

Chapter VI

Section I

COMPARATIVE ANALYSIS OF THE ACTION OF ACETYLCHOLINE
AND EPINEPHRINE ON THE DIFFERENT PARTS OF FISH GUT.

The hypersensitive response of the upper part of the fish intestine provoked us to study the relative sensitivity of the different parts of the gut towards acetylcholine. This study was extended with the action of epinephrine as it was reported to have various effects on the different parts of the teleost intestine. In this connection swim bladder was of special interest because of its two kinds of smooth muscle with difference in response to epinephrine (Fänge, 1953). Therefore, attempt was made to study the effects of acetylcholine and epinephrine on the smooth muscle of the different parts of intestine, stomach and swim bladder.

Methods :

Adult living fishes (*Ophicephalus punctatus*), weighing about 60 to 80 gm, maintained in the laboratory aquarium were used. Different parts of the digestive tract were isolated, 1 cm length being taken from each part, washed and cleared, and then perfused in 2 ml aerated fish Ringer bath as described previously. The bath, recording apparatus and perfusion assembly were same as described before. The normal spontaneous contraction as well as the tonus changes of the tissues were recorded kymographically with isotonic lever having a tension of 500 mg.

The following preparations were used :-

- a) Oesophageal strip.
- b) Stomach strip.
- c) Upper portion of the intestine.
- d) Mid portion of the intestine
(near the bend).
- e) Hind portion of the intestine
(about 1 cm above rectum).
- f) Pyloric caeca.
- g) Secretory part of the swim bladder
along with the pneumatic duct.
- h) Lower part of the swim bladder.

Drugs used :

1. Acetylcholine Iodide (Sigma Chemical Co).
2. Adrenaline Hydrochloride (May and Baker, Ltd).

Dilutions of the drugs were freshly prepared as described before and added into the bath with the help of micro-syringe. Drugs were kept in contact for 45 seconds, and then the bath fluid was replaced by washing the tissue with normal fish Ringer solution.

Results :

Spontaneous motility

The spontaneous rhythmic contraction was noted with different parts of the intestine. No spontaneous contraction was noted with isolated strip of oesophagus, stomach, pyloric caeca, upper portion of swim bladder with pneumatic duct or in the lower portion of the swim bladder.

The pattern of the rhythmic contraction of isolated parts of the intestine varied from place to place but no particular type of rhythm was specific to the individual part.

The pattern of the movement varied in contractile amplitude as well as in frequency and was associated with constant change of tonus. The detailed description of the spontaneous motility of the upper part of the small intestine below the caeca has been given later. However, from the gross analysis of the kymographic records it appeared that the spontaneous motility, both peristaltic and pendular, were markedly present in the upper part of the gut (Fig 41). In the mid portion and lower portion of the gut fine segmental movements were noted but the amplitude of contractions was much smaller.

The strip of either oesophagus or stomach, or the pyloric caeca did not show any contraction in a suspended perfused condition, but in situ within the body, stomach was seen to contract vigorously in response to pinching or any surgical manipulation. None of the tissues from the pyloric caeca and swim bladder showed any loss of tone while being equilibrated in normal Ringer solution for more than 30 minutes.

Actions of drugs :

Acetylcholine :

Various parts of the digestive canal including oesophagus, stomach, pyloric caeca, intestine and swim bladder showed marked contractile response, followed by increase in tone, towards acetylcholine. But the magnitude of the response of individual part varied widely according to the dosage used. Acetylcholine in the dose range of 10^{-6} gm/ml to 10^{-5} gm/ml produced contraction of the oesophagus and pyloric caeca, while it showed sustained contraction of low amplitude with stomach strip and upper portion of the swim bladder. In contrast, much lower doses

of acetylcholine (10^{-10} gm/ml to 10^{-6} gm/ml) caused immediate contraction of all the parts of intestine. Higher dose of acetylcholine (10^{-4} gm/ml) caused marked sustained contraction in oesophagus, stomach and upper portion of swim bladder. Comparatively, the sensitivity towards acetylcholine was maximum in the proximal portion or fore-gut and midgut while it was much lower in the hind gut. However, the overall sensitivity of the intestine was much higher than that of stomach, oesophagus and swim bladder. With pyloric caeca, acetylcholine in 10^{-4} gm/ml produced immediate contraction followed by return to base line within a very short time even when the tissue remained in contact with the drug. This spike like contractile activity towards acetylcholine response was short lasting and was abolished by repeated treatment. But this type of short lasting contraction was never observed in any other preparation except pyloric caeca (Fig. 33).

The contractile response of all the different portions of gut towards acetylcholine was greatly potentiated by physostigmine pretreatment (10^{-8} gm/ml to 10^{-6} gm/ml) and could be blocked by atropine (10^{-8} gm/ml to 10^{-6} gm/ml) pretreatment. The blocking effect of atropine towards acetylcholine response in those preparations was short lasting and recovery took place within 5 to 10 minutes.

Epinephrine :

Epinephrine in doses of 10^{-6} gm/ml to 10^{-5} gm/ml caused slight relaxation of oesophagus. The effect of the drug started within 1 minute and disappeared within 3 minutes. Both stomach and pyloric caeca produced contraction with epinephrine in a concentration range of 10^{-6} gm/ml to 10^{-5} gm/ml. Upper portion of swim bladder with pneumatic duct initially showed no response to graded doses of epinephrine (10^{-7} gm/ml to 10^{-5} gm/ml), but pretreatment with acetylcholine (10^{-6} gm/ml) it responded nicely by demonstrating sustained contraction simulating

acetylcholine response with the same doses of epinephrine. Recovery of such action took place within 3 to 4 minutes. In intestine, epinephrine produced relaxation with lowering of the tone in all the parts studied so far. Low dose of epinephrine (10^{-9} gm/ml to 10^{-7} gm/ml) immediately caused lowering of tone with relaxation and inhibition of rhythmic movements of the foregut, midgut and hindgut followed by recovery within 5 minutes after wash. (Fig. 33).

The lower portion of the swim bladder did not show any spontaneous contraction nor any response towards acetylcholine or epinephrine.

Section II

COMPARATIVE ANALYSIS OF THE ACTION OF ACETYLCHOLINE
AND SEROTONIN ON THE ISOLATED GUT OF ALLIED AIR -
BREATHING FISHES.

The high sensitivity of the intestine of *Ophicephalus punctatus* towards acetylcholine and serotonin response obviously led us to explore whether such response was specific to this particular species or not. Isolated intestines of allied fresh water and air-breathing fishes having common habitat with *Ophicephalus punctatus* were tested in this section .

The following fishes were used :

- (1) *Ophicephalus punctatus*-(order - Ophicephaliformes).
- (2) *Ophicephalus striatus*-(order - Ophicephaliformes).
- (3) *Clarias batrachus* -(order - Cypriniformes)..
- (4) *Anabas testudineus* -(order - Perciformes).

These fishes live in swamps, pond or muddy water in the tropical climate and according to the availability of supply of water and oxygen they move from water to land. They are very resistant to change of temperature from 10° to 40°C. The food, locomotory and breeding habits of those fishes are more or less the same. The external morphology of these fishes are shown in figure 1a, 34.

The acetylcholine and serotonin response of the isolated perfused foreguts of these fishes were studied for comparative analysis. The morphological features and microscopical anatomy of the guts of these fishes were compared grossly with those of *Ophicephalus punctatus*.

Methods :

Adult living specimens of each variety were collected from the market and maintained in laboratory aquarium. Their body weight and length are given below:

<u>Specimen</u> (with local name)	Weight of the body (in gm)	Length of the body (in cm)
O. punctatus (Lata)	60 to 80	18 to 22
O. striatus (Shol)	80 to 100	21 to 23
C. batrachus (Magur)	70 to 100	20 to 23
A. testudineus (Koi)	30 to 40	11 to 12

After decapitation of each fish, the abdomen was incised and the gross morphology of the gut of all those fishes were studied and compared. The measurement of the length and body weight versus length of intestine, number of caeca and position with other viscera were all noted. Isolated segments of upper part of intestine (1 cm in length) was perfused according to the method described before; the composition, pH, and temperature of the perfusing fluid remained the same. The effects of acetylcholine and serotonin at different doses were tested on these tissues and the responses were recorded kymographically.

Some portions of the intestine of all the specimens were used for the histomorphological study. The isolated tissues were fixed in 4% formol-saline. Following dehydration they were embedded in paraffin and sectioned at 5 μ , and were stained with haematoxylin - eosin for the subsequent microscopical observation.

Results :Gross morphology of the intestine of four species.

The general plan of the digestive tube of these four fishes was more or less the same with slight variations in the different individuals. Ophicephaliformes possessed comparatively shorter intestine than Cypriniformes and Perciformes. Among Ophicephaliformes though the gross morphology of the digestive tract was the same, in the species striatus it was longer in length and thicker in girth. The short wide muscular oesophagus of each fish opened in large anterior portion of the stomach. The stomach of the Perciformes (*Anabas testudineus*) was comparatively smaller than that of the other two orders. The distal end of the stomach of the *O. punctatus*, *O. striatus* and *A. testudineus* was tapering whereas that of *C. batrachus* was blunt. At the anterior aspect, just below the pyloric region at the lateral side of the stomach, two blind pouches arose in *O. punctatus* and *O. striatus* and three in case of *A. testudineus*. The caeca were absent in *C. batrachus*. In *O. striatus* and *A. testudineus* the pyloric caeca were longer than in *O. punctatus*. The intestines of *O. punctatus*, *O. striatus* and *A. testudineus* produced a U-shaped loop at the mid-region of intestine. The intestine of *C. batrachus* formed two or three bends before opening into anus. Intestine in Ophicephaliformes was comparatively shorter in relation to the body weight. Although intestine of Perciformes was longer it was comparatively slender. In all cases anal opening was just anterior to the anal fin. All these fishes possessed bi-lobed liver. The gall bladder opened just at the end of pyloric stomach. (Fig. 35). Sphincters were present in oesophagus, pyloric end of the stomach and rectal regions of all these fishes. Swim bladder was conspicuous on the left side of the visceral cavity.

The weight and length of the body along with the

length of the intestine of different fishes were measured and given in the table -V.

Spontaneous motility :

Normal spontaneous rhythmic contractions were present in the intestine of all the four varieties of fishes which were studied. The pattern, tone, rate and amplitude of contractions of the isolated intestine varied from species to species but there was no particular feature of rhythmicity which could be identified as species specific.

It appeared that the upper part of the intestine of Ophicephaliformes, Cypriniformes and Perciformes possessed both peristaltic and pendular movements. Among the four varieties, intestine of Ophicephaliformes showed pronounced rhythmicity of various patterns with relative changes in tone and amplitude of contraction whereas in Cypriniformes the amplitude and rate were lesser though its girth and volume were greater than in the former. The contraction of the intestine of Perciformes was of usually lower magnitude in relation with those of other fishes (Fig. 36).

However, the intestine of *O. punctatus* showed the best performances both in rate and amplitude of the spontaneous contraction when compared with its fellow species, *striatus*, or with other fishes like *C. batrachus* and *A. testudineus*.

Action of drugs :

Acetylcholine

The action of acetylcholine on the upper part of the intestine of all the fishes belonging to the four species was closely alike, being represented by immediate contraction following the addition of the drug. But the magnitude of response of a particular dose varied widely in different species. The

Table - V.

The weight and length of body in relation to
the length of intestine

Name of the species	Wt. of individual fish (in gm)	Length of individual fish (in cm)	Length of the intestine. (in cm)
Ophicephalus punctatus (10)	70 ± 5	18 ± 3	5 ± 1.2
Ophicephalus striatus (10)	140 ± 25	25 ± 5	15 ± 1.5
Clarias batrachus (10)	80 ± 11	20 ± 2	10 ± 1
Anabas testudineus (10)	30 ± 7	11.3 ± 1	7 ± 1

Number of animals in each species used are given in parenthesis. Figures are mean of 10 experiments with ± standard deviation.

intestine of *O. punctatus* showed appreciable contractile response with the lowest dose range of acetylcholine varying between 10^{-10} gm/ml and 10^{-8} gm/ml, while much higher dose was needed in cases of *O. striatus* and *C. batrachus* (10^{-7} gm/ml to 10^{-6} gm/ml) to get the response of identical magnitude. The intestine of *A. testudineus* only appeared to be more sensitive towards acetylcholine response (10^{-9} gm/ml to 10^{-7} gm/ml) in comparison with the latter. There was no change in the basal tone following washing of the intestine of any species. (Fig. 37) Similarly, the acetylcholine response of all the intestine was remarkably potentiated by pretreatment with physostigmine (10^{-8} gm/ml to 10^{-6} gm/ml). Further, the acetylcholine action could be blocked by atropine pretreatment (10^{-8} gm/ml to 10^{-6} gm/ml) in the intestine of all species.

Serotonin :

It has already been mentioned that serotonin caused some alteration in the pattern of spontaneous rhythm in *O. punctatus* intestine. Similar effect was noted in the intestine of *O. striatus* except little change in the *A. testudineus* and *C. batrachus* with some increase in basal tone. However, serotonin in all the dose range (10^{-12} gm/ml to 10^{-6} gm/ml) caused immediate contraction with increase of basal tone in the intestine of all the species. The contractile height was not lowered until the tissue was thoroughly washed with fish Ringer solution. After washing, the tone was lowered. Comparative analysis with particular dose response towards serotonin showed that the intestine of the *Anabas* was most sensitive and appreciable response was obtained at the dose range of 10^{-11} gm/ml to 10^{-12} gm/ml. But such effect was not reproducible after repeated treatment of the same dose. Minimum effective concentration of serotonin to evoke appreciable contraction was 10^{-12} gm/ml in *A. testudineus*, 10^{-10} gm/ml in *C. batrachus* and *O. punctatus*, and 10^{-8} gm/ml in *O. striatus*. However, the intestine of *O. punctatus* appeared

to be the most suitable preparation for demonstrating repeated response towards a particular dose of serotonin and a nice dose-response curve could be plotted with it while the sensitivity was being higher in the other two types, viz, *Anabas testudineus* and *Clarias batrachus*. The reproducibility in each case was much poorer in comparison with *Ophicephalus punctatus*.

Histomorphological observations:

The basic histological architecture of the intestine of above four fishes was more or less the same, i.e., each gut was composed of four layers, viz., mucous coat, submucous coat, muscular coat and serous coat, along with individual variations in the thickness of different layers, the distribution of different types of cells, and the number of villi. A gross observation indicated that the lumen of the intestine of *Anabas* was smaller than that of the other three fishes.

Mucous coat of intestine of all fishes exhibited elongated fine finger like, well organised villi, which were numerous in the *Clarias* but less in number in the other two species (*A. testudineus* and *O. striatus*). Mucosal epithelium of the intestine of all fishes were columnar. The submucosa of the *Clarias batrachus* and the *Anabas testudineus* was not so enriched with glands or nerve plexuses as that of *O. punctatus*, but the blood vessel and muscularis mucosae and connective tissues were all dispensed alike.

The muscle layer was composed of inner circular and outer longitudinal muscle fibers, thickness of the inner coat being double that of outer coat. Muscle cells possessed elongated deeply stained nuclei. In between the two coats network of connective tissue, blood vessels and sometimes nerve plexuses were present in all the intestines, and grossly no remarkable difference could be demonstrated amongst the muscular layers of the intestines of the different fishes. (Fig. 38, 39, 40).