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Studies on the Physiology of Digestion in *Sisor rabdophorus* HAMILTON

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Introduction

Sisor rabdophorus HAMILTON (Suborder, Siluroidei; family, Sisoridae) has been described as an ugly and deformed fish by CUVIER and VALENCIENNES (1840) due to its peculiar shape. A very long upper caudal filament and the presence of a row of bony scutes on the back make it conspicuous and indeed unique among the Indian fresh water fishes.

Sisor has a very restricted distribution being confined to a few tributaries of the main rivers of North India and Pakistan, mainly at the foot of the hills where the current may be fast but not too deep and the bottom is sandy. It tries to find a sheltered place where the water current is rather slow. It is essentially a bottom dweller and invariably keeps to sandy bottom; juveniles are found hiding below the stones on sandy bottom. It rises to the surface only when absolutely necessary.

It, perhaps originated in South China during Upper Eocene and spread at a later period, probably in early Pleistocene. It must have evolved from the same generalised stock of Bagarid fishes as have given rise to other cat fishes such as Schilbedae, Clariidae etc. During the early Pleistocene, a major upheaval of the Himalayas had occured and left the Pleistocene foredeep. Along this foredeep, the early stock of *Sisor* must have spread westwards. It appears that *Sisor* could not tolerate the strains and stress of that period and found its habitat disturbed every now and then by the frequent tectonic movements. It was thus forced to descend to a more congenial environment. This sudden evacuation naturally affected the population of

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the conservative species.

A number of workers have investigated the detailed anatomy and histology of the alimentary canal in fishes. Important references are those of MACALLUM (1884), VANAJAKSHI (1938), AHSAN-UL-ISLAM (1951), KAPOOR (1953), ISHIDA (1936), SARKER (1959), SEHGAL (1960) and MOHSIN (1962).

However, the references on the physiology of digestion in fishes are limited. BEAUVALET (1933) made such investigations on *Amiurus* and OYA and YOKOTA (1933) on *Parasiturus*. With this point in view, an attempt has been made to study the physiology of digestion of *Sisor* in relation to its feeding habit.

Material and Methods

A large number of living specimens of *Sisor rabdophorus* were collected and were kept in the aquarium for undertaking physiological experiments.

For making the measurements of the hydrogen-ion-concentration of the normal feeding fishes, live animals were dissected soon after they were collected; the different parts of the gut were carefully separated and were thoroughly cleared of any food contents. Both capillary indicator and indicator paper methods were used to determine the pH of the different parts of the gut. pH measurements were also made in starved fishes and in those fed after different intervals.

For preparing extracts of the different parts of the alimentary canal and the glands, about 20 fishes after being starved for about a week, were dissected and the different parts of the gut after being thoroughly washed were ground up with a little thymol and a few drops of glycerol so as to prepar a fine uniform emulsion. This was then diluted with 50% glycerine to about 10% concentration and was centrifuged at 3000 r. p. m.; the rest of the tube was filled with toluene. The different extracts were kept at room temperature for about 48 hours before being tested for different enzymes.

The Alimentary Canal

The fish feeds on such soft bodied animals as are available on the sandy bottom. Its food mainly consists of insects, insect larvae (especially dipteran larvae such as *Chironomus*), a few oligochaetes and occasionally algal filaments. Minute pebbles and sand particles have also been found in the stomach.

The alimentary canal of *Sisor* is comparatively small. In a fish about 100 mm. long, the gut is found to be about 90 mm. in length. In consists of the mouth, buccal cavity, pharynx, oesophagus, stomach, intestine, rectum and the digestive glands, liver and pancreas. The mouth is characteristic and is different from allied forms. It is crescentic in outline (not transverse as stated by DAY, 1878), the upper lip abutting against the lower lip. The food is engulfed by the protractile action of the upper lip.

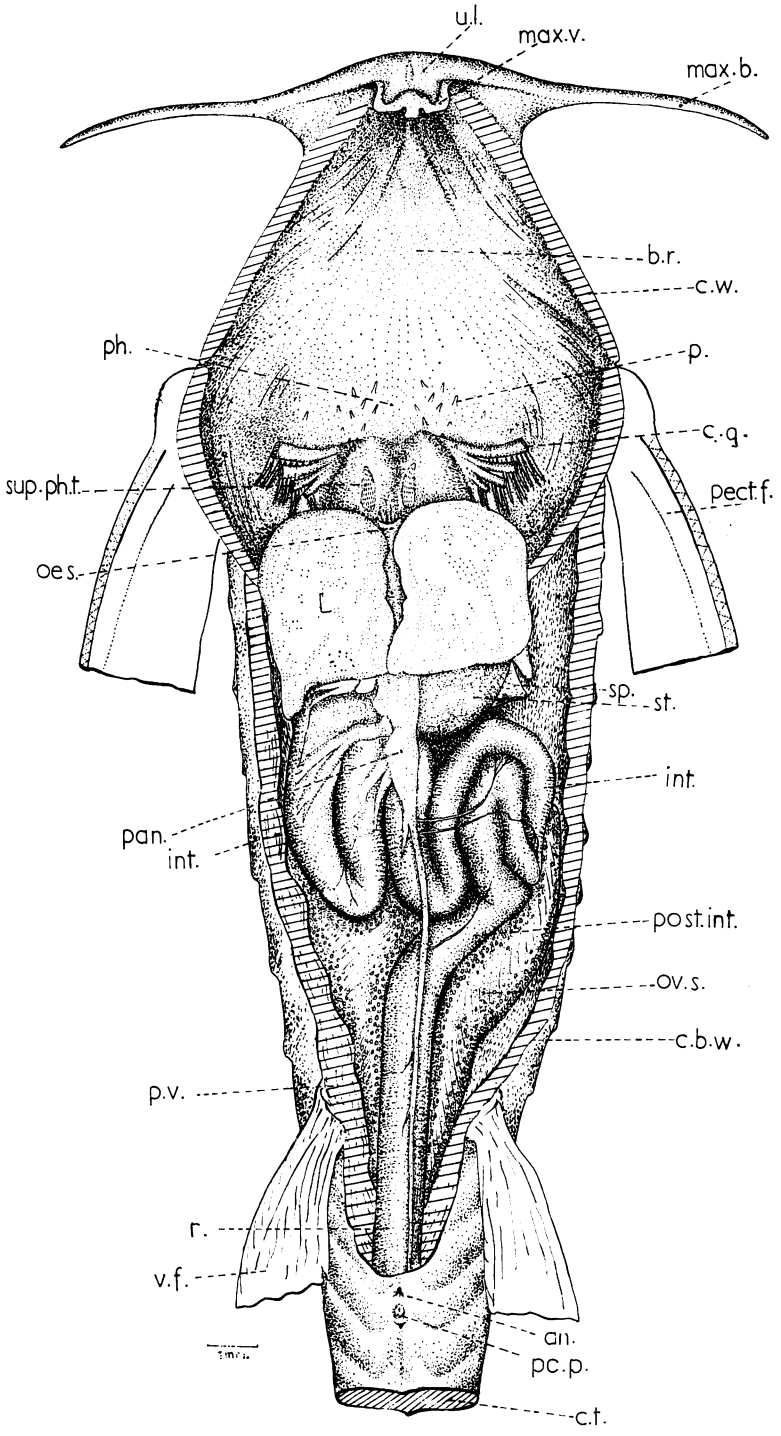

Fig. 1. Ventral view of *Sisor rabdophorus* showing the alimentary canal in situ. an., anus; b. r., buccal roof; c. b. w., cut body wall; c. g., cut gill; c. t., cut tail; c. w., cut wall of the buccal cavity; int., intestine; L, liver; max. b., maxillary barbel; max. v., maxillary valve; oes., oesophagus; ov. s., spent ovary; p., papillae on the buccal roof; pan., pancreas; pc. p., pseudocopulatory papilla; pect. f., pectoral fin; ph., pharynx; post. int., posterior part of intestine; r., rectum; sp., spleen; st., stomach; sup. ph. t., suprapharyngeal teeth; u. l., upper lip; v. f., ventral fin.

The buccal cavity has no teeth in the adult fish while small premaxillary teeth are present in the juveniles which are shed during growth and are replaced. The pharynx has horny pad teeth on the floor and pharyngeal teeth on the roof. The bucco-pharynx opens into the narrow muscular oesophagus. The stomach (Fig. 1) lies at right angles to the axis of the body. The opening of the oesophagus into the stomach is constricted by the presence of sphincter-like muscles. There is no external demarcation of the stomach into cardiac and pyloric parts. The intestine is a long narrow tube; its opening into the stomach is narrowed by the sphincter-like arrangement of the circular muscles. Posteriorly the intestine opens into the slightly wider rectum which opens to the exterior through ventral anus, situated in front of the pseudo-copulatory papilla.

Among the digestive glands, the liver is a large and compact gland. The hepatic ductules from the liver open into the gall bladder by a common duct, while the bile duct from the gall bladder opens into the duodenum. The pancreas is diffused in-between the liver and the stomach. It also extends posteriorly all along the intestine.

pH Measurement

The determination of pH in the different parts of the digestive tract of *Sisor rabdophorus* is important because different enzymes act optimally under different hydrogen-ion-concentrations.

In the following table the pH in the different parts of the gut of *Sisor* under different conditions is shown as an average of several observations.

Table 1. pH of the gut under different conditions.

S. No.	Buccal cavity	Pharynx	Oesophagus	Stomach	Intestine	Rectum	Liver	Pancreas
1. Immediately after collection	6.7	6.6	6.6	5.7	6.7	6.8	7.0	6.9
*2. 4 hours after feeding	6.7	6.6	6.5	5.2	6.8	6.8	6.8	7.0
3. 12 hours after feeding	6.8	6.7	6.6	5.6	6.8	6.8	7.0	7.0
4. 24 hours after feeding	6.8	6.7	6.6	5.7	6.8	6.9	7.1	6.9
5. 48 hours after feeding	6.8	6.7	6.6	5.8	6.9	6.9	7.1	6.9
6. 72 hours after feeding	6.9	6.7	6.6	5.8	6.9	6.9	7.0	7.0
7. One hour after feeding of fish not fed for 72 hours	6.5	6.4	6.4	4.2	7.0	6.8	6.9	6.9

* (i) The feeding was done after keeping the fish in an aquarium for 24 hours in filtered water.
(ii) The fish were fed on larvae of *Cironomus*. Algal filaments were also placed in the aquarium at the time of feeding.

The table above shows that the medium in the different parts of the alimentary canal is almost similar. However, it is distinctly acidic in the stomach while it is

only weakly acid in buccal cavity, pharinx, oesophagus, intestine and rectum. The condition in the liver and pancreas is nearer neutrality. In general, the acidity in the different parts of the gut decreases by starvation while it becomes normal on feeding.

Qualitative Estimation of Enzymes

Experiments were performed to investigate the site of enzyme secretion. The different extracts prepared from the different parts of the alimentary canal were tested for different enzymes, by performing the following experiments:

Amylase

A small quantity of tissue suspension were incubated at room temperature with a few drops of 1% boiled starch solution, the rest of the tube was filled with toluene; another tube containing some starch solution and a few drops of boiled extract acted as a control. After 24 hours the potassium-iodide-iodine test was performed in order to test whether the starch has been hydrolysed to sugar. These results were confirmed by the picramic acid test; 4 drops of incubated solutions, one drop of 10% sodium hydroxide solution and 2 drops of saturated aqueous solution of picric acid were kept in an oven at 50°C. After a short time, the yellow colour of picric acid had changed into reddish-brown colour of picramic acid, showing that the maltose has been formed as a result of the hydrolysis of the starch, catalysed by the presence of amylase. FEHLING's and BENEDICT tests confirmed the above observations. The control experiments in each case gave negative results.

Maltase

The incubated solutions of 2% maltose with the different extracts of the alimentary canal of *Sisor* were tested for any hydrolysis of maltose into glucose by the osazone test. The precipitated mixture with liver extract alone when examined under microscope, showed that most of the crystals formed were elongated needle-shaped crystals of glucose osazone, while in other mixtures as well as in control experiments, the crystals were those of maltose osazone. To confirm these results Barfoed's test for mono-saccaride was also performed.

Lactase

The incubated solutions of 2% lactose and the different extracts were similary tested by osazone and Barfoed's tests and it was found that lactase is absent in almost all the parts of the gut and glands.

Invertase

To investigate the presence of invertase, the incubated mixtures of 5% sucrose

and the different extracts were examined for reducing sugars by the Benedict and Fehling's tests.

Glycogenase

The incubated mixtures of the saturated solution of glycogen and the extracts were also tested by Benedict and Fehling's tests.

Similarly 1% solutions of raffinose, inulin and salicin were separately incubated with the different extracts. These mixtures tested after different intervals gave either positive or negative results for raffinase, inulinase or salicinase respectively as given in the tables.

Lipase

The presence of lipase was tested by the use of condensed milk. Two drops of bromo-thymol blue were added to 25 ml. of a 10% milk solution. To this was added 1% sodium hydroxide solution until the colour changed to light blue. One ml. of this blue milk solution was incubated with a few drops of different extracts. It was found that the colour of the milk in cases where lipase is present, had changed to yellow while in others and in control experiments the colour remained unchanged.

The above observations were confirmed by the olive oil test; 10 drops of olive oil were dissolved in 4 ml. of absolute alcohol, and to this was added 4 ml. of hot water. When this mixture had cooled, 10 drops of phenol red were added. Finally a few drops of 0.01 N NaOH were added till the emulsion became faintly pink. Two ml. of this mixture was incubated with 1 ml. of different extracts. After a short while the colour of the mixture, as in the case of blue milk solution changed to yellow and thus confiirmed the presence of lipase.

Proteases

To test the presence of proteases in the different parts of the gut of *Sisor*, 1 ml. of 10% gelatine solution was incubated with 1 ml. of the different extracts. It was found that after 4 hours, the gelatine incubated with the extracts containing proteases had completely liquified while in control experiments it remained solid.

To summarise these results, the following tables are given. The sign ++ means a vigorous reactions; + means a positive reaction; sign ± means traces of reaction and the sign — means no reaction. In all the instances the pH of the glycerine extracts was noted before and after the reaction which remained almost unchanged.

The tables show that most of the enzymes are present in the extracts of the liver and pancreas. However, weak amylase, glycogenase, protease and lipase are present in the extracts of stomach and intestine while none are present in the extracts of oesophagus and rectum.

Table 2. Reactions with liver extract (Including some pancreatic tissue).

Substrate	Duration of reaction and extent of digestion			Control experiment
	24 hrs.	48 hrs.	72 hrs.	After 72 hrs.
1% starch solution	+	++	++	−
Glycogen sat. solution	++	++	++	−
5% sucrose solution	−	−	−	−
2% maltose solution	+	+	+	−
2% lactose solution	±	±	±	−
1% raffinose solution	+±	+±	+±	−
1% inulin solution	−	−	−	−
1% salicin solution	±	±	+	−
10% gelatine solution	Dissolved after 8 hrs.			Remained solid
Condensed milk etc.	Changed to yellow colour after 4 hrs.			No change
Gum arabic	+	+	+	−

Table 3. Reactions with extracts of pancreas.

Substrate	Duration of reaction and extent of digestion			Control experiment
	24 hrs.	48 hrs.	72 hrs.	After 72 hrs.
1% starch soiution	+±	++	++	−
Glycogen sat. solution	+±	+±	+±	−
5% sucrose solution	−	−	−	−
2% maltose solution	±	±	±	−
2% lactose solution	−	−	−	−
1% raffinose solution	+	+	+	−
1% inulin solution	−	−	−	−
1% salicin solution	−	−	−	−
10% gelatine solution	Dissolved after 8 hrs.			Remained solid
Condensed milk etc.	Changed to yellow colour after 4 hrs.			No change

Table 4. Reactions with stomach extract.

Substrate	Duration of reaction and extent of digestion			Control experiments
	24 hrs.	48 hrs.	72 hrs.	After 72 hrs.
1% starch solution	+±	+±	+±	−
Glycogen sat. solution	+±	++	++	−
5% sucrose solution	−	−	−	−
2% maltose solution	−	−	−	−
2% lactose solution	−	−	−	−
1% raffinose solution	−	−	−	−
1% inulin solution	−	−	−	−
1% salicin solution	−	−	−	−
10 gelatine solution	Dissolved after 12 hrs.			Remaind solid
Condensed milk etc.	Slight change in colour			No change
Gum arabic	±	±	±	−

Table 5. Reactions with extract of intestine.

Substrate	Duration of reaction and extent of digestion			Control experiments
	24 hrs.	48 hrs.	72 hrs.	After 72 hrs.
1% starch solution	±	+	+	−
Glycogen sat. solution	+	+±	+±	−
5% sucrose solution	±	±	±	−
2% maltose solution	±	±	±	−
2% lactose solution	−	−	−	−
1% raffinose solution	−	−	−	−
1% inulin solution	−	−	−	−
1% salicin solution	−	−	−	−
10% gelatine solution	Dissolved ater 12 hrs.			Remained solid
Condensed milk etc.	Slight change in colour			No change
Gum arabic	±	+	+	−

Table 6. Reactions with extract of rectum.

Substrate	Duration of reaction and extent of digestion			Control experiments
	24 hrs.	48 hrs.	72 hrs.	After 72 hrs.
1% starch solution	±	±	±	−
Glycogen sat. solution	±	±	±	−
5% sucrose solution	−	−	−	−
2% maltose solution	−	−	−	−
2% lactose solution	−	−	−	−
1% raffinose solution	−	−	−	−
1% inulin solution	−	−	−	−
1% salicin solution	−	−	−	−
10% gelatine solution	Semi-dissolve			Remained solid
Condensed milk etc.	Slight change in colour			No change
Gum arabic	±	+	+	−

N. B. Tests with extracts from oesophagus gave all negative results.

Table 7. (Summary of Table 2-6).

	Carbohydrases								Protease	Lipase
	Amylase	Glycogenase	Invertase	Maltase	Lactase	Raffinase	Invertase	Salicinase		
Liver (+P*)	++	++	−	+	±	+±	−	±	++	++
Pancreas (+L**)	++	+±	−	±	−	+	−	−	++	++
Oesophagus	−	−	−	−	−	−	−	−	−	−
Stomach (+P)	++	++	−	−	−	−	−	−	+±	±
Intestine (+P)	+	+±	±	±	−	−	−	−	+	±
Rectum (+P)	±	±	−	−	−	−	−	−	±	±

* P=pancreatic tissue, ** L=liver

Discussion

YONGE (1937) has stated that there is a definite correlation between the food of any animal and the nature and relative strength of its digestive enzymes. An attempt, therefore has been made to correlate the feeding habit of *Sisor* with its enzymology.

It was found that liver and pancreatic tissue of *Sisor* have a slightly acidic medium, although in higher vertebrates, they are known to be distinctly alkaline. HERWEDEN (1908), VONK (1927 & 1929) and BAYLISS (1935) have pointed out that the acidity of the teleostean stomach is much less marked than in elasmobranchs. BABKIN and BOWIE (1928) recorded that pH in the bile of *Fundulus* lies between 6.8 and 7.2 while MACKAY (1929) reports a pH value of 5.4 to 6.2 in *Zoarces*. Pancreatic juice of *Raja* has been similarly found by BABKIN (1928) to have a pH of 6.6 to 7.2.

The present investigations also support the view of SULLIVAN (1907) that acidity in the stomach increases after feeding and as mentioned by DOBREFF (1927), the acidity tends to fall during fasting.

The presence of protease, lipase, amylase and a number of other carbohydrases show that fish can consume a large amount of protein and glycogen, a fair amount of fat and starch and small amount of other carbohydrates like raffinose, maltose, salicin etc. This is in accordance with its carnivorous diet. These findings agree with those of AL-HUSSAINI (1949) and SARBAHI (1951) who came to the conclusion that the concentration of carbohydrases was higher in herbivorous forms and lower in carnivorous fish, while for proteases and lipase, the condition is just the reverse.

Due to diffused condition of pancreases of *Sisor*, it is difficult to locate the exact place of enzyme secretion. According to BAYLISS (1935) and BARRINGTON (1957), pancreas alone is responsible for the secretion of proteolytic, lipolytic and amylolitic enzymes while the bile of the liver only suppliments the action of lipase. However, as stated by BARRINGTON (1957), it is not certain whether a separate route always exists for the discharge from the intrahepatic pancreas; the possibility that they may pass through the bile duct and gall bladder cannot therefore be excluded.

The presence of weak lipolytic and amylolitic enzymes in the extracts of stomach and intestine may also be due to the presence of diffused pancreas. In the present state of our knowledge it appears that pancreas, intestinal mucosa and liver may be collectively responsible for the secretion of enzymes. It is most likely, as also pointed out by ISHIDA (1936), CHESLEY (1934) and others that lipase is secreted by the pancreas. However, the greater intensity of reaction when the hepatic and pancreatic tissues are mixed shows that bile greatly helps the action of pancreatic lipase. BARRINGTON (1957) remarks that it is doubtful whether the evidence yet makes it possible to define with certainty the relative importance of the contribution of these two organs to the secretion of lipase or to the secretion of any other enzyme in fishes.

Thus the enzymological studies in *Sisor* show that the enzymes secreted are adapted for the digestion of predominantly carnivorous diet though some vegetable matter can also be made use of.

Summary

Sisor rabdophorus has a very restricted distribution, being confined to a few tributaries of the main rivers af North India and Pakistan.

The fish feeds on such soft bodied animals as dipteran larvae, mainly *Chironomus*, a few oligochaetes and occasional algal filaments.

The alimentary canal of *Sisor* is small. The buccal cavity has no teeth in the adult although small premaxillary teeth are present in the young fish.

The pancreas are diffused. The gall bladder is embedded within the pancreatic tissue.

The pH in the different parts of the alimentary canal and the glands is nearer neutrality while in the stomach, it is weakly acid. The acidity of the stomach increases immediately after feeding while it decreases on starvation.

Studies on the qualitative estimation of enzymes show that most of the carbohydrases are present in the liver and pancreatic extracts. The proteases and lipase are specially very active. A few enzymes are also present in the extracts of stomach and intestine.

These investigations show that the enzymes secreted are well suited to digest the kind of food, the fish takes.

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