THE ENZYMES OF SOME ELASMOBRANCHS FROM BOMBAY

IV. Lipases of *Scoliodon sorrakowah* and *Rhyncobatus djddensis*

BY D. V. BAL, F.A.SC. AND D. S. GHANEKAR

(*Department of Zoology, Institute of Science, Bombay*)

Received July 25, 1956

AT present a comprehensive literature is available on the lipases of plants and animals and this is particularly true of the esterase from castor bean and pancreatic lipase from Swine. Very little work has been done, however, on the lipases of elasmobranchii. Krukenberg (1877), Richet (1878), Young (1899) and Sullivan (1907) showed the presence of lipase in fish pancreas. Lucey (1942) in her effort to find a suitable method for the study of pancreatic lipase, selected the enzyme of *Carcharinus milberti.*

Since the investigation on the enzymes of elasmobranchs has been undertaken, the study of proteases (1955) the isolation of gastric proteinases (1955) and the study of amylases (1956) have been reported. The present paper deals with the lipases of *Scoliodon sorrakowah* and *Rhyncobatus djddensis.*

EXPERIMENTAL

M/S acetate buffer maintains pH 4–5·0, pH 6–8·0 is maintained by M/10 phosphate buffer and pH 9·0 by M/10 glycine buffer.

Preparation of olive oil substrate—5 c.c. of 0·5 M. NaOH are added dropwise to 100 c.c. of olive oil, which is stirred thoroughly and thus an olive milk is prepared. This olive milk mixed with equivalent amount of buffer of required pH is the substrate to be used.

The method employed here for the study of the action of the enzyme is based on the fact that during the enzyme action on the substrate fatty acids are liberated and these can be estimated titrimetrically.

Titrimetric methods have been described by Palmer (1922), Willstätter (1923) and Balls *et al.* (1937).

Reaction mixture " containing 2 *n* c.c. substrate + 2 *n* c.c. buffer + *n* c.c. enzyme prepartion (boiled in the case of control)" is kept at 39° C. for 120 minutes. The contents of the test-tube are shaken well by whirling the tubes at the intervals of four to five minutes. Reaction is stopped by adding 10 c.c. of alcohol; at the time of titration 5 c.c. of ether are introduced along with a drop of phenolphthalein (0·05% alcoholic), and the whole mixture is titrated

against N/20 NaOH till a permanent faint pink colour is obtained by the addition of the last drop of alkali. The subtraction of the control reading from the experimental one gave the measure of lipase activity in terms of c.c. of N/20 NaOH.

RESULTS

To make the general survey of lipolytic activity of various tissues of *S. sorrakowah* and *R. djddensis*, 6% aqueous extracts of viscera (stomach, intestine and pancreas), liver, spleen, kidney muscle and brain are prepared by grinding the tissue in each case with glass powder and extracting the homogeneous mass with water. The cloth filtrates of the tissue extracts are used as the source of enzyme. Reactions are run at pH 8·0 and the results are indicated by Table I.

In order to get a clear idea of distribution of lipases in various organs of the two varieties of fish activity, pH relationship of each tissue is studied. Cloth filtrate of 6% extract of tissue concerned is the enzyme source used. The reactions were run at different pHs varying from 4–9·0 and the results are given in Table II.

To study the solubility of the pancreatic and liver lipases of *S. sorrakowah*, 6% extracts of pancreas and liver are prepared in a usual way, using water, 10% (V/V) aqueous glycerol and 30% (V/V) aqueous ethanol as the extractants. Extracts are autolysed at 15° C. for 15 hours with toluene as the preservative, and then squeezed through muslin and filtered under suction. The residue in each case is suspended in the amount of solvent equivalent to the extract from which it is obtained. The filtrates and residue suspensions are tested for enzyme activity by carrying out the reactions at pH 8·0 in the case of pancreas and at pH 6·0 and 8·0 in the case of liver. The results are shown in Table III.

S. sorrakowah pancreatic lipase is prepared in powder form as follows:

Fish pancreas is stripped of connective tissue and fat. The gland is minced and treated with acetone and ether in a manner similar to that described by Willstätter and Waldschmidt-Leitz (1922). From 51·9 g. of crude gland, 9·8 g. of yellowish white defatted and dehydrated gland obtained is ground to a fine powder, sieved and stored in a dark bottle in refrigerator + 6·0 g. of this powder is extracted with 100 c.c. of 10% aqueous glycerol (V/V). The extract is kept at 5° C. for 4 hours and filtered. A water clear filtrate that results is the source of enzyme in subsequent experiments. The effect of varying H^+ concentration, time and enzyme concentration on the speed of enzymatic reaction have been studied and the results are represented by

TABLE I

General Survey of Lipolytic Activity of Different Organs of Fish

Reaction mixture "containing 4 c.c. Substrate + 4 c.c. Buffer pH 8·0 + 2 c.c. enzyme preparation" is set up at 39° C. for 120 minutes.

Batch Number	Description of fish	pH 8·0					
		Enzyme activity/100 mgm. dry tissue, in terms of c.c. of N/20 NaOH					
		Viscera	Liver	Spleen	Kidney	Muscle	Brain
	Scoliodon sorrakowah						
1	5 ♀ˢ February 1952	19·2	3·4	1·7	1·7	0·0	0·0
2	5 ♀ˢ Gravid March 1952	21·7	6·7	1·7	1·7	0·0	0·0
3	5 ♀ˢ Gravid March 1952	29·2	8·3	1·7	1·7	0·0	0·0
4	5 ♀ˢ Gravid March 1952	30·0	8·3	1·7	1·7	0·0	0·0
5	5 ♂ˢ April 1952	26·8	10·0	1·0	2·3	0·0	0·0
	Rhyncobatus djddensis						
1	2 ♀ˢ March 1952	33·3	9·2	2·3	1·7	0·0	0·0
2	1 ♀ˢ ,,	23·0	8·3	1·7	1·7	0·0	0·0
3	1 ♀ˢ ,,	16·7	3·4	1·7	1·7	0·0	0·0
4	1 ♀ˢ ,,	23·2	3·4	1·7	0·0	0·0	0·0
5	2 ♀ˢ ,,	25·4	6·7	1·7	1·7	0·0	0·0

TABLE II

Distribution of Lipases in Different Organs of Two Varieties of Fish

Reaction mixture "containing 4 c.c. Substrate + 4 c.c. Buffer of required pH + 2 c.c. enzyme preparation" is kept at 39° C. for 120 minutes.

Fish	Enzyme activity/100 mgm. dry tissue, in terms of c.c. of N/20 NaOH											
	Scoloidon sorrakowah						*Rhyncobatus djddensis*					
Tissue pH	4·0	5·0	6·0	7·0	8·0	9·0	4·0	5·0	6·0	7·0	8·0	9·0
Stomach ..	4·2	9·2	2·5	0·0	0·0	0·0	3·4	8·3	2·5	0·0	0·0	0·0
Intestine ..	0·0	0·0	1·7	3·4	4·2	0·0	0·0	0·0	0·0	3·4	3·4	0·0
Pancreas ..	0·0	0·0	3·4	10·0	28·3	8·3	0·0	0·0	1·7	6·7	34·3	16·7
Liver ..	0·0	3·4	6·7	4·2	10·0	4·6	0·0	2·5	4·6	2·5	13·3	6·7
Spleen ..	0·0	0·0	0·0	1·7	2·5	1·7	0·0	0·0	0·85	1·7	1·7	0·0
Kidney ..	0·0	0·0	0·0	1·7	1·7	0·0	0·0	0·0	0·0	0·85	2·5	1·7
Muscle ..	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Brain ..	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0

TABLE III

The Solubility of Pancreatic and Liver Lipases of S. sorrakowah

Reaction mixture "containing 4 c.c. Substrate + 4 c.c. Buffer of required pH + 2 c.c. enzyme preparation" is kept at 39° C. for 120 minutes.

Name of tissue	pH	Enzyme activity/100 mgm. dry tissue in terms of c.c. of N/20 NaOH					
		Aqueous extract		Glycerol extract		Ethanol extract	
		Filtrate	Residue suspension	Filtrate	Residue suspension	Filtrate	Residue suspension
Pancreas ..	8·0	16·7	8·3	39·9	0·0	36·7	0·0
Liver ..	6·0	6·3	2·5	8·3	0·0	4·2	0·0
	8·0	10·0	2·5	15·0	0·0	10·7	0·0

Table IV, Fig. 1 and Fig. 2 respectively. The influence of calcium chloride and bile salt on lipase activity at its pH optimum is studied and the results

TABLE IV

Activity of Lipase as the Function of pH

Reaction mixture "containing 4 c.c. Substrate + 4 c.c. Buffer of required pH + 1 c.c. H_2O + 1 c.c. enzyme solution" is set up at 39° C. for 120 minutes.

pH	5·0	6·0	7·0	8·0	8·5	9·0
Activity of 1 c.c. enzyme in terms of c.c. of N/20 NaOH	0·0	0·8	1·8	4·2	5·5	1·9
Enzyme Activity/100 mgm. dry weight in terms of c.c. of N/20 NaOH	0·0	4·0	9·8	22·9	27·3	10·4

are given in Table V. Experiment is carried out to study the comparative rates of hydrolysis of different fats by lipase, and the results are indicated by Table VI.

DISCUSSION

From Table I it will be seen that lipolytic activity is maximum in viscera (stomach, intestine and pancreas), minimum in spleen and kidney and that of liver ranges between the two, while muscle and brain exhibit no activity under the conditions of the experiment.

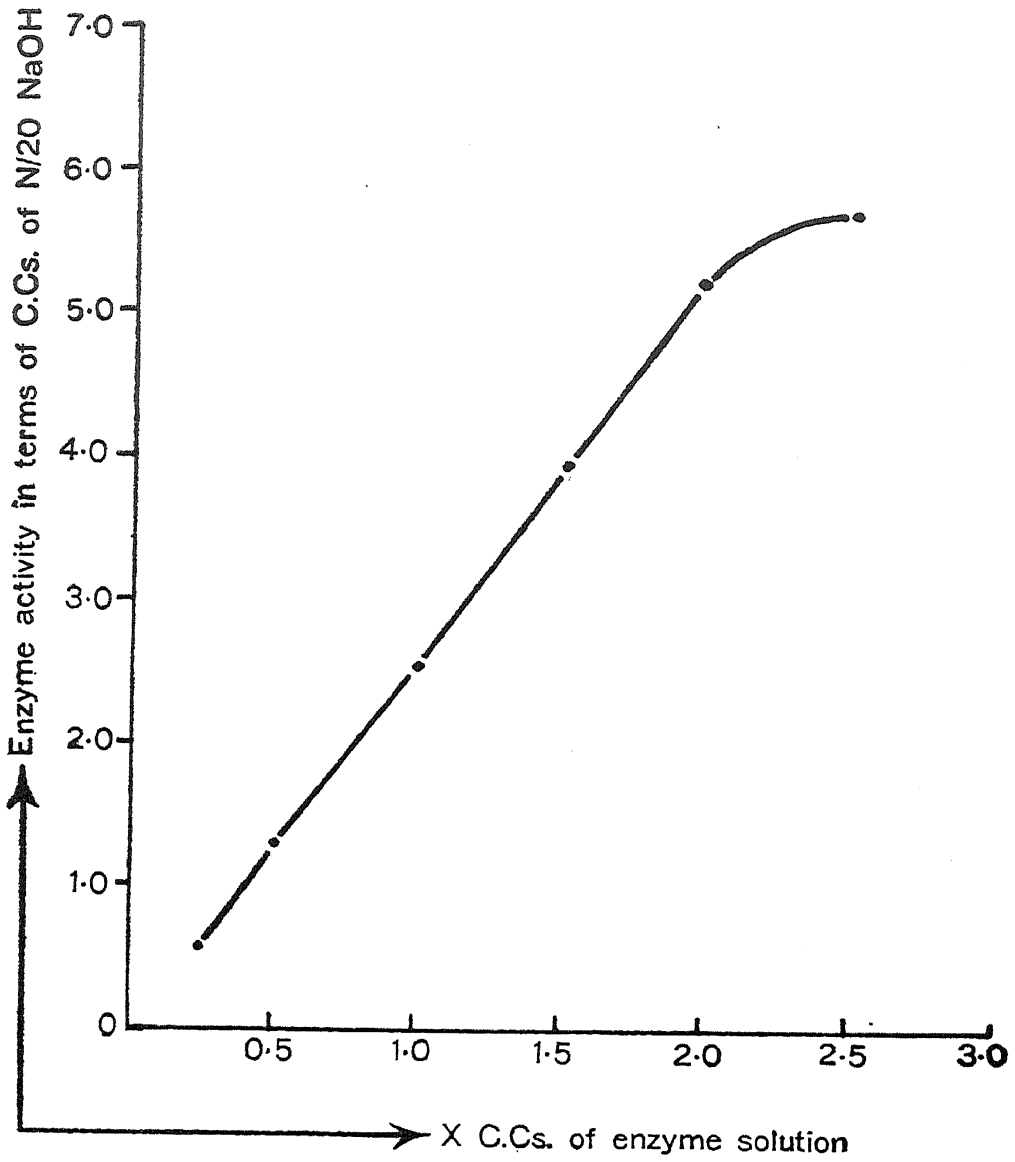

FIG. 1. Rate of digestion of olive oil by lipase. Reaction mixture "containing 4 c.c. Substrate + 4 c.c. Buffer (pH 8·5) + 1 c.c. H_2O + 1 c.c. enzyme solution," is kept at 39° C. for varying intervals of time.

The study of the distribution of lipases (Table II) shows that in *S. sorrakowah* and *R. djddensis*, gastric mucosa exhibits the presence of lipase acting best at pH 5·0. Pancreatic and intestinal lipases show optimum activity at pH 8·0, however the activity of pancreatic lipase is ten times greater than that of the intestinal mucosa. Among the various organs studied pancreas is found to be the most potent source of enzyme. Liver gives two activity pH optima one at pH 6·0 and the other at pH 8·0 thereby suggesting the presence of either two types of lipases or an isodynamic system. Spleen and kidney show low lipolytic activity ranging over pH 7–8·0.

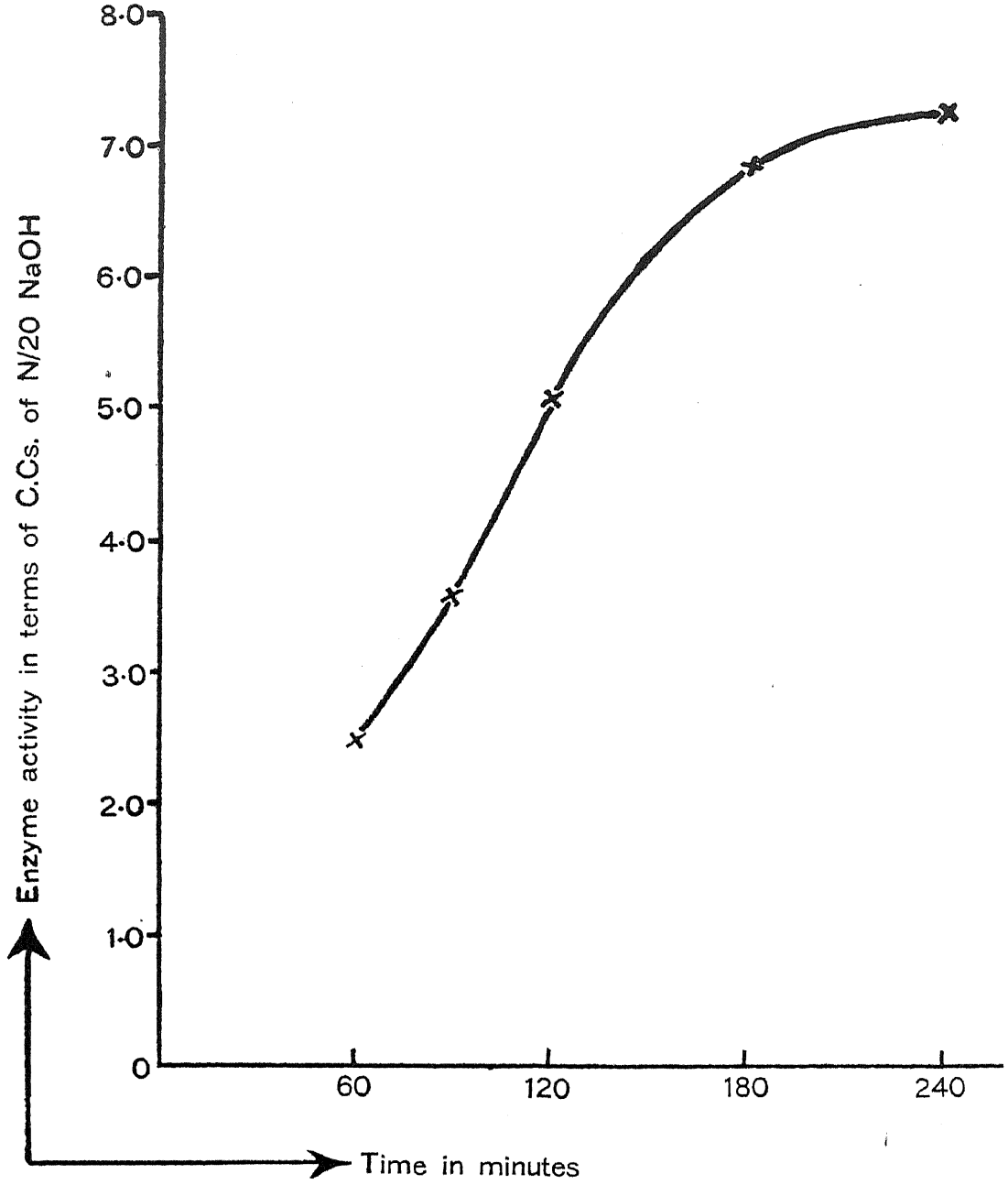

FIG. 2. Enzyme concentration curve. Reaction mixture "containing 4 c.c. Substrate + 4 c.c. Buffer (pH 8·5) + (2·5 − X) c.c. H_2O + X c.c. lipase solution", is set up at 39° C., for 60 minutes.

Observations on the solubility of pancreatic and liver lipases of *S. sorrakowah* (Table III) show that 10% aqueous glycerol and 30% aqueous ethanol are good extractants of pancreatic lipase. Aqueous glycerol is a good solvent of liver lipases, and aqueous ethanol is a good extractant of liver lipase acting best at pH 8·0.

TABLE V

Influence of $CaCl_2$ and Bile Salt on the Activity of Lipase

Reaction mixture "containing 4 c.c. Substrate + 4 c.c. Buffer (pH 8·5) + 1 c.c. $CaCl_2$ or bile salt of varying concentration + 1 c.c. enzyme solution" is set up at 39° C. for 120 minutes.

$10^4 \times$ Molar concentration of $CaCl_2$/ c.c. Re. Mix	Activity of 1 c.c. enzyme in terms of c.c. of N/20 NaOH
0·0	2·5
80·0	2·9
100·0	3·6
200·0	4·1
300·0	4·3
400·0	4·6
500·0	4·0
Concentration of bile salt in mgm./ c.c. Re. Mix.	
0·0	2·5
1·0	2·9
2·0	3·4
3·0	3·8
4·0	4·2
5·0	4·2
6·0	4·2

Study of the properties of the powder form lipase prepared from *S. sorrakowah* pancreas shows that powder when extracted with 10% aqueous glycerol yields whole enzyme in solution. The activity of the enzyme is a function of H concentration (Table IV), and is optimum at pH 8·5, it is a function of time

TABLE VI

Hydrolysis of Different Fats by Lipase

Reaction mixture "containing 0·8 g. fat dissolved in 6 c.c. glycerol + 40 mg. bile salt (2 c.c. of 2%) + 8 c.c. buffer of pH 8·5 + 1 c.c. H_2O + 3 c.c. enzyme solution" is set up at 39° C. for 120 minutes.

Name of fat hydrolysed	Activity of 1 c.c. enzyme in terms of c.c. of N/20 NaOH	% hydrolysis of each fat
Cow's ghee ..	4·7	100·00
Buffalo's ghee ..	3·9	82·99
Dalda ghee ..	1·8	38·30
Olive Oil.. ..	2·9	61·70
Trimyristine ..	1·5	31·90
Shark-liver oil ..	3·0	63·83

(Fig. 1) for a period of 120 minutes and of enzyme concentration (Fig. 2) till the maximum limit of 2 c.c. enzyme in reaction mixture is reached.

Both calcium chloride and bile salt (sodium taurocholate) (Table V) are activators of enzyme. Maximum activation occurs in the presence of 0·04 M $CaCl_2$/c.c. reaction mixture or 4 mg. bile salt/c.c. reaction mixture.

Table VI shows that the enzyme has the greatest affinity for cow's ghee as the substrate and lowest for dalda ghee and trimyristine as the substrates. Its affinity for buffalo's ghee stands next to that for cow's ghee, and for olive oil and shark-liver oil the affinity is in between the highest and lowest levels.

SUMMARY

1. In *S. sorrakowah* and *R. djddensis* lipolytic activity is maximum in pancreas, minimum in spleen and kidney, intermediate in liver and absent in muscle and brain.

2. Aqueous glycerol and aqueous ethanol are good extractants of *S. sorrakowah* pancreatic lipase.

3. *S. sorrakowah* pancreatic lipase is obtained as a yellowish white powder. Activity of enzyme is a function of H^+ concentration, time and

enzyme concentration. Both $CaCl_2$ and bile salt are activators of the enzyme. Comparative rates of hydrolysis of different fats by enzyme have been studied.

REFERENCES

1. Balls, A. K., Matlack, M. B. and Tucker, L. W. — *J. Biol. Chem.*, 1937, **122**, 125.
2. Ghanekar, D. S. and Bal, D. V. — *Ind. J. Fish.*, 1955, **2**, 349.
3. ———— ———— .. *J. Uni. Bom.*, 1955, **23** (5), 1.
4. Ghanekar, D. S., Bal, D. V. and Sohoni, Kamala — *Proc. Ind. Acad. Sci.*, 1956, **43B**, (2) 134.
5. Krukenberg .. *Unters des. physiol.*, 1877, **1**, 327, Inst. Heidelberg.
6. Lucey, M. R. .. *Contribution to the Catholic Univ. America*, 1942.
7. Palmer, L. S. .. *J. Ame. Chem. Soc.*,1922, **44**, 1527.
8. Richet .. *J. d'Anat. et physiol.*, 1878, **14**, cited by Young, p. 150.
9. Sullivan, M. X. .. *Bull. U.S. Bur. Fish.*, 1907, **27**, 3.
10. Willstätter, R. .. *J. Ame. Chem. Soc.*, 1923, **125**, 93.
11. ————, and Waldschmidt-Leitz — *Z. physiol. Chem.*, 1922, **125**, 132.
12. Young, E. .. *Arch. Zool. Exper. Series*, 1899, **3**, 7.