Digestive enzymes and their control in haematophagous arthropods

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Because of the role of haematophagous arthropods as vectors, the digestive physiology of these arthropods has attracted the attention of workers for many years. BUXTON (1955) reviewed the digestive physiology of tsetse flies and CLEMENTS (1963) discussed this aspect of mosquito physiology. A more general discussion of digestive physiology, including information on blood sucking insects is included in WIGGLESWORTH (1965). Three years ago I published a general review of digestive physiology in haematophagous insects (GOODING, 1972a). The present review will summarize in tabular form the distribution of digestive enzymes in the haematophagous arthropods and then discuss the regulation of proteinase activity in the midgut of several insects.

For many species of haematophagous arthropods information on digestive enzymes is restricted to an attempt to demonstrate the presence of one or more enzymes. Usually this is done by an *in vitro* assay using a crude homogenate of either salivary glands, midgut, or even whole animals. In this brief survey of the distribution of the digestive enzymes many pages of published information are reduced to plus or minus signs in tables 1 to 3 and the reader is cautioned that the process discards enormous amounts of information in the interests of brevity and in the hope of revealing patterns of enzyme distribution. The failure of a given attempt to find a specific enzyme may often reflect the use of a technique which is insensitive to the small quantities of enzyme present or the use of insects at a time when the enzyme is absent or present at very low concentrations. Variations in assay techniques also introduce a measure of variability which is lost in the summary procedure employed here.

Tables 1 to 3 summarize the distribution of digestive enzymes in the salivary glands and midguts of several blood sucking arthropods. The large number of blank spaces shows that we do not have a complete inventory of digestive enzymes for any species. Nor has a single enzyme been tested for in all of the species available. Since arthropods generally produce only a small amount of saliva, studies have generally used homogenates of the salivary glands rather than salivary secretions. In this respect it is interesting to note that in *Anopheles freeborni* and *Aedes aegypti* non-specific esterases were found in the cytoplasm of secretory cells but not in the salivary secretions (FREYVOGEL et al.,

Species	Proteinase: strong acid	Proteinase: mild acid	Proteinase: alkaline	Proteinase: milk clotting enzyme	dipeptidase	lipase	butyrase	lecithinase-A	amylase	invertase	maltase	melezitase	lactase	non-specific esterase	References
Cimex lectularius		–				–			–	–					1
Rhodnius prolixus		–				–			–	–					1
Triatoma infestans		–				–			–	–					1
Anopheles quadrimaculatus		–				–		–	–						2
Anopheles freeborni														+	3
Aedes aegypti														+	3
Culex tarsalis										+	+	+			4
Simulium venustum										–					5
Prosimulium fuscum										–					5
Chrysops silacea	–		–			?			–	–	–		–		6
Musca vitripennis		–	–						–						7
Stomoxys sitiens		–	–						–						7
Stomoxys calcitrans		–	–						–/+						7,8
Glossina morsitans	–		–		–	?			–	–	–		–		9
Glossina tachinoides	–		–		–	?			–	–	–		–		9
Gasterophilus intestinalis (l)	–		–	+/+		–			–/+	+	–/+		–		10 11
Protocalliphora avium (l)							+				+				12

Table 1. Enzymes in the salivary glands of haematophagous insects. Results are for adult insects except those designated (*l*) where larvae were used. Symbols: + indicates presence, – indicates that the enzyme has been looked for but not demonstrated, a blank indicates no information available, ? indicates the source of the information reported doubts about the demonstration of the enzyme or reported it as ±. References: 1 BAPTIST, 1941; 2 METCALF, 1945; 3 FREYVOGEL et al., 1968; 4 SCHAEFER & MIURA, 1972; 5 YANG & DAVIES, 1968c; 6 WIGGLESWORTH, 1931; 7 ROSTOM & GAMEL-EDIN, 1961 8 CHAMPLAIN & FISK, 1956; 9 WIGGLESWORTH, 1929; 10 ROY, 1937; 11 TATCHELL, 1958; 12 ROCKSTEIN & KAMAL, 1954.

1968), and one wonders how many of the enzymes reported elsewhere are in the cells and how many are in the salivary secretion. The data for salivary glands indicates that there are very few digestive enzymes found in the salivary glands or their secretions (table 1).

Table 2 summarizes the distribution of several digestive enzymes as determined by *in vitro* assays in a variety of haematophagous arthropods. Nutritional studies also indicate the presence of invertase, α-glucosidase(s) and melibiase (α-galactosidase) in *A. aegypti* (GALUN & FRAENKEL, 1957; NAYER & SAUERMAN JR., 1971) and in *Aedes*

Species	lipase	non-spec. esterase	alk. phosphatase	acid phosphatase	amylase	α-glucosidase(s)	invertase	β-glucosidase(s)	α-mannosidase	melibiase (α-galact.)	lactase (β-galact.)	References
Aedes aegypti		+			+		+	–	–	–	–	1,2,3
Anopheles freeborni		+										1
Anopheles stephensi		+										1
Culex pipiens					+							2
Simulium venustum					+		+					2,4,5
Simulium vittatum					+							2,5
Prosimulium fuscum							+					4
Chrysops silacea					+	–	+				–	6
Stomoxys calcitrans	+						+				–	7
Gasterophilus intestinalis (ℓ)	+				+	+	+				–	8
Argas persicus		+	+		–							9
Reighardia sternae		+		+								10

Table 2. Miscellaneous enzymes from the digestive tract of haematophagous arthropods. Symbols as in Table 1. References: 1 FREYVOGEL et al., 1968; 2 YANG & DAVIES, 1968b; 3 GALUN & FRAENKEL, 1957; 4 YANG & DAVIES, 1968c; 5 DAVIES & YANG, 1968; 6 WIGGLESWORTH, 1931; 7 CHAMPLAIN & FISK, 1956; 8 TATCHELL, 1958; 9 TATCHELL, 1964; 10 RILEY, 1972.

taeniorhynchus (NAYER & SAUERMAN JR., 1971). However, GALUN and FRAENKEL (1957) were unable to demonstrate a melibiose hydrolyzing enzyme from *A. aegypti.*

Since proteins are the most obvious nutrient in blood most workers have concentrated on studying the proteinases in haematophagous arthropods. A simple way to characterize a proteinase is on the basis of its pH optimum. Figure 1 shows the pH/activity curves for proteinases from several arthropods. I have taken these data from a variety of sources and in each case have redrawn the pH/activity curves as a percentage of the activity at the pH optimum. Data are available for other species but those species selected are representative of the entire range of pH/activity curves. The proteinase from the tick *Argas persicus* has the lowest pH optimum (TATCHELL, 1964) and in this respect is similar to mammalian pepsin. Maximum activity occurs around pH 5

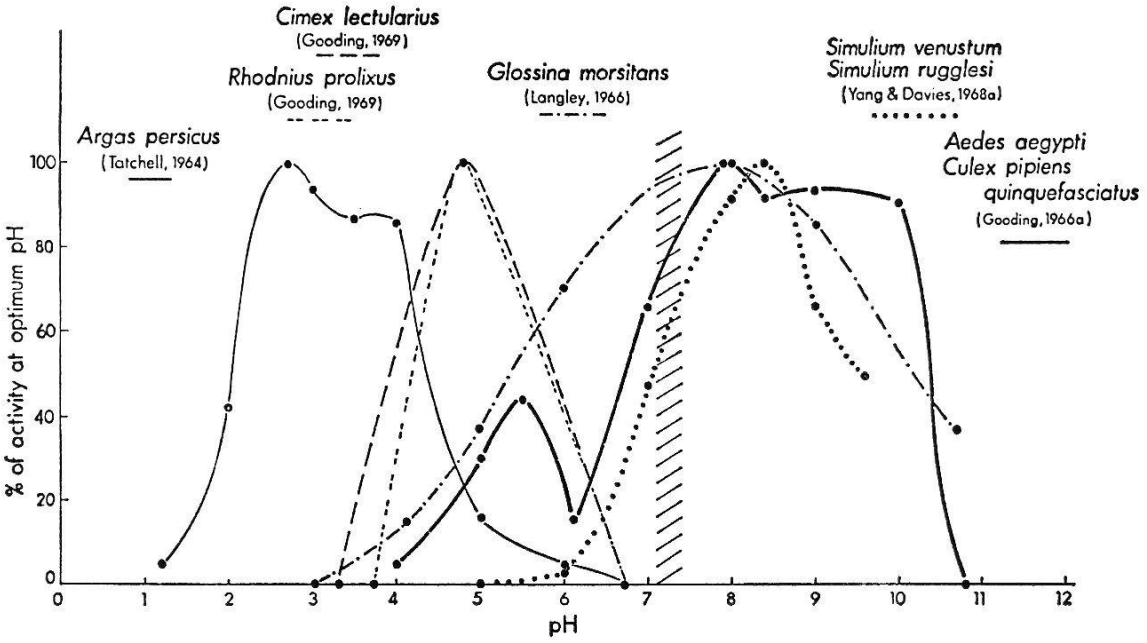

Fig. 1. Effect of pH upon proteolytic activity of midgut homogenates of haematophagous arthropods. The shaded region indicates the pH values reported for vertebrate blood (SPECTOR, 1956).

for the proteinases from *Rhodnius prolixus* (GOODING, 1969; PERSAUD & DAVEY, 1971) and *Cimex lectularius* (GOODING, 1969) and these proteinases are thus similar to mammalian cathepsins. OKASHA (1964, 1968) reported the pH optimum of *R. prolixus* was 3.55. The most frequently encountered pH optimum for insect midgut proteinases is in the alkaline range as shown with the tsetse fly (*Glossina morsitans;* LANGLEY, 1966), black flies (*Simulium venustum* and *Simulium rugglesi*; YANG & DAVIES, 1968a) and mosquitoes (*A. aegypti* and *Culex pipiens quinquefasciatus;* GOODING 1966a). In figure 1 the pH values reported for vertebrate blood (SPECTOR, 1956) are indicated by a shaded area to draw attention to one aspect of *in vivo* control of proteinase activity. For insects with proteinases which function well in a slightly alkaline medium there may be no requirement to adjust the pH of the gut contents. However, for arthropods like *Rhodnius, Cimex* and *Argas* it would be necessary to lower the pH of the meal, or at least that portion of it that is being digested, in order to bring it into the pH range where their enzymes function well. In *Argas* this appears to be accomplished by intracellular digestion of small portions of the meal (TATCHELL, 1964).

Table 3 summarizes attempts to further characterize the proteolytic enzymes of haematophagous arthropods. In several cases characterization has been limited to determination of the pH optimum, however, by use of synthetic substrates the alkaline proteinases have been further characterized as trypsin, chymotrypsin, carboxypeptidase-A, carboxy-

Species	Proteinase: strong acid	Proteinase: mild acid	Proteinase: alkaline	Proteinase: coagulin	plasminogen activator	trypsin	chymotrypsin	carboxypeptidase-A	carboxypeptidase-B	aminopeptidase	dipeptidase	References
Cimex lectularius		+										1
Rhodnius prolixus		+			+			+	–	–		1-5
Pediculus humanus						+	+					1
Pthirus pubis				+								6
Anopheles maculipennis				–								7
Aedes aegypti						+	+	–	–	–		1,8,9,10
Culex pipiens quinquefasciatus				+		+	+	–	–	–		1,7
Culiseta annulata				+								7
Simulium venustum						+						11
Simulium rugglesi						+						11
Simulium vittatum						+						11
Prosimulium fuscum	–					+						11
Prosimulium decemarticulatum						+						11
Cnephia dacotensis						+						11
Chrysops silacea	–		+								+	12
Tabanids (3gen. 9sp.)						+						13
Musca crassirostris				+								14
Stomoxys calcitrans	–		+									15,16
Glossina morsitans			+	+		+	+		+			17-21
Glossina tachinoides				+								18
Melophagus ovinus						+	+	–	–	–		1,22
Gasterophilus intestinalis (l)			+									23
Protocalliphora avium (l)			+									24
Argas persicus	+									+		25,26
Argas arboreus	+											26

Table 3. Proteolytic enzymes from digestive tracts of haematophagous arthropods. Symbols as in Table 1. References: 1 GOODING, 1969; 2 PERSAUD & DAVEY, 1971; 3 HELLMANN & HAWKINS, 1964; 4 HAWKINS & HELLMANN, 1966; 5 OKASHA, 1964; 6 GRUSZ, 1923; 7 DE BUCK, 1937; 8 WAGNER et al., 1961; 9 YANG & DAVIES, 1971; 10 HUANG, 1971; 11 DAVIES & YANG, 1968; 12 WIGGLESWORTH, 1931; 13 A. W. THOMAS (unpublished); 14 CORNWALL & PATTON, 1914; 15 CHAMPLAIN & FISK, 1956; 16 PATTERSON & FISK, 1958; 17 LANGLEY, 1966; 18 LESTER & LLOYD, 1928; 19 GOODING, 1974a; 20 GOODING, 1974b; 21 WIGGLESWORTH, 1929; 22 GOODING, 1972b; 23 ROY, 1937; 24 ROCKSTEIN & KAMAL, 1954; 25 TATCHELL, 1964; 26 TATCHELL et al., 1972.

peptidase-B or aminopeptidase. By far the commonest, and generally the most active, enzyme is trypsin.

It is a general feature of animals that the digestive enzyme activity rises after feeding, reaches a maximum and then declines as the meal is digested. Although there is considerable variation among the haematophagous arthropods with respect to the relative increase in the amount of proteinase after feeding and the duration of the elevated levels, this pattern has been demonstrated in mosquitoes (FISK & SHAMBAUGH, 1952; GOODING, 1966b), black flies (YANG & DAVIES, 1968a), *R. prolixus* (OKASHA, 1968; PERSAUD & DAVEY, 1971), *G. morsitans* (LANGLEY, 1967a), *A. persicus* and *Argas arboreus* (TATCHELL et al., 1972).

The questions concerning the control of enzyme activity within the digestive tract are: what is the state of enzyme synthesis prior to ingestion of the blood meal; what initiates the rise in enzyme activity; what terminates this rise.

The information stored in the DNA of the secretory cell passes to the m-RNA which serves as a template for synthesis of the protein. The arthropod then could synthesize either an active enzyme which is secreted by the cell or it could synthesize an inactive zymogen. In the latter case the zymogen would subsequently be activated and secreted or alternatively secreted and activated within the gut lumen. Within the midgut the active enzyme may be affected by other chemicals either activators or inhibitors.

Using *A. aegypti* FISK (1950) and SHAMBAUGH (1954) looked for, but failed to find, an inactive precursor of the proteinase which could be activated by the salivary glands, crop, or vertrebrate blood. As far as I am aware there are no examples in the literature demonstrating conversion of a zymogen from a haematophagous insect to an active proteinase.

There is a doubling or tripling of the proteinase activity in *A. persicus* and *A. arboreus* immediately after feeding which may represent activation of pre-existing enzyme (TATCHELL et al., 1972) or it may represent an addition of a salivary proteinase to the gut contents (Tatchell, pers. comm.). In *A. aegypti* there is a transitory decline in midgut proteinase activity immediately after feeding on blood which may be due to either a substrate depletion of enzymes or inhibition by specific proteinase inhibitors in the serum (FISK & SHAMBAUGH, 1952). After feeding on blood *Stomoxys calcitrans* shows neither an immediate rise nor a transitory decline in midgut proteinase activity (CHAMPLAIN & FISK, 1956) and the steady rise in proteinase content of the midgut could be interpreted as activation of pre-existing material or *de novo* synthesis of enzyme.

Attempts to find activators and inhibitors of arthropod proteinases are summarized in table 4. The activators of *A. persicus* proteinase gave

Species	Enzyme	Activators (+ effective, – not effective)	Inhibitors: Ions	Inhibitors: Sera	Misc.
Aedes aegypti	alk. pr'ase	– salivary glands[1] – crops[1] – vertebrate blood[2]	Ca, Mn, Mg[3]	chicken[3]	
	alk. pr'ase (non-trypsin)	± cysteine[4]	Ca, Hg Mn, Mg[4]		
	trypsin		Ca, Hg Cd, Zn[4]	17 vertebrate[5]	honey[6]
Culex pipiens quinquefasciatus	alk. pr'ase		Ca, Mn, Mg[3]	chicken[3]	
	trypsin				honey lily nectar[6]
Melophagus ovinus	trypsin			5 mammals[7]	
Glossina morsitans	trypsin			5 mammals[8]	G.m. salivary glds. anterior midguts malpighian tubules[8]
Argas persicus	acid pr'ase	+ ascorbic acid + cysteine + Pb[9]			

Table 4. Effect of activators and inhibitors upon proteinases from haematophagous arthropods. References: 1 FISK, 1950; 2 SHAMBAUGH, 1954; 3 GOODING, 1966a; 4 WAGNER et al., 1961; 5 HUANG, 1971; 6 GOODING et al., 1973; 7 GOODING, 1972b; 8 GOODING, 1974a; 9 TATCHELL, 1964.

in vitro activation of about 30 % (TATCHELL, 1964), which is not enough to account for the rise in proteinase activity observed *in vivo*. This strengthens Tatchell's suggestion that some of the rise may be due to ingestion of saliva and its proteinase. Inhibitors are more commonly reported and of particular interest is the inhibition of trypsin by serum (GOODING, 1966a, 1972b, 1974a; HUANG, 1971). Serum, however, is not always an inhibitor. Gooding and Thomas (in prep.) found two trypsins in several species of tabanids; one of these trypsins is inhibited in a normal manner and the other is activated by several mammalian sera. We have yet to determine what contribution this activation makes to control of digestive rate in tabanids.

The data on *in vitro* activation and inhibition of proteinases do not account for all the changes in the levels of enzyme activity which occur *in vivo* after feeding. Proteinase levels are depressed in blood-fed *A. aegypti* fed 5-fluorouracil (AKOV, 1965) and trypsin levels are depressed if either D-actinomycin or puromycin are included in the first blood meal (GOODING, 1973). Recently I found that inclusion of D-actinomycin in the second blood meal (0.4 μl D-actinomycin/ml rabbit blood) reduces the amount of trypsin from a control level which hydrolyzed 16.5 μ moles Benzoyl-DL-arginine-p-nitroanilide per min per gut at 30 °C to a level which hydrolyzed only 1.6 μmoles BApNA/min/gut. These results suggest that synthesis of both m-RNA and protein takes

place after ingestion of the first and second blood meal. When radioactive amino acids are included in the first blood meal taken by *A. aegypti* the trypsin purified from the midguts of these insects is radioactive (GOODING, 1973). This is further evidence that trypsin is synthesized *de novo* after ingestion of the blood meal.

Puromycin but not D-actinomycin depresses synthesis of trypsin and carboxypeptidase-B after ingestion of a serum meal by *G. morsitans* (GOODING, 1974c). This indicates *de novo* synthesis of both these enzymes after ingestion of the meal. The failure of D-actinomycin to inhibit enzyme production may indicate that m-RNA has been synthesized before the meal was taken, or it may indicate a failure of the D-actinomycin to penetrate the gut cells, or it may indicate some other mechanism by which the system is insensitive to D-actinomycin. This point requires further work.

I turn now to the question of the mechanism by which the levels of proteinase within the midgut are regulated. Figure 2 presents a diagrammatic scheme of two possible mechanisms: a hormonal mechanism and a secretogogue mechanism. In the hormonal mechanism some factor in the blood meal is detected and a message sent to an endocrine gland which releases a hormone into the haemolymph. The hormone then stimulates the secretory cells of the gut to synthesize and release proteinase. Experiments to demonstrate such a mechanism include cutting nerves from the gut to the central nervous system, removal or destruction of the appropriate endocrine gland and transfusion of haemolymph from fed to unfed individuals. The secretogogue mechanism requires that a chemical in the blood meal stimulates the secretory cells causing them to secrete the appropriate enzyme. Evidence in support of a secretogogue mechanism comes partly from failure to establish a hormonal mechanism as well as demonstration of a specific chemical stimulating the secretory cells. One should also be able to obtain stimulation of the secretory cells in a preparation which is isolated from any possible sources of hormone.

The relative merits of these two hypotheses have been evaluated with mosquitoes, *R. prolixus,* and tsetse flies.

Work with *Anopheles maculipennis* by DETINOVA (1962) and with autogenous *Aedes atropalpus* by HUDSON (1970) indicates that mosquitoes with mature or nearly mature eggs are incapable of normal digestion of the blood meal. This suggested to Detinova the possibility of hormonal involvement in digestion. One direct piece of evidence consistent with this is that decapitation of *A. aegypti* after feeding depresses the proteinase level by about 60% (GOODING, 1966b). However, ablation of the median neurosecretory cells of mosquitoes *(A. taeniorhynchus, Aedes sollicitans* and *A. aegypti)* does not reduce the rate of net synthesis of triglycerides after ingestion of a blood meal

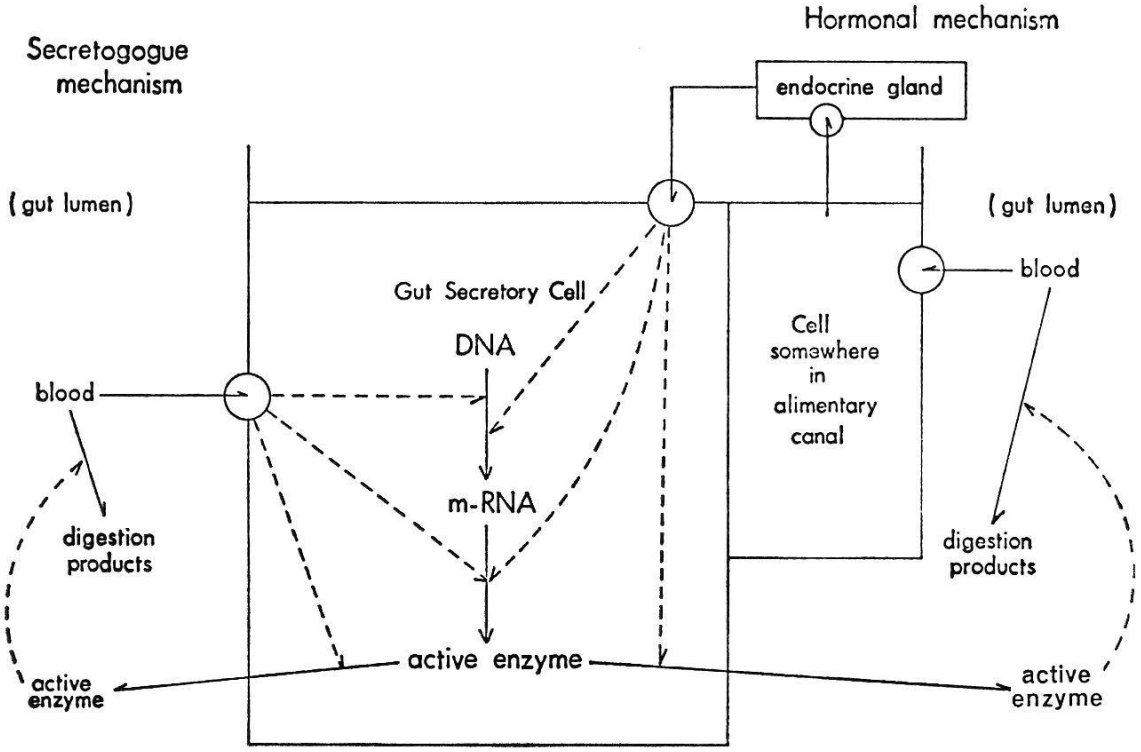

Fig. 2. A diagrammatic representation of a secretogogue and a hormonal mechanism for controlling secretion of digestive proteinases in haematophagous arthropods.

indicating that digestion is proceeding at a normal rate (LEA, 1967). Injecting haemolymph from fed to unfed *A. aegypti* does not increase proteinase content of the gut of unfed *A. aegypti* (FISK & SHAMBAUGH, 1952; SHAMBAUGH, 1954). However, serum proteins fed to *A. aegypti* stimulated proteinase production and there was a correlation between meal size and proteinase production (SHAMBAUGH, 1954). This latter relationship has been confirmed for trypsin but not chymotrypsin in the midguts of *A. aegypti* (GOODING, 1973). Attempts to get increases in proteinase levels by incubating blood with gut homogenates failed (SHAMBAUGH, 1954) and midguts dissected from fed *A. aegypti* failed to produce trypsin when maintained in a tissue culture medium (GOODING, 1973). Despite these last two failures I think the weight of the evidence favours a secretogogue mechanism in mosquitoes.

Thermal shock and decapitation after feeding reduces the amount of midgut proteinase in *R. prolixus* (OKASHA, 1964, 1968). These manipulations affect the endocrine functions and this suggested a possible endocrine control of digestion; Okasha concluded that "The initial rise in protease during the first two days following feeding is believed to be due to the effect of the brain hormone which is released during feeding from the corpus cardiacum . . ." (OKASHA, 1968). However, he pointed out that high temperatures interfere with protein synthesis in *R. pro-*

lixus and that at high temperatures blood is only slowly released from the crop into the digestive part of the midgut. Therefore his results did not distinguish between a hormonal and a secretogogue mechanism.

PERSAUD and DAVEY (1971) showed that decapitation, or removal of the corpora allata from *R. prolixus* depresses the level of proteinase, but does not abolish the cyclic change in the amount of proteinase after feeding. They also found that the proteinase level in the digestive part of the midgut paralleled changes in the contents of the gut. Removal of the corpora allata depresses the amount of material in the digestive section of the midgut as well as depressing the amount of proteinase there. These results suggested to Persaud and Davey that proteinase levels are probably controlled via a secretogogue mechanism. If there is a hormonal component to digestive physiology in *R. prolixus* it is probably by influencing the distribution of material within the midgut.

LANGLEY (1966) found that in *G. morsitans* fed upon guinea pigs there was a linear relationship between meal size and the amount of midgut proteinase. This same relationship held with flies fed various dilutions of ox blood. Feeding flies on saline alone did not increase the amount of midgut proteinase, but feeding upon serum did increase the levels of proteinase in the midgut. These findings led LANGLEY (1966) to propose the following hypothesis: "The size of the meal ingested controls the liberation of hormones into the haemolymph by causing impulses to pass from the stretch receptors of the crop duct along the oesophageal nerves to the neuroendocrine system. The hormone or hormones stimulate the production of an enzyme precursor in the middle segment of the midgut, which is then activated in the lumen of the gut by some factor in the blood serum." Further evidence concerning hormonal control of digestion was obtained when LANGLEY (1967b) showed that during the 24 hours after *G. morsitans* emerges there was a rise in the midgut proteinase level, and that if the ptilinum were punctured and the crop did not become inflated there was no rise in proteinase level. Ligaturing flies between the head and thorax caused high mortality in the flies but those which survived 24 h had low midgut proteinase; injection of macerated brain from a 24-h-old fly into a ligatured fly resulted in a significant rise in midgut proteinase activity. LANGLEY and ABASA (1970) reported that midgut proteinase in *Glossina austeni* is correlated with meal size 24 h after feeding, indicating that the control mechanism may be the same in both *G. morsitans* and *G. austeni*.

Not all evidence concerning control of digestion in the tsetse flies supports LANGLEY's (1966) hypothesis. The amount of midgut proteinase is not invariably correlated with the size of the blood meal in *G. morsitans* (GOODING, 1974b, 1974d) nor in *G. austeni* (AKOV, 1972). Although a meal of serum stimulates an increase in midgut proteinase

the amount of midgut proteinase is not correlated with the size of the meal (LANGLEY, 1966; GOODING, 1974b). AKOV (1972) reported that serum depressed the amount of midgut proteinase but I feel this result may have been due to dissecting technique (GOODING, 1974b). Injection of 4 μl of haemolymph from a 24-h-old fly into a ptilinum-punctured, unexpanded fly did not cause a rise in the midgut proteinase level, nor was there any histological evidence to indicate impairment of the normal function of the neuroendocrine system of flies which did not produce elevated levels of midgut proteinase (LANGLEY, 1967b). Although cutting the ventral nerve cord prevented a rise in midgut proteinase during the first 24 hours after emergence, the same result was obtained in several sham-operated controls (LANGLEY, 1967b). Ablation of the medial neurosecretory cells of *G. austeni* does not affect the level of midgut proteinase (FOSTER, 1972) indicating at least this portion of the endocrine system is not involved in controlling the midgut proteinase. Although serum fed to *G. morsitans* stimulates a rise in the level of midgut proteinase there is no evidence to indicate that this rise is due to activation of a precursor and, in fact, vertebrate sera inhibit *G. morsitans* trypsin *in vitro* (GOODING, 1974a) and *G. austeni* proteinase (Langley, pers. comm.; AKOV, 1972).

An alternative hypothesis is that the proteinase level in the midgut of tsetse flies is controlled by a secretogogue mechanism, with one or more serum proteins providing the normal stimulus to the midgut secretory cells. The first published evidence in support of this hypothesis is LANGLEY's (1966) demonstration that a meal of serum stimulates a rise in midgut proteinase in *G. morsitans* while meals of saline or washed erythrocytes do not. AKOV (1972) reported that diluted sheep blood, haemoglobin and casein fed to *G. austeni* each stimulated proteinase production to a different extent, and concluded that "any protein fed stimulates protease". Using *G. morsitans* of various ages and fed various kinds of meals I have found a statistically significant correlation between the amount of trypsin in the digestive part of the midgut and the amount of protein there (GOODING, 1974b, 1974c, 1974d). The amount of trypsin secreted is also influenced by the nature of the protein fed to the tsetse fly (GOODING, 1974b, 1974c). However, in unfed *G. morsitans* the amount of midgut trypsin is not correlated with the amount of protein in the digestive part of the midgut (GOODING, 1974b). Although there are a number of points that remain to be established I feel that the trypsin levels in *G. morsitans* are probably regulated by a secretogogue mechanism.

Above, I have tentatively concluded that after feeding a secretogogue mechanism controls the level of digestive proteinase in the midgut of mosquitoes, *R. prolixus* and the tsetse flies, and I would like to be able to extend this to haematophagous arthropods in general. There

are, however, a number of reports in the literature (some of which I have already cited) indicating that the level of digestive proteinase is influenced by some factor from outside the digestive tract. Field-caught *G. morsitans* have more midgut proteinase than do laboratory reared flies and this has been interpreted as indicating differences in the secretory activity of the neuroendocrine system (LANGLEY, 1967a). *R. prolixus* virgin females have more proteinase prior to feeding than do mated females, however, both mated and virgin females have a maximum proteinase level 4 days after feeding (PERSAUD & DAVEY, 1972). The subsequent decline in the proteinase level is faster in mated than in virgin females. The effect of mating upon proteinase level may be through a series of events rather than a direct hormonal influence upon proteinase synthesis since virgin females retain food in the crop longer than do mated females (PERSAUD & DAVEY, 1972). One would like to know whether the amount of food in the digestive part of the midgut is the same in virgin and mated females before speculating upon the mechanism by which mating has influenced digestion. In Argasid ticks digestion of the blood meal is faster in mated than in virgin females. This has been demonstrated with *A. persicus* (TATCHELL, 1964), *Ornithodoros tholozani* (GALUN & WARBURG, 1968) and *Ornithodoros moubata* (AESCHLIMANN & GRANDJEAN, 1973; GRANDJEAN & AESCHLIMANN, 1973). For a few days after feeding approximately the same amount of gut proteinase occurs in mated and virgin female *A. persicus,* but the latter maintain an elevated enzyme level longer (TATCHELL et al., 1972). This would appear to contradict the finding that mated females digest their meal faster than virgins unless the proteinase and the blood proteins are not mixed. If blood proteins are not being absorbed by the digestive cells then it is possible to have both an elevated proteinase level and slow digestion of the blood meal. An alternative suggestion is that if the products of digestion were not absorbed from the gut, product inhibition of the proteinase may result.

As far as I know no information has been published on the mechanism by which production of the digestive enzymes is turned off in haematophagous arthropods. It is possible that the secretory cells are stimulated and that the m-RNA is produced for a short period of time after which it serves as a template for enzyme synthesis until the m-RNA is destroyed. The balance between the rates of protein synthesis and of m-RNA destruction within the cell and loss of enzyme from the gut could determine the maximum amount of proteinase found in the midgut. It is also possible that the products of digestion act directly upon the secretory cells causing them to stop the sequence of events which had begun earlier. If the latter is the mechanism it would provide an explanation for some of the observations which appear to involve the endocrine system. Whatever the mechanism it seems likely that the

post-secretory cell is at the same level of information transmission found in the resting (pre-secretory) cell.

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