THE DIGESTIVE ENZYMES OF THE ONYCHOPHORA (*PERIPATOPSIS* SPP.)

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(With Eight Text-figures)

INTRODUCTION

The various species of Onychophora living to-day are limited almost entirely to the southern hemisphere; they show little variation in structural features, habits and habitats, in contrast to the other great arthropod groups, such as the Arachnida and Crustacea, which present a great variety of forms. The Onychophora represent the most annelid-like group of the Arthropoda, but are undoubtedly very highly specialised along their own lines. As terrestrial animals they are only partially capable of resisting desiccation, and have not become adapted to dry atmospheres as have the higher Arthropoda. Previous work on the Onychophora has been almost entirely morphological; an investigation into the biology and physiology of *Peripatus* should be of interest in view of the systematic position of the group and for comparison with other terrestrial invertebrates, particularly the arthropods.

Several species of *Peripatopsis* have been kept alive in Cambridge over the period of two years during which this work has been in progress. An investigation into the food and feeding mechanism, the digestion, food reserves, excretion and general biology has been carried out jointly with Dr S. M. Manton. This work could not be exhaustive, as only a limited supply of material was available, but it was pursued as far as was possible. The results, apart from those given below, will be published shortly in the *Journal of the Linnean Society*.

The work, of which an account follows, was done with *Peripatopsis Moseleyi*, *P. Balfouri*, *P. Sedgewicki* and *P. capensis*, which were being killed for embryological purposes. The viscera other than the genitalia were available for biochemical work, and from one to three animals were killed at a time.

The animals appear to be exclusively carnivorous in their habits. They feed infrequently, often not more than once a fortnight, and for their size may take a large meal which is completely digested within about 18 hours. The alimentary canal is straight and unbranched, and the mid-gut has no appendages or different histological regions. The salivary glands are large paired tubes extending two-thirds of the length of the body, and opening ventrally into the buccal cavity. These salivary glands and the gut are the only organs which may be expected to elaborate true

digestive enzymes. Salivary juice may be poured out of the mouth to carry out extensive external digestion of the soft parts of small arthropods, the partially digested food being sucked into the mouth and swallowed; food which can be swallowed whole, with or without being cut up by the mandibles, may be covered by salivary juice to a lesser extent. Within the mouth, the food passes at once through the foregut, which is lined by thick chitin and devoid of obvious glands, into the intestine, where it is separated from the epithelium by a delicate chitinous peritrophic membrane. There digestion and absorption proceed, and the indigestible remains, still enclosed in the contracted peritrophic membrane, are passed out of the body. For further details reference may be made to the full account mentioned above.

I. PRELIMINARY INVESTIGATION

*p*H *of the gut*

This was difficult to measure, because with the exception of two cases, there was practically no fluid in the gut—not enough to moisten a filter paper, and certainly not enough to collect in a capillary tube. After a number of different methods of determining the *p*H had been tried, the following procedure was finally adopted:

The entire gut is removed as quickly as possible and placed under liquid paraffin. It is then split open longitudinally and a drop of indicator solution is placed on its surface by means of a Pyrex capillary tube, and after a few seconds the fluid is drawn up the capillary again and compared with a standard. By this method contact with air, with attendant loss or gain of carbon dioxide, is avoided.

The almost invisible contents of the gut were apparently sufficiently well buffered for a reliable *p*H value to be obtained in this way, since the same final colour was obtained whether the indicator was added in the almost acid or almost alkaline form.

The indicators used were 0·04 per cent. aqueous solutions of brom cresol purple, brom thymol blue and thymol blue, and a 0·02 per cent. aqueous solution of phenol red; the standards used were those supplied with the B.D.H. Capillator set.

In no case was there any evidence that the *p*H differed in different regions of the same gut, though it varied from animal to animal. It was never below 6·0 nor above 8·2, while the mean value seemed to be about 7·0. In the two cases mentioned above, in which there was sufficient fluid to collect in a capillary tube, the *p*H was determined electrically, by means of a micro-hydrogen electrode: the values found were 6·8 and 7·8 respectively.

Owing to the small size of the salivary glands, it was found impossible to collect any of their natural secretion, but when indicator was introduced into the lumen through a drawn-out capillary, the *p*H seemed to lie between 6·4 and 7·0.

Qualitative examination for enzymes

Investigations into the nature of the enzymes present were made with a brei of the salivary gland or gut. Filtered and centrifuged extracts made with glycerol, alcohol and water were also tried. Boiled controls were rigidly set up, and great care was taken to exclude human saliva from the tubes.

Owing to the scantiness of the material, the volumes were always kept as low as possible; toluol was used as an antiseptic, and the tubes were corked and incubated at 28° C. Solutions were lightly buffered at *p*H 7·0 with phosphate buffer, but if a negative result was obtained under these conditions the test was repeated with buffer of a different *p*H.

Amylase and glycogenase were detected by the disappearance of the substrate (no colour with iodine), and by the production of substances reducing Fehling's and Benedict's reagents.

Maltase and invertase were detected by the formation of monosaccharides, as shown by reduction of Barfoed's reagent.

Cellulases were tested for by incubating the enzyme extract with cotton-wool, filter paper and sawdust, and estimating the reducing power of the filtrate after incubating for at least a week. No increased reduction was ever obtained.

The liquefaction of gelatin, the solution of fibrin and the breakdown of casein to substances not giving a precipitate with acetic acid, were all used to detect proteases.

The hydrolysis of the dipeptide, leucyl-glycine, was followed by estimating the free amino nitrogen by means of the van Slyke gas analysis apparatus, and that of leucyl-glycyl-glycine and chloracetyl tryosine by formol titration.

Fat-splitting enzymes and esterases were detected by the change of *p*H, as shown by an internal indicator, on incubating the extract with substrate in the absence of buffer; if no change occurred with, say, phenol red, the experiment was repeated with indicators working over different *p*H ranges. The substrates used were ethyl acetate, ethyl butyrate and olive oil.

The results of the preliminary qualitative tests are shown in Table I.

Table I

Substrate	Extract of salivary glands	Extract of gut
Glycogen	+	−
Starch	+	−
Sucrose	−	+
Maltose	−	+
Sawdust	−	−
Cotton-wool	−	−
Filter paper	−	−
Chitin	−	−
Ethyl acetate	−	+
Ethyl butyrate	−	+
Olive oil	−	+
Casein	+	−
Fibrin	+	−
Gelatin	+	+ (At *p*H 3·0 only)
l-Leucyl-glycine	−	+
Chloracetyl tyrosine	−	+
Leucyl-glycyl-glycine	+	+

While the animal was being chloroformed, body-wall contractions caused slime to pour out passively from the slime papillae, and sometimes a colourless drop of fluid, distinct from the slime, collected at the mouth. This usually had a *p*H of about 7·0, and it was thought that it might be salivary secretion, but whereas the extracts of the salivary glands contained a powerful amylase, the mouth secretion contained little or none. The fluid was found to contain blood corpuscles, and it is suggested

that the mouth secretion is an artefact caused by the strong body-wall contractions rupturing the salivary gland end-sac, so liberating blood. A similar flow of blood may be forced through the nephridia under identical conditions.

II. CHARACTERISTICS OF INDIVIDUAL ENZYMES

As has been said, the difficulty of obtaining the material was a great handicap, and in spite of working on a small scale there was insufficient material to deal properly even with one set of enzymes. With the proteolytic enzymes in particular, very little has been done; though no quantitative evidence on the point was obtained, it seemed that the relative activity of the proteases fell off more rapidly on dilution than that of the carbohydrate-splitting enzymes.

Technique

The enzyme extracts were prepared in the same way throughout the course of the work, viz.:

(1) *Gut.* The gut was split open longitudinally and washed with a very gentle stream of distilled water. It was then rubbed up in a small amount of water with a glass rod, and strained through glass-wool. This gave a brownish fluid containing in suspension the protein food reserve spheres and the fat globules. Both these components were removed by centrifuging at 3500 r.p.m. for 20 min., the spheres being thrown down and the fat forming a cake on the top of the almost clear centrifugate. The latter, which constituted the enzyme extract, was removed by syphoning off through a bent glass capillary.

(2) *Salivary glands.* These were dissected out as free as possible from adventitious tissue, and ground up in a small glass mortar with about 0·5 ml. of distilled water and a drop of toluol. (The extract was either used within half an hour, or else stood in the ice chest for some hours, though there was no marked difference in activity in the two cases.) Before use, the extract was filtered through a very small filter paper, the total volume, including rinsings, being about 1·0 ml.

Owing to the small amount of material available, all the experiments had to be done on a micro or semi-micro scale. The apparatus described by Linderstrøm-Lang and Holter (1931) was used in a number of cases, and the microburette described by the author (Heatley, 1935) was convenient and sufficiently accurate for most of the work.

In the *p*H optimum experiments, the *p*H of the actual solutions was always measured initially and finally, and sometimes also in the middle of an experiment. With the amylases the *p*H was determined colorimetrically, by means of the B.D.H. Capillator, but in most of the other cases it was measured electrically. Usually only small amounts of fluid were available, and the double micro quinhydrone electrode of Kuntara (1932) was found to be very satisfactory, and accurate when checked against an ordinary quinhydrone electrode.

All tubes were incubated at 28° C., and toluol was used as antiseptic.

As the removal of samples from all tubes could not be made simultaneously, it was found convenient to keep the tubes in ice, till they were all ready to be incu-

bated. Similarly, when it was desired to withdraw samples during the incubation, all tubes were plunged simultaneously into ice, and then simultaneously replaced in the incubator after the samples had been taken.

Proteases

*p*H *optimum.* The effect of *p*H on the salivary protease was first roughly investigated by the gelatin plate method of Gates (1927) as modified by Pickford and Dorris (1934), which showed that there was activity only in alkaline or neutral solution. The *p*H optimum was then studied more exactly by the three following methods:

(1) Liquefaction of gelatin.

(2) Formol titration.

(3) Precipitation of undigested protein, followed by an estimation of the total nitrogen in the washed precipitate.

In the first method the enzyme extract was incubated with a series of tubes containing 5 per cent. gelatin, buffered to different *p*H's, and the degree of liquefaction of the gelatin (as observed after cooling in ice for exactly 10 min.) was noted (Dernby, 1918). Owing to the high viscosity of a 5 per cent. gelatin solution at 30–40° C. it was not possible accurately to measure out small quantities by means of a pipette, but this difficulty was easily overcome by weighing out the required quantity of gelatin powder for each tube, and then adding water and buffer. After a few minutes the tubes were warmed to about 35° C. and thoroughly mixed, and again thoroughly mixed after the enzyme extract had been added.

In order that the full range of states between solid jelly and liquid might be recognised by shaking, it was necessary to work with tubes no smaller than 1·0 cm. internal diameter, and the volume of fluid, for the same reason, could not well be less than 1·0 ml. Even under these conditions the enzyme was apparently too dilute, for in all the experiments tried, at least 3 days of incubation were necessary to produce any appreciable change in the state of the jelly. It was impossible to work on a much smaller scale on account of the difficulty of observing the degree of liquefaction in a tube much less than 1·0 cm. in diameter. Eventually this difficulty, too, was overcome by working with small tubes measuring 4 × 120 mm. containing about 0·1–0·2 ml., and gauging the solidity of the contents by the ease with which a small glass sphere filled with iron filings could be drawn through the medium by an electromagnet.

The following four states were adopted as a rough quantitative guide to the degree of liquefaction:

(1) Glass sphere practically immovable.

(2) Glass sphere moves a short distance, but returns to original position.

(3) Sphere can be drawn anywhere by electromagnet.

(4) Quite liquid; sphere falls through medium by its own weight.

The gelatin was usually completely liquid in a few hours under suitable conditions, when the volume was kept small as in these experiments. The method was

not really satisfactory, however, owing partly to the difficulty of determining the *p*H accurately, and partly to the difficulty of properly mixing the contents of the tubes, but the results of one experiment are shown in Fig. 1.

The second method—the formol titration—was no more successful, though a considerable amount of material was used in testing it out. Here again, the change brought about in a given time was small, but the chief difficulty, and that which caused this method to be abandoned, was to control the *p*H adequately and at the same time get a sharp end-point. The tubes had to be strongly buffered in order to maintain the *p*H at all constant, and in the presence of any appreciable amount of buffer it was impossible to titrate accurately to an end-point lying within the range of that buffer.

The third method not only overcame these difficulties, but proved more sensitive. The principle was to incubate enzyme extract with well-buffered casein solutions at different *p*H's, and after a certain time to precipitate the casein in a portion, filter off, wash, and estimate the total nitrogen in the precipitate by the

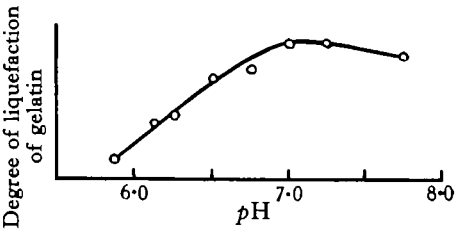


Fig. 1. Effect of *p*H on the liquefaction of gelatin by salivary extract.

micro-Kjeldahl method. The following detailed account of one experiment will indicate the general procedure:

A 4 per cent. solution of casein was made by shaking 1·0 gm. of Hammarsten casein with some water and 10·0 ml. of 0·1 *N* sodium hydroxide in a 25 ml. volumetric flask. When the casein had dissolved, the solution was made up to the mark, and then added to 25 ml. of the double strength buffer base used by Northrop (1922) which has the following composition:

23·7 gm. anhydrous disodium hydrogen phosphate,
60·7 gm. sodium citrate,
10·3 gm. boric acid,
Made up to 1000 ml. with distilled water.

A series of solutions of different *p*H were made by taking 5·0 ml. of this solution in a 10·0 ml. volumetric flask, adding a known amount of 0·2 *N* sodium hydroxide or hydrochloric acid, and making up to the mark with 0·2 *N*-sodium chloride. The *p*H of portions of these solutions were measured by the double hydrogen electrode (Cole, 1933) and 1·0 ml. was taken from each sample and placed in a small tube. The tubes were cooled in ice, and 117 μl. of salivary extract were added to each (Linderstrøm-Lang and Holter, 1931; pipette type 2). After a thorough mixing, samples of 241 μl. were removed from each tube. A small drop of toluol was added to the remainder, and all tubes were placed simultaneously in the incubator at 28° C.

After a certain time the tubes were removed and placed in ice, and a further sample of 241 μl. was removed from each; the *p*H of each tube was also redetermined.

The casein was precipitated from the samples in the following way: a drop of brom cresol green was added to the sample, then sufficient hydrochloric acid to give a greenish tint. 1·0 ml. of 0·5 *N* half-neutralised acetic acid-sodium acetate mixture was blown into the solution from a pipette, and the precipitate of casein was filtered off under suction on a small filter paper on a filter disc. The tube was rinsed out with a further 1·0 ml. of acetic-acetate mixture, the rinsings being put through the paper; the latter was then rolled up and placed in a micro-Kjeldahl incinerating flask. A certain amount of precipitated casein, which still adhered to the sides of the vessel in which the precipitation had been carried out, was dissolved up in two drops of *N* soda, and transferred, with washings, to the incinerating flask. The Kjeldahl estimation was carried out in the usual manner, blanks being set up with plain filter paper.

No controls were set up for the digestion; this would have used more enzyme, and it had already been shown that casein was not attacked by boiled enzyme. Table II gives the results, which have been plotted in graphical form in Fig. 2.

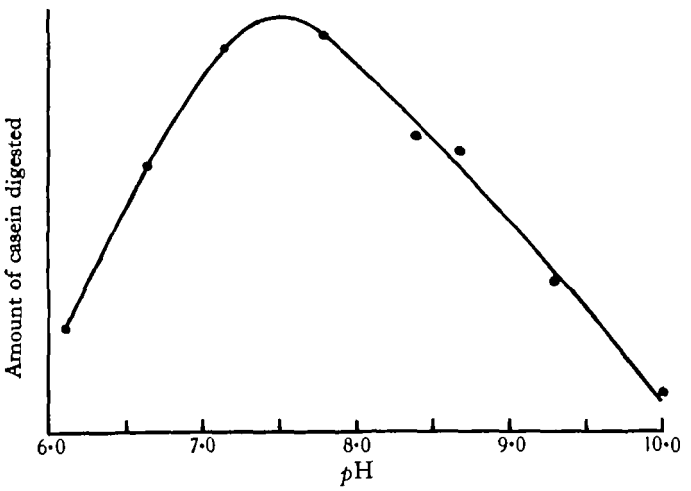


Fig. 2. *p*H-activity of salivary protease; substrate, casein.

Table II

Tube No.	*p*H			μl. of 0·01 *N* soda ≡ N in casein		
	Initial	Final	Mean	Initial	Final	Difference
1	8·23	7·53	7·78	1·38	0·35	1·03
2	7·37	6·93	7·13	1·40	0·38	1·02
3	6·73	6·53	6·63	1·40	0·69	0·71
4	6·09	6·13	6·11	1·43	1·11	0·32
5	8·63	8·13	8·38	1·34	0·61	0·73
6	8·88	8·48	8·68	1·40	0·64	0·76
7	9·48	—	9·28?	1·42	0·99	0·43
8	10·28	—	10·0?	1·30	1·28	0·02

Activation of salivary protease. Owing to the scarcity of material it was possible to perform only one, not entirely satisfactory, experiment to determine the effect of various activators on the salivary protease.

Samples of 120μl. of salivary extract were mixed with 150μl. of the following solutions:

(1) 2 per cent. hydrocyanic acid.
(2) 0·2 per cent. cysteine.
(3) Extract of *Peripatus* gut.
(4) Enterokinase. (Prepared and purified by the method of Waldschmidt-Leitz, 1925.)
(5) Water. Hydrogen sulphide was passed slowly through this tube for 1 hour.

The tubes were allowed to stand at room temperature for 1 hour, whilst controls of enterokinase alone, and raw and boiled salivary extract alone, were set up. The solutions were then neutralised to litmus, the volume in all tubes being kept the same, as nearly as possible, by the addition of water where necessary.

1·0 ml. of a 2 per cent. casein-phosphate solution, 0·2 *M* with respect to phosphate, and adjusted to *p*H 7·4, was added to each tube and thoroughly mixed.

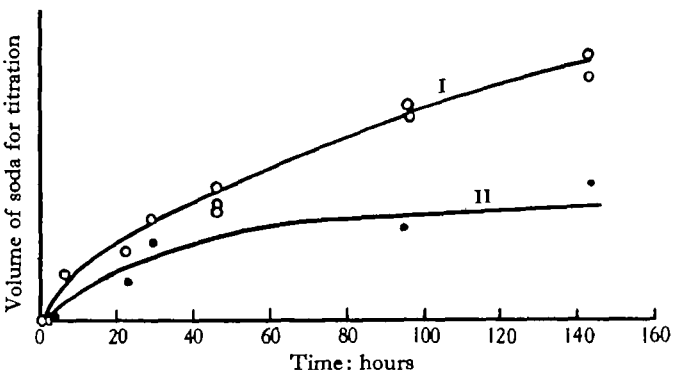


Fig. 3. Relation of "formaldehyde acidity" (I), to "free acidity" (II), during digestion of casein by salivary extract.

A sample of 135μl. was withdrawn from each, a drop of toluol was added, and the tubes were incubated for 3 days, samples being withdrawn at the end of this time and also at the end of 2 days.

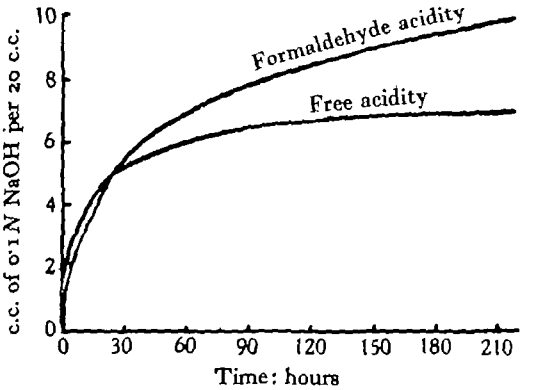
The 135μl. samples were titrated to a standard *p*H with 0·1 *N* soda with phenolphthalein as indicator; then 120μl. of neutral 30 per cent. formaldehyde was added, and the titration was carried on to the same end-point.

The enzyme must have been diluted too much, for after 3 days of incubation, the increase in the formol titration fell almost within the limits of experimental error. There was certainly no evidence of activation by any of the substances tried.

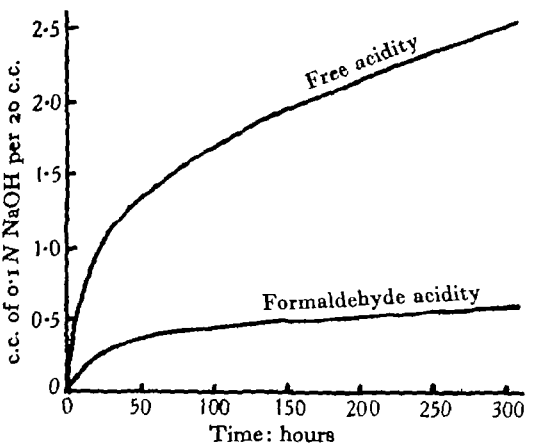
Relation of "formol acidity" to "free acidity" in the digestion of casein. Wigglesworth (1928) has recently drawn attention to an observation made some years ago by S. W. Cole which was communicated to the Biochemical Society, but never published. If a protein is digested by pepsin or trypsin, the "free acidity" increases during the course of the digestion, as does also the "formaldehyde acidity", but the

relative shape of the two curves is characteristically different for the two ferments (Fig. 4).

It can scarcely be considered profitable nowadays to compare invertebrate proteases with pepsin and trypsin, especially as the latter has recently been fractionated into a number of enzymes. However, the results of a rough experiment carried out on these lines, of the digestion of casein by the salivary protease of *Peripatopsis*, is



4·5 per cent. gelatin by trypsin at *p*H 8·1 (Cole)



4·5 per cent. gelatin by pepsin at *p*H 1·4 (Cole)

Fig. 4. The relation of "formaldehyde acidity" to "free acidity" in digestion of 4·5 per cent. gelatin by (i) trypsin, (ii) pepsin. (After Cole.) [See *Biochem. J.* **22**, 152.]

shown in Fig. 3. I am indebted to Mr Cole for permission to reproduce the curves in Fig. 4.

It will be seen from Table I that the gut is capable of liquefying gelatin at *p*H 3·0, though none of the other proteins were attacked by it at this or any other *p*H. This observation was repeatedly confirmed, with rigorous controls; the gelatin plate method of Pickford and Dorris (1934) showed that the gelatinase was very sensitive to the hydrogen-ion concentration, as it was practically inactive at *p*H 2·5 or 3·5.

Carbohydrate-splitting enzymes

*p*H *optima.* Attempts were made to determine the *p*H optima of the gut invertase, and of the salivary amylase and glycogenase. The procedure was to incubate about 100μl. of enzyme extract with 1·0–2·0 ml. of buffered substrate, and to estimate, after a certain time, the increase in reducing power of an aliquot portion, as measured by the method of Hagedorn and Jensen, modified by Boyland (1928).

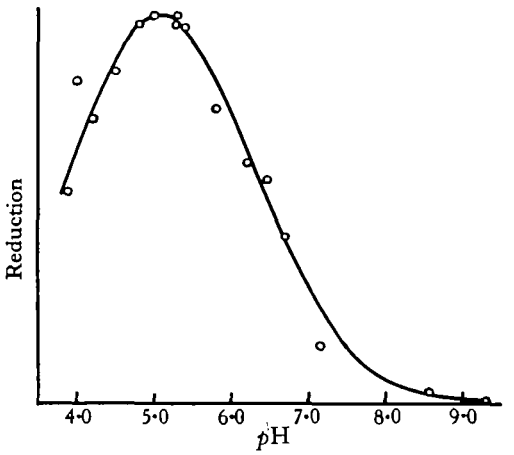


Fig. 5. *p*H-activity of gut invertase.

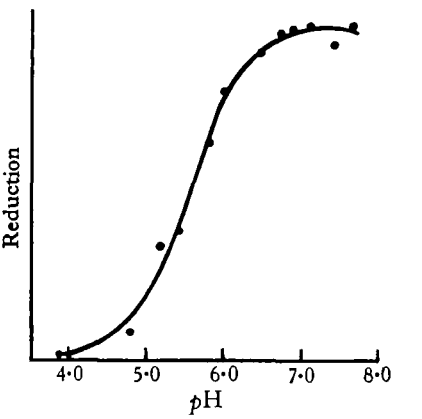


Fig. 6. *p*H-activity of salivary amylase.

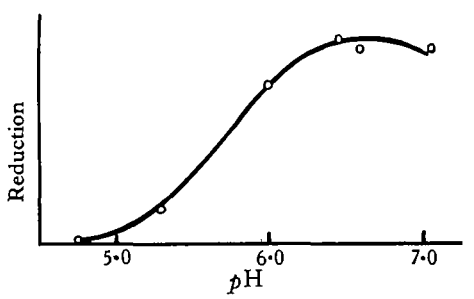


Fig. 7. *p*H-activity of salivary glycogenase.

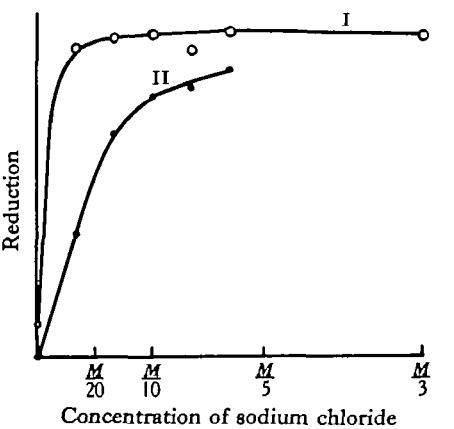


Fig. 8. Effect of sodium chloride concentration on amylase I, and glycogenase II.

The *p*H of the mixture was determined initially, and at various stages of the incubation, by means of the B.D.H. Capillator. The final strength of the buffers was approximately 0·05 *M*, phthalate, phosphate and borate buffers being used. Unlike the proteases, the activity of the carbohydrate-splitting enzymes did not fall off rapidly with dilution, and under the conditions employed (*e.g.* extract from one animal divided between six or eight tubes, each containing 2·0 ml. of buffer substrate), measurable hydrolysis had usually occurred within a few hours.

Fig. 5 shows the effect of *p*H on the gut invertase, the initial concentration of sucrose being 1·0 per cent.; two experiments have been superimposed on one graph. Figs. 6 and 7 show the results of experiments in which salivary extract had been allowed to act on 1·0 per cent. starch, and on 1·0 per cent. glycogen respectively. Though smooth curves have been drawn, it is probable that different buffers have varying activating or inhibiting effects on the enzyme.

Sodium chloride optimum. It was found that dialysed salivary extract did not hydrolyse starch or glycogen. The addition of sodium chloride, however, restored the activity of the enzyme, though the sodium phosphate of the buffer was without effect.

Fig. 8 shows how the activity of the enzyme varies with the concentration of sodium chloride, when acting on starch (curve I) and glycogen (curve II). Both curves were obtained with the same extract, which had been dialysed in a small collodion sac for 2 hours beforehand. The starch and glycogen had also been dialysed, their initial concentration in the incubating mixture being 1·0 per cent. The solutions were buffered to *p*H 7·2 with 0·02 *M* phosphate, and the incubation was carried on for 3 days at 28° C., with toluol as antiseptic.

Lipases

The qualitative tests had shown that whereas the esters ethyl acetate and ethyl butyrate were split fairly readily by the gut, olive oil was hydrolysed only with difficulty. In fact it was obvious that the latter substrate could not be used to determine the *p*H optimum of the lipase by ordinary chemical methods.

Dr A. H. Hughes of the Colloid Science Department, very kindly undertook to measure the activity of the lipase at different hydrogen-ion concentrations, using, as a measure of activity, the decrease of surface potential of a monomolecular film of triolein when spread over a solution containing the enzyme and a suitable buffer (Schulmann and Rideal, 1933).

The enzyme extract was prepared by splitting open the gut, washing it, and grinding it up with successive amounts of acetone. The dried residue was shaken up with phosphate buffer, *p*H 6·7, and centrifuged, the clear centrifugate being used for the experiment.

The results showed that there was no action at *p*H 5·0, that there was a slight action at *p*H 6·0, and that there was an optimum between *p*H 7·0 and 8·0.

Some months after these results had been obtained, further advances were made (Schulmann and Hughes, 1935) which showed, amongst other things, that the surface potential of a monomolecular film of triolein might fall in the complete absence of an enzyme as long as there was a trace of fatty acid present. Furthermore this would show an optimum *p*H effect similar to that found in the case of a true enzyme.

Dr Hughes then reinvestigated the lipase of *Peripatus*. An extract was prepared in the same way as previously and divided into four parts, one of which was used at once. Another was made strongly alkaline for 1 hour, then neutralised, and another was boiled; the fourth part was kept for 24 hours, during which it lost nearly half of its activity. In the two controls the surface potential of a monomolecular film of

triolein fell gradually, and to the same extent in both cases. In the test experiment the surface potential fell more rapidly than in the controls.

The only justifiable conclusions which can be drawn from this and the previous experiment, are that a true lipase is present, which can hydrolyse triolein between *p*H 6·0 and 8·5; that its optimum appears to lie between *p*H 7·0 and 8·0; and that its activity falls off rapidly on standing at room temperature at *p*H 7·2.

One experiment was performed to study the effect of *p*H on the hydrolysis of ethyl acetate by the gut, between *p*H 5·8 and 7·8. A 0·4 *M* solution of acid potassium phosphate, containing 40 gm. of ethyl acetate per litre, was prepared. 5·0 ml. of this were measured into a 10·0 ml. volumetric flask; *x*.ml. of 0·5 *N* sodium hydroxide were added and the solution was made up to the mark with boiled distilled water. A number of buffer-substrate solutions of different *p*H's were prepared in this way and 1·0 ml. of each was placed in a small tube. The tubes were cooled and 60μl. of gut extract were added to each, boiled extract being added to one tube. Toluol was added and the tubes were mixed and incubated at 28° C. for 6 hours, after initial samples had been withdrawn for titration and *p*H determination. For the former, samples of 117μl. were withdrawn and titrated to a standard pink colour with phenolphthalein against 0·01 *N* soda. Table III shows the results.

Table III

Tube No.	Mean *p*H	Initial titration	Final titration	Difference
1	5·8	224·3	242·5	18·2
2	6·1	180·5	203·5	23·0
3	6·5	130·6	150·9	20·3
4	7·1	58·0	80·8	22·8
5	7·5	28·5	52·3	23·8
6	7·8	19·3	42·3	23·0
7*	7·2	57·0	57·5	0·5

* Boiled control.

Thus the activity of the gut esterase acting on ethyl acetate, would appear to be independent of the hydrogen-ion concentration between *p*H 5·8 and 7·8.

DISCUSSION

In *Peripatopsis* as in other Arthropoda, there is no cytological evidence of phagocytosis or intracellular digestion. The presence of a peritrophic membrane separating the food from the gut epithelium indicates that digestion must occur in the lumen, and in view of the complete set of enzymes which has been demonstrated, this appears to be the case.

Although a variety of enzymes has been found, there is no corresponding histological differentiation, and gland cells throughout the gut show a similar structure, as do the secreting cells throughout the salivary glands.

The nature of the salivary juice enables considerable external digestion of the prey to take place, when the latter, because of its size, cannot be eaten whole. It will

be seen from Table I that the salivary extract contains the enzymes which act on the large molecules, reducing the food to a semi-fluid state, which is then acted on by the gut enzymes.

External digestion is a phenomenon of frequent occurrence in the Arthropoda, *e.g. Pseudogenia*, and the larvae of *Carabus*, *Myrmeleon* and many Diptera (Lengerken, 1924). Among the Annelida, *Lumbricus* is stated (Darwin, 1881) to cover its food with a juice containing amylase and protease before devouring it, though here the food is generally vegetable matter, though sometimes animal matter is also taken. In the arthropod examples mentioned above, as in the case of *Peripatopsis*, it is the salivary glands which elaborate the juices which accomplish the external digestion.

A striking point which has emerged from the work on *Peripatopsis* is the apparent weakness of the proteolytic enzymes compared with the amylases, yet the animal appears to be entirely carnivorous; a meal of meat one-tenth of the animal's body weight is digested fairly rapidly by the living animal, the indigestible remains being evacuated usually within 18 hours. The slow action of the proteolytic enzymes *in vitro* compared with the rapid digestion in the living animal cannot be due to the imperfect extraction of the protease in the enzyme preparations, because an unfiltered brei of the tissue, or extracts prepared in different ways, are no more active (cf. A. O. Weese, 1926; alcoholic or glycerol extracts of the gut of *Strongylocentrotus* contain a strong protease, whereas watery extracts contain practically none). Three explanations are possible:

(1) The protease has to be activated in some way.

(2) The enzyme does not have to be activated, but is only synthesised during, or immediately before, a meal.

(3) The relative activity falls off very rapidly on dilution.

No exact quantitative evidence of the latter suggestion was obtained, but it was observed more than once, that whereas the extract from one animal divided amongst a total of 8·0 ml. of 5 per cent. gelatin required 2 or 3 days for any noticeable change to occur, the same amount of extract would completely liquefy 1·0 ml. of 5 per cent. gelatin in 3 or 4 hours. (For a somewhat similar effect in the case of a deaminating enzyme, and a discussion on the problem, see Krebs, 1935.) This behaviour of the salivary protease may be due to the dilution of an activator, or more probably to the combined effect of dilution on activator and enzyme. It is much to be regretted that the supply of material was exhausted before a proper investigation of the effect of various activators could be made.

It is true that the food that the animal usually eats (small Arthropoda, etc.) may contain a certain amount of glycogen, but it is difficult to see why the glycogenase should be so powerful. In most animals the nature of the digestive enzymes is correlated directly with the type of food eaten; the enzymes present in each case may be interpreted as the result of the specialisation of each species, and do not appear to be influenced by the phylogeny of the animal in question; an excellent example of this is afforded by the work of Bounoure (1911), who determined the

relative activity of various digestive enzymes in five species of Coleoptera with five different feeding habits. A few exceptions to such generalisations are found, but they are rare; an invertase is present in the gut of the Oriental Fruit Moth (Swingle, 1928), which does not feed in the adult state, and the Septibranch Mollusca, although ingesting solid particles of food containing protein, are unable to digest it except phagocytically, since no extracellular protease is elaborated (Yonge, 1928).

It may be noted that carboxypolypeptidase is found in both gut and salivary glands, whereas aminopolypeptidase is found only in the gut. The salivary protease can therefore be regarded as a "trypsin-like" enzyme both in this sense and from the fact that its *p*H optimum lies on the alkaline side of neutrality.

With regard to the carbohydrate-splitting enzymes, it is interesting that the sodium chloride optimum of glycogenase is several times greater than that of amylase.

With the possible exception of the gut invertase, the *p*H optima of the enzymes present would seem well adapted to the needs of the animal, in view of the actual *p*H of the gut.

Nothing is at present to be gained from a discussion of the above work in relation to the digestive enzymes of invertebrates in general, since it appears that the equipment of digestive enzymes possessed by an animal has little or no phylogenetic significance. However, excellent summaries of much of the work have been made by Uvarov (1928) for insects, Kruger (1933) for all invertebrates, Yonge (1931) for marine invertebrates, and others.

SUMMARY

1. An investigation has been made of the digestive enzymes of *Peripatopsis* spp.

2. The *p*H of the gut varies between 6·0 and 8·2; it is usually about 7·0.

3. The salivary glands elaborate amylase, glycogenase, protease and carboxypolypeptidase.

4. The gut digestive enzymes consist of invertase, maltase, lipase, esterase, amino- and carboxypolypeptidase and dipeptidase. Gelatin is also liquefied by the gut, but at *p*H 3·0 only.

5. The properties of some of the individual enzymes have been examined.

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