HYDROGEN ION CONCENTRATIONS WITHIN THE ALIMENTARY TRACT OF INSECTS.

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There has been a good deal of discussion as to the digestive and absorptive functions respectively pertaining to the several divisions of the intestinal tract of insects. The actual acidities prevailing in the various regions of the gut are of special moment. Among other points of interest, questions arise as to the conditions of enzyme activity within the gut, and as to the possible rôle of such conditions in connection with the absorption of drugs. Possible relations to the transformations of intestinal parasites (e.g. Herptomonads) are likewise suggestive and important.

Chiefly as a result of qualitative experiments involving ingestion of foodstuffs containing indicators such as litmus powder, it has been stated that the anterior portion of the intestinal lumen is in general alkaline in reaction, the midgut acid, the hinder portion of the intestine faintly acid or neutral in reaction. Much variation is evident, however, in the reports of different observers\(^1\) using widely divergent sources of insect material. Thus Sanford\(^2\), repeating and extending observations and experiments of Petrunkevitsch, was able to show that the crop of the cockroach was alkaline to litmus, "neutral" to phenolphthalein (except after ingestion of olive oil, when it was sometimes found acid to litmus), while the stomach, corresponding to the condition reported by a number of observers, was acid. On the other hand, Jameson and Atkins\(^3\) find in the

\(^1\) A general summary of older work, with citations, is given by Jordan, H., Vergleichende Physiologie wirbelloser Tiere. I. Die Ernährung, Jena, 1913.


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silkworm that the contents of the fore part of the midgut are highly alkaline (pH = 9.0 to 9.8, full fed or fasting), the contents of the hindgut less alkaline (pH = 8.4); in the mature moth the midgut was distinctly acid (pH of contents = 5.2 to 5.8), perhaps due to waste products excreted into the intestine, since the imago does not feed. The reaction of the intestinal contents is difficult to interpret in the case of some insects, since the fluid food juices imbibed may largely determine the result; in Flata, for example, the mesenteric reservoir is acid to litmus, but so are the food juices, while in Siphanta the whole gut is said to be slightly alkaline to the same indicator.4 Some further confusion in comparing different accounts seems to be due, in addition, to the lack of uniformity in naming parts of the insect digestive tract.

Particularly instructive observations can be made with certain translucent aquatic larvae, such as young Chironomus larvae and the early larval instars of Psychoda (diptera). These may be maintained for days in solutions of non-toxic sulphonephthalein indicators. The indicators quickly permeate the alimentary tract. The larvae may be removed to clear water for observation under the microscope, and the condition of the indicator determined in the living larva by inspection of the juice in the anterior and posterior portions of the gut, and of the fluid surrounding the peritrophic membrane which encloses the food in the mesenteron. By dissection with needles the intestine is readily extracted and examined separately; the peritrophic membrane with its contents may be removed and opened, and the malphigian tubules isolated and dissected in a drop of indicator. The effect of feeding, the result of varying the external alkalinity over a wide range, the action of agents affecting salivary discharge, and similar points may be in this way studied directly.

Larvae of Psychoda alternata were chiefly used. The hemoglobin in Chironomus larvae, seen greenish in hue when viewed in thin layer, may interfere with the judgement of indicator tints, and the Psychoda larvae are of superior transparency. These two kinds of larvae, however, gave results essentially identical. A "two color" indicator is

4 Kershaw, J. C., The alimentary canal of Flata and other Homoptera, Psyche, 1913, xx, 175.
particularly serviceable. Brom-thymol blue was most convenient. Its non-toxicity is evidenced by the ecdysis and pupation of larvae growing in concentrated solutions of the dye, and their final emergence as imagines. Observations made with brom-thymol blue were checked quantitatively by others independently made with phenol red, neutral red, and brom-phenol blue; possible errors due to the color of food juices were thus eliminated.

In the actively feeding larva the most anterior region of the alimentary tract contains fluid of pH 7.0 to 7.2. The secretion of the salivary glands is on the whole faintly alkaline, rather than neutral. The lumen of the cardiac chamber of the stomach shows tints connoting pH 6.2 to 6.7, in different cases. The variation in pH I take to be possibly indicative of varying degrees of secretory activity. There is a sharp line of demarcation at the posterior border of the cardiac chamber between this zone of pH 6.2 to 6.7 and the immediately caudad mesenteron ("stomach" of textbooks); in the mesenteron the pH usually observed, in the fluid bathing the peritrophic membrane, is 7.5 (range, 7.2 to 7.8). The sharpness of this division is made vividly obvious by the yellow and blue colors of brom-thymol blue on either side of the pyloric sphincter. With Chironomus especially it frequently happens, also, that food is regurgitated from mesenteron into cardiac chamber; in such circumstances the blue material from the mesenteron is instantly turned yellow. When food from the cardiac chamber is ejected from the mouth into a medium of pH about 7.4 containing brom-thymol blue, it is seen yellow, and the diffusion of acid from it can be watched.

The hindgut ("intestine") is acid in reaction, the pH observed varying from 6.0 or less to 6.6. But this, as will be pointed out, is true only of young larvae.

The observations are summarized in Fig. 1.

*The numerical pH values obtained with this indicator may be subject to "salt correction" of unknown but probably quite small magnitude. The tints observed in the larvae, or in the freshly isolated intestine, were compared with those in test-tube standards. A convenient method of doing this, in the case of minute objects under the microscope, has been described by Pantin, C. F. A., Nature, 1923, iii, 81.
The magnitudes of the pH values, and the succession of alterations in these values down the course of the gut, do not differ significantly among larvae maintained in solutions of pH 6.0, 6.4, 6.8, 7.4, 7.6, or 8.0. Therefore they represent the normal, independent condition of the intestine. It will be noticed that in these larvae the ingested food is subjected first to a faintly alkaline salivary juice, passes then through a distinctly acid cardiac chamber, thence to a no less distinctly alkaline portion of the gut. This alkaline region, the mesenteron, often termed "stomach" (ventriculus) is the portion of the gut through which food passes most slowly, and seems in fact to be the region of most active absorption. The hindgut (proctodeum), often labelled "intestine," appears of greatest importance as an excretory channel. From this standpoint the course of alimentation in these larvae is not dissimilar to that in a vertebrate.

That the mesenteron is a major locus of absorption is shown by several additional facts. The peritrophic membrane is freely permeable to the indicators used, as indeed Eidmann's results would have suggested. The contents of the peritrophic tube, when the isolated tube is dissected out, appear to have the same alkalinity as that noted in the fluid which normally encloses it. The cells composing the wall of the mesenteron are the only cells in the gut which appear to absorb the dye. The stained cells show a color indicating (brom-thymol blue) pH 6.4. In Chironomus larvae the

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\[ \text{Fig. 1. Diagram of intestinal tract of dipterous larvae (Psychoda, Chironomus) showing pH values observed in the several divisions. Where a range of acidities is indicated, the commonest value is underlined. The pH of the malphigian tubules is as a rule less than 6.0.} \]

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fluid surrounding the peritrophic membrane is often confined to a narrow annulus at the anterior end of the mesenteron, from which level it is rapidly run over the peritrophic membrane posteriorly and then anteriorly again by a kind of peristalsis.

The hindgut of the larvae studied is probably of minor significance for digestion and absorption. Its acidity (pH 6.0 to 6.6) is due to the discharge from the malphigian tubules. The contents of the tubules are quite markedly acid. Older larvae, preparing for pupation, show the tubules plugged proximally by urate deposits. In such larvae the lumen of the hindgut is less acid, showing pH 6.8 to 7.1.

**SUMMARY.**

Larvae of *Psychoda* and of *Chironomus* (diptera) maintained in solutions of appropriate indicators show that the typical acidities (pH) prevailing within the several regions of the digestive tract are: esophagus 7.1; cardiac chamber 6.2; mesenteron 7.5; the latter being functionally an intestine. The acidity of the hindgut, pH 6.4, is due to the discharge of the malphigian tubules.