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CONTRIBUTION TO THE PHYSIOLOGY OF DIGESTION IN *TILAPIA MOSSAMBICA* PETERS: DIGESTIVE ENZYMES AND THE EFFECTS OF DIETS ON THEIR ACTIVITY

By

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With 8 Figures in the Text

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I. Introduction

Fish living in various habitats show as a rule different feeding habits. They may often utilize different kinds of food. Great variations occur in the degree to which various digestive organs develop. In some fish, such as sharks, the pancreas is compact, but in most teleosts it appears to be a diffuse organ. ARIMA and KUBOTA (1931), who made a detailed and thorough study of the pancreas of 72 species of fish, stated that it may be found either inside or outside of the liver adhering to it, outside of the digestive tract, or inside of the spleen (SUYEHIRO, 1942).

Pyloric caeca, which are the embryological equivalents of the exocrine pancreas, may be its physiological equivalent in certain cases (menhaden, *Brevoörtia tyrannus*; and scup, *Stenotonus chrysops*) where the pancreas does not show a high degree of morphological differentiation. In these fish the caeca are important as a source of proteases (CHESLEY, 1934). These structures are, however, absent in many fish.

Some of the fish do not have a "true" stomach. *Mugil cephalus*, for instance, has a stomach without gastric glands but with a gizzard-like pyloric region (ISHIDA, 1935). In *Cyprinus*, *Rutilus*, *Gobio* (AL-HUSSAINI, 1949, according to FISH, 1960) and *Fundulus* (BABKIN and BOWIE, 1928) a "true" stomach is also missing.

Herbivorous fish have long coiled intestines, whereas carnivorous fish have short ones.

AL-HUSSAINI and KHOLY (1953) described the distribution of amylase, protease, and lipase in the digestive tract of *Tilapia nilotica*, *Clarias lazera* and *Sargus vulgaris*. Extracts of the buccopharynx of the three species showed an amylolytic activity. It was higher in *Tilapia* and *Sargus* than in *Clarias*. In general, the concentration of the amylolytic enzyme seemed to increase towards the anal orifice, showing that it was strongest in the middle and posterior parts of the intestine. Proteases

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were completely absent in the buccopharynx. They were more concentrated in the stomach and posterior third of the intestine than in the rest of the tract. With regard to lipase, this was also found in the buccopharynx. Here it was more concentrated in *Tilapia* and *Sargus* than in *Clarias*. In *Tilapia* it was found throughout the tract but especially in highest concentration in the stomach. It was not known, however, whether the enzyme was secreted in the stomach or originated in the intestine or pancreas and subsequently passed on to the stomach. The fact that the differences in concentration of the enzyme of the three species were only slight might be due to the similarity of their food.

According to a recent study by FISH (1960) on the comparative activity of some digestive enzymes in the alimentary canal of *Tilapia mossambica* and the perch, *Perca fluviatilis*, amylase is found throughout the tract, including the buccal cavity and mesenteric tissues (pancreas) in *Tilapia*: whereas in the perch it can apparently be extracted from the diffuse pancreas in the connective tissues covering the intestine. Differences in the proteolytic activities of the two species seem to be relatively slight. The stomach contains a protease with a pH optimum of 2.0, which may therefore be pepsin or a pepsin-like enzyme. From the mesenteric tissues (pancreas) a very active protease can be obtained. Its optimum lies at the alkaline side of pH 7 and hence it may be of tryptic nature.

Comparatively little is known of the effects of special and natural diets on enzyme production, and this is particularly true in fish. CHESLEY (1934), comparing the mehhaden, *Brevoörtia*, which feeds on fatty food, with other fish which normally consume far less fats, could find no differences in the lipase activity. AL-HUSSAINI (1949) claimed that in the predominantly herbivorous *Cyprinus*, an appreciably more active carbohydrase and a definitely less active protease are present than in the carnivorous *Gobio* (BARRINGTON, 1957). VONK (1941) found that the differences in the activity of carbohydrases are much greater than those of the proteases when omnivorous fish are compared with carnivorous ones. He showed, for instance, that the amylolytic activity of the pancreas of the carp was more than a thousand times greater than that of the carnivorous dogfish and pike. In contrast to this big difference, the "tryptic" activity of the pancreas of the dogfish and pike was only eight times greater than that of the carp (BARRINGTON, 1957). FISH's (1960) investigations showed that the digestion of starch is more efficient in the herbivorous *Tilapia* and that digestion of protein is a little less than that found in the carnivorous perch.

With respect to the problem of the extent to which a particular organism can adapt its enzyme production to changes in diet, the picture is not so clear. SCHLOTTKE (1939) did find variations in the relative proportions of carbohydrase and protease activity in the carp, but these

variations were not clearly correlated with the nature of the diet (PROSSER and VAN WEEL, 1958).

SQUIRES (1953) stated that the African native on a carbohydrate-rich diet shows a salivary amylase activity several times higher than that of the native who has been on a protein diet. When put on a carbohydrate-rich diet, the latter showed, after several weeks, an increase in amylase activity of the saliva.

GROSSMAN *et al.* (1943, 1944) found that a high carbohydrate diet produced a pronounced increase in the amylase activity of the pancreatic tissues of rats. A high protein diet resulted in a greatly increased trypsin activity, but a high fat diet caused no important alterations in that of lipase.

SHAMBAUGH (1954) discovered a positive correlation between the blood ingested and the subsequent protease activity in the midgut of the mosquito, *Aedes aegypti*. However, GUTH *et al.* (1956) found no such correlation between specific pancreatic enzymes in dogs and the kind of food they were fed within a few hours after feeding. This negative result may be due to the fact that an adaptation to the diet was not possible because of the short interval of time (PROSSER and VAN WEEL, 1958).

VAN WEEL (1958) found a positive adaptation of carbohydrase activity to the diet in the African snail, *Achatina fulica*, after several weeks of dieting. The proteases, on the other hand, showed a negative adaptation: Less proteolytic enzymes seem to be produced in protein-fed animals than in starch-fed ones. Lipase activity does not seem to be affected by the diets.

From the literature it is apparent that the distribution of enzymes in *Tilapia* corresponds with that of most teleosts (BARRINGTON, 1957), except that the buccal cavity shows a certain amount of amylase and lipase activity. Since fish do not have salivary glands, these enzymes may have their origin in other parts of the intestinal tract and appear secondarily in the mouth as a result of regurgitation.

Concerning the effects of different natural diets on enzyme production in fish, the herbivorous fish seem to produce more carbohydrases, especially amylase, and a little less proteases than the carnivorous fish. As far as lipase activity is concerned, no significant differences have been found between fish which feed on fatty food and those which consume far less fats. No definite conclusion can be drawn yet with respect to the problem: "Does a particular individual fish adapt its enzyme production to changes in its diet?"

Tilapia mossambica PETERS was chosen to experiment with because being an omnivorous fish it may be expected to be equipped with a full complement of enzymes. If special diets do have a definite and specific

effect on their activity, it would be expected to become more noticeale in such animals than in those which have a limited enzyme complement and which are, on account of that, already specialized to a certain diet.

I am indebted to Dr. P. B. VAN WEEL for his critical interest in these investigations and his help in preparing the manuscript, and to Dr. A. L. TESTER for his comment on the statistical treatment of the results.

II. Material and methods

Tilapia mosambica PETERS, 15—20 cm in body length, were obtained from a private pond in Honolulu, Hawaii. For the experiments on the effects of diets on enzyme activity, three groups, each of which consisted of six fish, were kept separately. Each group was fed daily for a period of six weeks: The first group exclusively with rabbit meat (protein-rich diet), the second group with bread (carbohydrate-rich diet), and the third group was fed with ground beef, containing a high percentage of fat. The amount of food given to the fish was regulated so that it was all consumed by the next feeding time. At the end of the six-week period, all of the fish were killed by a blow on the head, and the digestive tracts quickly dissected. The mesenteric and fatty tissues covering the stomach and intestine were carefully removed and discarded. Since the intestine shows an anterior-, middle-, and posterior part, characterized respectively by zigzag-, longitudinal-, and transverse mucosal folds (AL-HUSSAINI and KHOLY, 1953), the digestive tract could be divided into the following five parts: esophagus, stomach, anterior-, middle- and posterior part of the intestine.

The contents of these parts were carefully removed by rinsing and the tissues mashed with a pair of scissors and weighed. The brei was extracted with 50% glycerol (3 ml of 50% glycerol: 1 g of brei) in a refrigerator for 24 hours, with a few drops of toluene added as a preservative. The extract was then filtered through glass wool and immediately assayed for enzyme activity.

Digestion was carried out at 36° C for a specific period for each enzyme (see Section III). A 3% gelatin solution was used as a substrate for proteases, a 3% starch solution for amylase, and a tributyrin emulsion for lipase. The following buffer mixtures were used in determining the pH curves: K-biphthalate-HCl; K-biphthalate-NaOH; KH_2PO_4—NaOH; and H_3BO_4—NaOH.

To determine amylase activity, SCHOORL's sugar titration method was used (PROSSER and VAN WEEL, 1958).

Protease activity was determined electrometrically by SÖRENSEN's formaldehyde titration to pH 10 with a "Photovolt" pH meter (VAN WEEL, 1959).

RONA's and MICHAELI's stalagmometric method was employed to measure the lipase activity (PROSSER and VAN WEEL, 1958).

Details as to the amount of extracts, substrate, and buffer used in the experiments are given in "Digestive enzymes".

III. Digestive enzymes

The pH optima of amylase, lipase, pepsin and trypsin were determined:

a) Amylase. 1 ml of extract, 1 ml of buffer solution, 5 ml of 3% starch solution and 0.5 ml of 2% NaCl solution were mixed and a few drops of toluene added. The controls were made in the same way, but heated extract was used. After incubation for 3.5 hours at 36° C,

the enzyme activity was arrested by heating, and the amount of reducing sugar was determined.

As can be seen from Fig. 1, an optimum was found to occur at pH 6.71.

b) Lipase. 1 ml of extract, 1 ml of buffer solution, and 15 ml of the aqueous tributyrin solution were mixed and a few drops of toluene added. The controls were made in the same way using heated extract. After incubation for 1 hour at 36° C, the enzyme activity was arrested by heating and the percentage of residual tributyrin determined.

The optimum appears to be about pH 7.15 (Fig. 2).

c) Proteases. Because two different enzymes (pepsin- and trypsin-like) were probably present (see Introduction), a more detailed study was necessary. The digestive tracts were divided into five parts (see Material and methods); and two experiments were carried out, one with a part including, the other with the corresponding part deprived of its contents.

1 ml of extract, 1 ml of buffer solution, and 5 ml of 3% gelatin solution were mixed and a few drops of toluene added. The controls were made in the same way using heated extract. After incubation for 4 hours at 36° C, the enzyme activity was arrested by heating.

The result of the protease activity determinations (Fig. 3, 4) show that there are two different proteases, one with an optimum at pH 2.8, which might be pepsin, and the other showing an optimum at pH 8.0—8.2, which conceivably might be a tryptic enzyme.

IV. Effects of diets on enzyme activity

To study the effects of special diets on the activity of each enzyme, three experiments were carried out.

Corresponding parts of the digestive tracts of six fish from each diet group were washed together in order to minimize individual differences and to give a sufficient amount of extract to carry out a determination.

Enzyme activity using the same mixtures as given in "Digestive enzymes", was determined at the pH optima found previously; pH 6.71 for amylase, pH 7.15 for lipase, pH 2.8 for pepsin, and pH 8.0—8.2 for the tryptic protease. The results are tabulated in Tables 1—4.

The data of these tables, except for those on "normal diets"[1] were treated statistically, using a "split-plot" analysis of variance (SNEDECOR, 1959, Section 12, 11) to determine if there were significant differences

[1] Fish which were obtained fresh from the pond and which had therefore lived on "normal", but uncontrolled diet, were found to be unsuitable for this statistical treatment.

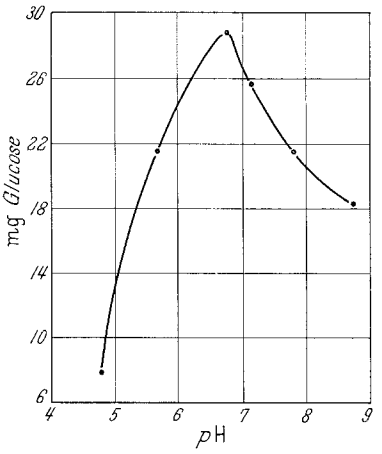

Fig. 1

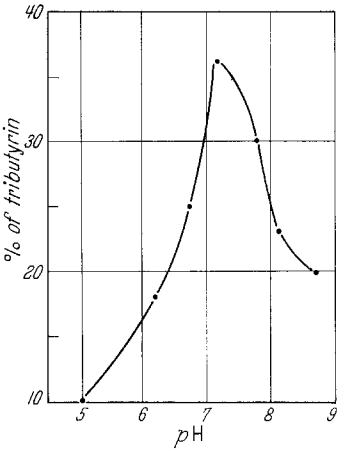

Fig. 2

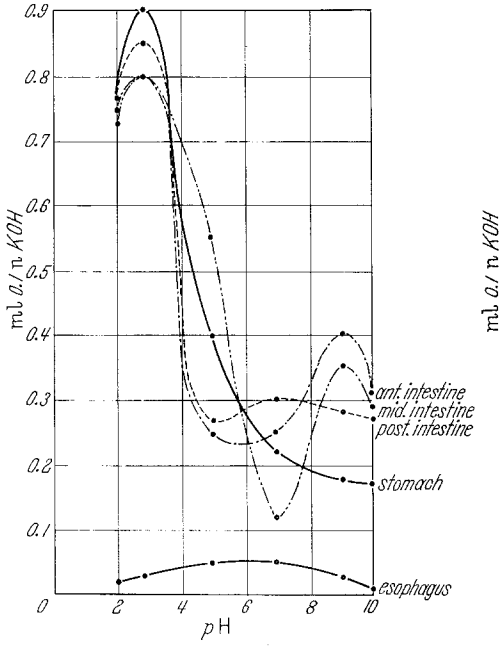

Fig. 3

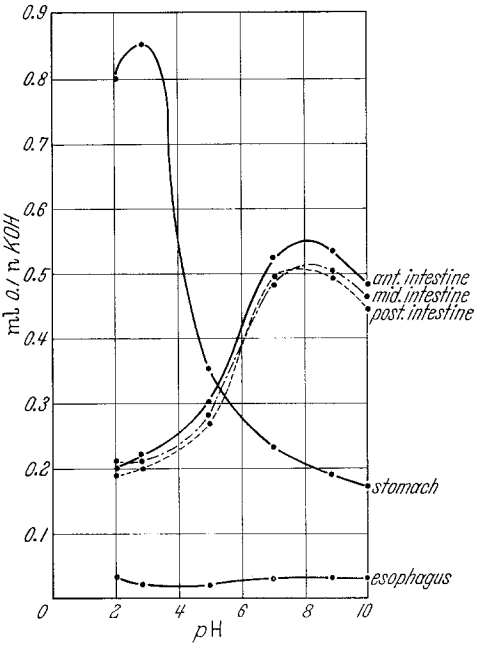

Fig. 4

Fig. 1. pH-optimum curve of amylase

Fig. 2. pH-optimum curve of lipase

Fig. 3. pH-optimum curve of proteases. (Each part of the alimentary tract with contents)

Fig. 4. pH-optimum curve of proteases. (Each part of the alimentary tract without contents)

between the means for enzyme activity depending on the different diets and the different parts of the gut (Table 5).

Table 1. *Amylase activity with different diets*

Diets Parts	Carbohydrate-rich	Protein-rich	Fat-rich	Normal
Esophagus	31.2[1]	28.6	29.7	28.9
	30.8	28.5	28.3	28.7
	31.9	28.4	29.0	28.9
Stomach	28.6	25.9	25.0	25.1
	28.0	25.1	24.8	25.0
	28.4	25.5	24.8	24.9
Anterior part of intestine	34.1	32.5	33.6	33.3
	33.9	33.1	33.2	32.5
	34.2	32.9	33.2	32.9
Middle part of intestine	36.7	34.2	35.1	35.2
	36.3	33.8	35.7	35.0
	36.5	38.9	35.3	34.9
Posterior part of intestine	34.9	33.5	33.0	33.2
	34.7	33.4	33.3	33.2
	34.9	33.1	33.2	33.1

[1] Expressed in mg glucose.

Table 2. *Lipase activity with different diets*

Diets Parts	Carbohydrate-rich	Protein-rich	Fat-rich	Normal
Esophagus	2[1]	1	1	2
	3	5	3	4
	6	2	4	5
Stomach	38	37	45	40
	40	40	40	39
	45	41	39	42
Anterior part of intestine	35	33	35	33
	30	30	30	29
	31	29	29	34
Middle part of intestine	33	34	29	30
	32	32	30	29
	34	30	31	28
Posterior part of intestine	25	25	25	25
	23	24	23	24
	29	28	27	21

[1] Expressed in % of digested tributyrin.

For the gut as a whole, the three diets produced significant differences in amylase and tryptic activity, but not in that of lipase and pepsin.

The significance of the individual means for diets was investigated by a series of *D*-tests (SNEDECOR, 1959, Section 10.6). From this analysis, it is apparent that:

1. Considering the entire digestive tract as a unit, there is a significant difference in amylase activity between the fish fed on a carbohydrate-rich diet and those fed in either of the other two diets, but there is no such significant difference between the fish fed on protein-rich and on fat-rich diets.

2. There is a significant difference in tryptic activity in the intestines between fish fed on a protein-rich diet and those fed on either of the other two diets. No significant differences are found between esophagus and stomach. Between fish fed on carbohydrate-rich and those fed on fat-rich diets, no significant differences are apparent.

As might be expected, enzyme activity differed significantly between the parts of the digestive tract for fish fed on each of the three diets. In

the case of amylase and trypsin, there was a small but significant interaction between the two factors (diets and parts). Despite this interaction, which reflects primarily differences in level rather than direction of change, it seems justifiable to draw the following conclusions from the data, which illustrated graphically in Fig. 5, 6, 7, and 8.

1. The amylase activity in the intestine is greater than in the esophagus and stomach: but in the esophagus, it surpasses that of the stomach.

2. The lipase activity in the esophagus is smallest. The stomach appears to contain more of this enzyme than any other part of the digestive tract.

3. The pepsin activity in the esophagus is smallest of all. The stomach contains much more pepsin than any other part of the intestinal tract.

4. The tryptic activity in the intestine is greater than in the esophagus and stomach. The esophagus contains the smallest amount of tryptic enzyme of all. There seems to be no significant difference in the enzyme activity between the three parts of the intestine.

Table 3. *Pepsin activity with different diets*

Diets / Parts	Carbohydrate-rich	Protein-rich	Fat-rich	Normal
Esophagus	0.02[1]	0.04	0.01	0.02
	0.03	0.02	0.01	0.01
	0.01	0.03	0.02	0.02
Stomach	1.00	1.00	1.00	0.90
	0.95	0.90	0.99	0.88
	0.92	0.80	0.97	0.89
Interior part of intestine	0.26	0.25	0.25	0.22
	0.24	0.23	0.20	0.20
	0.23	0.22	0.21	0.24
Middle part of intestine	0.22	0.21	0.20	0.23
	0.20	0.20	0.21	0.24
	0.21	0.23	0.20	0.21
Posterior part of intestine	0.23	0.19	0.20	0.21
	0.23	0.20	0.23	0.20
	0.25	0.22	0.24	0.23

[1] Expressed in ml 0.1 n KOH.

Table 4. *Tryptic activity with different diets*

Diets / Parts	Carbohydrate-rich	Protein-rich	Fat-rich	Normal
Esophagus	0.02[1]	0.03	0.01	0.02
	0.01	0.02	0.01	0.02
	0.02	0.02	0.01	0.01
Stomach	0.18	0.20	0.17	0.19
	0.16	0.18	01.6	0.16
	0.20	0.18	0.16	0.18
Anterior part of intestine	0.52	0.60	0.50	0.50
	0.50	0.62	0.52	0.52
	0.51	0.61	0.52	0.51
Middle part of intestine	0.50	0.58	0.49	0.50
	0.49	0.56	0.48	0.49
	0.49	0.59	0.50	0.50
Posterior part of intestine	0.47	0.56	0.48	0.48
	0.46	0.55	0.48	0.47
	0.45	0.55	0.46	0.46

[1] Expressed in ml 0.1 n KOH.

V. Discussion and conclusions

From the experimental results it is obvious that *Tilapia* appears to have an enzyme complement comparable to that of other fish. The pH optima found are also comparable with those found in other fish.

Amylase with its optimum at pH 6.71 compares favorably with that of *Acanthurus triostegus sandvicensis* (RANDALL, 1961), but its optimum is slightly lower than that of *Pleuronectes* (pH 7.5—8.0, BAYLISS 1935) and a few other fish such as the puffer, *Spheroides maculatus* and toadfish, *Opsanus tau* (optima at pH 7.2, CHESLEY 1934).

Table 5. *Analysis of variance of split-plot experiment on special diets and enzyme activity*

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Amylase	
Main-plots:			
Replications .	2	0.54	0.27
Diets	2	41.38	20.69**
Error	4	0.30	0.08
Sub-plots:			
Parts	4	486.83	121.71**
Parts × diets .	8	8.93	1.12**
Error	24	2.25	0.09
		Lipase	
Main-plots:			
Replications .	2	13.73	6.87
Diets	2	10.00	5.00
Error . . .	4	25.07	6.27
Sub-plots:			
Parts	4	7208.5	1802.13**
Parts × diets .	8	16.3	2.04
Error	24	129.20	5.38
		Pepsin	
Main-plots:			
Replications .	2	0.0037	0.0019
Diets	2	0.0025	0.0013
Error	4	0.0015	0.0004
Sub-plots			
Parts	4	4.6177	1.1544**
Parts × diets .	8	0.0122	0.0015
Error	24	0.0237	0.0010
		Trypsin	
Main-plots:			
Replications .	2	0.0004	0.0002
Diets	2	0.0349	0.0175**
Error	4	0.0002	0.00005
Sub-plots:			
Parts	4	2.0615	0.5154**
Parts × diets .	8	0.0153	0.0019**
Error	24	0.0026	0.0001

Lipase has practically the same pH optimum as the one found in *Acanthurus* (RANDALL, 1961).

The optimum of pepsin (pH 2.8) compares favorably with that of herring (pH 2.5—2.8, ALMY 1926) but is slightly higher than that of pepsin found in *Pleuronectes* (pH 1.5—2.5, BAYLISS 1935) and perch (pH 1.65—1.8, HAYKES, MAZANET and SZÉCSÉNYI, 1934 cited from BARRINGTON, 1957).

The pH optimum of trypsin (pH 8.0—8.2) is in general agreement with the optimum found in pancreatic extracts of *Anguilla japonica* (pH 7.0—8.0, OYA *et al.*, 1927), and *Pleuronectes* (pH 7.5—8.5, BAYLISS 1935).

Amylase in *Tilapia mossambica* was detected throughout the digestive tract with the greatest activity in the intestine. Amylase may be secreted by intestinal glands of *Tilapia* and by the pancreas (pancreatic tissue contained some amount of the enzyme according to FISH's investigation in 1960).

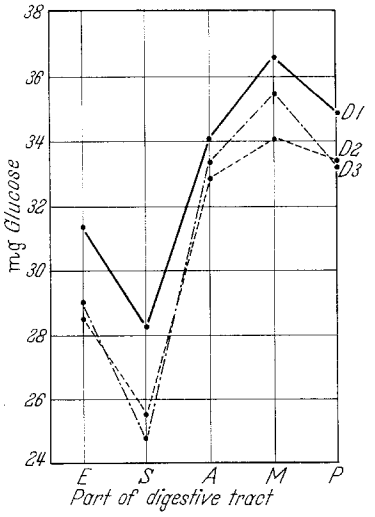

Fig. 5

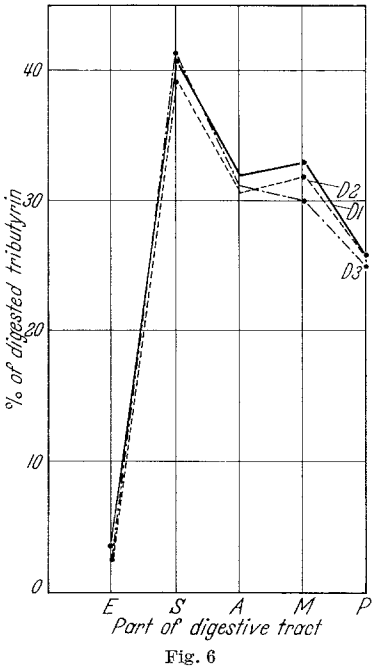

Fig. 6

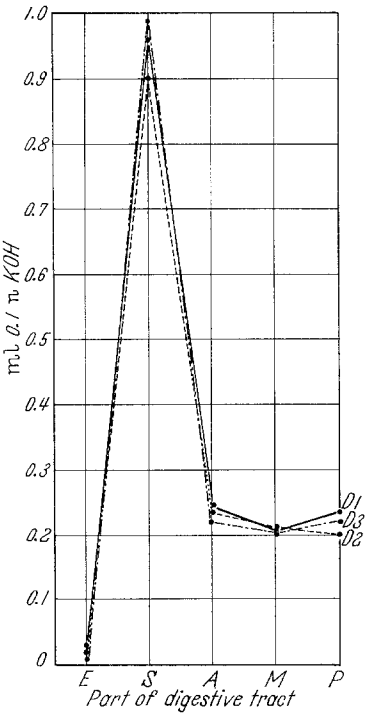

Fig. 7

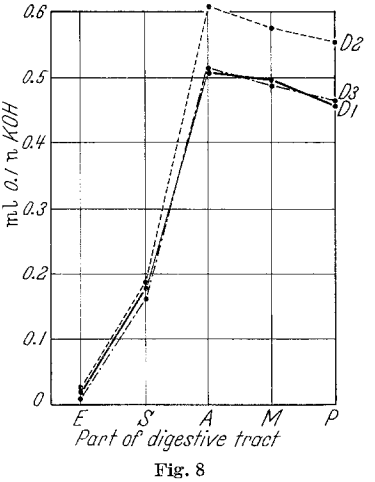

Fig. 8

Fig. 5. Amylase activity with different diets. Abscisse: part of digestive tract. Ordinates: mg glucose, in this and the following figures: *D 1* = carbohydrate-rich diet, *D 2* = protein-rich diet, *D 3* = fat-rich diet, *E* = esophagus, *S* = stomach, *A* = anterior-, *M* = middle-, and *P* = posterior part of the intestine.

Fig. 6. Lipase activity with different diets

Fig. 7. Pepsin activity with different diets

Fig. 8. Tryptic activity with different diets

The presence of amylase in stomach and esophagus is unusual, but AL-HUSSAINI and KHOLY (1953) and FISH (1960) found the same in *T. nilotica*, and in *T. mossambica* respectively. Carbohydrase has also been found in the stomach of *Mugil* by ISHIDA (1935). There is a remote possibility that intestinal secretions get into the stomach and esophagus by way of antiperistaltic movements, hence the presence of amylase in these parts. No direct evidence exists of carbohydrases being secreted by either stomach or esophagus. However, SARBAHI (1951) described racemose glands in the mucosa of the esophagus of *Labee rohita* and suggested that they might be concerned with the production of a carbohydrase. Thus amylase found in esophagus and stomach of this species may originate in these glands. However, as the activity of amylase in *Tilapia* appears to be very small as compared with that found in other parts of the digestive tract, its physiological importance (as to digestion of carbohydrates) appears to be small. That the activity of amylase in the esophagus is greater than in the stomach is understandable. The pH of the gastric contents proved to be 3.0—5.0 (as determined with pH test paper), therefore too low for amylase to show an appreciable activity (Fig. 1). From the experimental evidence, it may therefore be concluded that the secretions produced in intestine and pancreatic tissue are of primary importance in carbohydrate digestion in *Tilapia mossambica*.

In telosts, lipase has been found in the intestinal mucosa of *Fundulus heteroclitus* (BABKIN and BOWIE, 1928), *Zoarces anguillaris* (MACKAY, 1929), and *Pleuronectes platease* (BAYLISS, 1935). BAYLISS could not find any lipase in the pyloric caeca and the investing pancreatic tissue in *Pleuronectes* and believed that this enzyme was secreted by the intestine. However, CHESLEY (1934) found that the pancreas was also associated with lipase secretion in several teleosts, and according to BARRINGTON (1954), ISHIDA (1936) detected lipase activity in the pancreas of *Salarias*, in which this organ is separable from the liver.

The data (Fig. 6) show that the stomach apparently produces most of the lipase in *T. mossambica*. This unexpected result finds corroboration in AL-HUSSAINI'S and KHOLY'S investigations (1953) who found the highest activity of lipase in the stomach of *T. nilotica*. MACKAY (1929) also reported that in *Zoarces anguillaris* the lipase of the intestinal mucosa is weaker than that found in the gastric mucosa. It is therefore possible that the main seat of lipase secretion is the gastric mucosa. It does not mean, however, that lipolytic action is strong here. The gastric pH is too low to allow a marked fat digestion. However, when the enzymatic secretions arrive in the intestine, where a more favorable pH range (pH 7.0—9.0) occurs, the lipase should show activity. The fat digestion will occur mainly in the intestine, although lipase secretion seems to be much weaker here than in the stomach. Whether or not these data may be generalized in fish, is still questionable. More data

are needed before such a general statement as to the site of lipase production can be made.

Relating the physiological data with the histological ones, there seems to be good evidence that the production of pepsin is associated with the granular secretory cells of the gastric glands (BARRINGTON, 1954). It is usually assumed that pepsin and hydrochloric acid are produced in the same cells but there seems to be no clear cytophysiological evidence for this.

In *Tilapia mossambica*, pepsin activity is greater in the stomach than in any other part of the gut. This agrees with the above-mentioned view by BARRINGTON. When the contents of the intestine are carefully rinsed off, there is little activity of this enzyme at pH 2.8 in the rest of the gut (Fig. 4); but when the extract is made of intestine with its contents, then at pH 2.8 pepsin activity in the intestine becomes apparent (Fig. 3). Judging from these data and the fact that FISH (1960) found no pepsin activity in the pancreas, the enzyme in the intestine may very well arrive from the stomach. Considering the pH range found in the intestine (7.0—9.0), pepsin activity here will in all probability be nil.

As far as trypsin is concerned, the situation in the teleosts is frequently complicated by the diffuse form of the pancreas. BAYLISS (1935) attempted to determine whether the pancreas was the sole source of trypsin, or whether some production also occurred in the intestine. Unfortunately the results as a whole were inconclusive, and the source of the enzyme could not therefore be defined. In *Tilapia mossambica*, FISH (1960) found that most active trypsin preparations were obtained from the mesenteric tissues as compared with those from the other tissues. The investigation reported on in this paper shows that tryptic activity is highest in the intestine. Since this investigation does not include the pancreatic tissue, no conclusion can be drawn as to whether or not this enzyme is produced only by the pancreas or produced by the intestine and the pancreas as well.

As mentioned previously, herbivorous fish seem to produce more carbohydrases and a little less protease than carbivorous fish; and there seems to be no correlation with respect to lipase between fish which feed on fatty food, and those which consume little fat.

As to the problem of the adaptation of enzyme production by an individual fish to changes in diet (for which no data have been found in the literature), no significant differences were found in lipase and pepsin activity between the groups of *Tilapia mossambica* on these different special diets after a six-week period of feeding. On the other hand, there appears to exist a positive adaptation of amylase and tryptic activity to the diets.

No explanation can be offered as to why pepsin and lipase activities are not correlated with the diet, although it is generally accepted that the physiology of digestion is closely related to the diet of animals. This non-adaptation to the diet in lipase activity is also seen in the African snail, *Achatina fulica* (VAN WEEL, 1959) and in rats (GROSSMAN *et al.*, 1943, 1944). In addition, no explanation can be offered as to why in *Tilapia mossambica* the tryptic activity is correlated with diet and pepsin activity is not. It may be due to a difference in the time required for each type of secretion to become adapted to a particular diet, although nothing is really known about such an effect.

As was found in different animals (SQUIRES, 1953; GROSSMAN et *al.*, 1943, 1944; VAN WEEL, 1959) amylase production seems to be correlated with diet to a greater degree than protease production. However, in the cockroach, *Blattela germanica*, the opposite has been reported (DAY and POWNING, 1949).

Summary

1. *Tilapia mossambica* PETERS was used as an experimental animal in studies of the pH optima of amylase, lipase, pepsin and trypsin. They were 6.71, 7.15, 2.8, and 8.0—8.2 respectively. The optima were in general agreement with those from studies on other teleosts.

2. A weak amylolytic activity was found in esophagus and stomach.

3. In the entire digestive tract, lipolytic activity (at its pH optimum) appeared to be strongest in the stomach. However, since the prevailing pH (3.0—5.0) here is so low, lipase will in all probability not show a marked activity in this organ.

4. The effects of special diets (protein, carbohydrate, fat-rich) on the activity of these enzymes were studied: amylase and trypsin showed a positive correlation with the diet, whereas pepsin and lipase did not show such a correlation.

Zusammenfassung

Bei *Tilapia mossambica* PETERS wurden die pH-Optima der Amylase (6.71), Lipase (7.15), des Trypsins (8.0—8.2) und Pepsins (2.8) bestimmt. Sie stimmen mit den Ergebnissen an anderen Teleosteern überein.

Im Oesophagus und Magen wurde eine schwache Amylase-Aktivität gefunden.

Die Lipase-Aktivität (bei ihrem pH-Optimum) ist am größten im Magen. Da jedoch der pH (3.0—5.0) hier niedrig ist, ist die Lipase-Wirksamkeit im Magen wahrscheinlich gering.

Die Wirkung von eiweiß-, kohlenhydrat- bzw. fettreicher Nahrung auf die Aktivität der Fermente wurde untersucht: Amylase und Trypsin zeigten eine positive Korrelation mit der Nahrung, Pepsin und Lipase nicht.

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