17 SURVEY OF DIGESTIVE ENZYMES IN COCKROACH

17.1 INTRODUCTION

In unit of Block 1 of LSE-05 course, you have learnt about the digestive enzymes of various animal groups. You are aware that the food of the organisms consists of carbohydrates, proteins and lipids. These large molecules are broken down by enzymes to simpler ones in the digestive system. The simpler molecules are then absorbed across the intestine to be carried to the different tissues and cells of the body. In this experiment you shall make a survey of the enzymes which digest carbohydrates, proteins and lipids in four different regions of the digestive system of cockroach. The digestive enzymes that you will be examining are **amylase** and **invertase** for carbohydrate digestion, **protease** for protein digestion and **lipase** for lipid digestion.

Objectives

At the end of this experiment you should be able to:

- perform simple tests for testing the presence of digestive enzymes in any organism.

17.2 MATERIALS REQUIRED

cockroaches,
insect Ringer solution
30% glycerine
1% starch solution
5% sucrose solution
iodine solution
Benedict's reagent
developed photographic film (several pieces)
fresh milk
bromothymol blue
0.1 N NaOH
mortar and pestle
test tubes
test tube holder
porcelain tile
Pasteur pipettes
graduated pipettes (5 ml.)

17.3 PROCEDURE

1. Obtain ten cockroaches and quickly dissect out the alimentary canal along with salivary glands in insect Ringer solution. Divide the parts of digestive system as follows (Fig. 17.1).
   (i) salivary glands
   (ii) foregut (consisting of oesophagus, crop and gizzard)
   (iii) midgut (consisting of caeca and mesenteron) and
   (iv) hindgut consisting of ileum, colon and rectum.
2. Pool each one of these regions from ten cockroaches. In a small mortar grind the pooled regions of alimentary canal separately with a pestle in 3 ml of 30% glycerine and transfer the contents to a test tube.

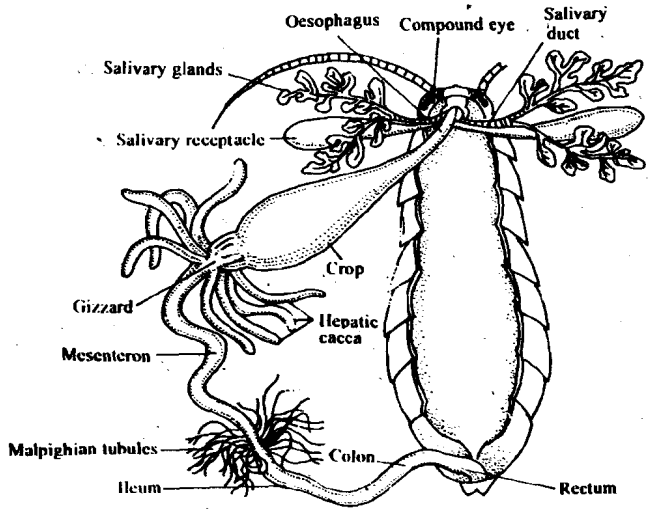

Fig. 17.1: Digestive system of cockroach.

Add another 2 ml of 30% glycerine to the mortar, rinse well and transfer this solution also to the test tube. In this way collect the extracts of four regions in test tubes and label them 1 to 4. Thus you will have following enzyme sources.

Tube 1 salivary gland extract

Tube 2 foregut extract

Tube 3 midgut extract

Tube 4 hindgut extract.

3. Divide the contents of each tube equally in two parts by transfering to another test tube. Label these test tubes as 1c, 2c, 3c and 4c respectively. Heat the contents of these tubes (1c to 4c) over a flame for a minute.

a) What does the heating do to the enzymes?

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A. Test for amylase

Amylase is the starch digesting enzyme. Amylase acts on starch and releases glucose molecules. Starch gives a blue colour with iodine solution. When amylase acts on starch and all the starch is converted into glucose, addition of iodine solution does not result in the develpment of blue colour. This confirms the completion of starch digestion by amylase.

1. Take eight test tubes and add 1 ml of starch solution to each. Mark 4 test tubes 1E to 4E and the 4 others 1C to 4C.
2. To the first four test tubes add ten drops of enzyme source from the four regions of the gut and to the other 4 test tubes add 10 drops of heat inactivated enzyme from the four regions of the gut. Mix well and incubate at 37°C in a waterbath.
3. Every two minutes over the next 10 to 20 minutes remove a drop of the mixture from each tube to a porcelain tile and add a drop of iodine solution. Note the colour developed.

Colour developed initially indicates the presence of starch. Towards the end of the starch digestion, the blue colour does not appear any more. Record your results in the Table 17.1.

b) Does amylase require any cofactor for its activity?

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c) Do you think that you have provided the cofactor in your test?

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B. Test for invertase

Invertase is a sucrose digesting enzyme and forms fructose and glucose as products. The test for the presence of reducing sugars namely glucose and fructose would indicate the occurrence or otherwise of invertase in different regions of the gut of cockroach.

1. Take eight test tubes and pipette out into each one of them 1 ml of 5% sucrose solution.
2. Label the first 4 tubes from 1E to 4E and the rest from 1C to 4C. To each of the first 4 tubes add 10 drops of enzyme source from salivary glands, foregut, midgut and hind gut respectively. To the four control tubes add 10 drops of heat-inactivated enzyme from the four regions of gut.
3. Incubate the reaction mixture (enzyme and substrate mixture) for 20 minutes at 37°C in a water bath.
4. At the end of the incubation period add 1 ml of Benedict's reagent to each of the tubes and leave in a boiling water bath for 5 to 10 minutes. Record the colour changes that you have observed.

The development of red colour indicates the presence of reducing sugars, glucose and fructose. Identify the test tubes in which red colour is formed and record your results.

a) What is the basis for the above reaction?

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b) In which regions of the gut do you observe invertase activity?

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c) Does the red colour appear in reactions in which heat inactivated enzyme was used? Explain your results.

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C. Test for protease

In this test the gelatin (a protein) coated as an emulsion on the film strip would be used as substrate to test the protease activity.

Proteases, as you are aware, are protein digesting enzymes. Perform the test for protease as follows.

1. Take several pieces of 1 inch square, blackend, exposed and developed (black and white) film and wash them well. You will require four such pieces.
2. Place a drop of salivary gland extract on the film (emulsion side) on one half of it and a drop of heat inactivated extract from the same source on the other half. Repeat this procedure for the other three pieces of film with extracts from foregut, midgut and hindgut as well as heat inactivated extracts from these regions.
3. Leave the film pieces in a moist chamber. You may use a petridish with water soaked cotton kept in it as a moist chamber.

4. At 30 minutes intervals, over a period of 2 hours, examine the film strips and note the degree of digestion of gelatin emulsion as an index of protease activity.

Record the results of all the eight observations in the Table 17.1.

1) What is the site of action of a protease in a protein molucule?

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2) Where does the protein digestion occur in the gut of the cockroach?

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D. Test for lipase

Lipase is a lipid digesting enzyme and the digestion products are fatty acids and glycerol. You will use milk fats as the substrate in this test and milk fats on hydrolysis yield free fatty acids. The formation of free fatty acids is identified by a change in the pH of the reaction mixture by using a suitable pH indicator solution.

1. Take eight test tubes and mark the first four as experimental (IE to 4E) and the rest as controls (1C to 4C).
2. To each of the test tubes add 1 ml of fresh milk and a few drops of bromothymol blue dye solution. The milk will now be in blue colour. If it is not sufficiently blue, add a drop of 0.1N NaOH solution.
3. To each of the four experimental tubes add 10 drops of enzyme source obtained from the respective regions of the gut. To the control test tubes add 10 drops of heat inactivated enzyme.
4. Incubate both the sets of test tubes at 37°C for two hours. Note the colour changes in experimental and control test tubes.

1) What colour changes do you observe in control and experimental test tubes?

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2) How would you explain the colour changes in the test for lipase activity?

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17.4 RESULTS

Prepare a table as shown below in your note book to record your results.

Table 17.1

	Regions of digestive system							
	Salivary glands		Foregut		Midgut		Hindgut	
Enzyme	C	E	C	E	C	E	C	E
Amylase								
Invertase								
Protease								
Lipase								

C : Control E : Experiment

Note: *Whenever you identify the presence of an enzyme in a specific region of the digestive system, you may indicate its presence in the table by a (+) sign; a (–) sign would indicate the absence of the particular enzyme in the given region.*