Studies on Digestive Enzyme in Whale

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I. Introduction

In this country where medicinal digestive enzymes are lacking due to shortages of land animals, materials for such use must be sought elsewhere. Luckily, Japanese is surrounded on all sides by the sea where resoures for marine product abounds and to which we can turn for material. It is natural, therefore, that we should turned to one of its biggest animal, the whales, not only for madicinal material but also for the solvation of our foodstuff problem.

Although biological researches on whales have been made to a large amount in the past and many bibliographical data are available, very few references can be found of chemical studies of whales, either in fundamental chemistry or in its application. The author, therefore, undertook to make some chemical experiment with whales with the object of obtaining fundamental knowledge as basis for their utilization. The first report, herewith, present some observations obtaind regarding digestive enzymes contained in whales.

II. Digestive enzymes of a whale (Balaenoptera borealis Lesson)

Researchs into pepsin like substance.

Stomachs of whales differ in structure according to species but they are generally divided into 4 sections. For the sake of convenience, the author and others named then as follows: 1st stomach the one directly connected with cesophagus. 2nd, 3rd and 4th stomachs-stomachs connected to the 1st in that order.

In order to determine how pepsin is distributed in each stomach, mucous membrane of the stomach was separated from its sinews and its dried powder was prepared.

1) No enzyme was found in the 1st stomach, very little in the 2nd, and large amount were found in the 3rd and 4th stomachs.
2) Contrary to the existing idea of protein decomposing enzyme of animals, pepsin, which acts in acid medium, the substance found in whales work best in neutral or alkaline media.

3) Certain concentrated product can be obtained from the mucuous membrane of the stomach by a simple process.
4) The substance is activated by common salt.

EXPERIMENTAL

1) Preparation of the material

Mucous membrane from each stomach of a whale (Baleanoptera borealis Lesson) dissected at Ayukawa in Miyagi prefecture, were soaked in acetone and sent to Tokyo. The concentration of acetone was about 50%. Time elapsed in transportation to Tokyo was about 10 days. The membrane was then cut into small pieces with scissors, put through a mincing machine once and soaked in two to three volumes of acetone. This was left for one hour with occasional shaking and then filtered by suction. This procedure was repeated 3 to 4 times. The membrane was finally shaken with an equal mixture of acetone and ether for dehydration and degreasing, filtered, dried in the open and finally dried in vaccuum desiccator over calcium chloride. The dried powder here obtaind was ground in mortar and put through a sieve.

2) Determination of suitable pH for activity

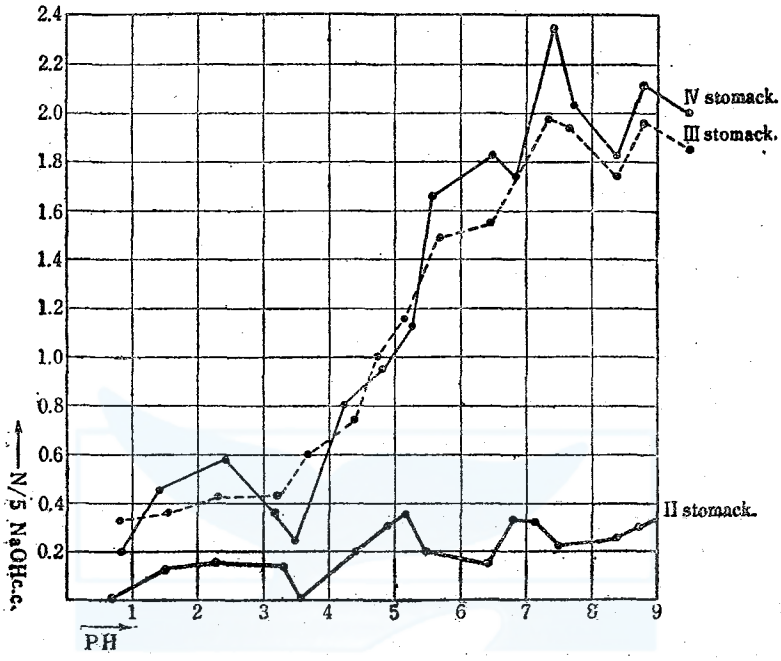
a) Determination of suitable pH by the liquefaction of gelatine.

3 g gelatine was added to 10 cc each of $^{N}/_{10}$ buffer solutions[1] of different pH values, dissolved at 50°C and placed in a thermostat of 40°C. 0.5 g of the above dried stomach powder was added to each gelatine solution and kept for 4 hours in a thermostet at 40°C. The gelatine solutions were taken out, cooled by water and their solidification observed.

The protein decomposing enzyme present in stomach is called pepsin which, as is well known, is active in acid medium of pH 1 to 2. According to bibliography, the most suitable pH values for pepsin to work on gelatine is at pH 2—3. However, according to the results obtained in the above experiment, it takes 48—72 hours for the powder of 3rd and 4th stomachs to liquefy gelatine if the pH is kept at 2 to 3. On the other hand, if the pH is maintained at 5.0 to 6.2, powder from 3rd and 4th stomachs will liquefy gelatine completely in 4 hours. Liquefaction occurs even at ph 8.5.

1) For buffer solution, Sörensen's hydrochloric acid sodium hydroxide, and Michaelis acetic acid-sodium acetate, were used. (Experimental Chemistry, II, 12, p. 127)

Table 1



b) Determination of suitable pH value by titration

0.5 g Gelatine was added to 10 cc of hydrochloric acid or sodium hydroxide of various strengths, melted at 50°C and put into thermostat at 40°C. 0.1 g Dried stomach powder was added to each of these solutions, held for 4.5 hours at 40°C in the thermostat. They were then taken out, 5 cc Formol solution[2] added and titrated with N/5 NaOH solution, the end point being coloration of the solution to red. Titration under identical conditions were made with each of the solution of various acid or alkaline gelatine without the addition of enzyme and the difference in the two values were taken as the increase in acidity at each pH value. Following graph shows the titration values at various pH values. No change in pH value was observed before and after the reaction. pH values were determined by the test paper of Toyo Filter paper Co.

On the other hand, Biuret reaction was tested with the reaction mass after reaction and obtained results coinciding well with the results of Formol titration showing deep blue coloring on acidic side and light pink in neutral

2) 1 cc 0.5% phenolphthalein (0.5 g phenolphthalein dissolved in 100 cc 50% alcohol) added to 50 cc formalin (J. P.) and made into a pink solution with N/5 NaOH solution. (Biochem. Z. 7 (1907), 45 : Z. phys. C. 64 (1910), 120.

and alkaline portions.

3) **Concentration of enzyme**

500 cc 0.2% hydrochloric acid solution was added to 50 g dried stomach powder and left for 5 hours at room temperature with occasional stirring. This was then filtered and a solution of $(NH_4)_2SO_4$ was added the filtrate to 50% saturation and the white precipitate that salted out was filtered and dried under reduced pressure over calcium chloride. This was taken as a sample and enzymic action observed.

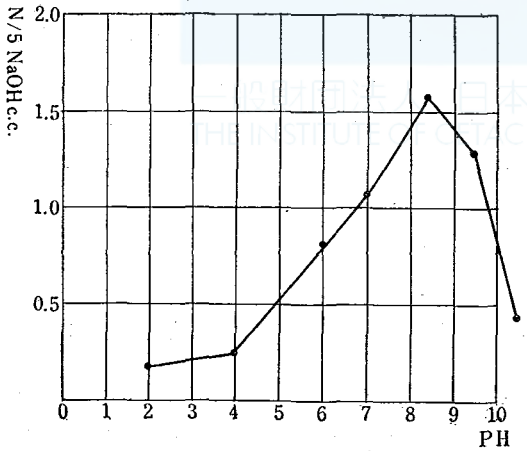
a) Pancreatin testing (as in J. P.). Method of testing by means of modification in clause [6] for casein solubility.

0.1 g Casein dried over sulphuric acid was taken in 50 cc measuring bottle, 1 cc N/10 KOH added and dissolved, and water added to make the whole volume 50 cc of this solution was taken, 4.75 cc water added and held in a thermostat at 40°C. 0.1 g purified enzyme powder mentioned above was taken in a 500 cc measuring bottle, water added to make the whole volume 500 cc with solvation of the enzyme. 0.25 cc of this enzyme solution was added to the casein solution and held for 1 hour in a thermostat at 40°C. This was then taken out and 3 drops of acetic acid alcohol solution (1 cc acetic acid, 9 cc water and 10 cc alcohol) was added but no opalescence or precipitation occured. This shows that at 40°C and at pH 7, the enzyme digests 200 times its quantity of casein in 1 hour.

b) Determination of suitable pH value

50 mg of the above mentioned salted-out enzyme was taken in a 25 cc measuring bottle and dissolved in 50% glycerine. 1 cc of this solution was taken as the enzyme solution. For a base material, 10 cc 6% gelatine solution was made into a 14 cc solutions with the addition of a suitable amount of N/5 NaOH or N/5 HCl with further addition of water. The enzyme solution was added to each of the acid or alkaline test solutions, reacted for 1 hour at 40°C and titreted with Formol solution. As a control, a solution with 1 cc of

Table 2



distilled water instead of enzyme solution was used and the difference in titration value was taken as the amount dissolved at each pH value. Determination of pH was mas made as in the above (2) b). Results are shown in the following graph.

4) **Activation by common salt**

0.5 g Gelatine (I) was dissolved in 10 cc distilled water (II) and 10 cc 0.3% NaCl, 0.1 g dried powder of 3rd (or 4th) stomach added and reacted for 4 hours at 40°C. It was found that the Formol titration value of (II) was 30% larger than of (I). In this case, the titration values of the basic material are the same for both (I) and (II) when no addition of enzyme is made. It is assumed that enzymic action is somewhat activated by commom salt.

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