These discrepant observations may perhaps be due to differing conditions of feeding and amounts of food taken. It is clear, however, that with continued starvation discharge of enzyme ceases and allows epithelial reserves to be accumulated. This separation of the processes of synthesis and discharge contrasts markedly with the condition in *Tenebrio*.

DISCUSSION

Information on the development of digestive function in relation to the moulting cycle of insects is meagre. Where extensive reorganization of gut tissues is involved secretion might be expected to decrease or cease as in *Tenebrio*. Uvarov (1928) mentions similar changes in some lepidopterous pupae. Spontaneous secretion after moulting or emergence is readily understood in *Tenebrio* where food is normally always available. Enzyme activity increases after moulting in larvae of *Limnophilus* and *Bombyx* (Roques, 1909; Roeder, 1953), which likewise live near their food. The newly emerged female *Aëdes*, on the other hand, has negligible protease until blood has been taken (Fisk, 1950; Fisk & Shambaugh, 1952). In this mosquito the absence of enzyme preparatory to feeding may be of significance in relation to the sporadic occurrence of blood meals.

Many authors have been concerned to relate digestive secretion to feeding behaviour. Though quantitative studies have usually demonstrated increased enzyme activity after feeding, it has been argued that in insects which feed continuously, particularly in larvae with food always available, a mechanism for the stimulation of secretion at the time of feeding may not be required (Haseman, 1910; Schlöttke, 1937*b*; Pradhan, 1939). Particular interest therefore attaches to the stimulation of secretion by food in the *Tenebrio* larva, where, superficially, feeding seems continuous. Crowell (1943) considered most cases of reputed continuous feeding to be doubtful. Critical examination usually reveals that though the gut is kept full ingestion is intermittent, and this appears the case with *Tenebrio* larvae (Murray, 1956).

On the available information secretory situations are more profitably related to the amount of food normally maintained in the gut. On this point Day & Powning (1949) distinguish between 'effectively continuous' feeders which maintain a more or less full gut, and discontinuous feeders. In the former, continuous secretion subject to regulation in rate might be anticipated, as in the roaches they studied. Intermittent secretion is most likely where the gut may frequently be empty, as in predators dependent for meals on occasional prey.

Tenebrio and *Dytiscus* illustrate this distinction in feeding habit and have been shown to possess distinctive secretory mechanisms. Secretion in *Tenebrio* may be supposed a continuous process initiated at moult or emergence and accelerated after feeding. This seems especially likely in the larva, where it is difficult otherwise to account for the high level of protease maintained during protracted starvation. In *Dytiscus*, on the other hand, the independence of discharge from synthesis, by allowing the intra-epithelial accumulation of enzyme during starvation, results in secretion as a whole being markedly intermittent. The work of Fisk & Shambaugh (1952) on *Aëdes* supports the correlation of intermittent secretion with irregular feeding, though in detail the secretory mechanism involved is very different from that of *Dytiscus*. As a working hypothesis, the view that intermittent secretion and irregular feeding are associated has some use, but it is clear that wider study will show diversity in detail.

Thus far it has been tacitly understood that considerable information may be obtained from an examination of single enzymes by supposing them to indicate secretion of the digestive juice as a whole. Duspiva (1939) considered secretion in *Dytiscus* to occur as a single process, and the observation of concurrent increases in various midgut enzymes argues a similar situation in some Orthoptera (Schlöttke, 1937*b*, *c*; Day & Powning, 1949). A uniform digestive juice seems probable in *Tenebrio*. Although in preceding sections only proteolytic activity has been described, amylase values were determined for some extracts, and were high where protease was high. Further, the similar results obtained by feeding either flour, cellulose powder or water to adults suggest a general secretory response rather than the separate stimulation of individual enzymes. Differential secretion of protease and amylase in response to their appropriate substrates occurs in *Aëdes*, and may be related to the need for occasional blood meals for ovarial maturation in an insect whose main diet consists of plant juices (Shambaugh, 1954). In *Tenebrio* and other insects whose normal diet always requires their full complement of enzymes differential secretion would effect no improvement in digestive efficiency.

Of great interest in this connexion is the postulation of different modes of stimulation of the secretory cells. Shambaugh (1954) shows secretogogues to be active in *Aëdes*, whereas Day & Powning (1949) suggest that a hormone-like blood factor provides the immediate stimulus in *Tenebrio* and roaches. As substances lacking the chemical qualities of normal food are effective in stimulating secretion in *Tenebrio* adults, the response seems to be dependent on some mechanical factor involved in ingestion or feeding behaviour rather than a gustatory or secretogogue effect elicited by specific nutrients. This accords with the hypothesis of Day &

Powning. Further, as endogenously initiated secretion occurs at moult and emergence in the absence of food and therefore of secretogogues, its initiation may well be an integral part of the hormone-regulated events of metamorphosis.

SUMMARY

1. In *Tenebrio* secretion of protease occurs spontaneously after moult and adult emergence, and in response to feeding in the active larva and mature adult. Damp cellulose powder or water are effective in increasing secretion in the adult.

2. Since little enzyme is accumulated in the epithelial tissue when the total midgut enzyme is greatly increased, it is inferred that synthesis and discharge are interdependent. When synthesis (as indicated by comparatively high tissue enzyme) is accelerated, growth of the midgut epithelium occurs.

3. In starved *Dytiscus* protease is accumulated in the midgut tissue. Within one hour of feeding it is largely discharged into the crop. Protease recurs in the midgut tissue in a few hours, but remains low so long as the crop contains undigested material. When the crop is empty, discharge ceases and enzyme is again accumulated in the epithelium. Thus the process of discharge appears to be independent of synthesis.

4. The secretory mechanisms of *Tenebrio* and *Dytiscus* are discussed in relation to their feeding habits.

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