STUDIES ON THE DIGESTION OF WOOL BY INSECTS

VI. THE pH AND OXIDATION-REDUCTION POTENTIAL OF THE ALIMENTARY CANAL OF THE CLOTHES MOTH LARVA (*TINEOLA BISSELLIELAL* (HUMM.))

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Summary

The pH and oxidation-reduction potential of the contents of the alimentary canal of *Tineola* larvae were determined by adding suitable indicators to their food.

The contents of the foregut and the introductory portion of the midgut have a pH of 8.0 to 8.4. Then follows a region of pH 8.5 to 9.0, which leads into the middle region of the midgut with a pH of 9.8 to 10.0. In the posterior midgut the pH drops first to 8.5 to 9.0 and then to 7.8 to 8.0. The hindgut contents have a pH of 4.6 to 5.8.

The oxidation-reduction potential of the foregut and introductory region of the midgut lies in the range —20 to +32 mV. Then follows a region with a potential of about —200 mV. The middle region has a potential in the range —250 to —280 mV. In the posterior midgut the potential rises first to —150 to —190 mV. and then to above +62 mV. The hindgut contents have a potential higher than +250 mV.

The pH of the contents of the goblet cell cavities in the anterior and posterior regions is 6.2 to 6.5 and the potential +31 to +80 mV. Because these values are very different from those of the contents of the digestive tract it is unlikely that the goblet cells function principally in the production and accumulation of digestive secretions. It is suggested, instead, that important functions of these cells are storage and active excretion. These functions may be correlated with the absence in Lepidoptera of replacement of epithelial cells between moults, a process characteristic of many other insects.

I. INTRODUCTION

The current theory of the mechanism of digestion of wool by larvae of the clothes moth *Tineola* is that the alkaline, highly reducing, midgut digestive juices reduce the disulphide bonds of wool cystine to sulphydryl groups. This reduced wool is far less resistant than normal wool to enzymic attack and is degraded by the larval proteolytic enzymes (Linderstrøm-Lang and Duspiva 1936). Some other animals, which do not possess the capacity to reduce wool (and hence cannot digest it) can, however, digest previously reduced wool. It is clear, therefore, that, for the theory of the mechanism of digestion to be soundly based, detailed information is required on the pH and oxidation-reduction potential of the digestive tract of *Tineola* larvae.

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Many years ago Sitowski (1905) reported that the foregut and midgut contents of clothes moth larvae were alkaline to litmus, but that the hindgut contents were acid. Hindgut acidity was due to the presence of an organic acid (uric acid), since congo red failed to turn blue. These results were confirmed and extended by Titschack (1922) who, in addition, reported that phenolphthalein took on its alkaline coloration in the midgut, thereby indicating a pH greater than about 9.7. Confirmatory tests were also reported by Schulz (1925) using litmus. In 1936, Duspiva re-investigated the problem. He found, using thymol blue, that the midgut secretions were more alkaline than pH 9 and by glass electrode measurements that they averaged 9.9, with a range in different larvae from 9.44 to 10.15. Fore- and midgut alkalinity and hindgut acidity are, therefore, well established. However, it was considered desirable to learn more about the variation in pH along the digestive tract and, in particular to define the zone of extremely high pH by means of a wider range of pH indicators than previously employed. Furthermore, information was also required on the pH of the goblet cavities, which have been held to act as reservoirs for digestive enzymes (see Waterhouse 1952*b*).

Linderstrøm-Lang and Duspiva (1936) determined the oxidation-reduction potential of the contents of the alimentary canal to be in the vicinity of -300 mV. by feeding six redox indicators. They also recorded dye uptake by the midgut epithelium. The results of Day (1951*a*) with triphenyltetrazolium chloride confirmed the existence of reducing conditions in the midgut although the results obtained were rather confusing. The dye experiments are extended in the present series of tests, which indicate that the conditions in the most reducing region of the midgut are probably slightly less reducing than previously thought. They also provide the first information on the potential in other regions of the gut and in the goblet cell cavities.

II. Methods

Larvae of *Tineola bisselliella* were transferred after feeding for 3-4 weeks on a casein-yeast diet at 27°C. to woollen fabric which had been impregnated with saturated aqueous solutions of indicators. A small amount of alkali was required to bring some of the pH indicators into solution. At times indicators were also fed with silk and with the yeast-casein diet.

Larvae were examined after one or two days on these treated diets and dissected, where necessary, to determine more accurately the regions of colour change. Major differences in coloration could be seen quite readily through the transparent body wall of the living larva. Because of the solubility of the indicators in fluids used for histological preparations no sections were prepared except of methylene-blue-fed larvae. Methylene blue was rendered insoluble by the ammonium molybdate procedure. pH indicators of the sulphonphthalein series,* which are relatively free from salt and protein errors

* BDH water-soluble indicators.

were used except for the range above pH 9.0, where recourse had to be made to other indicators to extend the range. The presence of the reduced leuco form of some of the oxidation-reduction indicators was confirmed by exposing the gut to oxidation by air following dissection.

III. Results

(*a*) *pH*

Table 1 lists the pH indicators that provided useful information when fed to *Tineola* larvae and Figure 1 illustrates the results obtained. The contents of the foregut and the short introductory portion of the midgut had a pH of 8.0-8.4. Then followed an equally short second region of the midgut with alkalinity increasing to about pH 9. Commencing with the middle region of the midgut (Waterhouse 1952*a*, Fig. 1) the alkalinity rose sharply to pH 9.8-10. From the end of the middle region and throughout the posterior midgut the alkalinity decreased, first to pH 8.5-9.0 and then to 7.8-8.0. In the hindgut the contents had a pH of 4.6-5.8.

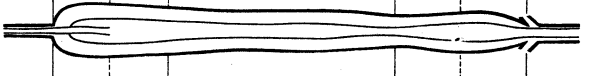
	FOREGUT	MIDGUT			HINDGUT
		ANTERIOR A | B	MID C	POSTERIOR D | E	
pH					
LUMEN CONTENTS	8·0-8·4	8·5-9·0	9·3-10·0	8·5-9·0 | 7·8-8·0	4·6-5·8
GOBLET CAVITIES	-	6·2-6·5	-	6·2-6·5	-
E_h					
LUMEN CONTENTS	-20 TO +32	ABOUT -200	-220 TO -300	-150 TO -190 | >+62	> +250
GOBLET CAVITIES	-	+30 TO +80	-	+30 TO +80	-

Fig. 1.—Diagram showing pH and oxidation-reduction potential (mV.) of various regions of the digestive tract of *Tineola* larvae.

In the alimentary canal epithelium pH indicators were present in sufficient concentration to be visible only in the cavities of the cigar-shaped goblet cells of the anterior and posterior regions. No accumulation was observed in the flask-shaped goblet cells of the middle region (Waterhouse 1952*a*). The contents of the cigar-shaped goblet cell cavity had a pH of 6.2 to 6.5. When larvae that had accumulated many of the pH indicators in the goblet cavities were transferred to control fabric the accumulations disappeared in the course of one or two days, indicating that these accumulations are not static, unlike those of the metal sulphides (Waterhouse 1952*a*).

(*b*) *Oxidation-Reduction Potential*

The oxidation-reduction potential indicators used and the potentials recorded in the lumen of the digestive tract are shown in Table 2 and Figure 1. The potential at which these indicators are 50 per cent. oxidized (their E'_0

TABLE 1

pH OF THE CONTENTS OF THE ALIMENTARY CANAL AND GOBLET CELL CAVITIES OF *TINEOLA* LARVAE

Indicator	Gut Contents						Goblet Cavity Contents		
	Foregut and Midgut *A*	Midgut Region (See Fig. 1) *B*	*C*	*D*	*E*	Hindgut	*A* + *B*	*C*	*D* + *E*
Brom-phenol blue	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0		> 4.0
Brom-cresol green	> 4.6	> 4.6	> 4.6	> 4.6	> 4.6	> 4.6	> 4.6		> 4.6
Chlor-phenol red	> 5.8	> 5.8	> 5.8	> 5.8	> 5.8	< 5.8	> 5.8		> 5.8
Brom-cresol purple	> 6.2	> 6.2	> 6.2	> 6.2	> 6.2	< 6.0	> 6.2		> 6.2
Brom thymol blue	> 6.9	> 6.9	> 6.9	> 6.9	> 6.9	< 6.5	< 6.5		< 6.5
Phenol red	> 7.6	> 7.6	> 7.6	> 7.6	> 7.6	< 7.2	< 7.6		< 7.6
Cresol red	> 7.8	> 8.0	> 8.0	> 8.0	> 7.8	< 7.6	< 7.6		< 7.6
Meta-cresol purple	> 8.0	> 8.0	> 8.0	> 8.0	< 7.8	< 7.8	> 7.6-< 7.8		< 7.6
Thymol blue	< 8.4	8.4-8.8	> 9.0	8.4-8.8	< 8.4	> 8.4	< 8.0		< 8.0
Phenol violet	< 8.5	About 9.0	> 9.5	About 9.0	< 8.5	< 8.5	< 8.5		< 8.5
O-cresol-phthalein	< 9.0	< 9.0	> 9.5	< 9.0	< 9.0	< 9.0			
Phenol-tetrachloro-phthalin	< 8.2	> 8.5	> 9.0	> 8.5	< 8.2	< 8.2			
Phenol-phthalein	< 8.3	< 9.0	> 9.7	< 9.0	< 8.3	< 8.3			
Phenol-thymol-phthalein	< 9.4	About 9.6	> 9.8	About 9.6	< 9.4	< 9.4			
BDH universal indicator	About 8.0	8.5-9.0	9.5-10.0	8.5-9.0	About 8.0	6.0-6.5	About 6.5		About 6.5
Range	8.0-8.4	8.5-9.0	9.8-10.0	8.5-9.0	7.8-8.0	4.6-5.8	6.2-6.5		6.2-6.5

value) varies with pH (with the exception of benzyl viologen (Michaelis and Hill 1933)) and it is to be expected therefore that different potentials will be recorded for each of the six zones of varying pH. The E'_0 values of the

TABLE 2

OXIDATION-REDUCTION POTENTIAL OF THE CONTENTS OF THE ALIMENTARY CANAL OF *TINEOLA* LARVAE

Indicator	E'_0 (V.) at pH 5	8	8.8	10	Colour Change (oxidized to reduced)	Foregut and Midgut *A*	Midgut Region (see Fig. 1) *B*	*C*	*D*	*E*	Hindgut
1-naphthol 2-sulphonate indophenol	*c.* + 0.250	+ 0.062	+ 0.010	— 0.055	Pink to colourless	Colourless	Colourless		Colourless	Pink > + 0.062	Pink > + 0.250
Toluylene blue	+ 0.0221	+ 0.082	+ 0.057		Blue to colourless	Colourless	Colourless		Colourless		Blue
Thionine	+ 0.138	+ 0.032	+ 0.008	— 0.025	Blue to colourless	Colourless < + 0.032	Colourless		Colourless	Light blue	Blue
Methylene blue	+ 0.101	— 0.020	— 0.044	— 0.08	Blue to colourless	Blue > — 0.020	Colourless		Colourless	Light blue	Blue
Indigo tetrasulphonate	+ 0.065	— 0.083	— 0.108	— 0.13	Blue to yellow	Blue or greenish blue	Yellow		Yellow	Blue	Blue
2,3,5-triphenyltetrazolium chloride		(— 0.080 at pH 7)			Colourless to red	Pink	Pink		Pink	Pink	
Indigo trisulphonate	+ 0.032	— 0.121	— 0.146	— 0.175	Blue to yellow	Blue to green	Yellow		Green < — 0.146	Blue	Blue
Indigo disulphonate	— 0.010	— 0.167	— 0.193	— 0.22	Blue to yellow	Light blue	Greenish yellow — 0.220 to — 0.300		Bluish green > — 0.193	Blue	Blue
Janus green		(— 0.225 at pH 7)			Blue to colourless	Pink	Pink		Pink	Pink	Pink
Phenosafranin	— 0.160	— 0.259	— 0.295	— 0.350	Pink to colourless	Pink	Pink		Pink	Pink	Pink
Rosindulin	— 0.161	— 0.340	— 0.385	— 0.438	Red to colourless	Dark pink	Light pink		Dark pink	Dark pink	Dark pink
Benzyl viologen	— 0.359	— 0.359	— 0.359	— 0.359	Colourless to violet Range	Colourless — 0.020 to + 0.032	Colourless About — 0.200	— 0.220 to — 0.300	Colourless — 0.150 to — 0.190	Colourless > + 0.062	Colourless > + 0.250

dyes at different pH values are taken from Butler (1935), Linderstrøm-Lang and Duspiva (1936), Michaelis (1931), and Hewitt (1950).

The contents of the foregut and introductory region of the midgut had a value near zero potential (in the range —20 to +32 mV.). The short second region of the anterior midgut was considerably more reducing (—200 mV.). Commencing with the middle region of the midgut there was a further decrease in potential, which reached its lowest level in the digestive tract (—220 to —300 mV.) in this region. At the beginning of the posterior midgut the potential first rose to —150 to —190 mV. and then to +62 mV. or higher. In the hindgut the conditions were oxidizing (at least +250 mV.).

TABLE 3

OXIDATION-REDUCTION POTENTIAL IN THE MIDGUT EPITHELIUM OF *TINEOLA* LARVAE. COLOUR CHANGES OF THE DYES ARE SHOWN IN TABLE 2

Indicator	E'_0 at pH 6.4	Eh of Goblet Cavities	Midgut Region (See Fig. 1)			
			A	*B*	*C*	*D* and *E*
1-Naphthol 2-sulphonate indophenol	+ 0.159		No colour			
Toluylene blue	+ 0.141		No colour			
Thionine	+ 0.080	< + 0.80	Very occasionally turns blue on oxidation		No colour	
Methylene blue	+ 0.031	> + 0.031	Deep blue in goblet cells: few blue granules in columnar cells	Colourless		Sometimes very light blue
Indigo tetrasulphonate	— 0.017	> — 0.017	Light blue in goblet cavities; general diffuse blue			No colour
2,3,5-Triphenyl-tetrazolium chloride	> — 0.181		Entire epithelium pink			
Indigo trisulphonate	— 0.051	> — 0.051	Goblet cavities blue			
Indigo disulphonate	— 0.092	> — 0.092	Goblet cavities blue			
Janus green	> — 0.225	> — 0.225	Purple, mainly in goblet	Diffuse salmon		
Phenosafranin	— 0.235	> — 0.235	Entire epithelium a light pink			
Rosindulin	— 0.245	> — 0.245	Faint pink in goblet cells			
Benzyl viologen	— 0.359	> — 0.239	Colourless			

Unlike the pH indicators, some redox indicators were accumulated in detectable amounts in the goblet cells of all regions and, to a lesser extent, also by the columnar cells (Table 3). Although it is not possible in the absence of sections to establish the precise location of the indicators, they generally appeared to be accumulated in the goblet cavities. However, sections did indicate that fully coloured methylene blue occurred principally in the cytoplasm of

the anterior goblet cells and in the lining of the goblet cavities of the middle region. Small scattered granules of dye also occurred in the columnar cells (Waterhouse 1952*a*).

From available data a potential within the range +30 to +80 mV. is indicated in the anterior and posterior goblet cells. Because of the absence of information on the pH of the contents of the goblet cavities in the middle region of the midgut it is not possible to assign a potential value to them. Nevertheless some inferences can be drawn from the facts that indigo trisulphonate is fully reduced in the lumen of the mid midgut (Table 2), but is oxidized in the goblet cell cavities of this region (Table 3). This suggests that the potential in the cavities is higher than that in the lumen. An alternative possibility is that the pH of the cavity contents is considerably higher than that of the lumen of the gut (i.e. greater than 10.0). It is conceivable that the goblet cells of this region produce the very alkaline secretion which is responsible for the high midgut alkalinity, although their relatively infrequent occurrence in this region suggests that this is unlikely. If they do produce a secretion having a pH of 10 or higher, they certainly differ very markedly in function from the goblet cells of the anterior and posterior regions to which they show some functional resemblance in accumulating (albeit less frequently and less readily) metal sulphides (Waterhouse 1952*a*) and redox indicators.

IV. Discussion

Although the very much greater precision of electrical methods over the indicator method for measuring pH is clear, nevertheless the value of the indicator method is well illustrated in the present experiments. It is now possible to say, for example, that only in the middle region of the midgut is a pH as high as 10.0 attained. In fact, it is rather surprising that pooling the contents of the anterior and posterior midguts with those of the middle region, as was done for the glass electrode measurements of Duspiva (1936), still gave an average pH reading of 9.9. It is possible, therefore, that the pH of the middle region actually lies between 10 and 11, in which range it is not possible to determine the pH more accurately with available indicators.

One rather unexpected feature of the pH records is the value of 8.0-8.4 for the foregut contents, even when wool having a pH of 6-7 was fed. Unless it can be explained on the basis that the salivary secretion is copious and alkaline, it would appear that fluid from the midgut is passed forward regularly into the foregut. The well-developed oesophageal invagination (Waterhouse 1952*a*, Fig. 1) apparently does not preclude this movement. Alkaline foregut contents can be observed before dissection in the feeding larva and during dissection the forward movement of fluid from the beginning of the midgut into the foregut can sometimes be observed.

The materials responsible for midgut alkalinity, which is characteristic of all Lepidoptera (Waterhouse 1949), have not yet been identified. However, it was suggested many years ago (Kirkland and Smith 1898) that potassium

phosphate was largely responsible for the alkalinity of the midgut of the gypsy moth larva. This view receives support from Japanese workers on *Bombyx* larvae who found that the ash of the digestive juices contains 46 per cent. K_2O, 35 per cent. Na_2O, and 5 per cent. P_2O_5 (Itaya 1936). Furthermore, relatively large amounts of inorganic phosphate have been reported to occur in *Deilephila euphorbiae* (Heller 1949; Heller, Karpiak, and Zubikowa 1950).

Hindgut acidity is due, partly at least, to the presence of uric acid and urates which form some 30-40 per cent. of the weight of the faeces (Holland and Cordebard 1926; Powning, unpublished data). Since approximately the same pH is maintained on a diet of silk or yeast and casein as on wool, gut pH is not greatly influenced by the nature of the food. Silk is not digested by the larva although it is ingested. On this diet hindgut acidity is probably maintained by excretion of uric acid produced by larval fasting metabolism.

The establishment of very reducing conditions only at the posterior end of the anterior midgut agrees well with the observations that visible digestion of wool fibres can first be detected with polarized light in this region and that this region is poorly tracheated compared with most other insects (Day 1951*a*, 1951*b*). The precise value of the oxidation-reduction potential in the middle region of the midgut, if indeed it is accurately poised, is of some interest since it influences the selection of possible systems that may be responsible for its maintenance. The fact that gallophenine shows its oxidized coloration in this region (Table 14 of Linderstrøm-Lang and Duspiva 1936) and that indigo disulphonate is partly (probably more than 50 per cent.) reduced (Table 2; see also Linderstrøm-Lang and Duspiva 1936) suggests that the potential (if the pH is 10) lies somewhere between —220 and —290 mV., and probably in the range —250 to —280 mV. Additional accuracy will be possible only when further indicators are available in this range. Whereas this range is little different from "in the neighbourhood of —0.3 volts" (Linderstrøm-Lang and Duspiva 1936), it is of value to indicate that the potential is probably somewhat more positive, rather than more negative, than —300 mV. Since the potential of the cystine-cysteine system is —350 mV. at pH 10 (Fruton and Clarke 1934) it appears that this system is not alone responsible for the maintenance of the reducing potential in the middle region of the midgut, although sulphydryl groups are abundant in the food undergoing digestion. It is of interest to record that xanthine oxidase has been detected in the midgut of *Tineola* larvae (Day 1951*b*), since the hypoxanthine-uric acid reaction has a very low oxidation-reduction potential (Green 1934).

The value of +250 mV. or higher for the hindgut contents, which are rich in uric acid, is somewhat surprising at first sight in view of the fact that uric acid has some reducing properties. However, the high positive potential is partly the result of the low pH (4.6-5.8) and would probably not be influenced much by the small amount of uric acid in solution. It might be influenced more by any soluble urates present. The hindgut is not particularly richly tracheated, although it is relatively better supplied than the midgut.

Some information on the probable function of the goblet cells can be derived from the present tests. In the anterior and posterior regions of the midgut the contents of the cavities have a pH (6.2-6.5) considerably lower than that of the lumen contents (7.8-9.0). Certainly in the foregut, therefore, the goblet cavity contents cannot be responsible for the maintenance of an alkaline pH in the digestive tract. Furthermore, the cavity contents have an oxidation-reduction potential more oxidizing than the mass of food undergoing digestion. If, therefore, the contents play any part in the maintenance of the potential in the lumen of the gut, it must be in the production of precursors of the systems responsible rather than in the production of the actual systems operative. However, in view of the fact that the goblet cells occur least frequently in the middle region of the midgut, where the potential is lowest and the pH highest, it appears improbable that they play any important role in the maintenance of these conditions. There is good reason to believe therefore that, just as in other insects, the columnar cells function actively both in secretion of digestive enzymes and poising and buffering agents and in absorption.

The reason that goblet cells have been regarded as secretory is partly because of their superficial resemblance to the mucus-producing goblet cells of vertebrates and partly because they expose so little of their surface to the lumen that they would not be expected to be effective in absorption. Although there is no information available, there is no more reason to believe that the cavities are reservoirs for the accumulation of digestive enzymes than for enzymes responsible for maintenance of gut potential or for materials responsible for maintenance of gut pH. Other possibilities are that the goblet cells are specialized for intermediary metabolism or that they are primarily concerned with storage or active excretion. The presence of a cavity is difficult to reconcile with the specialized functions of intermediary metabolism, but is not at all inconsistent with the latter suggestions. Furthermore, the goblet cells have been demonstrated to have an important function in storage excretion, not only under the somewhat unusual conditions of high metal and dye intake, but also under normal conditions, such as in storing the brown material accumulated in the goblet cells of grass-fed *Heteronympha* larvae (Waterhouse 1952*a*).

With regard to active excretion the observation is relevant that masses of nickel sulphide, which by their shape had evidently accumulated in the goblet cell cavities, could occasionally be seen in the gut lumen of *Tineola* larvae (Waterhouse 1952*a*). This suggests either the discharge of solid material from the cavities, or the casting off of entire goblet cells under certain conditions. Furthermore, the disappearance of some accumulated pH indicators from the goblet cavities after transfer to control fabric indicates that movement of materials out of the goblet cavities occurs in the feeding larva. The fate of these indicators was not determined. However, as it is not improbable that they were discharged into the gut lumen, both active and storage excretion can probably be regarded as among the important functions of the goblet cells of Lepidoptera.

Perhaps goblet cells have been developed in conjunction with the method of epithelial regeneration in Lepidoptera. In this order no inter-moult replacement is apparent. Either the entire epithelium is cast off and completely regenerated at each moult or it is not completely degraded but is largely reorganized (Lotmar 1941). On the other hand, there is no reason to believe that the malpighian tubules of lepidopterous larvae are any less efficient in excretion than those of other insects. Furthermore, an exclusively excretory function does not adequately explain the frequent occurrence of goblet cells in some regions and their sparse occurrence in others, unless their density is, perhaps, related to the amount of absorption and intermediary metabolism proceeding in the various regions.

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