THE HYDROGEN ION CONCENTRATION IN THE ALIMENTARY CANAL OF LARVAL AND ADULT LEPIDOPTERA

By D. F. Waterhouse*

[*Manuscript received August* 26, 1949]

Summary

The pH of the midgut digestive juices of two species of carnivorous lepidopterous larvae and of 40 species of adult Lepidoptera belonging to 16 families was determined. In all species it was alkaline. From these records, and from those available from the literature for phytophagous, wool-eating, and wax-eating lepidopterous larvae, it is possible to generalize that midgut alkalinity is characteristic of the order Lepidoptera and that it is not dependent upon feeding habits.

For those species for which it was determined, the blood was neutral or slightly alkaline, the crop contents had the same pH as that of the food offered, and the hindgut contents were slightly acid.

I. Introduction

The pH of the digestive juices has a very great influence on the activity of digestive enzymes and on the absorption of foodstuffs and poisons. Any generalizations that can be made for the midgut pH of insects, therefore, will help to provide a clearer picture of the extremely varied processes of digestion and absorption which occur in insects.

In most insects the midgut digestive juices vary only slightly from neutrality, generally falling within the pH range 6.0 to 8.0. Strong midgut acidity has seldom been recorded, but occurs in blowfly larvae, in blowfly adults, and in housefly larvae (Hobson 1931; Waterhouse 1940). It has also been reported for aphids (Bramstedt 1948) and for adult mosquitoes (MacGregor 1931), although the latter record is suspect in view of more recent studies (Fisk 1949; Popow and Golzowa 1933). At the other extreme, larvae of Lepidoptera (see Table 3) and Trichoptera (Shinoda 1930*b*) have weakly to strongly alkaline midgut contents, these generally varying from pH 8.0 to 10.0. In Coleoptera an alkaline reaction also occurs in the midgut of many foliage or wood-feeding species, whereas in many predacious species this region is weakly acid (Shinoda 1930*b*; Staudenmayer 1940). It is worthy of consideration, therefore, whether the occurrence of alkaline digestive juices in Lepidoptera is correlated with phytophagous habits or whether it is an attribute also of non-phytophagous forms and, therefore, a general characteristic of this order.

From records already available for lepidopterous larvae it is clear that distinct alkalinity is not limited to strictly phytophagous forms, since the midgut

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

contents of larvae of the clothes moth (*Tineola bisselliella*) have a pH of 9.9 and those of the wax moth (*Galleria mellonella*) have a pH of 8.4 (Duspiva 1935, 1936; Linderström-Lang and Duspiva 1936). However, it is known that both wool and wax are more readily degraded under alkaline than under acid conditions. Alkaline midgut juices would, therefore, favour digestion of these materials and may even have been responsible for permitting these species to adapt themselves successfully to their food. From available records, therefore, there is really no evidence upon which to decide whether alkalinity of the midgut contents in lepidopterous larvae is an adaptation to their food or whether it is a character of the order.

Two lines of approach to this problem were adopted. In the first, the pH of the digestive tract of two species of predacious lepidopterous larvae was investigated. Carnivorous insects frequently have neutral or acid midgut contents, and a pH above 8.0 would be regarded as unusual. In the second, the pH of the midgut of adult Lepidoptera was determined. If adults feed at all (and there are many species which do not) it is generally only on plant juices, such as nectar. There would not seem to be any advantage biochemically in having alkaline conditions for any of the enzymes required to split sugars, which alone can be utilized (Stober 1927). If, therefore, carnivorous larvae and nectar-feeding adults have alkaline midgut contents it may be concluded that midgut alkalinity is a characteristic of the order Lepidoptera. If, on the other hand, the digestive juices are not alkaline, this would be strong evidence that the midgut reaction was correlated with type of food.

The only available record of the pH of the midgut of adult Lepidoptera is that of Jameson and Atkins (1921), who reported a pH of 5.8 for the forepart of the midgut of the adult silkworm (*Bombyx mori*) when one gut was immersed in a solution of brom-cresol purple. Reasons for considering that this record is misleading are given later. Stober (1927) reported that the pupal residues still present in the midgut of several adult butterflies on emergence were acid. The indicators he used (which all changed at pH 7.0 or less) did not enable him to decide if, when the residues had been discharged, the digestive juices were neutral or weakly alkaline.

II. Methods

Indicator paper and indicator feeding methods were used since the amounts of fluid available were often extremely small and since the latter method enables localized regions of different pH to be readily recognized. Although the use of indicators is subject to certain errors (Waterhouse 1940), their magnitude is not sufficiently great to alter any of the conclusions of this paper.

It was not found possible to induce the carnivorous larvae to ingest indicators. They were therefore carefully dissected in their own haemolymph and the alimentary canals transferred to indicator paper and blotted dry. The midgut was then punctured and the contents allowed to spread out into the surrounding paper. This method appeared to be the only one feasible, at least for the smaller of the two species (*Stathmopoda melanochra*), which contained a large amount of dark red pigment derived from the bodies of the coccid on which it preys. Whereas the midgut fluid diffused out into the indicator paper the pigment was retained near the alimentary canal, thus permitting observation of indicator changes which would otherwise have been obscured by the pigment.

Adult Lepidoptera were starved for some hours and then allowed to feed on sugar solutions saturated with indicators. As a rule, when the tarsi were brought into contact with the sugar solution, an immediate uncoiling of the haustellum resulted and feeding followed. The adults were dissected at various intervals after feeding and the indicator coloration observed immediately through the walls of the alimentary canal. The indicators used in the feeding experiments all belonged to the sulphonphthalein series (thymol blue, cresol red, phenol red, brom-thymol blue, brom-cresol purple, chlor-phenol red, brom-cresol green, and brom-phenol blue). One member of this series, meta-cresol purple, gave aberrant colour changes in preliminary tests and was not used. All indicators were readily accepted except chlor-phenol red, which alone of the series has a definite phenolic smell.

The nomenclature of G. A. Waterhouse (1932) was followed for the butterflies examined, and the author is indebted to Mr. I. F. B. Common for identifying the moths.

III. Results

(*a*) *Larvae*

The two species used were *Stathmopoda melanochra* Meyr. (Heliodinidae) the larvae of which prey upon the scale *Eriococcus coriaceous* Maskell, and *Titanoceros thermoptera* (Low.) (Pyralidae) the larvae of which eat the eggs of the Boree or bag shelter moth, *Ochrogaster contraria* (Walker).

As may be seen from Table 1, the larval midgut of both species is quite alkaline, pH 8.3 to 8.6 for *Stathmopoda* and 8.4 to 8.6 for *Titanoceros*.* The fluid regurgitated by *Stathmopoda* larvae, consisting of crop contents and saliva, had the same pH as the midgut contents. The blood of the two species was neutral or slightly alkaline.

(*b*) *Adults*

Forty species of adult Lepidoptera belonging to 16 families were examined and, in all species, the midgut contents were alkaline (Table 2). Both sexes gave similar results. The midgut pH of many species is shown in the Table as 8.4. In these species, the contents caused both phenol red and cresol red to exhibit their alkaline coloration, although thymol blue failed to do so, thus indicating a pH of about 8.4. There was some suggestion, however, from experiments in which the alimentary canal was immersed in buffers that thymol blue was not changing colour visibly at as low a pH as it should. It is possible,

* Since this paper went to press, *Catoblemma* larvae (Noctuidae) predacious on the scale *Cryptes* have been found to have alkaline (pH 8.2 to 9.6) midgut contents.

therefore, that the pH of the midgut contents in these species is actually higher than 8.4. The cabbage white butterfly (*Pieris rapae*) was the only species in which thymol blue gave some indication of its alkaline coloration. Here, the midgut contents are apparently slightly more alkaline than those of the other species tested.

TABLE 1

THE pH OF THE LARVAL MIDGUT AND BLOOD OF TWO SPECIES OF MOTH

Indicator Paper	*Stathmopoda*		*Titanoceros*	
	Midgut	Blood	Midgut	Blood
2-Cresol phthalein*			< 9.6	
Sodium 2,4-dinitrobenzol-azo-4′-napthol-8-sulphonic acid*	< 8.8	< 8.8	8.2 to 8.8	
8410 wide range†	< 8.7	< 8.7	< 9.0	
Thymol blue	< 8.6	< 8.6	< 8.6	< 8.6
Universal†	8.0 to 9.0	7.0 to 7.5	*c.* 9.0	7.0 to 8.0
Disodium diamidostilbene-azophenol disulphonate*	*c.* 8.4	< 7.2	> 8.4	
6883 wide range†	> 8.3	< 7.4		*c.* 7.1
Cresol red	> 8.2	< 7.8	> 8.2	< 7.8
Disodium 2,4-dinitrobenzol-azo-1-naphthol-3, 6-disulphonate*	> 8.0	6.5 to 7.5		*c.* 7.0
Phenol red	> 7.6	< 7.4		< 7.4
Brom-thymol blue	> 6.7	> 6.7		< 6.7
Range	8.3 to 8.6	*c.* 7.0	8.4 to 8.6	*c.* 7.0

* Bayer (Leverkusen) indicator papers.
† Johnson and Sons (London) indicator papers.

All except three of the butterflies examined had quite alkaline midguts. These three belonged to the family Satyridae and, although each possesses a well-developed haustellum, great difficulty was experienced in inducing them to take the sugar solutions. In fact, the majority of individuals starved to death after several days without feeding. In their natural habitat they are certainly not nectar-loving species; they are generally found in shady situations, and they are not associated with the presence of flowers. The slightly alkaline pH (7.6) of the midgut in these species may, therefore, be associated with their habit of feeding rarely, if at all.

The pH of the contents of the crop was always the same as that of the food offered, and the hindgut contents were acid, falling within the range

TABLE 2

MIDGUT AND HINDGUT pH IN ADULT LEPIDOPTERA

Species	Mid-gut	Hind-gut
Hesperiidae		
Toxidia peroni (Latr.)	8.4	
Padraona flavovittata flavovittata (Latr.)	8.4	5.8-6.2
Taractocera papyria papyria (Bois.)	8.4	5.8
Satyridae		
Heteronympha merope merope (Fabr.)	8.4	6.0-6.9
Heteronympha philerope (Bois.)	8.4	
Oreixenica lathoniella herceus Wat. and Lyell	7.6	
Hypocysta pseudirius Butl.	7.6	
Hypocysta metirius Butl.	7.6	
Nymphalidae		
Precis villida calybe God.	8.4	6.4
Pyrameis cardui kershawi McCoy	8.4	
Pyrameis itea (Fabr.)	8.4	
Danaidae		
Danaida plexippus (L.)	8.4	
Danaida chrysippus petilia (Stoll.)	8.4	
Papilionidae		
Papilio sarpedon choredon Feld.	8.4	6.4
Papilio aegeus aegeus Don.	8.4	
Pieridae		
Pieris rapae (L.)	8.4-9.0	6.4
Terias smilax (Don.)	8.4	
Delias nigrina (Fabr.)	8.4	
Delias harpalyce (Don.)	8.4	
Lycaenidae		
Zizeeria labridas labridas (God.)	8.4	5.7-6.0
Lucia lucanus (Fabr.)	8.4	
Lampides boeticus damoetes (Fabr.)	8.4	
Lycaenidae (continued)		
Candalides acasta Cox	8.4	6.4
Neolucia mathewi Misk.)	8.4	6.4
Plutellidae		
Plutella maculipennis (Curtis)	7.6	6.0-6.5
Gelechiidae		
Sitotroga cerealella (Oe.)	7.6	5.8
Gnorimoschema operculella (Zell.)	8.4	4.0-5.8
Oecophoridae		
Endrosis lactella (Schiffermüller)	*c.* 7.5	
Machimia carnea (Zell.)	8.4	
Tortricidae		
Cydia pomonella (L.)	8.4	
Tortrix postvittana (Walk.)	8.4	
Acropolitis dryinodes Meyr.	*c.* 7.5	
Heliodinidae		
Stathmopoda melanochra Meyr.	7.6-7.8	
Zygaenidae		
Pollanisus apicalis Walk.	8.4	
Arctiidae		
Thallarcha lochaga (Meyr.)	*c.* 7.5	
Asura lydia (Don.)	*c.* 7.5	
Noctuidae		
Cruria donovani Bois.	*c.* 7.5	
Agrotis infusa Bois.	*c.* 7.5	
Heliothis armigera (Fabr.)	8.4	
Sterrhidae		
Scopula rubraria (Dbld.)	8.4	

5.7 to 6.9. When examining the hindgut it was observed that the contents were generally less acid at the anterior end than at the posterior end. This was particularly noticeable when indicator solution had recently passed on from the alkaline midgut. Similarly, indicator solution which had recently passed from the anterior into the middle and posterior segments of the hindgut was less acid than when it had been retained in these latter segments for some time. In several instances the excreta were slightly more acid than the hindgut contents, as in *Precis villida* (Nymphalidae), where the hindgut pH was generally about 6.4, whereas drops of excreta had a pH of about 5.8.

The rate of passage of food down the alimentary canal varied in different species, although in most it appeared to be fairly rapid and, in all, indicator solution filled the midgut within 30 minutes. The rate of passage was most rapid in starved individuals. In *Pieris rapae* food had already reached the middle segment of the hindgut in several instances 30 minutes after feeding commenced, whereas in *Precis villida* the corresponding time was about 60 minutes.

In 7 of the 16 species of moths the midgut pH was 8.4 or higher and in the remaining species it was about 7.5, the turning point of phenol red. In some of these species (e.g. *Stathmopoda melanochra*) the midgut often appeared to be slightly more alkaline at the posterior than at the anterior end, although in others (e.g. *Gnorimoschema operculella*) the reverse was true.

In most moths, and particularly those with a midgut pH about 7.5, the digestive tract appears to be very poorly buffered. Thus, food passed recently from the crop to the midgut does not change rapidly to the characteristic alkaline pH of this region and, if adults are examined within an hour or two after feeding, the pH of the midgut is seen to be variable. However, several hours after feeding, the midgut contents have generally become alkaline, although at any time they may be altered by further passage of food from the crop. Similarly, the pH of the hindgut contents of moths is more variable than that of the butterflies examined, the rectal contents of the potato moth *Gnorimoschema*, for example, varying in different individuals from about 4.0 to about 5.8. In dissected individuals the acidity of the hindgut contents can be observed to diminish rapidly with the passage of alkaline fluid from the midgut. In recording results in Table 2, it has been assumed that the highest alkalinity observed in the midgut and the greatest acidity in the hindgut are typical of the secretions of these regions.

IV. Discussion

It is clear from these results and from the published records summarized in Table 3, that the alkalinity characteristic of the midgut contents of larval Lepidoptera cannot be regarded simply as an adaptation to phytophagous habits, for alkalinity is characteristic also of carnivorous larvae and of nectar-feeding adults. The degree of alkalinity varies within a rather wide range, from species

TABLE 3

RECORDS OF MIDGUT AND HINDGUT pH IN LEPIDOPTERA
(FIGURES GIVEN TO NEAREST 0.1 pH UNIT)

Species	Stage	Midgut	Hindgut	Author
Nymphalidae				
Vanessa urticae	Larva	8.7		Staudenmayer and Stellwaag 1940
Pieridae				
Pieris brassicae	,,	9.4		Skrjabina 1936
,, ,,	,,	8.0		Staudenmayer and Stellwaag 1940
P. napi	,,	9.3		Skrjabina 1936
Tineidae				
Tineola bisselliella	,,	9.9		Duspiva 1936
,, ,,	,,	9.6-10.2		Linderström-Lang and Duspiva 1936
Hyponomeutidae				
Hyponomeuta malinella	,,	9.2		Skrjabina 1936
Tortricidae				
Cydia molesta	,,	7.2-7.3	7.2	H. S. Swingle 1928
C. pomonella	,,	8.5-8.7	7.8	Marshall 1936
Polychrosis botrana	,,	7.7		Staudenmayer and Stellwaag 1940
Clysia ambiguella	,,	7.6		Staudenmayer and Stellwaag 1940
Galleriidae				
Galleria mellonella	,,	8.0-8.4		Duspiva 1935
,, ,,	,,	8.4		Duspiva 1936
,, ,,	,,	7.0-8.0	6.4-6.6	Roy 1937
Notodontidae				
Datana integerrina	,,	9.5		H. S. Swingle 1938
Noctuidae				
Heliothis obsoleta	,,	8.0		M. C. Swingle 1931
,, ,,	,,	8.0		H. S. Swingle 1938
Panolis flammea	,,	7.6	7.3	Trappmann and Nitsche 1933
Barathra brassicae	,,	9.6		Skrjabina 1936
Euxoa segetum	,,	9.7		Skrjabina 1936
Lymantriidae				
Lymantria dispar	,,	8.5	8.2	Trappmann and Nitsche 1933
,, ,,	,,	9.4		Skrjabina 1936
L. monacha	,,	8.3	7.3	Trappmann and Nitsche 1933
Euproctis chrysorrhea	,,	9.6		Skrjabina 1936

TABLE 3 (continued)

Species	Stage	Midgut	Hindgut	Author
Geometridae				
Cheimatobia brumata	Larva	9.5		Skrjabina 1936
Sphingidae				
Protoparce sexta	"	9.6		H. S. Swingle 1938
Ceratomia catalpa	"	9.5	5.7	M. C. Swingle 1931
Deilephila euphoribae	"	> 7.5	< 5.0	Stober 1927
" "	"		5.8-6.3	Heller and Aremowna 1932
Lasiocampidae				
Malacosoma neustria	"	9.3		Skrjabina 1936
Bombycidae				
Bombyx mori	Adult	5.8	5.2	Jameson and Atkins 1921
" "	Larva	9.0-9.8	8.4	Jameson and Atkins 1921
" "	"	8.0, fed 9.6, starved		Suzuki 1924
" "	"	10.3		Gamo 1928
" "	"	10.0		Gamo *et al.* 1933
" "	"	9.8		Shinoda 1930
" "	"	9.1	8.9	Trappmann and Nitsche 1933
" "	"	9.8		Skrjabina 1936
" "	"	9.2 to 9.8, fed 10.0, starved		Itaya 1936

in which the contents are only slightly alkaline to those in which a pH of 10.0 or higher has been recorded, e.g. 10.3 for *Bombyx mori* larvae (Gamo 1928).

Although it is possible that the midgut of some lepidopterous larvae and adults yet to be investigated may be found to be neutral or acid, the fact remains that, of 26 species of larvae and 41 species of adults for which records are available (Tables 1, 2, and 3), there is only one record of midgut acidity and this is for the silkworm adult (Jameson and Atkins 1921). This record may be questioned on a number of grounds. It is based on one individual only; the method used of breaking the gut in a drop of indicator is not a particularly reliable one since the pH may be influenced by that of the cell contents; and, most important of all, adult *B. mori* do not take food and, therefore, the record is not for functional digestive juices. Furthermore, the midgut is said to be the site of secretion of an alkaline fluid which the emerging adult uses to soften the cocoon (Itaya 1936). One may conclude, therefore, that the record of Jameson and Atkins (1921), even if valid, is not an exception to the generalization that the midgut digestive juices in both larval and adult Lepidoptera are alkaline irrespective of their food.

No generalization can be made about the reaction of the hindgut except that it is never as alkaline as the midgut. In some species it is quite alkaline (e.g. pH 8.4 to 8.9 in *B. mori* larvae), although in most it is mildly acid.

V. Acknowledgment

The work described in this paper was carried out as part of the research programme of the Division of Entomology, C.S.I.R.O.

VI. References

Bramstedt, F. (1948).—Über die Verdauungsphysiologie der Aphiden. *Z. Naturforsch.* **3b**: 14-24.

Duspiva, F. (1935).—Ein Beitrag zur Kenntnis der Verdauung bei Wachsmottenraupen. *Z. vergl. Physiol.* **21**: 632-41.

Duspiva, F. (1936).—Die Verwendung der Glaselektrode zur Bestimmung der H+ Konzentration im Darmsaft der Kleider und Wachsmottenlarven. *C. R. Trav. Lab. Carlsberg* **21**: 167-75.

Fisk, F. W. (1949).—Studies on digestive enzymes in adult mosquitoes. Ph.D. Thesis, University of Minnesota, U.S.A.

Gamo, T. (1928).—Nogaku Kwai Ho. *J. Sci. Agric. Soc. Tokyo* **305**: 123-49 (quoted from Shinoda 1930*a*).

Gamo, T., Yamaguchi, S., and Nagai, S. (1933).—Studies on respiratory injuries in the silkworm (*Bombyx mori L.*). III. On the change of the permeability of the enteric epithelium due to some respiratory injuries. *Bull. Seric. Silk. Industr. Uyeda* **5**: 1-2.

Heller, J., and Aremowna, H. (1932).—Über den Harn der Schmetterlinge. *Z. vergl. Physiol.* **16**: 362-70.

Hobson, R. P. (1931).—Studies on the nutrition of blowfly larvae. I. Structure and function of the alimentary tract. *J. Exp. Biol.* **8**: 109-23.

Itaya, K. (1936).—"Theoretical and Experimental Physiology of the Silkworm." (Meibundo: Tokyo.)

Jameson, A. P., and Atkins, W. R. G. (1921).—On the physiology of the silkworm. *Biochem. J.* **15**: 209-12.

Linderström-Lang, K., and Duspiva, F. (1936).—Studies on enzymatic histochemistry. XVI. The digestion of keratin by the larvae of the clothes moth (*Tineola bisselliella* Humm.). *C. R. Trav. Lab. Carlsberg* **21**: 53-82.

MacGregor, M. E. (1931).—The nutrition of adult mosquitoes. Preliminary contribution. *Trans. R. Soc. Trop. Med. Hyg.* **24**: 465-72.

Marshall, J. (1939).—The hydrogen ion concentration of the digestive fluids and blood of the codlin moth larva. *J. Econ. Ent.* **32**: 838-43.

Popow, P. P., and Golzowa, R. D. (1933).—Zur Kenntnis der Wasserstoffionenkonzentration im Darmkanale einiger blutsaugender Arthropoden. *Archiv. Schiffs- u. Tropenhyg.* **37**: 465-6.

Roy, D. N. (1937).—On the nutrition of larvae of the bee-wax moth *Galleria mellonella*. *Z. vergl. Physiol.* **24**: 638-43.

Shinoda, O. (1930*a*).—Contributions to the knowledege of intestinal secretion in insects. III. On the digestive enzymes of the silkworm. *J. Biochem. Tokyo* **11**: 345-67.

Shinoda, O. (1930*b*).—Contributions to the knowledge of intestinal secretion in insects. IV. A comparison of the pH optima of the digestive enzymes from different groups of insects. A preliminary note. Anniversary Volume to Prof. Masumi Chikashige, Kyoto: 8-23.

SKRJABINA, E. (1936).—pH of the insect intestine and blood and its modification at poisoning by arsenic and fluorine compounds. *Bull. Pl. Prot. Leningr.* **7**: 9-23. (In Russian.) (From Staudenmayer 1940.)

STAUDENMAYER, T. (1940).—Die Wasserstoffionenkonzentration der Insekten. *Anz. Schädlingsk.* **16**: 114-19, 125-32.

STAUDENMAYER, T., and STELLWAAG, F. S. (1940).—Über die Wasserstoffionenkonzentration und das Pufferungsvermögen des Darmes von *Clysia ambiguella, Polychrosis botrana* und einigen anderen Insekten sowie ihres Futters. Z. *Angew. Ent.* **26**: 589-607.

STOBER, W. K. (1927).—Ernährungsphysiologische Untersuchungen an Lepidopteren. Z. *vergl. Phys.* **6**: 530-65.

SUZUKI, H. (1924).—*Rep. Jap. Seric. Ass.* **33**: 554-8. (From Shinoda 1930*a*).

SWINGLE, H. S. (1928).—Digestive enzymes of the oriental fruit moth. *Ann. Ent. Soc. Amer.* **21**: 469-75.

SWINGLE, H. S. (1938).—Relative toxicities to insects of acid lead arsenate, calcium arsenate, and magnesium arsenate. *J. Econ. Ent.* **31**: 430-41.

SWINGLE, M. C. (1931).—Hydrogen ion concentration within the digestive tract of certain insects. *Ann. Ent. Soc. Amer.* **24**: 489-95.

TRAPPMANN, W., and NITSCHE, G. (1933).—Methoden zur Prüfung von Pflanzenschutzmitteln. V. Beiträge zur Giftwertbestimmung und zur Kenntnis der Giftwirkung von Arsenverbindungen. *Mitt. biol. Anst.* (*Reichsanst.*) *Berl.* **46**: 61-89.

WATERHOUSE, D. F. (1940).—Studies of the physiology and toxicology of blowflies. 5. The hydrogen ion concentration in the alimentary canal. Coun. Sci. Industr. Res. Aust. Pamph. No. 102.

WATERHOUSE, G. A. (1932).—"What Butterfly is That?" (Angus and Robertson: Sydney.)