A CONTRIBUTION TO THE STUDY OF THE DIGESTIVE GLAND IN MOLLUSCA AND DECAPOD CRUSTACEA

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Since the time of Krukenberg many observations have been made on the digestive processes in lower animals. The literature of the subject is extensive but for the purposes of the present paper it is only necessary to mention some of the papers more directly concerned with the experiments described herein. Krukenberg¹ stated that the digestive gland of the crab contained both tryptic and peptic ferments. His reason for stating this was that the tryptic enzyme could be destroyed by the peptic enzyme in acid solution and likewise the peptic enzyme was destroyed by digestion in two per cent. soda solution at 38°—40° C. Frenzel² said that a glycerine extract of the gland acted on fibrin like trypsin. Hoppe-Seyler³ found proteolytic, amylolytic and lipolytic ferments in the digestive gland of the crab. He considered the proteolytic ferment to resemble or to be identical with ordinary trypsin. Jordan⁴ claims that the proteolytic ferment of the fresh water crayfish (*Astacus fluviatilis*) is similar to trypsin and that lipolytic and amylolytic ferments are also present.

Frédéricq,⁵ in two species of molluscs, found a ferment which dissolved fibrin best in acid reaction whilst in other molluscs the ferment acted best in alkaline media. He also found a diastatic

1. *Untersuchungen a. d. physiol. Institut, Heidelberg*, Vol. II, 1879.
2. *Mittheil. a. d. zoolog. Stat. Neapel*, p. 50, 1883.
3. *Arch. f. d. ges. Physiol.*, Vol. XIV, p. 395, 1876.
4. *Arch. f. d. ges. Physiol.*, Vol. CI, p. 263, 1904.
5. *Bulletins de l'acad. roy. de Belgique*, 2 ser., Vol. XLIV, 1878.

ferment in most of the animals. Griffiths¹ stated that the 'liver' of *Patella vulgata* contains proteolytic, amylolytic, and lipolytic ferments, and MacMunn² states that in *Aplysia* there are peptic, tryptic, and amylolytic ferments.

The following species have been investigated during the course of these experiments :—Crustacea : the Edible Crab (*Cancer pagurus*) and the Swimming Crab (*Portunus puber*) ; Mollusca : the Limpet (*Patella vulgata*), the Periwinkle (*Littorina littorea*), the Dog-whelk (*Purpura lapillus*), and the Hard-whelk (*Fusus antiquus*).

EXPERIMENTAL METHODS

The digestive gland was minced, weighed, and mixed with a definite volume of distilled water to which a suitable preservative had been added. After allowing to stand for some time it was strained through muslin and a series of solutions were made up using a definite quantity of the extract. The digestive action was tested for the various ferments by the following methods.

Proteid digestion was sought for by using coagulated egg white (Mett's tubes), fibrin and peptone solution. In no case was there any action on the egg white, but fibrin was acted on in most cases. The degree of action on fibrin was shown by loss of weight and by tests for the products of digestion. Three tests were employed for digested products. (1) Albumoses or peptone (biuret test), (2) tyrosin (testing filtrate from addition of Millon's reagent), (3) tryptophane (addition of either bromine or chlorine water). The effect of reaction was shown by using mixtures which were either acid, alkaline or neutral. For the neutral solutions distilled water was employed. The acid solution, which resulted from mixing a definite quantity of standard acid with the extract of the gland, was 0·18% hydrochloric acid and the corresponding alkaline solution was 0·2% sodium carbonate. The peptone solution was employed in the solutions of varying reaction, to test for ereptic ferments, and the same three

1. *Proc. Roy. Soc.*, Vol. XLII, p. 392, 1887.

2. *Royal Soc. Phil. Trans.*, B., Vol. CXCIV, p. I, 1900.

tests were used only in this case the diminution of the biuret pink showed increased digestion, whilst with fibrin the more active the digestion the greater the pink colouration. Carbohydrate digestion was shown by hydrolysis of starch solution and inversion of cane sugar. The tests employed were the non-appearance of the blue colour on the addition of iodine to the starch solution and the presence of a reducing sugar by Trommer's test.

Fat digestion was at first tested by an emulsion of olive oil in most cases, but as the results were uniformly negative, methyl acetate was used in the later experiments and the titration figure with decinormal sodium hydrate in presence of phenol-phthalëin was compared with a control. This latter method gave a marked hydrolysis in every case in which it was used.

As a preservative toluol was employed at first but later on it was found that proteid digestion, as pointed out by Vines,¹ proceeded better when hydrocyanic acid was used. When testing for reducing sugar hydrocyanic acid cannot be used as it decolourizes the copper solution and holds cuprous oxide in solution. A number of experiments were performed and the results were used to check one another, thus individual variations were excluded and a good average obtained.

RESULTS

Amongst the crustacea it was found that digestion of fibrin proceeded most actively in alkaline reaction, and least in acid solution, neutral mixtures being intermediate in their rate of action. Starch was converted into reducing sugar and cane sugar was inverted in practically every case. Although no hydrolysis of olive oil was found, methyl acetate was readily split into methyl alcohol and acetic acid.

There was no increase in the action on fibrin when intestinal extract was added to the extract of the gland, thus there is no evidence of a substance like enterokinase which can convert an inactive proferment into an active ferment. It is, however, difficult to exclude contamination, and the least trace of enterokinase can

1. *Annals of Botany*, Vol. XVII, p. 597, 1903.

activate a large quantity of inactive juice. Both peptolysis and proteolysis were found to occur and both were most active in alkaline media. Lastly, the proteolytic ferment was destroyed by acid but not by alkali, but the presence of fibrin protects the ferment from the injurious action of the acid, so that some digestion can occur in acid media if the fibrin is added at the same time as the acid.

All the crustacea used gave practically identical results so there is no need for details of the individual species. The following protocols will serve as an example of the method in which an experiment was performed.

May 16th, 2.30 p.m.—57 g Hepato-pancreas of *Cancer pagurus* + 2 c.c. of 2% HCN + 55 c.c. distilled water (added to each and kept at 13° C.)

May 17th, 3 p.m.—The following solutions were made up and two drops 2% HCN added :—

May 18th, 10.30 a.m.—

1. 5 c.c. $\frac{N}{10}$ -HCl + 3 c.c. distilled water + 2 c.c. extract + 0.5 g fibrin.	Swollen and gelatinous, all strains through muslin; fair biuret, marked tyrosin, no tryptophane.
2. 5 c.c. $\frac{N}{10}$ Na ₂ CO ₃ + 3 c.c. distilled water + 2 c.c. extract + 0.5 g fibrin.	All digested; marked biuret, fair tyrosin, slight tryptophane.
3. 8 c.c. distilled water + 2 c.c. extract + 0.5 g fibrin.	0.03 g fibrin left; strong biuret and tyrosin, slight tryptophane.
4. 5 c.c. $\frac{N}{10}$ -HCl + 3 c.c., 2% peptone solution + 2 c.c. extract.	Good biuret, strong tyrosin, fair tryptophane.
5. 5 c.c. $\frac{N}{10}$ Na ₂ CO ₃ + 3 c.c., 2% peptone solution + 2 c.c. extract.	Good biuret, very strong tyrosin, strong tryptophane.
6. 5 c.c. distilled water + 3 c.c., 2% peptone solution + 2 c.c. extract.	Fair biuret, strong tyrosin, medium tryptophane.
7. 5 c.c., 1% starch solution + 3 c.c. distilled water + 2 c.c. extract.	Blue colour with iodine gone, decolourization of Trommer's test.
8. 5 c.c., 1% cane sugar solution + 3 c.c. distilled water + 2 c.c. extract.	Trommer's solution decolourized.
9. 8 c.c., 1% methyl acetate solution + 2 c.c. extract.	With phenol-phthalëin = 3.2 c.c. $\frac{N}{10}$ -NaOH.

10. 8 c.c., 1% methyl acetate solution
+ 2 c.c. boiled extract.	With phenol-phthalëin = 1.4 c.c. $\frac{N}{10}$ NaOH.
11. 5 c.c. $\frac{N}{10}$ HCl + 2 c.c. distilled water
+ 2 c.c. extract after standing 3
hours, 1 c.c. N Na_2CO_3 and 0.5 g
fibrin added.	0.36 g fibrin left; slight biuret, fair tyrosin,
very slight tryptophane.
12. 5 c.c. $\frac{N}{10}$ Na_2CO_3 + 2 c.c. distilled
water + 2 c.c. extract, after
standing 3 hours 1 c.c. NHCl and
0.5 g fibrin added.	0.13 g fibrin left; strong biuret and tyrosin,
slight tryptophane.
13. 5 c.c. $\frac{N}{10}$ HCl + 2.5 c.c. distilled
water + 2 c.c. extract, after
standing 3 hours 0.5 c.c.
N Na_2CO_3 and 0.5 g fibrin added.	0.38 g fibrin left; fair biuret, medium tyro-
sin, no tryptophane.
14. 5 c.c. $\frac{N}{10}$ Na_2CO_3 + 2.5 c.c. distilled
water + 2 c.c. extract, after
standing 3 hours 0.5 c.c. N HCl
and 0.5 g fibrin added.	All fibrin digested, very strong biuret,
strong tyrosin, medium tryptophane.

The extract itself gave strong biuret, tryptophane and tyrosin reactions evidently by auto-digestion, but these effects were negligible when the solution was diluted in the proportions used in the experiments. The digestion of starch and cane sugar are invalidated by the presence of hydrocyanic acid, but similar experiments when toluol was used as a preservative showed hydrolysis of starch and inversion of cane sugar.

The last four solutions (11-14) are very interesting as showing that the ferment is destroyed by acid and is thus rendered incapable of acting on fibrin when rendered alkaline (11) or neutral (13); it is not destroyed by alkali and can act on fibrin after being rendered neutral (14), but not after being rendered acid (12).

With the mollusca the results were very much the same, the main difference being that the digestion of fibrin proceeded most actively in acid media and was practically absent in alkaline reaction. Starch was hydrolysed and cane sugar inverted as in the crustacea. With *Fusus* the 'stomach' was carefully separated from the 'liver' and an extract of it was found to digest fibrin markedly in acid medium, slightly in neutral, but not at all in alkaline reaction, thus corresponding with the results obtained by the extract of the digestive gland.

The following experiment shows these points :—

May 14th, 5 p.m.—35 g Liver of *Fusus antiquus*+1 c.c. 2% HCN + 34 c.c. distilled water (and kept at 13° C.) Extract of stomach also made.

May 15th, 10.30 a.m.—Following solutions made up, and two drops 2% HCN added to each :—

May 17th, 10 a.m.—

1. 5 c.c. $\frac{N}{10}$ HCl + 3 c.c. distilled water + 2 c.c. liver extract + 0.5 g fibrin.	0.2 g fibrin; very strong biuret, tyrosin and tryptophane.
2. 5 c.c. $\frac{N}{10}$ Na ₂ CO ₃ + 3 c.c. distilled water + 2 c.c. liver extract + 0.5 g fibrin.	0.47 g fibrin; strong biuret, fair tyrosin, no tryptophane.
3. 8 c.c. distilled water + 2 c.c. liver extract + 0.5 g fibrin.	0.35 g fibrin; strong biuret, marked tyrosin, no tryptophane.
4. 5 c.c. $\frac{N}{10}$ HCl + 3 c.c., 2% peptone solution + 2 c.c. liver extract.	Very strong biuret and tyrosin, medium tryptophane.
5. 5 c.c. $\frac{N}{10}$ Na ₂ CO ₃ + 3 c.c., 2% peptone solution + 2 c.c. liver extract.	Very strong biuret, very slight tyrosin, no tryptophane.
6. 5 c.c. distilled water + 3 c.c., 2% peptone solution + 2 c.c. liver extract.	Fair biuret, slight tyrosin, no tryptophane.
7. 8 c.c., 1% methyl acetate solution + 2 c.c. liver extract.	With phenol-phthalëin = 6.8 c.c. $\frac{N}{10}$ NaOH.
8. 8 c.c., 1% methyl acetate solution + 2 c.c. distilled water (HCN as others).	With phenol-phthalëin = 0.1 c.c. $\frac{N}{10}$ NaOH.
9. 5 c.c. $\frac{N}{10}$ HCl + 3 c.c. distilled water + 2 c.c. stomach extract + 0.5 g fibrin.	Fibrin gone; very strong biuret, medium tyrosin, no tryptophane.
10. 5 c.c. $\frac{N}{10}$ Na ₂ CO ₃ + 3 c.c. distilled water + 2 c.c. stomach extract + 0.5 g fibrin.	0.5 g fibrin; fair biuret, very slight tyrosin, no tryptophane.
11. 8 c.c. distilled water + 2 c.c. stomach extract + 0.5 g fibrin.	0.37 g fibrin; fair biuret, very slight tyrosin, no tryptophane.

Starch and cane sugar were not tried in this experiment because of presence of hydrocyanic acid. No. 7 smelt strongly of acetic acid, showing that the titration figure was due to splitting of the methyl acetate. The fact that no tryptophane was found in No. 9 whilst markedly present in No. 1 might be due to there being two ferments, one splitting fibrin to the peptone stage and the other splitting peptones to amido-acids.

CHEMICAL EXAMINATION OF THE GLAND

A chemical examination of the gland was made in *Cancer pagurus*, *Patella*, and *Fusus*. The method employed was to mince the gland and extract with alcohol. After several extractions, the residue was next extracted several times with ether, and the ethereal extracts added to the alcoholic extracts. The alcohol and ether were evaporated off and the solid residue taken up in fresh dry ether. This was called the ethereal extract. The residue left after the removal of the ether soluble portion was treated with absolute alcohol and the solution called the alcoholic extract.

The portion left after treatment with alcohol consisted of substances soluble in water but which had dissolved in the first instance because the alcohol was diluted by the water contained in the tissues; this was taken up in distilled water and called the watery extract.

Cancer pagurus: Ethereal extract = 8.53% of the fresh gland, golden-yellow colour, due to lipochrome.¹ This was redissolved in ether and precipitated by excess of acetone to find if any lecithin were present. The acetone precipitate and the fraction not precipitated by acetone were weighed, after drying, and saponified by alcoholic potash. The portion not precipitated by acetone consisted entirely of neutral fat, whilst the acetone precipitate gave a higher saponification value that would correspond to the weight of the material if it were considered to consist of neutral fat. After incineration the acetone precipitate showed the presence of phosphates.

1. Miss Newbiggin, *Journ. Physiol.*, Vol. XXI, p. 237, 1897.

Neutral fat = 7.77% of fresh gland, whilst acetone precipitate = 0.77% of the same.

Alcoholic extract = 0.32% of fresh gland.

Watery extract gave following reactions: pink biuret, tyrosin, faint tryptophane, no sugar, creatinin. No bile pigments by Gmelin's test, no bile acids by either Pettenkoffer's or Oliver's tests.

Patella vulgata: Ethereal extract = 7.49% of the fresh gland, dark greenish brown mass which gives when dissolved in alcohol the absorption bands of enterochlorophyll.¹

Alcoholic extract = 0.81% of the fresh gland.

Watery extract shows biuret, tryptophane, very faint tyrosin and creatinin, some reduction of Fehling's solution. Gmelin and Pettenkoffer tests negative, but slight cloudiness with Oliver's reagent for bile acids.

Fusus antiquus: Ethereal extract golden brown (probably due to a lipochrome), with no absorption bands. Gives a precipitate with acetone which yields tests for phosphates after incineration.

Watery extract: Creatinin and tyrosin present, no biuret tryptophane or reduction of Fehling. Gmelin's test negative. Faint cloudiness with Oliver's reagent.

The glands show evidence of the products of digestion (albumoses, tyrosine and tryptophane). A large amount of fat and some lecithin. No bile pigments or acids but the faint cloudiness with Oliver's reagent suggests the presence of some substance in the mollusca which, like bile acids, is capable of precipitating albumoses. The presence of 'enterochlorophyll' in *Patella* (herbivorous) whilst it is absent in *Fusus* (carnivorous), supports MacMunn's view that enterochlorophyll is an indirect derivative of the chlorophyll contained in the animal's food.

Finally, I wish to express my thanks to Professor Herdman for his assistance and advice during the course of this research, and also to Professor Vines, of Oxford, for advice in connection with the digestion experiments.

1. Miss Newbiggin, *Q. J. M. S.*, Vol. XLI, p. 391, 1898, and MacMunn, *loc. cit.*