CV. DIGESTION IN THE COCKROACH.

I. THE HYDROGEN ION CONCENTRATION IN THE ALIMENTARY CANAL.

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ALTHOUGH there is a considerable literature on the chemistry of digestion in insects this contains little more than records of the presence or absence of particular enzymes and there have been few attempts to investigate the properties of such enzymes more closely. The present work has been undertaken as a preliminary to the study of digestion in blood-sucking and disease-carrying insects; for it seemed desirable before dealing with such specialised material to make a more or less detailed study of the digestive enzymes in some insect of generalised structure and omnivorous habits, to compare the properties of these enzymes with those from other sources which have been the subject of detailed examination in the past and to relate these properties with the conditions under which digestion is normally carried on. It was hoped that such an investigation would serve as a useful point of reference in subsequent work, while at the same time the experiments have afforded an opportunity for the development of a technique for obtaining quantitative results with very small amounts of material.

The insect selected for this purpose was the small German Cockroach (*Blatella germanica*), but in most cases the experiments have been repeated and confirmed on the large American Cockroach (*Periplaneta americana*).

The present paper deals with the reaction of the intestinal contents of this insect and the factors by which this reaction is influenced.

THE REACTION OF THE INTESTINAL CONTENTS OF THE COCKROACH.

From the small amount of work which has been done on the reaction of the intestinal contents of insects [Crozier, 1924; Bodine, 1925; Senior-White, 1926], it appears that they do not as a rule show the extremes of acidity and alkalinity which are found in higher animals, though Jameson and Atkins [1921] record a p_H of 9.2 in the mid-gut of the larva of the silkworm.

The reaction of the crop contents of the cockroach has been variously stated to be weakly acid or neutral, the acidity when noted being usually attributed to changes in the food during digestion [Plateau, 1873]. Bodine

[1925], using the method of Felton [1921], measured the p_H of the gut contents of various grasshoppers (which belong to the same natural Order as the cockroach) and gives the p_H of the crop as 5.0–5.9 and of the mid-gut as 5.75–6.9, becoming less acid towards the hinder end. The p_H of the hind-gut and rectum varies from 6.4–7.2.

Methods.

The fluid available for the estimation of p_H in the gut of *B. germanica* amounts only to a few cubic millimetres. Approximate results may be obtained on this material by the rough method of Jameson and Atkins [1921]. But by the use of waxed plates and pipettes and the consequent elimination of the capillarity of the fluids a more exact colorimetric method has been developed.

Fig. 1.

A capillary pipette (Fig. 1) is lined with a thin film of paraffin wax and two arbitrary graduations made at *B* and *C*, the volume *A-B* being about 2 mm.³. The pipette is filled from *A-B* with the fluid to be tested, and then a solution of an appropriate indicator suitably diluted with boiled distilled water is run in until the fluid reaches *C*, the volume *B-C* being about 10 mm.³. The accurate manipulation of these minute quantities of fluid requires a little practice, but is most readily effected as follows. A piece of rubber tubing is attached to the end of the pipette. The end *E* of this tubing is held in the teeth and the point *D* between the finger and thumb. Fluid is drawn in by compressing *D*, closing the teeth on *E*, relaxing *D* until the required volume has been sucked up and then releasing *E*. Fluid is expelled by the reverse process of closing *E*, compressing *D* and releasing *E* at the required moment. It is of course of the utmost importance *never to expel the fluid by blowing*. If the pipette has been well lined with wax and the fluid is expelled slowly and evenly the latter will be transferred quantitatively from the pipette. The fluid should be mixed by drawing it in and out several times.

The p_H of the fluid is determined by comparing it with mixtures made up with the same indicator solution in the same pipette, using standard buffer solutions in place of the test fluid in *A-B*. The colour comparison may be effected by expelling the drops of fluid on a white tile covered by a film of wax, where they form little spherical globules. But the evaporation of such drops is rapid and their exposure to the air is undesirable. A more satisfactory

method of comparison is the following. A length of hard glass tubing of about 1.5 mm. bore is tested by means of a short column of mercury and those pieces of uniform bore are selected. It is then well washed with distilled water and tested for alkali by standing in a dilute solution of phenol red for 12 hours [Martin and Leppér, 1926]. Finally, the selected portions are dried, cut into lengths of about 1 inch and their ends sealed by dipping them in melted paraffin wax (*F*, Fig. 1). When required for use the ends are perforated with a fine needle. They are filled with the series of standard buffer mixtures or with the test mixtures by means of a rubber tube attached to one end, and the ends are then sealed by touching them with a heated glass rod. The closure of the ends of the tubes with paraffin caps has the twofold advantage of allowing them to be readily sealed and of enabling one to take up the fluid quantitatively from the waxed tile. Using the volumes indicated in the above description the column of fluid to be observed is about 5 mm. long.

With practice the whole procedure from the dissection of the insect to the colour comparison can be effected in half a minute. The standard buffers employed were of the Clark and Lubs series; colour differences of 0.1 p_H can be detected readily. The indicators used were the phenol red, bromothymol blue, bromocresol purple, methyl red and bromophenol blue of Clark and Lubs. The standards were made up fresh each day. The waxed tiles and other apparatus require very careful washing with distilled water between each experiment.

The method is a dilution method and as such is applicable only when the solution to be tested is adequately buffered. That this is the case with the saliva and gut contents of the cockroach was demonstrated as follows. A series of cockroaches fed upon different diets was dissected and the crop contents made up with suitable indicators as above described; but from each specimen several samples were taken. In one, the volume *A-B* would be filled with the test fluid; in another, it would be partly filled and made up to *B* with distilled water. In this way dilutions varying from 1:6 to 1:12 were compared. In the great majority of cases the results obtained were identical and in few cases did they differ by more than about 0.1 p_H unit.

A rough check on the results has been obtained by the method of Jameson and Atkins [1921] and, where the amount of fluid available has permitted, the results have been further controlled by using more than one indicator.

The dissection of the cockroach is very easily accomplished. The insect is anaesthetised with chloroform, laid on its back, and, while the body is steadied between the finger and thumb, the head is seized with forceps and gently drawn forwards. Almost invariably the salivary glands with the salivary receptacles distended with fluid and the entire gut with its contents as far back as the malpighian vessels may be drawn through the neck of the insect and placed upon the waxed plate. The various regions of the gut are then punctured and the escaping fluid rapidly taken up into the pipette.

Results.

Table I shows the results obtained from a series of freshly caught cockroaches which had been feeding on a varied mixed diet. The "saliva" in each case was the clear fluid distending the salivary receptacles.

Table I.

Region of gut	No. of estimations	Range of p_H	Average p_H
Saliva	22	6.7-7.4	6.9
Crop	12	4.6-6.0	5.2
Mid-gut	9	6.0-6.4	6.2

These results are in fair agreement with those obtained by Bodine on grasshoppers. There is a definite production of acid in the crop.

It was of interest to determine the source and nature of this acid. The digestion of the cockroach takes place mainly in the crop, the digestive juices coming from the salivary glands and the mid-gut. The reaction in both these regions is less acid than in the crop and this suggests that the acid is produced in the crop itself. The degree of acidity is too slight to be due to a strong acid such as hydrochloric and Gunzberg's test proved negative. Biedermann [1898], who noted a similar mild acidity in the mid-gut of mealworms, considered that this was due to an acid salt of phosphoric acid.

A series of *B. germanica* was fed on a mixture of 5 % gelatin and sucrose and dissected 5 hours later. The fluid from the crops of these insects was collected and showed a p_H of about 5.0. A portion of this fluid was mixed with appropriate quantities of Briggs' [1922] phosphate reagents in a capillary tube, but no trace of phosphorus could be demonstrated, although very small quantities can be detected by this method. On the other hand, the fluid gave a bright lemon yellow colour with dilute ferric chloride and there is little doubt that the acidity is due to lactic or other organic acids. It seemed probable that these acids are produced during digestion, and if such be the case the reaction should vary with the food taken. This point is the subject of the experiment given in Table II.

Table II.

Series	Food (mixed with water)	No.	p_H of crop		No.	p_H of mid-gut	
			Range	Average		Range	Average
A	Caseinogen	13	5.6-6.9	6.3	6	6.2-7.0	6.4
"	Sucrose	10	4.4-5.6	4.8	7	6.2-6.4	6.3
B	Caseinogen	5	6.2-6.6	6.4	2	6.0-6.6	—
"	Fibrin	2	5.8-6.4	—	1	6.2	—
"	Gelatin	3	6.2-6.6	6.4	1	6.2	—
"	Starch	3	5.2-5.8	5.5	—	—	—
"	Maltose	2	5.6-5.8	—	1	6.4	—
"	Lactose	2	5.6-6.0	—	—	—	—
"	Sucrose	5	4.4-5.6	4.7	3	6.2-6.4	6.3
"	Glucose	3	4.4-4.6	4.5	2	6.2	—

Series A starved for 2 days and dissected 3 to 18 hours after feeding. Series B given nothing but water for 20 days and dissected 4 to 6 hours after feeding.

It is clear from these figures that acid is being produced from carbohydrate and that this acid production is much more intense with sucrose and glucose than with the other carbohydrates. It will be shown in a subsequent communication that sterile extracts from the gut of the cockroach have no glycolytic action; it is therefore highly probable that the acidity is due to the activity of micro-organisms.

Microscopic examination of smears prepared from the crop contents of wild cockroaches showed a great variety of bacilli, cocci and small yeasts, and it was noted that yeasts were far more abundant in crops with a high acidity (p_H 4.6) than in those with a more nearly neutral reaction.

Finally, an emulsion of the crops of wild cockroaches was incubated with various sugars. The details of this experiment are given in Table III, and it will be seen that acid is produced most readily from glucose, less readily from maltose and least so from lactose. No gas was produced in any of the cultures. Stained films from the cultures showed the presence of yeasts, bacilli and streptococci.

Table III.

A. Equal quantities of an emulsion in distilled water of the crops of *B. germanica* incubated with 10 cc. of 2 % solutions of sugars and 10 drops of methyl red, under sterile conditions. Controls (*B*) are the same using boiled emulsion. Y = yellow to methyl red. The figures give the approximate p_H .

Time	Glucose		Maltose		Lactose	
	<i>A</i>	<i>B</i>	<i>A</i>	<i>B</i>	<i>A</i>	<i>B</i>
11.0 a.m.	Y	Y	Y	Y	Y	Y
3.0 p.m.	5.2	Y	Y	Y	Y	Y
4.0 p.m.	4.8	Y	5.8	Y	Y	Y
9.30 a.m.	4.4	Y	4.6	Y	4.8	Y

DISCUSSION.

It is evident from the foregoing experiments that the acidity commonly observable in the crop of the cockroach is not a physiological constant for the animal but is dependent on bacterial action. None the less, since the cockroach feeds mainly on carbohydrates, a certain degree of acidity represents the normal reaction of that part of the gut where digestion mainly occurs; and no matter what the origin of this acid the digestive enzymes must be capable of acting over that range of p_H observed in the living animal. This subject will be considered in subsequent communications.

The variability of the reaction in the crop is in striking contrast with the relative constancy in the mid-gut. This is doubtless due to the neutralisation of the acid by the secretion of the mid-gut and perhaps also of the malpighian tubes. The reason for the failure of acid fermentation to continue in the mid-gut is not clear. Most probably it is due to the rapid absorption of sugar. For example, it was found that after a meal of sucrose the crop contents always reduced Fehling's solution strongly, whereas a much less intense reduction was obtained with the contents of the mid-gut. Nothing is known of the regulating mechanism of the sphincter of the proventriculus (gizzard) by which the passage of food from the crop to the stomach is controlled.

SUMMARY.

1. A colorimetric method for the determination of p_H in about 0.002 cc. of fluid is described. The essential feature of the method is the use of waxed plates and pipettes for the manipulation of the fluid.

2. In wild cockroaches (*Blatella germanica*) the average p_H of the saliva is 6.9, of the crop contents 5.2, and of the stomach 6.2.

3. The acidity of the crop is shown to be due to the action of micro-organisms, particularly yeasts, upon sugar. After protein food the average p_H is 6.3 in the crop and 6.4 in the stomach. After carbohydrate food these figures are 4.8 and 6.3 respectively.

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