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DIGESTION

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I. INTRODUCTION

Fishes are the dominating vertebrate group as far as number of species is concerned, and in their immense variety have adopted many nutritional habits. One can distinguish piscivores, insectivores, molluscivores, large plant feeders (herbivores), phyto- and zooplanktivores, mud feeders (detritivores), cleaner fish, and, especially in the primitive Cyclostomata, parasites and feeders on carcasses. Some species are extremely specialized in their feeding habits while others are omnivorous. As described elsewhere (Chapter 3) fishes have specific amino acid, lipid, carbohydrate, vitamin, inorganic ion, and water requirements. A wide variety of structural and physiological adaptations permit fishes to capture, digest and absorb these requirements from their food. Several previous surveys deal with digestion and digestive organs in fishes: Biedermann (1911), anatomy, digestive physiology; Pernkopf and Lehner (1937), anatomy of the intestine; Jacobshagen (1937), anatomy of the stomach; Suyehiro (1942), anatomy, feeding habits; Al-Hussaini (1949a,b), functional anatomy; Bernard (1952), digestion; Barrington (1957, 1962), digestion; Bertin (1958), anatomy; Creac'h (1963), proteolytic enzymes; Smit (1968), gastric digestion in lower vertebrates; Barnard (1973), comparative biochemistry; Prosser (1973), comparative physiology; Kapoor *et al.* (1975a,b), digestion, gustatory system.

Our aim in this article is to review the current information on the structure and physiology of the fish alimentary canal but particularly to stress the mechanisms controlling the movement and digestion of food. The rate at which fish digest their food is of primary importance in determining the rates of feeding and growth (see Chapters 3 and 11).

II. FEEDING MECHANISMS

Feeding mechanisms in the jawless Cyclostomata are different from those of other vertebrates. The round suctorial mouth of the adult *Petromyzon* and *Lampetra* is armed with horny teeth. Anticoagulant secretions allow tissue fluids and blood to be ingested and passed directly to the intestine, since cyclostomes possess no stomach. Juvenile (ammocoete) lampreys survive in freshwater for several years by microphagous feeding. At metamorphosis pouchlike gills are formed inside the gill arches which generate a tidal water flow quite

independent of the mouth to facilitate respiration while the fish is attached to the host. Hagfish, which possess barbels, have horny teeth on the palate and tongue.

In the gnathostomata, the anterior visceral arches have formed jaws which are relatively simple in the Chondrichthyes. Ectodermal folds inside the jaws produce a series of teeth which move upward to replace those that are lost. The teeth may be homodont (*Raja*) or heterodont (*Heterodontus*) in relation to the diet (Reif, 1976).

In the osteichthyes, the membrane and cartilage bones which form the jaws have a more complex structure provided with an equally complex arrangement of muscles, nerves, and ligaments. Jaw movements in both groups are associated with respiration, biting, scraping, chewing, and rejection of particles. Ballentijn *et al.* (1972) gave a detailed description of the architecture of the jaws of the carp, showing how the movements of the premaxilla and maxilla allow flexible protrusor movements of the mouth to change its shape and position under the influence of the adductor mandibulae 1 alpha and beta, 2 and 3. The mouth can be turned ventrally for feeding and ejection of particles but can be closed without compressing the buccal cavity when full of food. Similar analyses of jaw movements are given in Alexander (1970) and Osse (1969). Keast and Webb (1966) made a detailed comparison of the mouth and body structure of fifteen species of teleosts of one Canadian lake; Hatanaka *et al.* (1954) made a similar study of flatfish, while Hobson (1974) undertook a survey of the feeding relationships among more than 100 species of marine teleosts of the Coral Reefs at Hawaii. The latter author concluded that, in this marine community, the carnivorous habit is central to teleostean evolution. The relatively unspecialized carnivores have limited prey species which are vulnerable to attack. These are mainly nocturnal in activity as are the fish predators. After the final development of modern lithothamnion–scleractinian coral reefs some 50 million years ago, an explosive radiation of acanthopterygian teleosts occurred allowing them to become diurnal carnivores and planktivores, coral eaters, benthos foragers on large echinoids and mollusks or cleaner fish. In contrast to freshwater evolution, herbivorous species probably appeared relatively late. Recognizable adaptations to the new feeding niches are the following.

1. New positions of the paired fins for increased maneuverability
2. Reorganization of the premaxilla/maxilla for greater flexibility and protrusibility

3. Changes in jaw and snout shape such as elongation for snipping off coral polyps (*Chaetodon*) or other sessile invertebrates (*Forcipeger*) leading to the evolution of cleaner fishes (*Labroides phthiriphagus*)
4. Changes in tooth shape such as the delicate incisors of *Chaetodon*, the crushing teeth of blennies, or even the fused plates of the tetraodontiformes
5. Development of accessory structures such as the barbels of *Mullus* to detect prey buried in the substratum
6. Behavioral changes allowing *Coris* to roll over stones to detect small prey or *Sufflamen* to expose buried prey with water jets from fins or gills.

These are only a few examples of adaptations of feeding mechanisms. Lips may be present (*Catostomus, Mugil*) or completely absent (*Sparus*). Accessory external organs bearing taste buds and used for detection and location of food are found as barbels on the snout or lower jaw (Cyprinidae, Siluridae, Gadidae, Mullidae, and others) or as sensory fin areas (Gadidae, Triglidae) (Kapoor *et al.*, 1975a). Teeth may be found not only on the jaws (premaxilla, maxilla, mandible) but also on the prevomer, vomer, palatine, sphenoid, tongue, and the dorsal and ventral regions of the pharynx. Among the teleosts, a bewildering array of tooth and gill raker adaptations are encountered which allow successful ingestion of the preferred food. The teeth may be absent from the jaws (Cyprinidae), minute (planktivorous clupeids), flat and molariform (*Raja, Brama*), incisiform (*Blennius*), pointed or serrate (*Sphyraena*, sharks) or fused into crushing plates (*Tetraodon*). Gill rakers may form blunt "teeth" (most carnivores) or a filter basket (*Dorosoma, Alosa, Polyodon, Labeo*) (see, e.g., Suyehiro, 1942; Weisel, 1973). A tongue is not always present (*Labeo*). It is rarely freely movable, yet in *Dorosoma* it is protrusible and in *Plecoglossus* it forms flaps producing mucus to entrap algal particles scraped off by the comblike teeth. In the pharynx, dorsal and ventral pharyngeal pads may be developed to crush the food, or to compress the algal or detrital ingesta before swallowing (Cyprinidae, Catostomidae, Cobitidae). Pharyngeal and epibranchial organs, with a lumen entering the esophagus, are believed to consolidate food particles and are found in several genera among the Osteoglossiformes, Cypriniformes, Gonorhychiformes, and Clupeiformes (Nelson, 1967). There are no multicellular salivary glands in fish, but solitary mucus-producing gland cells (goblet cells) lubricate the food to facilitate swallowing.

III. ANATOMY AND HISTOLOGY OF THE ALIMENTARY CANAL

The major divisions of the vertebrate alimentary canal are mouth, buccal cavity, pharynx, esophagus, stomach, intestine, rectum, and related organs. In some fishes the digestive canal constitutes a straight tube from the mouth to the anus. More often, however, the canal makes loops and is structurally divided into functionally different parts. Thus one can usually distinguish esophagus, stomach, and intestine and often subdivisions of these. Valves or sphincters often separate different parts of the digestive canal. The principal layers of the gut wall are the mucosa (inner epithelium and adjacent tissues), submucosa, muscularis (usually double layered), and serosa. Associated with the canal are two glands, the liver and the pancreas, which deliver their secretions into the intestinal lumen through special ducts.

It is generally agreed that the structure of the regions of the alimentary canal in a given species is related to its diet but that modifications are superimposed on the basic gut plan of the group to which the species belongs. An example of this is given by Weisel (1962) who examined the cyprinid *Ptychocheilus oregonense*, which preys on young salmon, but which has inherited the toothless and stomachless condition from ancestors assumed to be catostomid suctorial feeders on fine particles.

A number of comprehensive articles have appeared in which the morphology, histology, and cytology of the fish alimentary canal have been described. There are many studies in addition to the reviews mentioned in the Introduction (Ishida, 1935; Kirtisinghe, 1940; Girgis, 1952; Burnstock, 1959a,b; Weisel, 1962; Mohsin, 1962; Hale, 1965; Bishop and Odense, 1966; Keast and Webb, 1966; Bullock, 1963, 1967; Chaichara and Bullock, 1967; Schmitz and Baker, 1969; Frantsusova, 1971; De Groot, 1971; Bucke, 1971; Vegas-Velez, 1972; Chakrabarte *et al.*, 1973; Kayanja *et al.*, 1975). Tanaka (1973) has investigated the structure and function of the digestive system of teleost larvae.

A. Esophagus

Posteriorly, the pharynx passes into a short, wide, muscular esophagus. In elasmobranchs, the esophageal mucosa is often provided with cone-shaped or branched papillae directed backward. Without marked boundaries the esophagus merges caudally with the

stomach. In many species the submucosa contains voluminous masses of lymphomyeloid tissue ("organ of Leydig"). In teleosts the mucosa is dominated by characteristic large mucous cells (goblet cells) which may give the epithelium a "frothy" appearance in histological sections. The mucosal epithelium is said to be typically stratified (Kapoor *et al.*, 1975b), although Vegas-Velez (1972) found it to be simple in the species he examined. The mucosa, including the basement membrane, and the stratum compactum are usually thrown into folds which allow distension during swallowing. The muscular coat is typically of striated muscle. If a circular layer is present (*Gadus, Labeo*) it lies outside the longitudinal coat; *Gasterosteus* has only the circular coat. The muscles are innervated by the Xth (vagus) nerve. Glands similar to gastric glands have been observed in the caudal esophagus of some species such as *Mugil capito* (Ghazzawi, 1935), *Cottus gobio* and *(Par)enophrys bubalis* (Western, 1969), and *Dorosoma cepedianum* (Schmitz and Baker, 1969). In a variety of fishes examined by Isokawa *et al.* (1965) and Khanna and Mehrotra (1970), esophageal sacs are reported with (*Ariomma, Pampus*) or without (*Tetragonus, Iticus*) teeth. The esophagus may terminate in a cardiac sphincter or valve (*Labeo*) although such demarcation is not invariable (Odense and Bishop, 1966; Schmitz and Baker, 1969). In stomachless fishes the esophagus enters the intestine directly.

B. Stomach

Within the gnathostomata, the stomach is claimed to be a concomitant development with the jaws to receive and store newly ingested food, which may be of large size, and to initiate digestion with pepsin in an acid medium. In elasmobranchs, the stomach is present as a J-shaped organ consisting of a descending *pars cardiaca* and an ascending *pars pylorica* (Fig. 1). The inner epithelium of the mucosa is simple and consists of cylindrical cells which stain with the periodic acid Schiff (PAS) reagent and which probably secrete mucin. Tubular multicellular glands running perpendicular to the luminal border open into mucosal foveolae. The gland cells are of one type only containing acidophilic granules. They may be designated oxyntic cells (Hogben, 1967a,b). A muscularis mucosa is present in the cardiac region only. The muscularis (externa) consists of an inner circular and an outer longitudinal layer of smooth muscles. The circular muscles are strongly developed in the pyloric region to form the pyloric sphincter (Petersen, 1908–1909; Oppel, 1896–1900). In some elasmobranchs, a chamberlike enlargement, the *bursa entiana*, is formed in the pyloric

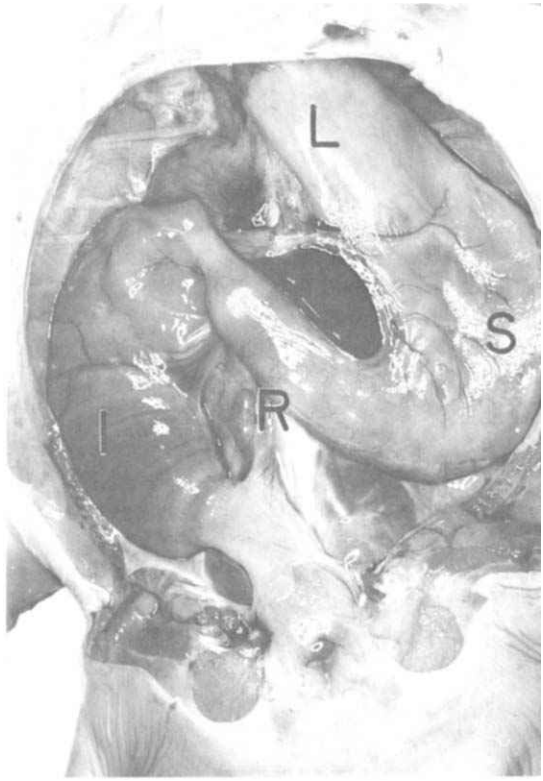

Fig. 1. *Raja radiata.* Abdominal viscera after removal of the liver. S, stomach; I, spiral intestine; L, esophagus with the organ of Leydig; R, rectal gland.

part of the stomach. Doughnut-shaped sphincters are found around veins in the gastric wall of rays (*Raja*) (Weinland, 1901), but similar sphincters occur also elsewhere in the body of rays.

In the osteichthyes the stomach is usually well developed, although in some forms it is reduced or absent (e.g., Cyprinidae, Cyprinodontidae: Rauther, 1940). Tanaka (1969) reported that the stomach is absent from larvae of young fish such as *Cyprinus* and *Salmo,* so the stomach develops (if at all) late in the ontogeny. The loss of stomach may therefore be neotenous and is frequently associated with microphagous habits. The stomachless fishes are stated to have no gastric glands and no pyloric sphincter. However, stomachless predators (labrids, *Ptychocheilus, Scomberesox*) as well as planktivores (*Tilapia, Syngnathus*) prove exceptions to the above generalization. Bertin

(1958) and Vegas-Velez (1972) point out that earlier reviews, tabulating families all of whose members are "stomachless," may be in error since histological examinations have demonstrated the presence of gastric glands in some species (e.g., *Syngnathus, Mugil*). Gupta (1971) observed cells resembling gastric gland cells in the digestive canal of the stomachless carnivorous fish, *Xenentodon cancila.*

Many of the stomachless fishes are provided with pharyngeal chewing devices which permit the ingested food to reach the intestine in a fragmented condition (Rauther, 1940; Bertin, 1958).

Where present, the stomach of teleosts varies greatly in shape. A straight tubelike stomach is found in Gobiidae, Gasterosteiformes, and Symbranchi (*Pomatoschistus, Gasterosteus, Spinachia, Symbranchus*). In flatfish and esocids (*Limanda, Pleuronectes, Esox*) it is curved, whereas in most fishes the stomach may be shaped like one of the letters U, J, or Y (Suyehiro, 1942). In cottids (*Myoxocephalus, Enophrys*) and *Tilapia* the stomach is saclike while in elopids, clupeids, *Gymnarchus, Ophiocephalus, Anguilla* and many others a gastric cecum extends caudally. This cecum sometimes is very long (*Regalecus, Stomias*, Rauther, 1940).

The teleost stomach typically has a lining of columnar epithelial cells, without a striated border. Goblet cells are scattered through the epithelium. Tubular glands, sometimes in groups, are found in the cardiac and fundic regions. The tubules may be simple or occasionally branched, their presence thickening the depth of the mucosa. They open into foveolae. Mucus-producing neck cells may be distinguished. As in elasmobranchs (and in fact in nonmammalian vertebrates generally) the main part of the gastric glands are made up of one type of cell only. This contains abundant secretory granules, probably pepsinogen (Tan and Teh, 1974), but the cells are believed at the same time to be producers of HCl (Barrington, 1957; Iro, 1967). According to Weinreb and Bilstad (1955), gastric gland cells in the rainbow trout (*Salmo gairdneri*) structurally resemble chief cells in other animals. Changes in the microscopic structure of the gastric glands during secretion were reported by Arcangeli (1908). The ultrastructure of gastric gland cells in teleosts has been studied by Ling and Tan (1975) in the coral fish, *Chelmon rostratus*, and by Noaillac-Depeyre and Gas (1978) in the perch, *Perca fluviatilis*. The gastric gland cells in their apical region contain a compact system of tubules. The tubules show resemblances to tubular structures of oxyntic cells of amphibians and higher vertebrates and to structures in the chloride cells of cyclostomes (lamprey). At their bases the cells contain zymogenlike secretory granules and a rich rough endoplasmic reticulum. The secretory

granules are released apically by a process of exocytosis. The ultrastructural features of the gastric gland cells are consistent with the hypothesis that they are active both in acid production and in the synthesis of pepsinogen.

Supporting the gastric mucosa is a submucosa often containing a stratum compactum and smooth muscle fibers (Rauther, 1940; Burnstock, 1959a). The muscularis consists of an inner circular and an outer longitudinal layer of smooth muscles, but striated esophageal muscles may extend into the cardiac portion of the stomach (*Perca, Centropristes, Zeus, Solea,* Blake, 1930; Rauther, 1940). In the stomiatid *Cyclothone* the stomach wall contains two layers of diagonally-crossing striated muscle fibers (Nusbaum, 1923) and in the stomachless cyprinid *Tinca* a layer of inner circular and outer longitudinal striated muscle fibers surround the "normal" (smooth muscle) muscularis (Kilarski and Bigai, 1971). The inner circular layer of smooth muscles of the fish stomach is usually two to three times thicker than the longitudinal coat, and this is accentuated in fishes provided with a gizzard. The pyloric sphincter consists of thickening of the circular smooth muscle layer. In stomachless fishes the sphincter may be absent or replaced by an esophageo–intestinal valve formed by a fold of the mucosa and submucosa.

The capacity of the stomach in relation to the body weight varies between species and is reflected in the size of the meal that can be taken voluntarily. The flatfish *Limanda* for example has a gastric volume of 8 ml/100 g and can ingest up to 10% of its body weight in a meal. The stomachless *Leuciscus rutilus* can consume 15% of its body weight of chironomid larvae, and *Carassius carassius* 21% of its body weight. Sculpins may ingest 30–50% of their body weight at a single feeding.

Several teleosts possess a gizzardlike enlargement of the *pars pylorica* (*Mugil* spp., *Coregonus, Osphromenus, Chanos, Sardinella, Chatoessus, Citharinus, Mormyrus, Notopterus*). In *Dorosoma* the muscularis of the gizzardlike part of the stomach consists of three strongly developed layers of smooth muscles and has a thick mucous cuticula lining the lumen. Fish with gizzards usually are microphagous, detritivores, or herbivores. *Mugil cephalus* ingests microalgae and plant detritus together with mineral particles which act as a grinding paste (Rauther, 1940; Schmitz and Baker, 1969; Odum, 1970).

On of us (D.G.) has observed that when two species of small shore fish (*Blennius pholis* and *Ciliata mustela*) are offered barnacles (*Balanus balanoides*) as food, the fish ingest them readily although only the blenny takes this species in the wild. The blenny, however,

has no stomach and as the meal is digested the barnacle's calcareous plates are readily transferred to the intestine and defecated within 24 hr at 18°C. *C. mustela*, on the other hand, retains the plates long after the organic part of the meal is evacuated from the stomach and cannot transfer them to the intestine. Clearly the retention of the pyloric sphincter can limit the utilization of available food species.

C. Intestine

In elasmobranchs and holocephalans, the intestinal wall consists of the usual layers of mucosa, submucosa, muscularis, and serosa. In development, independent twisting of the mucosa and supporting tissues leads to the formation of a spiral valve which increases intestinal surface area (Fig. 1). The number of turns in the spiral may be as low as two or three (*Chimaera monstrosa*) or as many as fifty (*Cetorhinus maximus*) and reflects the diet of the species (Rauther, 1940; Bertin, 1958). Spiral valves are also found in the intestine of dipnoi, polypterids, holosteans, acipenseroids, and in the coelacanth, *Latimeria chalumnae*.

In teleosts the intestine may be short and straight or thrown into folds or loops. Its length varies from one-fifth to twenty times the body length and it is longest in microphagous and herbivorous fish (Bryan, 1975). This trend is supported by tabulated data on characinoid and cyprinoid species (Kapoor *et al.*, 1975b). de Groot (1971) reviewed the morphological variations in flatfish guts in relation to diet for 133 species and, in more detailed analysis of 31 species, found that the relative length is greatest in Soleidae (which ingest smaller polychaetes, mollusks, and crustaceans) and least in Psettodidae and Bothidae (which eat fish and larger invertebrates). A similar trend was described by Hatanaka *et al.* (1954). The surface area of the intestine in carnivorous teleosts is usually increased by folds of the mucosa but the intestine length is less than the body length. Al-Hussaini (1947) adopted the term "mucosal coefficient" to describe the relative surface area of the intestine in fishes to allow both for intestinal length and for mucosal foldings. Lange (1962) found that gut length increases with age in species of *Rutilus* as they graduate from yolk, through zooplankton to a diet of larger invertebrates. Angelescu and Gneri (1949) found that starvation of *Prochilodus* leads to intestinal shortening by 30–45%.

Many teleosts have blind tubes connected with the anterior end of the intestine. These intestinal ceca (often termed pyloric ceca) vary in number between 1 and more than 1000 (Suyehiro, 1942) (Figs. 2, 3). They may be relatively free and short (Pleuronectidae) or bound to-

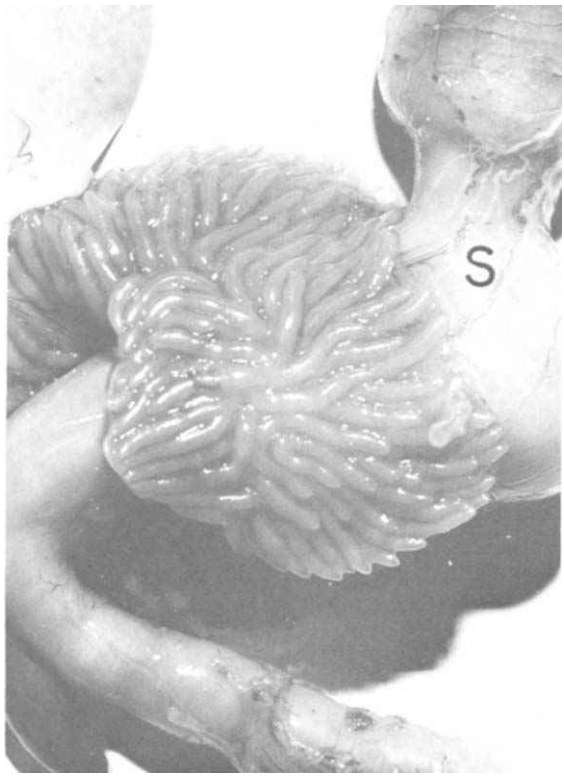

Fig. 2. *Cyclopterus lumpus.* Pyloric part of the digestive system in a freshly dissected specimen. In the middle of the figure are numerous intestinal (pyloric) ceca. A powerful ring-shaped muscle contraction is observed in the stomach (S).

gether to form a compact, glandlike mass (Thunnidae, Xiphidae). Very well developed ceca are found in certain malacopterygians (clupeids, salmonids), gadids, and coryphaenoids. Their presence is not related to the relative gut length nor to the general feeding niche of the species, yet within a chosen group of fishes (e.g., Heterosomata), it seems possible to relate the number of ceca to the diet (de Groot, 1971).

The intestinal ceca closely resemble the intestine in structure, with a well-developed muscularis consisting mainly of circular muscle fibers. The inner epithelium contains goblet cells, but light and electron microscopic studies indicate that they lack cells that secrete digestive enzymes (Jansson and Olsson, 1960; Luppa, 1966; Vegas-Velez, 1972). Between the ceca highly basophilic pancreatic exocrine gland cells may occur in the connective tissue. The function of the ceca is not clear. Since they originate from that region of the intestine

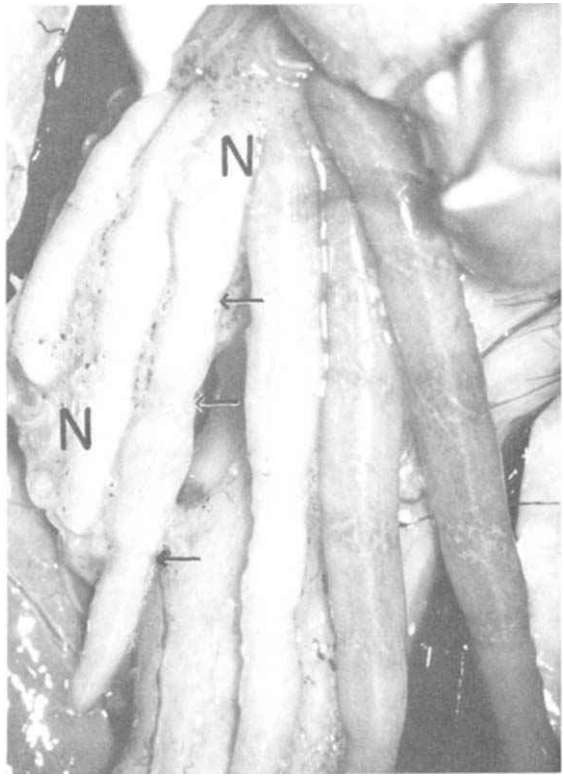

Fig. 3. *Micromesistius poutassou.* Intestinal (pyloric) ceca in a freshly dissected preparation. The ceca to the right in the figure are dilated with semitransparent walls. The ceca to the left show constrictions (arrows) due to contraction of circular smooth muscles. (N, parasitic nematodes in the connective tissue surrounding the ceca).

where bile and pancreatic juices are released, the ceca may form digestive compartments active in resorption of certain nutrients. It has been supposed that they are of especial importance for the resorption of fat and waxes (Greene, 1914; Janson and Olsson, 1960; Benson and Lee, 1975). The ceca often harbor remarkably large numbers of parasites. Thus in the ceca of *Coregonus lavaretus* Reichenbach-Klinke and Reichenbach-Klinke (1970) observed up to 300 individual cestodes.

Stomachless fishes have no intestinal ceca. Other fishes without ceca include *Amia, Chirocentrus, Symbranchus, Anguilla, Esox, Gasterosteus, Agriopus, Megalops, Batrachus,* silurids, and loricarids (Jacobshagen, 1915; Pernkopf and Lehner, 1937).

The mucosa of the intestine is lined by a simple columnar epithelium which possesses the brush border of microvilli (apical plate) typical of absorptive tissues (Odense and Bishop, 1966; Yamamoto, 1966; Krementz and Chapman, 1975). Cartier and Buclon (1973) estimated the number of microvilli per mm^2 as 34×10^6 in cyprinids. Mucus-secreting goblet cells occur scattered among the epithelial cells and show a positive PAS reaction (Gammon *et al.*, 1972). Multicellular glands are not usually found. A stratum compactum is frequently present, but the teleostean intestine seems to lack a muscularis mucosa. The submucosa is very thin and contains scattered collagen and elastic fibers, blood vessels, and nerves. The muscularis consists of inner circular and outer longitudinal smooth muscles. In cyprinids, cobitids, and *Syngnathus,* striped muscles may be found in the muscularis (Rauther, 1940). In the tench (*Tinca tinca*) the striated muscle layer extends over the whole intestine (Ohnesorge and Rauch, 1968; Kilarski and Bigai, 1971).

Ciliated epithelium has been reported in the intestine of a few teleosts (*Syngnathus*) and some primitive fishes (*Polypterus senegalis, Polyodon spathula*) (Ishida, 1935; Bucke, 1971; Weisel, 1973; Magid, 1975) although it may be difficult to differentiate cilia from long microvilli on the basis of structure alone. Observations should always be made on living epithelial cells (Odense and Bishop, 1966). Where present, cilia are probably a primitive character since they are found in *Amphioxus* and in ammocoete larvae. In the cyclostome *Myxine glutinosa* the intestinal epithelium produces a peritrophic membrane (Adam, 1960).

Rectum. The terminal part of the intestine is frequently differentiated as a wider "rectum," often demarcated from the intestine by an ileo–rectal valve largely formed by smooth muscles (*Gadus, Gambusia*), but in many species no valve is present (Mohsin, 1962). The mucosa is typically richly endowed with goblet cells. The mucosal epithelium may be provided with microvilli indicating strong absorptive properties (Bullock, 1967). In elasmobranchs the epithelium may be stratified. The rectal gland of elasmobranchs (Fig. 1) is now believed to secrete Na and Cl ions into the lumen as part of the osmoregulatory mechanisms. The muscularis of the rectum is similar to that of the intestine, but striated muscles occur near the anus.

D. Liver and Gallbladder

The liver lies anteriorly in the body cavity and receives blood from the hepatic artery and from one or more portal veins which drain the gastric and intestinal mucosa, the swimbladder (gas gland), the spleen,

and the pancreas. Embryologically, the liver develops as an epithelial outgrowth of the digestive tube, and in its simplest form is a branched tubular gland as in the cyclostome *Myxine glutinosa*. In some teleosts the liver is claimed to have a tubular structure (*Anguilla anguilla, Muraena, Pleuronectes*). However, in most vertebrates, including fishes, the liver is a complicated structure consisting of anastomosing epithelial lamellae separating blood sinusoids (Elias and Sherrick, 1969). This "muralium" has been found in fishes as diverse as lampreys (Bengelsdorf and Elias, 1950), *Salmo gairdnerii* (Scarpelli *et al.*, 1963), *Poecilia* (Hale, 1965), *Ictalurus* (Hinton and Pool, 1976), and *Micropterus* (Hinton *et al.*, 1972). As in other vertebrates, hepatocytes contain numerous mitochondria, rough endoplasmic reticulum (microsomes), Golgi apparatus, lysosomes, peroxisomes, lipid and glycogen deposits. Intracellular bile canaliculi are present, with the single exception to date of *Ictalurus* (Hinton and Pool, 1976), which link up with intercellular canaliculi. The lining epithelial cells of the canaliculi possess numerous microvilli (David, 1961; Yamamoto, 1965a,b). According to Welsch and Storch (1973) the teleostean liver contains two categories of hepatocytes, lipid-rich and glycogen-rich. In some species (*Tetraodon, Limanda*) lipid-rich cells predominate while in others (*Chromis, Corydoras, Amphiprion*) glycogen-rich cells are more common. Hinton and Pool (1976) concluded that, in *Ictalurus*, large protruding cells, which differ from hepatocytes lining the sinusoids, are Kupffer cells of the reticulo-endothelial system. Lipids may be present in large quantities in some fish livers. In elasmobranchs, squalene and other hydrocarbons accumulate in the liver, helping the fish to gain buoyancy (Corner *et al.*, 1969).

Due to the presence of a large fat vacuole the hepatocytes of elasmobranchs are larger than those of teleosts. In most fishes the exocrine secretion of the liver, the bile, is stored in a gallbladder. The bile duct opens into the anterior intestine or into the intestinal ceca (Western, 1969). The wall of the gallbladder consists of columnar or cuboidal epithelium, a thin submucosa and a muscularis of smooth muscle cells. The similarly constructed *ductus choledochus* is associated with the pancreatic duct in many fish, and its entrance to the intestine is guarded by the smooth muscle sphincter Oddi.

E. Pancreas

In the cyclostome *Myxine glutinosa* (hagfish) the pancreas consists of exocrine gland cells, containing zymogen granules, in the intestinal mucosa. The homologue of the endocrine pancreatic tissue of higher

vertebrates is concentrated around the bile duct. In elasmobranchs and holocephalans, the exocrine pancreas forms a discrete organ with a duct emptying into the anterior intestine (duodenum). The microscopic structure may be much like that of the mammalian pancreas [e.g., in *Chimaera monstrosa* (Fujita, 1962)] and numerous scattered islets of endocrine cells are present. In elasmobranchs the spleen and the pancreas are often closely associated. This is also the case in *Protopterus,* the African lungfish, in which the pancreas is a large black-pigmented organ at the posterior end of the spleen and, like the latter, embedded in the gut wall. In teleosts the exocrine pancreas is usually diffuse. It consists of ramified tubules or acini scattered in the connective tissue of the intestinal surface, the mesenteries, between the intestinal ceca or within the liver or spleen (Nagase, 1964; Bishop and Odense, 1966; Western, 1969; Gammon *et al.,* 1972; Hinton and Pool, 1976). In the mesenteries the pancreas forms sheaths around blood vessels. Several small pancreatic ducts open into the intestine or intestinal ceca, although in some cases a single duct may join the bile duct. In cyprinids (*Cyprinus carpio*), labrids (*Crenilabrus melops*), and certain other forms, strands of pancreatic tissue are found within the portal canals of the liver, the pancreatic ducts probably opening into the bile ducts. The combined liver and pancreatic tissue has been termed "hepatopancreas" but must not be confused with so-called hepatopancreas of invertebrates (Ito *et al.,* 1962; Elias and Sherrick, 1969). The exocrine cells of the teleostean pancreas are strongly basophilic and contain zymogen granules. In a minority of teleosts the pancreas forms a distinct organ which is embedded in fat and connective tissue. The compact pancreas accompanies portal vessels on the surface of the intestine (*Esox, Silurus, Parasilurus, Anguilla, Conger, Acerina, Pleuronectes,* Oppel, 1896–1900; Hill, 1926; Chesley, 1934; Suyehiro, 1942; Kukla, 1954, 1958). The secretory cells form acini with narrow lumina, and a common pancreatic duct opens into the anterior intestine. Blood reaches the pancreas from three arteries and drains into the v. portae (Kukla, 1958). For a discussion of the endocrine elements associated with the pancreas, see Section III,F.

F. Endocrine Cells and Hormones

The vertebrate alimentary canal and certain other organs contain large numbers of granulated endocrine cells. Two lines of these cells have been termed enterochromaffin (argentaffin) and enterochromaffinlike (argyrophil) cells. When supplied with amine precursors these cells produce and store amines. For that reason they have

been termed APUD cells [abbreviation for Amine Precursor Uptake and Decarboxylation (Pearse, 1977)]. Another term is gastro–entero–pancreatic (GEP) cells (Solcia *et al.*, 1978). A classification on ultrastructural grounds of mammalian GEP cells has been suggested (Solcia *et al.*, 1978). Several investigators have demonstrated GEP cells in nonmammalian vertebrates such as cyclostomes and true fishes (Uggeri, 1938; Read and Burnstock, 1968; Bucke, 1971; Gabe and Matoja, 1971, 1972; Ling and Tan, 1975; Tue, 1975; Östberg, 1976; Falkmer *et al.*, 1978). These endocrine elements are also found in protochordates (amphioxus: Van Noorden and Pearse, 1976).

The GEP cells are assumed to synthesize low molecular weight polypeptides which act as hormones. The various polypeptides show structural resemblances to each other and the hypothesis has been forwarded that different polypeptide hormones (glucagon, secretin, gastrin) evolved together with insulin from an ancestral proinsulinlike molecule (Track, 1973). Several gastrointestinal polypeptide hormones occur in fish (Table I). Nilsson (1970, 1973) obtained evidence for the presence of secretin- and cholecystokininlike substances in the

Table I

Hormones and Hormonelike Substances in Fish Gut and Pancreas

Substance	Tissue	Species	Reference
Insulin	Islets	All	
Gastrin	Islets	*Rhinobatus productus*	Hansen (1975)
Secretin	Intestine	*Myxine glutinosa*	Nilsson (1970, 1973)
Secretin	Intestine	Salmon, dogfish, ray	Bayliss and Starling (1903)
Secretin	Intestine	*Esox, Gadus*	Dockray (1975)
Cholecystokinin (CCK)	Intestine	*Myxine glutinosa*	Nilsson (1973)
CCK	Intestine	*Chimaera monstrosa*	Nilsson (1970)
Cholecystokininlike substance	Intestine	*Lampetra, Petromyzon*	Barrington (1972)
Cholecystokininlike substance	Intestine	*Esox, Anguilla*	Barrington and Dockray (1972)
Ceruleinlike substance	Stomach	*Gadus morhua*	Larsson and Rehfeld (1978)
Substance P	Intestine	*Gadus morhua, Squalus acanthias*	Dahlstedt *et al.* (1959)
Histamine	Stomach	*Salmo gairdnerii, Gadus morhua*	Reite (1969)
Histamine	Stomach	*Esox lucius*	Lorentz *et al.* (1973)
Histamine	Intestine	*Myxine glutinosa*	Reite (1965)

intestine of the hagfish (*Myxine glutinosa*) and the holocephalan *Chimaera monstrosa*. Gabe and Matoja (1972) demonstrated cells structurally resembling mammalian gastrin cells in the stomach mucosa of the teleost, *Mugil auratus*. Östberg *et al.* (1976) showed, in immunofluorescence studies, that the intestinal mucosa of cyclostomes contains cells that react with antisera against mammalian glucagon and gastrin. Larsson and Rehfeld (1978) found that the gastric mucosa of the cod (*Gadus morhua*) contains a rich population of cells demonstrable with antisera against mammalian gastrin and cholecystokinin (CCK), but the immunogenic reactivity was due to a ceruleinlike polypeptide. The authors concluded that, in teleosts, ceruleinlike molecules possibly function as gastrin.

The endocrine pancreas belongs to the GEP system. Islets of endocrine cells are found in the compact pancreas of the eel (*Anguilla anguilla:* Kukla, 1958) and the pike (*Esox lucius:* Bucke, 1971). In teleosts with a diffuse pancreas (*Gadus morhua, Lophius piscatorius, Myoxocephalus scorpius*) aggregations of endocrine cells, separate from exocrine pancreas tissue, form the Brockmann bodies. Within the teleostean islet tissue Falkmer and Olsson (1962) and Bishop and Odense (1966) detected inner B cells (insulin secreting) and more peripheral A cells (glucagon secreting) together with other cell types. According to Brinn (1973) the islets of some fish species contain, in addition to A and B cells, D cells (somatostatin secreting) and a fourth argyrophilic type of granulated cell.

The pancreatic islets are innervated by autonomic nerves, and nervous influence may be a factor in the regulation of endocrine pancreatic secretion. The polypeptide hormones produced by the pancreatic islets are regulators of nutrient homeostasis. The rates at which glucose, amino acids, and free fatty acids enter and leave the extracellular space are influenced by insulin, glucagon, and probably somatostatin (Unger *et al.*, 1978). However, most studies have been done on mammals and very little is known about fishes in these respects.

The peptide-producing endocrine cells of the vertebrate digestive system have been investigated by various methods: immunohistochemistry, silver staining, lead–hematoxylin staining, formaldehyde-induced fluorescence, radioactive labeling, and enzyme studies (Dawson, 1970).

Besides gastrointestinal polypeptides, other hormones or hormonelike substances occur in the digestive system of fishes, for example, histamine. Holstein (1975a) found diamine oxidase, a histamine-deaminating enzyme, in the intestine of teleosts. GEP cells are thought to be the source of the intrinsic factor in the mammalian

stomach. It is not known if this factor, needed for the intestinal absorption of iron, occurs in fishes.

G. Blood and Lymph Vessels

In the cyclostome, *Myxine glutinosa,* the intestine is supplied by segmental arteries and the venous drainage goes to the hepatic portal system. In elasmobranchs (sharks, rays) and dipnoans (lungfish) the intestine receives three or four arteries, whereas in teleosts one single celiaco–mesenteric artery supplies the digestive canal. In teleosts the arterial branches to the intestine are often closely followed by veins (Grodzinski, 1938), an arrangement reminiscent of the rete mirabile in the fish swimbladder. Well developed retia mirabilia were further described in the portal, hepatic, intestinal and splenic veins of tuna fish and the elasmobranch, *Alopias vulpes,* by Müller as early as 1840.

Lymphatic vessels and sinuses are found in the digestive system of both cyclostomes and true fishes. In the hagfish, *Myxine glutinosa,* a superficial lymphatic plexus is found in the intestine (Fänge, 1973). In some teleost species the intestine has a system of chyle vessels similar to those of mammals. The chyle vessels converge into a large vessel, the *vas lymphaticum intestinale.* In the marine catfish (*Anarrhichas lupus*) this intestinal lymph vessel is strongly developed and situated to the right of the esophagus (Glaser, 1933). It is not known whether intestinal lymph vessels in fishes are of importance for the transport of absorbed fat from the intestine.

H. Lymphoid Tissue

In most vertebrates lymphoid or hematopoietic tissue is associated with the digestive canal. Thus in the hagfish, *Myxine glutinosa,* the intestinal submucosa contains an extensive granulocyte-producing tissue, assumed to constitute a primitive spleen (Tomonaga *et al.,* 1973). Granulocyte-producing tissue is further found in the submucosa of the esophagus of elasmobranchs (organ of Leydig, Petersen, 1908–1909; Fänge, 1968). Lymphocytes and granulocytes have been observed to invade certain layers of the intestine of fishes, such as membrana propria and epithelium. This infiltration of the gut by leukocytes probably plays a role in a defense system against microbes and parasites. In elasmobranchs the granulocyte-producing tissue of the esophagus is rich in lysozyme (bacteriolytic enzyme) and chitinolytic enzymes. The latter perhaps give some protection against chitin-containing

parasites (Fänge *et al.*, 1978). Whether leukocytes of the gut are of any direct importance for the digestion is not known.

IV. DIGESTIVE FLUIDS AND ENZYMES

A. Gastric Secretion

Production of an acid gastric fluid probably occurs in most fishes (e.g., Norris *et al.*, 1973) except those which have no stomach (myxinoids, chimaeroids, many teleosts). In these neither HCl nor pepsin is formed in the gut.

1. Hydrochloric Acid

Large amounts of gastric fluid, usually distinctly acid, are found in elasmobranchs. Weinland (1901) used a glass cannula to obtain 50 ml gastric fluid from a nonfed *Scyllium*, and Yung (1899) took 500 ml from the large shark, *Lamna cornubica*. In the gastric fluid of *Scyllium stellare* pH 1.69 was measured (van Herwerden and Ringer, 1911), but the acidity of the fluid varies. Maximal acidity is observed a few hours after food intake, whereas in the absence of food the gastric fluid may be weakly acid or neutral (Bernard, 1952). Weinland (1901) found both acid and alkaline secretion in the ray, *Raja asterias*. Babkin *et al.* (1935a,b) obtained small quantities of gastric fluid with a pH of 3–3.8 from nonfed *Raja* sp.

Acid gastric fluid has regularly been found in teleosts (e.g., Western and Jennings, 1970). pH values of 3.0–5.0 were measured in the stomach of *Micropterus salmoides*, *Perca fluviatilis*, and *Tilapia mossambica* (Sarbahi, 1951; Fish, 1960). The acidity increases after food intake (MacKay, 1929). Western (1971) noted that in *Cottus scorpio* and *Enophrys* (*Cottus*) *bubalis* the gastric content was neutral in the absence of food, but 30 hr after ingestion of food a pH of 2.0 was measured. A pH of 2.0 is reached in the stomach of *Tilapia* a few hours after daily feeding begins (Moriarty, 1973).

2. Enzymes

The fact that optimal proteolytic activity has been reported at different pH's (2.0, 3.0, 5.0, 8.5) (Creac'h, 1963) indicates that the gastric fluid of fishes contains several types of protease. Pepsin undoubtedly is the major acid protease. It is secreted by the gastric gland cells as a

zymogen called pepsinogen, which is inactive. Conversion of pepsinogen into active pepsin is brought about by pepsin in an acid environment. During the activation process amino acids are split off from the NH_2-terminal end of the molecule as a mixture of peptides (Lehninger, 1971). Pepsin is an endopeptidase which cleaves peptide linkages formed by amino groups of aromatic and acidic amino acids. It attacks most proteins but not mucins, spongin, conchiolin, keratin, or low molecular weight peptides (Sumner and Somers, 1947).

Merret *et al.* (1969) isolated four different pepsinogens from the gastric mucosa of a dogfish (*Mustelus canis*). Crystalline pepsin has been prepared from the gastric mucosa of teleosts, the Pacific King salmon (*Oncorhynchus tschawitscha*) (Norris and Elam, 1940) and the halibut (*Hippoglossus hippoglossus*) (Eriksen, 1945). In these investigations the pepsinogen was extracted by alkaline solutions and then transformed into pepsin by the addition of acid. Extraction with an acid solution was used by Norris and Mathies (1953) in the isolation of pepsin from the tuna fish (*Thynnus albacores*). The purified tuna fish pepsin differed crystallographically and in its amino acid composition from porcine pepsin.

A few nonproteolytic digestive enzymes may occur in the gastric fluid of fishes. Amylase has been found in the stomach of some fishes (*Clupea harengus*, Battle, 1935; *Dorosoma cepedianum*, Bodola, 1966). Lipase is reported from the stomach of *Tilapia* sp. (Al-Hussaini and Kholy, 1953; Nagase, 1964) and *Dorosoma cepedianum* (Bodola, 1966), and esterases with pH optima at 5.3 and 8.0 in the stomach of the rainbow trout (*Salmo gairdnerii*, Kitamikado and Tachino, 1960). Chitinase has been demonstrated in the gastric mucosa of elasmobranchs, insect-feeding teleosts, and the dipnoan *Polypterus* (Okutani, 1966; Micha *et al.*, 1973). Remarkably strong chitinolytic activity was found in the gastric mucosa of the marine deep water teleost, *Coryphaenoides rupestris*, and the elasmobranchs, *Etmopterus spinax* and *Raja radiata* (Fänge *et al.*, 1978). These three species largely feed on crustaceans and other invertebrates with a chitinous integument.

Hyaluronidase, a mucopolysaccharidase which cleaves beta-*N*-acetyl-glucosaminidic bonds of hyaluronic acid, has been prepared from the gastric mucosa of the Japanese mackerel, *Scomber japonicus* (Yamamoto and Kitamikado, 1971).

Among 148 fish species, a few estuarine fishes and one freshwater fish (*Ictalurus punctatus*) but no offshore marine species were found to contain cellulase in their stomach or anterior intestine. Because no cellulase activity was detected in fishes exposed to streptomycin, it

was concluded that the cellulase probably was produced by microorganisms of the gut content (Stickney and Shumway, 1974).

B. Pancreatic Secretion

Due to difficulties in collecting pure pancreatic juice in most fishes the chemical composition of this fluid in representative fish species is not known. Undoubtedly it is rich in enzymes (mostly as zymogens) which serve in digestion of proteins, carbohydrates, fat, and nucleotides. Probably, as in higher vertebrates, the pancreatic juice contains bicarbonates that neutralize hydrochloric acid entering the intestine. Extracts containing pancreatic enzymes have been obtained from the isolated compact pancreas of elasmobranchs and holocephalans, from tissues containing scattered pancreatic acini (intestinal ceca, mesenteries, the "hepatopancreas" of certain fishes), and from the intestinal content.

1. PROTEASES

Trypsin, chymotrypsin, carboxypeptidase, and elastase are stored in the pancreatic cells as inactive zymogen (proenzyme) granules. When arriving in the intestinal lumen, trypsinogen is transformed into trypsin by proteases produced by intestinal mucosal cells (enterokinases). Other pancreatic zymogens are activated by trypsin.

Trypsin is formed by removal of a hexapeptide from the trypsinogen molecule as a result of the hydrolysis of a lysine–isoleucine bond. Trypsin is an endopeptidase with optimal action at a pH of about 7. It cleaves peptide linkages whose carbonyl groups come from arginine or lysine (Lehninger, 1971). Appropriate substrates for estimation of trypsin activity are synthetic peptides such as benzoyl-L-arginine ethyl ester (BAEE) and *p*-toluenesulfonyl-L-arginine methyl ester (TAME). Trypsinogen, or trypsin, has been demonstrated in the intestinal wall of the cyclostome *Myxine glutinosa* (Nilsson and Fänge, 1970), the pancreas of the holocephalan *Chimaera monstrosa* (A. Nilsson and Fänge, 1969), elasmobranchs (*Ginglymostoma cirratum*, *Squalus suckleyi*, Zendzian and Barnard, 1967), and the African lungfish (*Protopterus aethiopicus*, Reeck *et al.*, 1970; Reeck and Neurath, 1972), and from intestinal ceca of various teleosts (Creac'h, 1963; *Oncorhynchus*, Croston, 1960, 1965; *Gadus morhua*, Overnell, 1973; *Dicentrarchus* (*Morone*) *labrax*, Alliot *et al.*, 1974). The lungfish (*Protopterus aethiopicus*) pancreas also contains a trypsin inhibitor (Reeck *et al.*, 1970). Jany (1976) studied digestive endopeptidases in the

stomachless teleost *Carassius auratus gibelio*. Trypsin and chymotrypsin of pancreatic origin were found but no pepsin, no elastase, and no collagenase.

Chymotrypsin is formed by the action of trypsin on chymotrypsinogen. It is an endopeptidase which attacks peptide bonds with carbonyl from aromatic side chains (tyrosine, tryptophan, phenylalanine). Benzoyl-L-tyrosine-ethylester (BTEE) is an example of a synthetic peptide useful as a substrate in assays of chymotrypsin activity. Chymotrypsin has been found in most fishes in which trypsin has been found.

Elastase is formed when the zymogen, proelastase, is activated by trypsin. This enzyme is especially active on peptide bonds in the protein elastin and may be assayed on purified elastin or specific ester substrates (De Haën and Gertler, 1974). Elastase probably does not occur in the cyclostome *Myxine glutinosa* (A. Nilsson and Fänge, 1970) but has been found in the pancreas of the holocephalan *Chimaera monstrosa* (A. Nilsson and Fänge, 1969), the elasmobranch *Dasyatis americana*, and the teleosts, tuna (*Thynnus secundodorsalis*) (Zendzian and Barnard, 1967) and the angler, *Lophius piscatorius* (Lansing *et al.*, 1953). A new type of pancreatic elastase, proelastase A, has been reported to occur in a dipnoan, the African lungfish (Walsh, 1970).

Carboxypeptidases are exopeptidases which hydrolyze the terminal peptide bonds of their substrates. Carboxypeptidases A and B, differing in their specificities, are formed by activation of procarboxypeptidases by trypsin. Mammalian carboxypeptidase A is a zinc-containing enzyme (Vallee, 1955). Carboxypeptidase A, but not carboxypeptidase B, was found in intestinal extracts of the cyclostome *Myxine glutinosa* (A. Nilsson and Fänge, 1970). Carboxypeptidases have further been found in an elasmobranch (*Squalus acanthias*, Prahl and Neurath, 1966a,b) and in the teleost, *Dicentrarchus labrax* (Alliot *et al.*, 1974). Zendzian and Barnard (1967) showed carboxypeptidase B-like activity in the tuna fish, and Ooshiro (1968, 1971) found carboxypeptidase A-like activity in the intestinal ceca of another teleost, the mackerel (*Scomber japonicus*). In the Japanese mackerel the carboxypeptidase A-like activity appeared to depend on Co^{2+} ions rather then Zn^{2+} ions.

Evolution of pancreatic proteases. Vertebrate trypsin, chymotrypsin, and elastase are structurally related to each other and, together with a few other proteolytic enzymes, are called serine proteases as they contain serine in the active site of the molecule. On the basis of analyses of amino acid sequences of purified trypsinogen from differ-

ent vertebrates, Reeck and Neurath (1972) suggested a possible scheme for the evolutionary changes in the structure of the activation peptide of trypsinogen. The lungfish (*Protopterus*) trypsinogen at the molecular level shows some resemblance with invertebrate trypsins. It has been speculated that different serine proteases have evolved from a common ancestor molecule (Walsh, 1970; Stryer, 1974).

2. Amylase

In some plant-feeding teleosts such as *Tilapia*, amylase has been found in all parts of the digestive tract (Nagase, 1964; Fish, 1960). In a carnivore, the perch (*Perca fluviatilis*), on the other hand, digestive amylase is confined to the diffuse pancreas of the connective tissues surrounding the intestine. According to Nagase (1964) the *Tilapia* amylase has a pH optimum at 6.71, and the pH of the stomach is too low for the enzyme to show any appreciable activity. Pancreatic and intestinal amylase probably are more important than gastric amylase in carbohydrate digestion in *Tilapia*.

3. Chitinase

Chitinase occurs in the digestive system of many fishes and other vertebrates (e.g., Yoshida and Sera, 1970), notably in forms feeding on insects (Micha *et al.*, 1973; Dandrifosse, 1975) or crustaceans. Exceptionally high chitinase activity was found in extracts of the pancreas of the stomachless holocephalan *Chimaera monstrosa*, a fish that feeds largely on shrimps (Fänge *et al.*, 1976, 1978). Little or no pancreatic chitinase was detected in some other marine fish species with similar diets, but these instead possessed strongly active chitinase in the gastric mucosa. The *Chimaera* pancreatic chitinase has a strong optimum around pH 8–10, while gastric chitinases from other species show pH optima at 1.25–3.6. Chitinase splits chitin into dimers and trimers of *N*-acetyl-D-glucosamine (NAG), which may be further broken down by glucosaminidase (NAGase). This enzyme occurs together with chitinase in the digestive tract. NAG, the end product of the chitinolytic process, probably is of nutritive value since it is resorbed faster than glucose by the intestine (Alliot, 1967). Although chitinase may be produced by intestinal bacteria, most chitinases are synthesized by gastric or pancreatic gland cells.

4. Lipases

Lipases are esterases which split ester bonds. Triglyceride fats, phospholipids, and wax esters are hydrolyzed by lipase. Although

lipase activity has been demonstrated in various parts of the fish digestive system the pancreas is probably the major source (Barrington, 1957; Kapoor *et al.*, 1975b). According to Chesley (1934) lipase is more abundant in fishes with a compact pancreas than in those with a diffuse pancreas. Brockerhoff (1966) by *in vivo* experiments found lipolytic activity in the intestinal content of cod (*Gadus morhua*) but Brockerhoff (1966) and Overnell (1973) both failed to find any lipase activity in extracts of the pyloric ceca or adjacent tissues. Leger (1972) partly purified a lipase from the rainbow trout (*Salmo gairdnerii*). Patton *et al.* (1975) found lipase activity in the bile of two marine fishes, the anchovy (*Engraulis mordax*) and the jack mackerel (*Trachurus symmetricus*). These authors suggested that in fishes not pancreatic lipase (EC 3.1.1.3), but another enzyme may function as the major fat-digesting enzyme.

5. Other Enzymes

Alkaline RNase and phosphodiesterase have been purified from the intestinal ceca of rainbow trout (*Salmo gairdnerii*, Imura, 1974a,b). An increased activity of carbonic anhydrase found in the gut of coral fishes is supposed to be an adaptation to ingestion of calcium carbonate (Smith, 1975).

C. Bile

Production of a detergent-containing fluid, bile, by the liver is found in all vertebrates. Usually the bile is stored in a gallbladder with contractile walls. By contraction of the smooth muscles of the gallbladder the bile is ejected into the lumen of the intestine. During its storage in the gallbladder the bile becomes more concentrated. Mammalian bile contains bile salts, cholesterol, phospholipids, bile pigments, organic anions, glycoproteins, and inorganic ions. Fish bile has a similar composition. It is weakly alkaline and has a high sodium and a low chloride concentration (Hunn, 1972). Bile salts are special types of steroids which are synthesized in the liver from cholesterol. In fishes (carp, *Cyprinus carpio*) as in other vertebrates, administration of ^{14}C-labeled cholesterol results in production of radioactive bile salts. In *Myxine glutinosa* (cyclostome), *Chimaera monstrosa* (holocephalan), elasmobranchs, dipnoans, and *Latimeria* (coelacanth) the bile contains bile alcohol sulfate esters (as sodium salts), but in teleosts as in higher vertebrates the bile contains salts formed with taurine conjugates of bile acids. However, in one group of teleosts, the

cyprinids (carp fishes) a bile alcohol sulfate is the principal bile salt (Haslewood, 1968). Bile salt molecules have hydrophilic and hydrophobic groups and in solution at a critical concentration they form aggregates called micelles.

In some teleosts the bile contains trypsin, lipase, amylase, or other enzymes from the intrahepatic pancreatic tissue (Babkin and Bowie, 1928). Bile which contains lipase is free from phospholipids (Patton *et al.*, 1975). In fishes as in mammals a large proportion of the secreted bile salts are presumably resorbed from the intestine into the blood and to a large extent returned to the liver. This so-called enterohepatic circulation concerns both bile salts and other bile components.

D. Intestinal Enzymes

Digestive enzymes produced by intestinal cells are located mainly in the brush border of the epithelium (Ugolev and Kooshuck, 1966; Matthews, 1975). However, enzymes in intestinal extracts may partly be pancreatic, as pancreatic enzymes have the tendency in the intestine to adsorb to the glycocalyx of the epithelial cells. Enzyme activities of the fluid of the intestinal lumen, with the exception of the anterior part of the intestine where pancreatic juice is delivered, are low. Cells or fragments of cells continuously released from the intestinal epithelium, extracellular enzymes from the stomach and the pancreas, and enzymes of the ingested food may contribute to enzyme activities in the lumen.

Enzymes thought to be produced by the intestinal mucosa include aminopeptidases, di- and tripeptidases (formerly termed erepsin), alkaline and acid nucleosidases (which split nucleosides), polynucleotidases (which split nucleic acids), lecithinase (which splits phospholipids into glycerol, fatty acids, phosphoric acid, and choline), lipase and other esterases, and various carbohydrate-digesting enzymes: amylases, maltase, isomaltase, sucrase, lactase, trehalase, and laminarinase. The knowledge of intestinal enzymes in fishes is fragmentary. Piavaux (1973) found laminarinase (β-D-1,3-glucan glucohydrolase, EC 3.2.1.58) in intestinal extracts of *Tilapia macrochira*, an African freshwater teleost, which feeds on plankton and plant detritus. Amylase activity is considerably higher in the intestine of herbivorous species such as the carp (*Cyprinus carpio*), than in the intestine and intestinal ceca of more carnivorous forms such as salmon (*Salmo*), cod (*Gadus morhua*), and flounder (*Pleuronectes*) (Phillips, 1969; Kapoor *et al.*, 1975b). Dipeptidase activity was investigated in the intestine of the white grunt (*Haemulon plumieri*) using synthetic dipeptides as

substrate. The highest activity was measured in the anterior half of the intestine.

E. Regulation of Secretory Activities

1. Gastric Acid Secretion

a. Elasmobranchs. Continuous secretion of very small amounts of gastric acid in fasting rays (*Raja* sp.) was not influenced by vagotomy or atropine but was inhibited by adrenaline or sympathetic nerve stimulation. Spinal destruction, probably due to elimination of tonic sympathetic nerve influence resulted in a "paralytic secretion" of gastric juice (Babkin *et al.*, 1935a). An influence of vascular sphincters on the composition of the gastric juice was assumed by Weinland (1901) who observed that in the ray, *Raja asterias,* treatment with an ergot preparation caused contraction of the vascular sphincters and alkalinity of the gastric juice. Ungar (1935), working with the isolated perfused stomach of *Torpedo, Squalus* and *Scyliorhinus,* found gastric secretion to be stimulated by acetylcholine and histamine. Hogben (1967a,b) found that the isolated gastric mucosa of the spiny dogfish (*Squalus acanthias*) secretes an acid juice spontaneously. Both histamine and the cholinergic agent carbachol increase the rate by 100–150%, but carbachol was 200 times more effective than histamine. No effect was obtained with a preparation of porcine gastrin, a peptide which causes secretion of gastric fluid in higher vertebrates.

b. Teleosts. In the living fish distension of the stomach serves as a powerful stimulus for gastric secretion (Smit, 1968; Norris *et al.*, 1973). Probably the effect is due to reflex activation of vagal cholinergic fibers. The secretion of gastric acid appears to be intermittent; that is, acid is produced only in connection with digestion or when otherwise stimulated (Gzgzyan *et al.*, 1968).

Histamine is an effective stimulus for gastric acid secretion in the European catfish (*Silurus glanis:* Gzgzyan *et al.*, 1968) and in the cod (*Gadus morhua:* Holstein, 1975b). The facts that exogenous histamine causes acid secretion and that the fish gastric mucosa contains considerable amounts of non–mast-cell histamine (Reite, 1969; Lorentz *et al.*, 1973) indirectly indicate that histamine has a physiologic function in the regulation of acid secretion.

In studies of gastric acid secretion in the cod (*Gadus morhua*) Holstein (1975b, 1976, 1977) found only a low basal output of acid

(5–10 μmol/kg hr). However, the method used to collect gastric juice involved ligating the pylorus, which seriously interferes with the water balance. In spite of their dehydrated condition, the fishes were able to secrete considerable amounts of gastric acid when injected with histamine or carbacholine. Both effects were blocked by the H_2-receptor antagonist metiamide. This provides evidence that histamine is physiologically important as a regulator of acid secretion in fish. Further experiments (Holstein, 1978 personal communication) showed that fishes kept in water balance either by perfusion of the intestine with 33% seawater or by intramuscular injection of hypotonic saline show a relatively high "basal" secretion of acid (50–100 μmol of H^+/kg hr). The intense secretory response after carbacholine is accompanied by vasodilation. The response is blocked by atropine (Holstein, 1977).

The question whether gastric secretion in fishes is influenced by gastrin or other GEP hormones is undecided. Holstein (1975b) found no stimulatory effect of pentagastrin in the cod (*Gadus morhua*), but the experiments were made on fishes not in water balance. Larsson and Rehfeld (1977) suggested that in nonmammalian vertebrates ceruleinlike peptides may serve as "gastrin." In isolated frog stomach (*Rana*) cerulein stimulates gastric secretion more powerfully than gastrin (Negri and Erspamer, 1973).

2. Pepsinogen Secretion

In mammals the secretion of pepsinogen is induced by vagal impulses (Hirschowitz, 1975). The stomach of fishes is richly supplied by vagal fibers, but it is not known whether these have any influence on the pepsinogen producing cells.

3. Pancreatic Secretion

In mammals the pancreatic juice is secreted as the result of stimulation of the exocrine pancreas cells by peptide hormones produced by cells in the anterior intestine and the stomach. The pancreas-stimulating principle of the anterior intestine was termed "secretin" by Bayliss and Starling (1903), but later investigations have shown the existence of more than one hormone which stimulate the pancreas. Thus, in mammals secretin produces a thin watery pancreatic fluid rich in bicarbonate, whereas cholecystokinin (CCK) stimulates a secretion rich in enzymes. Other hormones such as gastrin (regulates

gastric secretion) and cholinergic agents (acetylcholine, carbachol, mecholyl) also stimulate the mammalian pancreas.

Babkin (1929, 1933) was able to stimulate pancreatic secretion in a ray (*Raja*) by the introduction of hydrochloric acid into the anterior intestine. It seems plausible that in elasmobranchs as in mammals, introduction of acid stomach content into the intestine causes release of pancreas-stimulating humoral substances.

4. Secretion and Release of Bile

Release of bile into the intestine is produced by contraction of the smooth muscles of the muscularis of the gallbladder. In the hagfish (*Myxine glutinosa*) this contraction is probably brought about by cholinergic vagal influences (Fänge and Johnels, 1958). CCK-PZ, which causes contraction of the mammalian gallbladder is probably present in some fishes (see Table I). In a ray, *Raja erinacea*, the production of bile by the liver seems to take place continuously but in linear relation to the portal vein pressure. The isolated perfused liver continues to produce bile (Boyer *et al.*, 1974).

F. Intestinal Microorganisms

It has been suggested that in certain species of fish the decomposition of food components by microorganisms may be of importance for digestion. Nitrogen-metabolizing bacteria may explain the capacity to utilize urea in the food by the mullet (*Mugil auratus*) (Albertini-Berhaut and Vallet, 1971). Okutani (1966) found chitinolytic bacteria in the intestine of the marine teleost, *Lateolabrax*. These bacteria were gram-negative motile rods with a polar flagellum (*Vibrio*). The bacterial chitinase showed a pH optimum at 7.0, while the gastric mucosa produced a chitinase which was optimally active at a considerably lower pH. Probably in fishes chitinolytic bacteria play a negligible role for digestion in comparison with chitinases produced by the gut mucosa or the pancreas. Cellulase-producing microorganisms were found in the intestine of some estuarine fishes (Stickney and Shumway, 1974). From the little that is known about intestinal microbiology of fishes one is inclined to conclude that microorganisms are less important for the decomposition of food elements than they are in many mammals, especially ruminants. On the other hand, bacteria and other microorganisms are quantitatively an important food component in detritus-feeding fishes such as the mullet (*Mugil cephalus*) (Moriarty, 1976).

V. DIGESTION AND ABSORPTION

A. Digestion

By the action of enzymes of the digestive fluids and gut epithelial cell proteins, polysaccharides, lipids, and nucleic acids are degraded into smaller molecules, which can be absorbed and assimilated. Some proteins and polysaccharides, however, resist degradation.

Protein. Digestion of protein begins in the stomach in species which possess this structure. The endopeptidase activity of the gastric juice renders proteins soluble and more readily digested by pancreatic and intestinal proteases. In the intestinal digestion of proteins, trypsin and chymotrypsin from the pancreas are of major importance. Polypeptides formed by their interaction are further split by pancreatic carboxypeptidases and by intestinal peptidases. Enzymes such as elastase and collagenase may attack special proteins. The protein digestion leads to a mixture in the intestinal lumen of low molecular peptides and amino acids. Van Slyke and White (1911) found that in the dogfish (*Squalus acanthias*) during digestion of protein di- and tripeptides appear in the intestine.

Fat. Triglycerides, which are highly concentrated stores of metabolic energy, are important components of the food of many fishes. However, in addition to neutral fats, wax esters are a very abundant type of lipid in marine organisms such as certain crustaceans and fishes (Patton *et al.*, 1975). Lipases hydrolyze neutral fat (triglycerides) into diglycerides, monoglycerides, glycerol, and free fatty acids. Brockerhoff (1966) found that in the cod (*Gadus morhua*) after 2 days ingested triglycerides had transformed into the above mentioned decomposition products. Even phospholipids and wax esters are attacked by lipases, but fishes that consume waxes from marine organisms hydrolyze triglycerides four times faster than wax esters. Rahn *et al.* (1973) found that in the freshwater gourami (*Trichogaster cosby*) intestinal hydrolysis of wax esters is followed by oxidation of released alcohols to fatty acids. In the vertebrate gut, products from lipolysis are solubilized by bile salts, which form micelles with these products. Cholesterol and highly nonpolar lipids (hydrocarbons, sterols, fat-soluble vitamins) are particularly dependent on the presence of bile salts for micellar solubilization and subsequent absorption (Borgström, 1974).

Carbohydrates. Carbohydrate-digesting enzymes from the pancreas and in the intestinal epithelium transform oligo- and polysaccharides into hexoses and pentoses. Cellulose is probably utilized

only to a small extent and in rather few fish species (cellulose degrading intestinal bacteria, see Stickney and Shumway, 1974), but the presence of high activity of chitinase in the digestive system of many fishes indicates that in some species chitin in the food is broken down to *N*-acetylamino sugar. Muramic acid, the polysaccharide of bacterial cell walls, is split by lysozyme. This enzyme has a wide distribution in nature, but it is not known if it plays any digestive role in fishes. Undoubtedly an enzyme, which dissolves bacterial cell walls, would be useful in detritus-feeding fishes. For example in the detritus-feeding mullet (*Mugil cephalus*) bacteria make up to 15–30% of the organic material in ingested food (Moriarty, 1976).

B. Absorption

The general problem of intestinal absorption is treated by Davson (1970), and from a comparative physiological point of view by Prosser (1973). In the main the mechanisms of intestinal absorption in fish appear similar to those of mammals. Absorption of the products of digestion takes place by diffusion and by active transport. Everted and noneverted segments of the intestine or *in vivo* techniques have been used to study the transport of different substances through the intestinal epithelium of fishes (Farmanfarmaian *et al.*, 1972).

Protein. The degradation products of protein are absorbed from the intestinal content as amino acids or peptides (Matthews, 1975). Individual amino acids are readily absorbed against concentration gradients and their absorption appears to be coupled to transport of inorganic ions (Smith and Lane, 1971; Farmanfarmaian *et al.*, 1972). Proteins and peptides in the intestinal content are probably also taken up to some extent, without previous degradation, by pinocytosis or related processes. Thus in the goldfish intestine, administered protein (horseradish peroxidase) was found to be absorbed in the distal region, in which the epithelial cells seemed to be specialized for the uptake of large molecules.

Fat. In fishes lipids seem to be absorbed mainly by the epithelial cells of the anterior part of the intestine (Gauther and Landis, 1972). Jansson and Olsson (1960) found that in the perch (*Perca fluviatilis*) the mucosal epithelial cells of the intestinal ceca are strongly sudanophilic, indicating that fat absorption is a function of these cells. In mammals the absorbed fat is transported from the intestine mainly by lymph in chyle vessels (lacteals). Within the blood the lipids form chylomicrons (i.e., particulate complexes of proteins, triglycerides,

phospholipids, and cholesterol) or occur as albumin-bound free fatty acids (FFA). Lymph vessels resembling chyle vessels have been found in teleostean intestine (*Anarrhichas lupus,* Glaser, 1933) but chylomicrons have not been found in the blood plasma of fishes (Bilinski, 1974). Malins and Wekell (1970) suggested that in the spiny dogfish, *Squalus acanthias,* the absorbed fat is probably transported by the blood vascular system. The cyclostome *Myxine glutinosa* and certain elasmobranchs contain waxes in their blood plasma (Benson and Lee, 1975).

Carbohydrates. Absorption of glucose by the intestinal epithelium occurs by an active mechanism and can take place against considerable concentration gradients. At low temperature the transport of glucose diminishes. The transport of glucose is associated with electric potentials. Thus in the goldfish, *Carassius auratus,* the serosal side of the intestinal mucosa is positive in relation to the mucosal side. Addition of glucose produces a rise of potential, which is inhibited by phlorhizin (Smith, 1966) Farmanfarmaian *et al.* (1972) investigated the absorption of sugar *in vivo* in the toadfish, *Opsanus tau.* Glucose absorption, which occurs primarily in the anterior intestine, is linear with time and is blocked by phlorhizin.

Salt and water. While freshwater teleosts drink little water, marine teleosts continuously drink water. This was first demonstrated by Smith (1930), who added phenol red to the aquarium water. The quantity of seawater swallowed by marine teleosts per day varies from about 5 to 12% of the body weight (flounder, *Platichthys flesus,* sea perch, *Serranus scriba,* Motais *et al.,* 1969). The absorption of water by the fish intestine is secondary to active transport of sodium (House and Green, 1963). Water and salt movements in the eel (*Anguilla*) have been investigated with the use of isolated sacs of the intestine (Sharratt *et al.,* 1964).

Calcium. In mammals and probably other vertebrates the hormonelike substance 1,25-dihydroxycalciferol, a transformation product of vitamin D, is needed to stimulate the active uptake of calcium in the intestine. However, the physiological role of vitamin D in fishes is little known (Hay and Watson, 1976).

Iron. In mammals absorption of iron is promoted by low pH and by the presence of the reducing agent ascorbic acid. It has been suggested that facilitation of the absorption of iron is an important function of the vertebrate gastric glands, which secrete hydrochloric acid (Granick, 1953). The absorption of iron is an energy-requiring process and involves an iron acceptor in the brush border of the intestinal epithelial cells (Linder *et al.,* 1975).

Total assimilation. Assimilation efficiencies of the various nutrients discussed above are a fundamental part of dietary formulation (see Chapter 1). Assimilation efficiency can be studied by incorporating an inert reference material into the diet which can be readily measured in the subsequent feces. Thus the ratio of nutrient under study to chromic oxide in the food and in the feces is used to calculate the efficiency of assimilation:

$$\text{Assimilation efficiency (\%)} = 100 \times 1 - \frac{(Cr_2O_3\text{: Nutrient}) \text{ in food}}{(Cr_2O_3\text{: Nutrient}) \text{ in feces}}$$

This method has been successfully applied to the study of assimilation efficiency in rainbow trout (Nose and Mamiya, 1963; Nose and Toyama, 1966; Singh and Nose, 1967) and other aquatic animals (Nose, 1967).

VI. MOVEMENT OF FOOD THROUGH THE ALIMENTARY CANAL

A. Methods Used to Measure the Time for Gastric Emptying

The sequence of steps which leads to emptying of food from the stomach of fish has been examined by a number of workers using a variety of techniques. Fish have been given food and killed later to determine the extent of stomach emptying. Construction of the shape of the emptying curve with time requires large numbers of fish but this method has been extensively used (Method 1 in Table II). The degree of breakdown of the food can be measured visually (Fortunatova, 1955; Darnell and Meierotto 1962), by volume (Hunt, 1960), wet or dry weight (Daan, 1973), dry weight of contents after subtracting ash and chitin (Windell, 1966; Windell and Norris, 1969a,b) or calorific and biochemical analysis of the residue (Beamish, 1972; Gerald, 1973). Other workers have avoided killing the fish by causing the fish to vomit (Markus, 1932) or by using a stomach pump (Seaburg, 1956) to collect the residue (Method 2). X-radiography of the fish during its gastric phase of digestion has been successfully used by Molnár and Tölg (1962a,b), Molnár *et al.* (1967), and Edwards (1971, 1973) (Method 3). Hirao *et al.* (1960) incorporated ammonium phosphomolybdate containing ^{32}P into eel and trout diets, Kevern (1966) employed cesium isotope while Peters and Hoss (1974) preferred the poorly assimilated ^{144}Ce to monitor food translocation (Method 4). Other techniques include incorporation of dye in the food (Laurence,

1971), a change to distinguishable items (Blaxter, 1963) or even direct observation of food in the gut of transparent larvae (Rosenthal and Hempel, 1970). Various workers have estimated digestion rates in fish by observing the time between feeding and the production of feces. This technique is relatively straightforward in fish such as *Rutilus* and *Misgurnus* where a small meal subsequently appears as a single dropping (Scheuring, 1928; Bokova, 1938). However, most fish larvae and many microphagous adults feed continuously and it is helpful to label a "quantum" of the diet in such a way that this portion of the meal is detectable in the feces. Much of the work on larval and juvenile fish employs this technique (Lane and Jackson, 1969; Blaxter, 1963; Laurence, 1971). Predacious fish which consume large items of food, however, gradually erode the outer layers of the bolus and continuously pass food into the intestine for further digestion and assimilation. Feces from a given meal start to appear while part of the original meal is still in the gastric phase of digestion. Magnuson (1969) points out that some feces are extruded within 1–2 hr of feeding skipjack tuna. Rozin and Mayer (1961) observed that the first feces containing carmine appeared ca. 7 hr after ingestion by the goldfish but that most of the feces from the meal appeared between 8 and 24 hr after feeding. De Groot (1971) showed that in the turbot the completion of gastric emptying almost coincided with the complete voidance of the meal from the gut. Moriarty and Moriarty (1973a,b) studied the passage of food through the alimentary canal of *Tilapia* and *Haplochromis* which feed continuously on phytoplankton through much of the day. Only part of the ingested plant population is retained in the acid stomach and much of the meal, especially that taken in early morning, passes straight into the intestine and is poorly assimilated. Since the intestine in these species is almost completely emptied of the previous days' food, they were able to follow gastrointestinal motility by the weight of food in different gut segments in serial samples through the day.

As a result of this type of study, more detailed information on gastric emptying has been obtained. After the intake of a meal, there may be a delay of variable extent before the weight of the stomach contents begins to decrease. This delay is usually temperature dependent. Jones (1974) found that small pieces of *Pollachius* fed to gadoids remained unaltered for 3 hr at 6°C but for only 1.5 hr at 12°C. The duration of the delay also depends on the digestibility of the food item. The same author found that *Mytilus* meat starts to decrease in weight almost immediately whereas whole *Centronotus* or crustacea such as *Crangon*, *Nephrops* or *Carcinus* may require almost a day before disintegration is clearly initiated. Similarly, near the end of gastric emp-

Table II

Emptying Time of the Stomach or Intestinal Bulb in Fishes (Simplified from Various Authors)

Fishes	Temperature (°C)	Time to 100% evacuation (hr)	Fish size (cm or g)	Meal type	Method[a]	Reference	Natural diet[b]
Elasmobranchii							
Squalus acanthias	15	>48		Chopped beef	1c	van Slyke and White (1911)	C/F
Holostei							
Lepisosteus osseus	23–26	25	70–132 g	0.5–1.3 g *Gambusia* or *Molliensia*	2a	Hunt (1960)	F
Lepisosteus platyrhynchus	23–26	42	70–132	2.5 g *Gambusia, Molliensia*	2a	Hunt (1960)	
	26	24		6.7% *Gambusia, Molliensia*		Netsch and Witt (1962)	
Chaenobrytus gulosus	23–26	26	93 g	2.7% *Gambusia, Molliensia*	2a	Hunt (1960)	C/F
Amia calva	3	100–190	—	—		Riddle (1909)	C/F
	21	32	11–33	4.9% *Gambusia, Molliensia*	1a	Herting and Witt (1968)	
Teleostei							
Clupeiformes	7	27				Blaxter and Holliday (1963)	MC
Clupea harengus	20	10					
Engraulis encrasicolus	18	26				Okul (1941)	MC/MH
Clupeonella delicatula	9	4					
Engraulis mordax	16–19	1–4	2.7–18.5	*Artemia* nauplii	1a	Leong and O'Connell (1969)	MC/MH
Megalops cyprinoides	28	14–18	52 g	2% *Gambusia, Metapenaeus*	1b	Pandian (1967)	C/F
Esox lucius	18–23	50	40 cm		5	Seaburg and Moyle (1964)	F/HV
Salmo trutta	0	35	7–15 cm	*Gammarus pulex*	1b	Otto (1976)	
	2–4	12–18					
	6–8	10					
	12–15	3					
	5.2	49	90 g	1% *Gammarus*	2c	Elliott (1972)[c]	I/C/M/FF
	7.6	37					
	9.8	29					
	12	22					
	15	16					
Salmo gairdneri	8	27	30 g	1% *Lumbricus*	3a	Grove *et al.* (1976)	I/C/M/FF
	11	24					
	15	22					

	8.5	26.5					
	13.5	18.2	75–85 g	1% Paste	3a,b	Grove *et al.* (1978)	
	18	15					
	0.5	43					
	2	26					
	7	18	60–80 g	0.5 g *Gammarus*	1a	Reimers (1957)	
	10	13					
	12	30	30 g	1.7% Pellet	1c	Windell *et al.* (1969, 1972)	
	15	36	90 g	0.7–1% Capsules			
Salvelinus malma	13	24	95–213 g	2% *O. nerka* fry	1a	Armstrong and Blackett (1966)	I/C/M/FF
Salvelinus fontinalis	5	20–24		*Chaoborus, Chironomus,*	1a	Hess and Rainwater (1939)[c]	I/C/N/F
	7.5	30–30	9–11 cm	*Hydropsyche* or *Acroneuria*			
	11.5	95		(increasing exoskeleton)			
Oncorhynchus nerka	3.1	147					
	5.5	79					
	9.9	38	30–40 g	1.5–2.7% Pellet of canned	1c	Brett and Higgs (1970)	I/C/F
	14.9	23		salmon			
	20.1	18					
Cypriniformes							
Barbus liberiensis	22–25	3–5	3–10 cm	Green algae 3.19 g Juvenile	1b	Payne (1975)	I/D
Ptychocheilus oregonensis	6	111	230 g				
	10	38		*S. gairdnerii*	2a	Steigenberger and Larkin (1974)	I/FF
	15	14					
	20	10					
	24	8					
Misgurnus anguillicaudatus	20–27	20–24		*Viviparus* or *Penaeopsis*	1b	Tanaka (1955)	C/MI
Silurus glanis	5	206					
	10	87					
	15	49		1 *Acerina*	3a	Fábián *et al.* (1963)	F/HV
	20	28					
	25	20					
Ictalurus melas	24	6	4–6 cm	1% *Hyalella*	1b	Darnell and Meierotto (1962)	C/M/I
Ictalurus punctatus	10	24					
	16	24					
	22	7–10	380 g	3 g Pellets	1a	Shrable *et al.* (1969)	C/M/I
	27	3–4					
Anguilliformes							
Anguilla japonica	20	9.5		Minced sardine	4	Hirao *et al.* (1960)	I/M/FF

(*Continued*)

Table II—*Continued*

Fishes	Temperature (°C)	Time to 100% evacuation (hr)	Fish size (cm or g)	Meal type	Method[a]	Reference	Natural diet[b]
Mugiliformes							
Crenimugil labrosus	8–15	4–8	30–150 g	2–3% Paste	3b	Grove *et al.* (1976)	D/MH
Atherina pontica	26	4				Okul (1941)	MC/MH
Gadiformes							
Gadus morhua	2	72					
	5	58					
	10	25	150–375 g	0.45–0.64 g *Pandalus* tails	1b	Tyler (1970)	C/M/F
	15	20					
	19	20					
	12	72	1240 g	46 g *Clupea*	1b	Daan (1973)	
	8–10	48–130	50–55 cm	11–25% *Gammarus* or *Clupea*	1b	Karpevitch and Bokova (1936, 1937)	
Gadus morhua	6	12–45	18–527 g	0.5–2.5 g *Crangon*, fish, meat, polychaetes	1b	Jones (1974)	C/M/A/F
Melanogrammus aeglef.	10	12–26					
Merlangus merlangus	12	11–16					
Lota lota	1	288			1b	Gomazkov (1959)	C/F
	10	168					
Pleuronectiformes							
Pleuronectes platessa	1	36					
	5	25					
	9	16	280–320 g	1.3–1.5 g *Arenicola*	3a	Edwards (1971)	M/A/C
	14	12					
	20	10					
Platichthys flesus	10	24			1b	de Groot (1971)	M/A/C
	17–18	16					
Solea solea	10	24		5% *Arenicola*	1b	de Groot (1971)	A/C
	14–17	6					
Limanda limanda	8.5	18	100 g	1% Paste	3a	Jobling *et al.* (1977)	M/A/C/E
	16.5	12					
Scophthalmus maximus	10	96–100		5% *Sprattus*	1b	de Groot (1971)	F

	8.5	26.5					
	13.5	18.2	75–85 g	1% Paste	3a,b	Grove *et al.* (1978)	
	18	15					
	0.5	43					
	2	26					
	7	18	60–80 g	0.5 g *Gammarus*	1a	Reimers (1957)	
	10	13					
	12	30	30 g	1.7% Pellet	1c	Windell *et al.* (1969, 1972)	
	15	36	90 g	0.7–1% Capsules			
Salvelinus malma	13	24	95–213 g	2% *O. nerka* fry	1a	Armstrong and Blackett (1966)	I/C/M/FF
Salvelinus fontinalis	5	20–24		*Chaoborus, Chironomus,*	1a	Hess and Rainwater (1939)[f]	I/C/N/F
	7.5	30–30	9–11 cm	*Hydropsyche* or *Acroneuria*			
	12.5	95		(increasing exoskeleton)			
Oncorhynchus nerka	3.1	147					
	5.5	79					
	9.9	38	30–40 g	1.5–2.7% Pellet of canned	1c	Brett and Higgs (1970)	I/C/F
	14.9	20		salmon			
	20.1	18					
Cypriniformes							
Barbus liberiensis	22–25	3–5	3–10 cm	Green algae 3.19 g Juvenile	1b	Payne (1975)	I/D
Ptychocheilus oregonensis	6	111	230 g				
	10	38		S. *gairdnerii*	2a	Steigenberger and Larkin	I/FF
	15	14				(1974)	
	20	10					
	24	8					
Misgurnus anguillicaudatus	20–27	20–24		*Viviparus* or *Penaeopsis*	1b	Tanaka (1955)	C/MI
Silurus glanis	5	206					
	10	87					
	15	49		1 *Acerina*	3a	Fábián *et al.* (1963)	F/HV
	20	28					
	25	20					
Ictalurus melas	24	6	4–6 cm	1% *Hyalella*	1b	Barneli and Meierotto (1962)	C/M/I
Ictalurus punctatus	10	24					
	16	24					
	22	7–19	380 g	3 g Pellets	1a	Shrable *et al.* (1969)	C/M/I
	27	3–4					
Anguilliformes							
Anguilla japonica	20	9.5		Minced sardine	4	Hirao *et al.* (1960)	I/M/FF

(*Continued*)

Table II—*Continued*

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Atherina pontica	26	4				Okul (1941)	MC/MH
Gadiformes							
Gadus morhua	2	72					
	5	58					
	10	25	150–375 g	0.45–0.64 g *Pandalus* tails	1b	Tyler (1970)	C/M/F
	15	20					
	19	20					
	12	72	1240 g	46 g *Clupea*	1b	Daan (1973)	
	8–10	48–130	50–55 cm	11–25% *Gammarus* or *Clupea*	1b	Karpevitch and Bokova (1936, 1937)	
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Melanogrammus aeglef.	10	12–26					
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Lota lota	1	288			1b	Gomazkov (1959)	C/F
	10	168					
Pleuronectiformes							
Pleuronectes platessa	1	36					
	5	25					
	9	16	280–320 g	1.3–1.5 g *Arenicola*	3a	Edwards (1971)	M/A/C
	14	12					
	20	10					
Platichthys flesus	10	24			1b	de Groot (1971)	M/A/C
	17–18	16					
Solea solea	10	24		5% *Arenicola*	1b	de Groot (1971)	A/C
	14–17	6					
Limanda limanda	8.5	18	190 g	1% Paste	3a	Jobling *et al.* (1977)	M/A/C/E
	16.5	12					
Scophthalmus maximus	10	96–100		5% *Sprattus*	1b	de Groot (1971)	F

Cottus scorpius	9	Up to 168		50%	1b	Karpevitch and Bokova (1936, 1937)	C/F
Cottus gobio	10	72	5–6 cm	12% *Tubifex*	1b	Western (1971)	I/C
Enophrys bubalis	10	68–100	5–6 cm	32% *Tubifex*, insect larvae	1b	Western (1971)	C/F
Ophiocephalus punctatus	20	48	7 g	8% *Lepidocephalichthys*	1c	Gerald (1973)	C/F
	28	24					
	33	20					
Haemulon plumieri	24	25	19–20	3.9 g *Anchoviella*	1b	Pierce (1936)	F
Ocyurus chrysurus	24	30	19–20 cm	6.5 g *Anchoviella*	1b	Pierce (1936)	F
Micropterus salmoides	5	110	200–700 g	1 *Alburnus*	3a	Molnár and Tölg (1962a,b)	C/F
	10	50					
	15	37					
	20	24					
	25	19					
	20	12–27	91 g	2–8% *Notropis*	1c	Beamish (1972)	
	22	14–16	176 g	3% *Hyporhynchus*	2	Markus (1932)	
	24–25	17–18	89 g	2.7% *Gambusia, Molliensia*	2a	Hunt (1960)	
Prosopium williamsoni	6–11	8–10	22–28 cm	0.4–1.3 g *O. nerka* alevins	1a	McKone (1971)	I/FF
Pungitius pungitius	5	20	0.3–0.6 g	2% *Daphnia*	1b	Cameron *et al.* (1973)	I/MC
	15	7					

[a] Methods: (1) The fish is killed and the stomach contents removed by dissection for weighing or similar treatment. (2) Stomach emptied without killing the fish. (3) X-radiography. (4) Retention of radioactive isotope. (5) A number of fish captured from the wild are then killed at intervals (as in 1). Suffix: (a) force fed; (b) voluntarily fed; (c) digestible organic weight, biochemical or calorific values determined.

[b] Symbols indicate the preferred diet of adult fish: D, detritus; MH, microalgae; MC, microcrustacea and zooplankton; I, insects and larvae; FF, larval and juvenile fish; C, medium to large crustacea; M, molluscs; A, annelids; E, echinoderms; F, fish; HV, other vertebrates.

[c] Authors who have presented more extensive data on the effects of fish size and meal size on gastric evacuation time.

tying less digestible remains such as chitin may form the bulk of the residuum (Karpevitch and Bokova, 1936; Kionka and Windell, 1972).

Once digestion has been initiated, parts of the meal are transferred along the gut. The weight of the residuum decreases with time but the shape of this curve varies in the reports which have been published. In *Gadus* and related species (Jones, 1974; Daan, 1973), *Lepisosteus* (Hunt, 1960), *Stizostedion* (Swenson and Smith, 1973), *Katsuwonus* (Magnuson, 1969), *Megalops* (Pandian, 1967), and other species, the curve is adequately represented by a straight line. On the other hand, many workers have found that the decrease in contents is best described by an exponential curve. A relationship of this kind suggests that the rate of emptying (in grams per hour) is proportional to the instantaneous bulk of food in the stomach and can be expressed as

$$w_t = w_0 e^{-b(t-a)}$$

where w_t, content of the stomach at time t after ingestion; w_0, size of the meal; a, delay before disintegration begins; b, instantaneous rate of digestion. It must be remembered that a and b will vary, as described below, with temperature, meal size, food type, fish size, method of feeding, and feeding history of the fish. Exponential rates of emptying have been described for *Oncorhynchus* (Brett and Higgs, 1970), young cod (*Gadus morhua*) (Tyler, 1970), *Salmo trutta* (Elliott, 1972), *Ptychocheilus* (Steigenberger and Larkin, 1974) and others. In his report on gastric evacuation in *Micropterus*, Beamish (1972) points out that emptying at the early and late phases of gastric digestion is faster than predicted by the exponential equation. Hunt and Knox (1968) showed that in mammals a linear relationship exists between the square root of gastric volume and time, related theoretically to the radial distension of the stomach as by a cylinder of changing volume. Kariya *et al.* (1969) were able to show that stomach diameter increases linearly with the square root of meal size in the mackerel while Jobling (personal communication) has shown that in *Pleuronectes* the gastric evacuation curve is linear when the square root of the residuum (dry weight) is plotted against time.

B. Effect of Temperature

The speed with which food moves through the fish alimentary canal has been investigated by many workers, a simplified account of their results being presented in Tables II, III, and IV. The environmental temperature significantly affects the speed with which food is

Table III
Total Emptying Time for Meals from the Digestive Tract of Fish

Species	Temperature (°C)	Time to 100% empty (hr)	Reference
Clupeidae			
Sardinops caerulea	18	12	Lasker (1970)
Salmonidae			
Salmo gairdneri	8	49–51	Grove *et al.* (1978)
	11	46	
	13.5	35	
	15	40	
	18	30.5	
Salvelinus namaycush	12	60–108	Lane and Jackson (1969)[a]
Esocidae			
Esox lucius	12	72	Lane and Jackson (1969)
Cyprinidae			
Ctenopharyngodon sp.	9	7	Hickling (1966)
Barbus liberiensis	22–25	6–8	Payne (1975)
Rutilus rutilus	3	30	Karzinkin (1932); Bokova (1938)
	6	31	
	10	21–22	
	14	22	
	17	12	
	20	9–10	
	25	6–8	
Pimephales promelas	12	36	Lane and Jackson (1969)
	20	12–24	
Carassius auratus	12	36–48	Lane and Jackson (1969)
	20	60–72	
	25	60	
	25	8–24	Rozin and Mayer (1964)
Cyprinus carpio	12	60	Lane and Jackson (1969)
	23	48	
	12.5	22–50	Kevern (1966)
	25	16–25	
	10	18	Maltzan (cited in Hickling, 1970)
	26	4–5	
Leuciscus baicalensis	0.5	130	Pegel and Popov (1937)
	15	40	
	25	15	
Notemigonus chrysoleucas	20	36	Lane and Jackson (1969)
Catla catla	28–30	18–54	Renade and Kewalramani (1967)
Cirrhina mrigala	28–30	18–60	Renade and Kewalramani (1967)

(*Continued*)

Table III—*Continued*

Species	Temperature (°C)	Time to 100% empty (hr)	Reference
Catostomidae			
Ictiobus cyprinellus	20	24	Lane and Jackson (1969)
Catostomus commersoni	12	60	Lane and Jackson (1969)
Ameiuridae			
Ictalurus melas	12	84	Lane and Jackson (1969)
Ictalurus punctatus	12	24–36	Lane and Jackson (1969)
Ictalurus catus	20	48	Lane and Jackson (1969)
Ictalurus nebulosus	20	60	Lane and Jackson (1969)
Ictalurus natalis	24	72	Lane and Jackson (1969)
Cobitidae			
Misgurnus fossilis	10	40	Scheuring (1928)
	15	14	
	20	10	
Embiotocidae			
Brachyistius frenatus	23–26	10–12	Bray and Ebeling (1975)
Phanerodon furcatus	23–26	10–12	Bray and Ebeling (1975)
Coridae			
Oxyjulis californica	23–26	10–12	Bray and Ebeling (1975)
Sparidae			
Labeo rohita	28–30	24–54	Renade and Kewalramani (1967)
Percidae			
Perca flavescens	12	36–60	Lane and Jackson (1969)
Stizostedion vitreum	12	60	Lane and Jackson (1969)
Centrarchidae			
Micropterus salmoides	12	48–84	Lane and Jackson (1969)
	20	36–48	Beamish (1972)
	20	60	Lane and Jackson (1969)
Micropterus dolomieui	12	48–72	Lane and Jackson (1969)
Pomoxis annularis	20	60	Lane and Jackson (1969)
Lepomis cyanellus	12	60	Lane and Jackson (1969)
	20	48	
Lepomis gibbosus	12	84	Lane and Jackson (1969)
Lepomis megalotis	12	72	Lane and Jackson (1969)
Lepomis macrochirus	12	36–84	Lane and Jackson (1969)
	17	48	
	20	36–60	
	22	36	
	25	36	
Cottidae			
Cottus gobio	10	100	Western (1971)
Enophrys bubalis	10	100	Western (1971)

Table III—*Continued*

Species	Temperature (°C)	Time to 100% empty (hr)	Reference
Moronidae			
Dicentrarchus (Morone) labrax	16	36–74	Grove *et al.* (1976)
	19	20	
Carangidae	24	16	
Katsuwonus pelamis	23–36	14	Magnuson (1969)
Mugilidae			
Crenimugil labrosus	8	6–10	Grove *et al.* (1976)
	18–19	17–18	
Mugil cephalus	20–26	4–5	Odum (1970)
Labridae			
Tautogolabrus adspersus	10–15	10–14	Chao (1973)
Cichlidae			
Tilapia nilotica	25	7–15	Moriarty (1973)
	27	15–27	Moriarty (1973)
Blenniidae			
Blennius pholis	8	49	Grove *et al.* (1976)
	11	45	
	16	21	
	19	17.5	
Pleuronectidae			
Pleuronectes platessa	1	158	Edwards (1971)
	5	53	
	9	37	
	14	24	
	20	20	
	10	72	de Groot (1971)
Patichthys flesus	10	72	de Groot (1971)
	17.4	54	
Limanda limanda	17	19–24	Jobling *et al.* (1977)
Soleidae			
Solea solea	10	72	de Groot (1971)

[a] The study by Lane and Jackson (1969) (*Salvelinus namaycush, Esox lucius, Pimephales promelas, Carassius auratus, Notemigonus chrysoleucas, Ictiobus cyprinellus, Catostomus commersoni*, etc.) was limited to young fish (2.5–9 cm) which were allowed a long period of voluntary feeding before isolation and feces collection at 12 hr intervals. Tanaka (1955) noted that the time for gastric evacuation is close to 50% of that for the whole canal to be cleared.

processed (Table II). This effect is illustrated in Fig. 4, in which the time for a meal to be emptied from the stomach or intestinal bulb is plotted in relation to temperature. Jones (1974) found that the evacuation rate of the stomach changes in proportion to $10^{0.035(\Delta t)}$, where Δt is

Table IV

Time for Fish Larvae to Empty Food from the Alimentary Canal

Species	Temperature (°C)	Time (hr)	Remarks	Reference
Clupeidae				
Clupea	7	6–9	Smaller larvae	Various authors, cited
harengus	9	5–7.5	Smaller larvae	in Blaxter (1963)
	11	4–5	Smaller larvae	
	15	4	Smaller larvae	
	12	24–30	Larger larvae	
Clupea pallasi	9	12–19	Duration increased with meal size	Kurata (1959)
Brevoortia	16	5	Copepod diet,	Kjeldson *et al.* (1975)
tyrranus	15	5.1–7.4	*Artemia*	
Sardinops caerulea		3–11	Time increases with fish size	Arthur (1956)
Coregonidae				
Coregonus clupeaformis	14.4	24–48	Time increases with meal or fish size	Hoagman (1974)
Cyprinidae				
Abramis	14	3.5		Panov and Sorokin
brama	20	1.75		(1962)
Cyprinus	18–29	1–8	Continuous feeding	Chiba (1961)
carpio		20	Single meal	
Belonidae				
Belone	22	2.7–4.8	38–45 mm larvae	Rosenthal and Paffen-
belone		2.9–5.3	50–55 mm larvae	höfer (1972)
Sparidae				
Lagodon	16–17	4.7–6.4	Copepod diet	Kjeldson *et al.* (1975)
rhomboides	16	5.15	*Artemia*	
Sciaenidae				
Leiostomus	17	6.1	Copepod diet	Kjeldson *et al.* (1975)
anthurus	16	9.6–10	*Artemia*	
Centrarchidae				
Micropterus	17	3	Continuous feeding	Laurence (1971)
salmoides		5.5	Single meal	
	23	2	Continuous feeding	
		3.7	Single meal	
	20	8	Darkness	
Percidae				
Perca	21	2.1–2.9	Total evacuation for	Nobel (1973)
flavescens			17–19.5 mm fish	
Pleuronectidae				
Pleuronectes platessa	10	6		Ryland (1964)

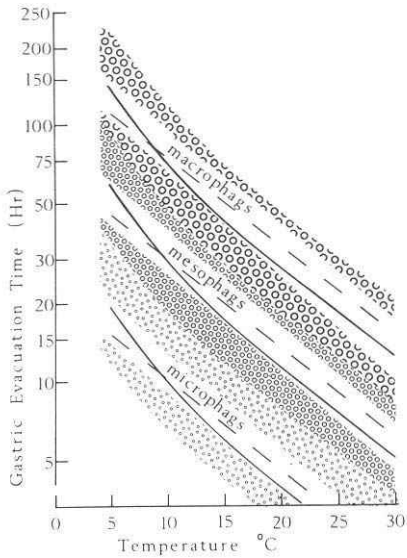

Fig. 4. Relationships between gastric or foregut emptying times and temperature in fishes. Data are taken from Table II and grouped into microphagous (D, MH, MC, I, FF), mesophagous (M, A, E, C), and macrophagous (C, F, HV) based on their preferred natural diet. Note that the time scale is on a logarithmic axis. The effect of temperature on digestion rate in each group is represented by a Q_{10} of 2.6 (Backiel, 1971; continuous lines) or in proportion to $10^{0.035t}$ (Jones, 1974; broken lines).

the temperature change in degrees centigrade. This trend is indicated in the figure by straight lines. Since the time to empty the stomach in many fish deviated from a straight line when log evacuation time is plotted against temperature, it may be more appropriate to represent the effect of temperature on rate in the form of Krogh's curve with a Q_{10} of ca. 2.6 as used by Backiel (1971). Fábián *et al.* (1963) plotted the log reciprocal of evacuation time against the reciprocal of temperature to demonstrate that their data on predatory fish follow the Arrhenius equation. At temperatures near the upper physiological limits of the species in question, the trend may cease or even reverse (Tyler, 1970; Gomazkov, 1959).

C. Feeding Niches

The data presented in Table II are derived from experiments in which a number of variables which affect gastrointestinal motility are confounded. Within the table however there is evidence that fish

which have dissimilar food in nature have dissimilar digestion rates. For example, *Prosopium* in nature feeds on insects and fish fry. When fed salmon fry at a meal size of 1% body weight (8–10°C) the stomach empties within 6–11 hr. The flatfish *Limanda*, which normally ingests marine bivalve mollusks, crustacea, and other invertebrates, requires fully 18 hr to empty the stomach when fed a 1% body weight diet of easily digestible flatfish plaice diet at the same temperature. The piscivorous *Lepisosteus* requires more than 24 hr to digest a meal of fish, also as a meal of 1% (wet weight) of body weight, even though the test was carried out at the higher temperature of 23–26°C. We suggest that the inherent rate of gastrointestinal motility and digestion has evolved to suit the natural diet of fish and that this factor is borne out in Tables II and III. With this in mind, Fig. 4 has been constructed in such a way that the data in Table II have been grouped into microphagous (planktivores, insectivores, and similar), mesophagous (eating larger invertebrates such as mollusks, annelids, and shrimps) and macrophagous (taking crabs, fish, or other vertebrates) species. In many cases the species fall into the appropriate group, related to their natural diet, even when the laboratory data has been obtained using unnatural food items. An interesting example of this pre-adaptation to the feeding niche has been found in our own studies. We fed mullet (*Crenimugil labrosus*) or the blenny (*Blennius pholis*) on the same artificial diet of fishmeal and additives used in flatfish rearing in Aberdeen. The mullet has a long convoluted intestine and normally ingests particulate detritus whereas the littoral blenny predates on barnacles and other invertebrates and has a relatively short intestine with no true stomach. When similar sized animals were fed the same meal size at the same temperature, the mullet completely emptied the gastrointestinal tract three to four times more rapidly than the blenny. The most notable exception to the pattern presented in Fig. 4 is the description by Moore and Beamish (1973) that the ammocoete larva of *Petromyzon marinus*, which feeds on unicellular algae using ciliary mechanisms, digests its food more slowly than teleostean microherbivores.

D. Other Factors That Influence Gastric Motility

It is important, when discussing the emptying rate of meals from the fish stomach, to remark that many workers use the terms "digestion," "evacuation," "emptying," "elimination," and "clearance" rate to describe the *total time* taken for the stomach to empty after a given meal. Others use the same terms to describe the disappearance of food (measured as grams per hour or percentage of the meal per hour) from

the stomach. Barrington (1957) stated in his review that, on the available evidence, "a smaller meal may be expected to be digested more readily than a larger one." This statement is based on the relatively larger surface area presented to digestive enzymes by smaller food items. In apparent contradiction to this, Windell (1966) points out that, in his studies, the rate of digestion of a larger meal (measured as the amount disappearing per unit time) is greater than that of a smaller meal.

Appropriate analysis of the gastric emptying process shows that both statements are true. Since enzymes typically attack the food bolus at the surface, the rate of digestion will obviously be proportional to the surface area, namely,

$$dV/dt = -aV^{2/3} \tag{1}$$

where V is the volume (or weight) of the food in the stomach, and a is a constant which depends on such factors as the species and size of the individual fish, temperature, and food type. Accordingly, it is to be expected, as pointed out by Windell, that a larger meal will have a faster digestion rate (grams per hour) and that a plot of log digestion rate (grams per hour) against log meal size will have a slope of 0.67 (for a given sized fish at a stated temperature using a standard food). If the above differential equation (1) is integrated and arranged for any meal size (V_s) to be reduced to $V = 0$, it is seen that the time required to empty the stomach (t_λ) is given by

$$t_\lambda = a' V_s^{1/3} \tag{2}$$

and Barrington's (1957) statement remains true: A smaller meal will be digested sooner. It follows that a plot of $\log t_\lambda$ against $\log V_s$ should produce a straight line of slope 0.33, again provided that the experiments are carried out with fish of the same size, at the same temperature, and using the same food.

This relationship may also hold when different sized fish of the same species are examined. If such fish are fed a stated percentage of their body weight, the food bolus will be larger in the bigger fish and will present a relatively smaller surface area to gastrointestinal secretions. Larger fish will digest, for example, a 1% body weight meal at a greater rate (g/hr) than a smaller fish but the time required to complete digestion will be prolonged.

1. Meal Size

In published accounts, it has been reported that a larger meal fed to a given size of fish does take longer to digest (Hunt, 1960; Beamish,

1972; Elliott, 1972; Swenson and Smith, 1973; Jobling *et al.*, 1977). Beamish (1972) found that a four-fold increase in meal size fed to *Micropterus* only doubled the time required to empty the stomach. Using X-radiography, Jobling *et al.* (1977) found that increasing meal size from 1 to 5% (100-g *Limanda* at 16°C) leads only to a fourfold increase in stomach emptying time. Clearly both the time to complete the meal and the rate of digestion increased as predicted by the model. Analysis of the published records for various fish shows that digestion rate, expressed in grams per hour, varies as the following exponents of the wet weight of the meal: 0.46 (gadoids, Jones, 1974), 0.5 (*Limanda*, Jobling *et al.*, 1977), 0.6 (*Prosopium*, McKone, 1971; (*Megalops*, Pandian, 1967), 0.7 (*Micropterus*, Beamish, 1972), and 0.75 (*Salmo gairdnerii*, Windell *et al.*, 1969). In a few fish digestion rate has been found to increase as (meal size)$^{1.0}$, such that overall evacuation time in a given fish is the same for different sized meals (*Lepomis*, Windell, 1966; *Oncorhynchus*, Brett and Higgs, 1970; *Blennius*, Crawford and Grove, unpublished observations). The arrival of larger meals in the fish stomach or foregut will initiate or accelerate peristalsis (Section VII) thereby raising the emptying rate above the level predicted by the simplistic model. Only Steigenberger and Larkin (1974), in a careful study of digestion in *Ptychocheilus*, have found that digestion rate decreases with increased meal size. Clearly more species must be examined before this result can be held anomalous. Elliott (1972) found that the instantaneous rate of gastric evacuation in *Salmo trutta* is not affected by increases in meal size but, since his evacuation curves are exponential, the early digestion rate (g/hr) increases with meal size. A similar conclusion applies to the data for *Oncorhynchus nerka* (Brett and Higgs, 1970).

2. Fish Size

Several workers have reported the digestion rate of fish of different sizes in a form which can be compared with the predictions of the model for gastric emptying time (t_λ). Jobling *et al.* (1977) (Figs. 5 and 6) fed *Limanda* of different body weights to the same percentage of their weight with a flatfish paste diet. The time required to empty the stomach varied as (fish weight)$^{0.386}$. Since the stomach volume is proportional to body weight in this species ($V = 0.081\,W - 0.39$ where the units are ml and g, respectively) a stated % body weight meal will fill the stomach to the same extent in different sized fish. A similar analysis of Pandian's (1967) data for *Megalops* fed on prawns shows that t_λ varies as (fish weight)$^{0.41}$.

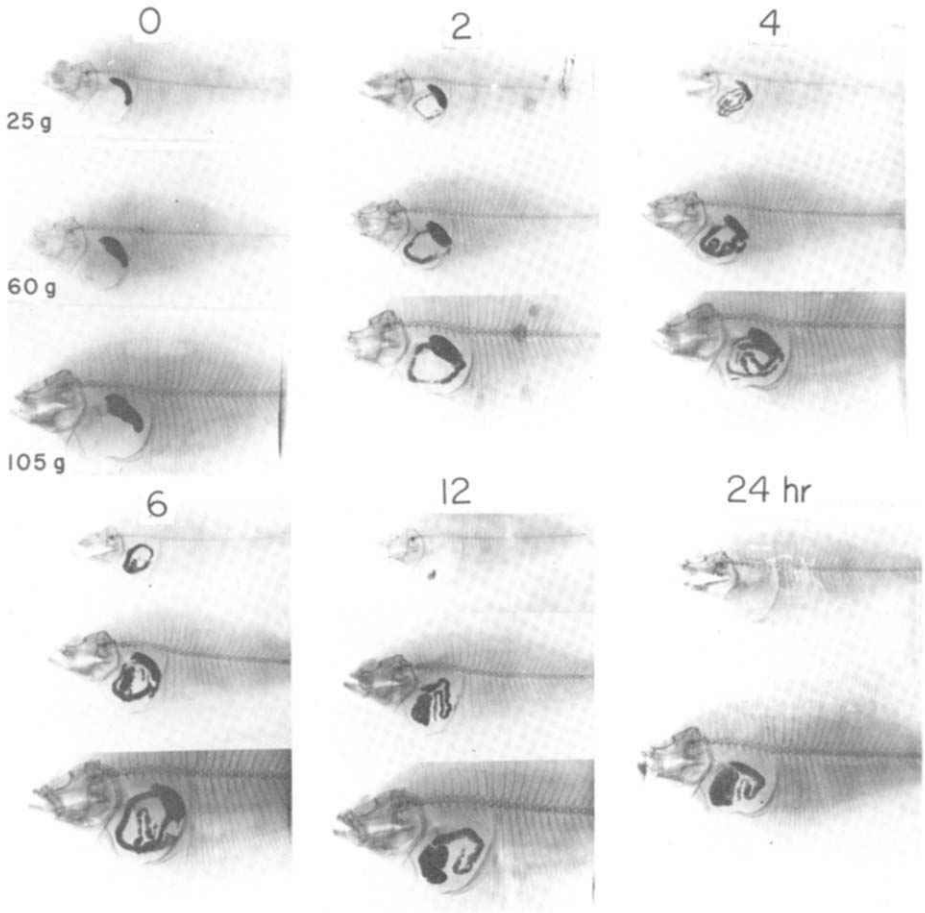

Fig. 5. X-Ray photographs of *Limanda limanda* fed a 1% body weight meal at 16°C. The fish weighed 25, 60, and 105 g. Exposures were taken at 0, 2, 4, 6, 12, and 24 hr after feeding. The delay in stomach and intestinal clearance with increasing size is clearly seen. (From Jobling *et al.*, 1977, *J. Fish Biol.* **10**, 291–298.)

The above reports not only offer reasonable support for the relationships proposed in Eqs. (1) and (2) but offer physiological explanations for the pattern of feeding by fish in their natural environment. It has been suggested (Kariya, 1969; Kariya and Takahashi, 1969; Brett, 1971; Ware, 1972; Elliott, 1972, 1975) that the appetite of fish returns as the stomach empties. It would be natural to expect that, for a given species, fish of different sizes which show the same feeding periodicity must voluntarily regulate their meal size so that digestion

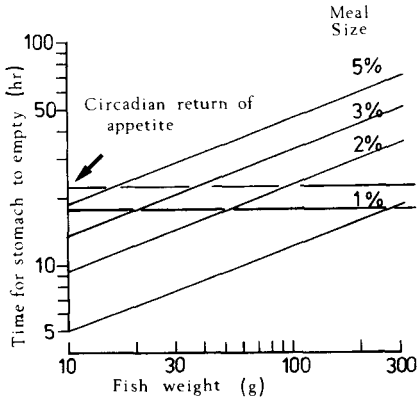

Fig. 6. Relationship between fish weight, meal size (as percentage of body weight) and gastric evacuation time for *Limanda limanda* at 16°C. The decrease in relative digestion rate with size shows that, if feeding is to return at regular intervals (e.g., daily) larger fish must necessarily ingest relatively smaller meals. Voluntary intake in grams will vary as (body weight)$^{0.5}$. (From Jobling *et al.*, 1977, *J. Fish Biol.* **10**, 291–298.)

is completed in time for the next feeding bout (Fig. 6). The stomach contents of freshly captured fish show a relative decrease in amount for bigger fish (Daan, 1973; Steigenberger and Larkin, 1974) and voluntary intake of food has been shown to decrease relative to body weight by many workers (Table V). It must be recognized, in design-

Table V

Magnitude of the Exponent in the Relationship Meal Size = a(Fish Weight)^b for Voluntarily Feeding Fish

Species	b	Reference
Petromyzon marinus (ammocoete)	0.72 (summer) 0.84 (winter)	Moore and Beamish (1973)
Salvelinus fontinalis	0.91	Baldwin (1956)
Salmo trutta	0.75	Elliott (1975)
Oncorhynchus nerka	0.75 (single meal) 0.65 (multiple meal)	Brett (1971)
Megalops cyprinoides	0.71	Pandian (1967)
Micropterus salmoides	0.47	Niimi and Beamish (1975)
Navodon modestus	0.52	Suzuki (1976)
Ophiocephalus punctatus	0.76	Gerald (1973)
Blennius pholis	0.38	Wallace (1973)
	1.00	Crawford and Grove (unpublished observations)
Trachurus japonicus	0.56	Hotta and Nakashima (1968)

ing experiments, that the amount taken at a meal by an individual fish will depend on the previous feeding regime (Kariya and Takahashi, 1969; Ishiwata, 1969; Brett, 1971). For example, young *Scomber japonicus* will eat 23% of their body weight of anchovy after several days of deprivation but, on a regular feeding regime, will ingest only 16%.

3. Type of Food and Its Presentation

A further variable with significant effects on the gastric emptying rate is the type of food ingested by the fish. Jones (1974) found that gadoids digested the following foods (after an appropriate delay) at the stated rate at 12°C: *Nereis/Nephthys*, 0.31 g/hr; *Pollachius* muscle, 0.26 g/hr; *Crangon*, 0.19 g/hr. Elliott (1972) found that brown trout completed 90% elimination of gammarids or oligochaetes in 22 hr at 12°C whereas *Protonemura* (26 hr), *Hydropsyche* (30 hr), and *Tenebrio* (49.5 hr) took longer. A similar observation was made by Reimers (1957) on rainbow trout, where *Helodrilus* (12 hr) was eliminated faster than *Gammarus* (13 hr) or *Arctopsyche* (16 hr) at 10°C when given to 12.4–20 cm fish in 0.5 g meals. Other workers who have detected decreased evacuation rates with less digestible foodstuffs are Pandian (1967) (*Megalops* fed *Gambusia* or *Metapenaeus*), Karpevitch and Bokova (1936, 1937) (Gadus fed *Clupea* or *Gammarus*), Western (1971) (*Cottus, Enophrys* fed on *Tubifex, Calliphora* or semifluid meals), Reshetnikov *et al.* (1972) (*Lutianus* fed on *Jenkinsia* or *Harengula*), Renade and Kewalramani (1967) (*Labeo, Cirrhina* or *Catla* fed on various algae, plant detritus, and zooplankton), and Kionka and Windell (1972) (*Salmo* fed various diets). The digestibility of the food will not only affect the emptying rate from the stomach, expressed as g/hr, but may also determine the time after ingestion before weight decrease of the meal can occur. Jones (1974) found that *Merlangus* or *Melanogrammus* start to digest shell-less *Mytilus* almost immediately but that meals of *Ophiopholis*, large crustacea or *Centronotus* require up to 10, 20, and 25 hr, respectively, before weight loss begins. Although in many cases the decrease in digestibility can be attributed to thick or inert casings (e.g., caddis larvae) Windell (1967) suggested that the presence of fat in the food may delay gastric emptying, possibly by a release from the intestinal wall of a hormone similar to enterogastrone which in mammals inhibits gastric motility (Hunt and Knox, 1968). Diets with increased fat levels clearly decrease gastric evacuation rate in the rainbow trout (Windell *et al.*, 1969). Finally, Swenson and Smith (1973) found that when *Stizostedion* were fed the same meal size at the same temperature but with smaller or larger food

items, the evacuation rate was faster with the smaller items (they used *Pimephales*).

A further complication to the measurement of gastric emptying rates in fish are the observations by Windell (1966) and by Swenson and Smith (1973) that force-feeding the test animal usually decreases the rate of evacuation of the meal from the stomach when compared with fish which consume the food voluntarily. Of even greater concern, however, are the reports from Kariya and Takahashi (1969), Ishiwata (1969), Tyler (1970), Brett (1971), Jones (1974), and, for larval fish, Laurence (1971), Blaxter and Holliday (1963), Rosenthal and Paffenhöfer (1972), and Nobel (1973) that fish which have been deprived of food for a time prior to feeding show a slower gastric emptying rate than fish tested under continuous feeding. Digestion rates measured in previously deprived fish are usually only 50–68% of those found in actively-feeding fish. In their study on feeding rate of *Tilapia* ingesting blue–green algae, Moriarty and Moriarty (1973a) found that the rate of stomach emptying during the feeding period was faster than that measured after feeding had ceased. This factor is a clear restriction when using laboratory digestion studies to estimate feeding rate in the wild (Healey, 1971).

E. Conclusions

If experiments on gastric evacuation are undertaken (whether to compute daily food intake in nature, to determine the relationship between stomach emptying and the return of appetite for fish culture, or to investigate the physiological control of gastric motility *in vivo*) the following procedures must be controlled and reported with the results.

1. The temperature at which observations are made, together with the previous acclimation history of the fish
2. The frequency of feeding, or the previous duration of food deprivation, since the fish will consume a meal size related to this regime if voluntary feeding is allowed
3. The meal size, as absolute wet and dry weight and as percentage of the body weight
4. The biochemical composition of the food, including the calorific value per unit dry weight of digestible food, both at ingestion and at later stages as stomach emptying proceeds

5. The method of feeding, especially whether the animals were force fed or ingested voluntarily; mention should be made if the fish were handled at intervals during the experiments (e.g., if several X-ray photographs were taken)
6. The length and weight of the fish used in the study

It is likely that other factors, not discussed in the present account, might affect gastric emptying. Reproductive cycles, photoperiod of long or short daylength, presence of parasties and/or the stocking density may all modify gastric motility. Many of the results listed in simplified form in Table II are taken from reports where factors 1–6 were not fully controlled. More recent studies (e.g., Elliott, 1972; Steigenberger and Larkin, 1974) have used factorial designs to test some of the variables likely to affect gastric emptying.

It has often been stated that the return of appetite is closely related to the time taken to reduce the stomach contents. Ware (1972), for example, defined hunger in newly caught fish in relation to the degree of stomach fullness. Magnuson (1969), Brett (1971), and Elliott (1975) all report that hunger returns before the stomach is completely empty. However, the observations of Tugendhat (1960) and Beukema (1968) suggest that stomach emptiness alone does not control the amount ingested. *Gasterosteus* with empty stomachs ingested more food at a faster rate if they had a longer time of deprivation before food was made available. Elliott (1975) found that a trout of stated size ate more in a meal if the temperature is raised. Rozin and Mayer (1961, 1964) found that the goldfish (*Carassius*) feeds continuously, but consumes a greater amount of food per day if the food is diluted with kaolin. A similar conclusion is reported for *S. gairdneri* by Lee and Putnam (1973). In an attempt to clarify this latter phenomenon of "calorie counting," we carried out a study on *Salmo gairdneri* (Grove *et al.*, 1978). As shown in Fig. 7, rainbow trout kept in continuous light exhibit regular periods of feeding activity similar to those described by Adron *et al.* (1973). The records from the demand feeders were subjected to periodogram analysis and showed that, as the food is diluted with kaolin, so the period between meals is progressively shortened and, as a consequence, the food intake per day is increased. Adron *et al.* (1973) suggested that the periodic feeding is determined by the rate of gastric emptying, so X-radiography tests were undertaken to see if dilution of the food with kaolin shortens the time required for the stomach to empty. The gastric evacuation times presented in Table VI clearly show that this is the case. The mechanisms responsible for the increased gastric motility are unknown but may

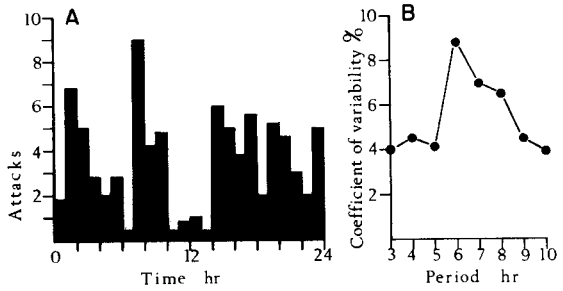

Fig. 7. (A) Activation of a "demand" feeder by a group of 60 g *Salmo gairdnerii* during a 24 hr period under continuous illumination at 18°C. (B) Periodogram analysis of (A) (Williams and Naylor, 1967) shows that appetite returns at 6-hr intervals. (From Grove *et al.*, 1978, *J. Fish Biol.* **12**, 507–516.)

either depend on a decreased production of enterogastrone from the intestine or a change in activity of extrinsic nerves which supply the stomach (see Section VII).

Digestion Efficiency: Pappas *et al.* (1973) added 0.37% chromic oxide to diets fed to channel catfish and observed the disappearance of nutrients (fat, protein, and carbohydrate) relative to the inert marker while the food remained in the stomach. Nearly 50% of these dietary components were removed before the food entered the intestine, al-

Table VI

"Calorie Counting" in *Salmo gairdnerii*: Control of Appetite by Changes in Gastric Motility[a]

Test food	Food (cal/g)	Mean daily intake (cal/100 g/day)	Mean feeding intervals (hr)	Mean gastric evacuation time (hr)
Salmon pellet	4.78	11.3	6	15
75% food/25% kaolin	4.20	15.9	5	14.5
67% food/33% kaolin	3.23	15.0	5	11.8
50% food/50% kaolin	2.18	13.1	4	10

[a] The fish obtained food from a "demand feeder" under continuous illumination at 18°C. Each week the control pellets (Cooper's salmon starter) were replaced by reconstituted pellets diluted with kaolin, and feeding activity was subjected to periodogram analysis to determine the time for return of appetite during the succeeding 7 days. Separate groups of fish were fed the same diets and X-rayed to determine the time for complete gastric evacuation. Note that the stomach empties more quickly when the food is diluted, that appetite returns more quickly and, if the evacuation curve is exponential, this occurs when 70–90% of the previous meal has left the stomach. From Grove *et al.* (1978, *J. Fish Biol.* **12**, 507–516).

though no conclusion was reached whether they undergo gastric absorption or are passed in solution into the intestine for assimilation. Beamish (1972) showed that protein was solubilized more extensively in *Micropterus* stomach than fats, which required intestinal digestion before assimilation occurred. Overall protein digestion is efficient in fish, more than 85% assimilation being the rule unless oxidation of oils occurs in the food employed (Nose and Toyama, 1966). Smith and Lane (1971) reported that protein assimilation of salmonids was impaired if α-cellulose was incorporated in the diet. Increase in the proportion of carbohydrates in the diet, or absolute decrease in the protein content of the diet, has been reported to decrease protein assimilation without explanation of the mechanism involved (see references in Nose, 1967). Nose demonstrated that this apparent decrease in protein assimilation arises from failure of previous workers to allow for the release of metabolic nitrogen into the feces, which becomes significant in biochemical determinations only at low levels of protein intake. A true decrease in protein assimilation was detected by Nose when rainbow trout were given egg albumin, which contains a trypsin inhibitor. The early work of Inaba *et al.* (1962) clearly demonstrates that the protein in whalemeat or soya bean residues is much less digestible than that in white fish meal, but the basis for this observation requires further study. A more extensive account for formulation of fish diets is given in Chapter 1.

VII. PHYSIOLOGICAL STUDIES ON FISH GASTROINTESTINAL MOTILITY

A. Introduction

1. Excitation of Gastrointestinal Smooth Muscles

Our understanding of muscular activity of the gastrointestinal tract in various fish is based on comparisons with higher vertebrates. The major contractile elements in the muscularis are hexagonally packed groups of smooth muscle cells which act as a contractile unit (Bennett and Burnstock, 1968). The cells affect each other electrotonically so that coordinated myogenic contractions, independent of the nervous system, can develop in response to stretch as a result of Ca^{2+} flux across their membrane. In the *in situ* gut, rhythmic myogenic contractions of the circular muscles temporarily divide the intestine into a series of segments to facilitate mixing of the luminal contents.

Superimposed on this activity is the peristaltic process, dependent on cell bodies in the nerve plexuses of the gut wall, usually seen as waves of relaxation and contraction which progress anally along the gut. The combination of these two processes, segmentation and peristalsis, may cause the segments of the intestine to swing slowly from side to side in pendular activity.

2. The Sites of Drug Action

In view of the difficulty in separating the roles of muscle and nerve cells, most workers have investigated the neurogenic components by using drugs which are believed to act at the receptor sites on tissues with which neurotransmitters are known to act. This approach is not without problems since few drugs are specific, and may only be considered *selective* within certain dose ranges (Daniel, 1968) which must be determined for each new tissue. It has become general to refer to nerve cells releasing a stated neurotransmitter (e.g., acetylcholine, noradrenaline) as cholinergic, adrenergic, and the receptor they occupy as cholinoceptor, adrenoceptor. When the transmitter, or a similar molecule (agonist), occupies the receptors of a tissue, a response is evoked in proportion to the agonist concentration. A dose–response curve can be constructed and the dose producing 50% of maximum response is a measure of the affinity of the agonist at that site (Ariens and Van Rossum 1957). Similar molecules which occupy the site without eliciting a response may combine with the agonist reversibly, or irreversibly by binding to the receptor chemically. The blocking action of reversible antagonists can be overcome by increasing the agonist concentration, and may be visualized as a parallel shift of the agonist dose–response curve to the right. A measure of the affinity of the antagonist is the observed shift of the dose–response curve in the presence of a stated antagonist concentration. Noncompetitive and irreversible antagonism is usually detected as an insurmountable blockade, accompanied by a depression of the dose–response curve (e.g., Guimaraes, 1969). Examples of these relationships are given in Fig. 8. In Table VII, the drugs which have been commonly used in studies of fish gastrointestinal motility are shown, together with the receptors that they occupy.

3. Intrinsic Neurons

Two main techniques for activating neurons in the gut wall are commonly used. Various workers have adapted the technique of Trendelenburg (1917) in which distension of the gut wall *in vitro* initiates

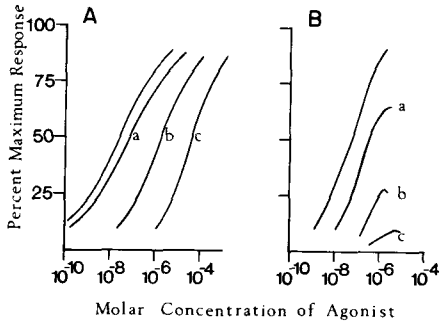

Fig. 8. Analysis of drug effects on *in vitro* Magnus preparations of plaice gastric longitudinal muscle. (A) Dose-related contractions induced by acetylcholine alone. Control $ED_{50} = 2 \times 10^{-8}$ *M* (and $pD_2 = 7.7$). The addition of atropine to the bath in doses of 3.5×10^{-x} *M* causes a parallel shift of the dose–response curve to the right in relation to the dose: curve a, $x = 9$; curve b, $x = 8$; curve c, $x = 7$. The concentration of atropine for which a control dose of acetylcholine must be *doubled* to obtain the control response is 1.6×10^{-9} *M* (and $pA_2 = 8.8$). (B) A similar study using 5-hydroxytryptamine as stimulant gives a control $ED_{50} = 4 \times 10^{-8}$ *M* (whence $pD_2 = 7.4$). Atropine here acts noncompetitively when the doses used above are employed. The concentration of atropine required to depress the control maximum by half is 1.2×10^{-8} *M* (whence $pD_2' = 7.9$). (From Edwards, 1972b, *Comp. Gen. Pharmacol.* 3, 345–358.)

activity. To study stimulant effects on inactive preparations, the gut segment is simply suspended in a bath under minimum stretch (Magnus preparation). The second method, which may be used on Trendelenburg or Magnus preparations, is to stimulate the intrinsic neurons by electrodes in the bath placed near (field stimulation) or on either side (transmural stimulation) of the gut wall. The frequency of stimulation may be between 1 and 50 Hz and the pulse duration of 1 msec or less to avoid direct activation of the muscle cells. In addition to recording the muscle responses mechanically (levers, strain gauges, pressure transducers), changes in muscle membrane potential can be monitored using the sucrose gap technique (Campbell and Burnstock, 1968; Ito and Kuriyama, 1971). The use of the latter technique has shown that, in low tone preparations, transmural stimulation may excite inhibitory neurons in the gut wall which hyperpolarize the muscle cell membranes without a clear relaxation being recorded. After cessation of the stimulus, the cells repolarize and may overshoot the resting potential leading to spontaneous electrical activity and "rebound contractions." This phenomenon led Burnstock (1969) and Campbell and Burnstock (1968) to reinterpret the conclusions reached in earlier studies of fish gastrointestinal motility; the vagus nerve was earlier claimed to excite the fish stomach whereas it is now believed to

Table VII

Sites of Action and Names and Abbreviations of Drugs Used to Analyze Gastrointestinal Motility in Fishes

Receptor/effect	Agonist	Antagonist
1. Muscarinic; cholinoceptor on smooth muscles	Acetylcholine (ACh) Pilocarpine Carbachol Methacol	Atropine (Atr) Hyoscine
2. "Nicotinic"; cholinoceptor on neurons	Acetylcholine Carbachol 1,1-Dimethyl-4-phenylpiperazonium (DMPP)	Nicotine Hexamethonium (C_6) *d*-Tubocurarine (dTC) Mecamylamine (Mec)
3. "Nicotinic"; cholinoceptor on striated muscle	Acetylcholine	*d*-Tubocurarine
4. Adrenoceptors		
α	Phenylephrine Adrenaline (A) Noradrenaline (NA)	Phenoxybenzamine (PBZ) Phentolamine (Phent) Dihydroergotamine (DHE) Piperoxane (Pip) Yohimbine
β	Isoprenaline (Iso) Adrenaline (A) Noradrenaline (NA)	Propranolol (Prop) Butoxamine
5. Tryptaminoceptors "dibenamine type" D	5-Hydroxytryptamine (5-HT)	Methysergide 2-Bromo-lysergic acid diethylamide (BOL) Phenoxybenzamine (PBZ) Ergotamine
6. Purinoceptor	Adenosine triphosphate (ATP)	
7. Histamine receptor (H_1)	Histamine	Mepyramine
8. Cholinesterase inhibition	Eserine	
9. Neuron blocking		
All nerves	Tetrodotoxin (TTX), procaine	
Cholinergic nerves	Morphine, hemicholinium	
Adrenergic nerves	Reserpine, bretylium, guanethidine	

inhibit it in the majority of fishes. It has been proposed that intramural neurons exist in vertebrate viscera which excite or inhibit the smooth muscles by releasing a purinergic transmitter, possibly adenosine triphosphate (Burnstock, 1972; Burnstock *et al.*, 1970, 1972).

4. Extrinsic Neurons

The extrinsic nerves to the fish gut originate from the autonomic nervous system. The simplistic account of this system in mammals, attributed to Langley (1921), is that "parasympathetic" nerve tracts from the craniosacral region innervate the intrinsic neurons of the gut wall by way of vagus and pelvic nerves. A "sympathetic" outflow in the thoraco-lumbar region innervates ganglion cells in the sympathetic chain. In both divisions, preganglionic medullated cholinergic nerves leave the CNS. The postganglionic parasympathetic neurons are cholinergic. Activity in the parasympathetic division enhances gastrointestinal motility whereas sympathetic activity is inhibitory.

It is now acknowledged that adherence to this model is likely to cause misconceptions, and many mammalian exceptions have been reported. Vagal fibers may activate either cholinergic or purinergic intrinsic neurons. The vagus may be joined by postganglionic axons (adrenergic or cholinergic) from the sympathetic division, or itself send preganglionic cholinergic fibers along the nearest convenient "sympathetic" tract. The postganglionic adrenergic nerves of the "sympathetic" system may not innervate gut muscle directly, but enmesh ganglion cells in the gut wall (Campbell, 1970a,b). These conclusions, reached for higher vertebrates, are equally likely to complicate studies on the less understood fish groups.

B. Cyclostomes

Relatively little information is available on the physiology of intestinal motility in hagfish and lampreys. The intestinal muscles of both groups are poorly developed and food movements may depend on contractions of the body wall. Fänge (1962) and Burnstock (1969) in their reviews report that the intestine in hagfish is contracted by acetylcholine (10^{-8} to 10^{-5} g/ml) but relaxed by adrenaline (10^{-7} to 10^{-5} g/ml), and by atropine. Peristalsis is induced in *Lampetra* rectum by 10^{-4} g/ml 5-hydroxytryptamine.

In the hagfish *Myxine glutinosa,* the two intestinal rami of the vagus nerve unite dorsally to the intestine forming an unpaired nerve-like structure (*ramus impar*) which contains numerous ganglion cells. Cholinergic and adrenergic neurons are present (Fänge and Johnels, 1958; Hallbäck, 1973; Fänge *et al.,* 1963). Patterson and Fair (1933) found that vagal stimulation slightly relaxes the hagfish gut. von Euler and Fänge (1961) detected catecholamines in the gut of *Myxine* and Honma (1970) used the fluorescent histological technique of Falck

and Owman (1965) to show that adrenergic neurons and terminals are present in the enteric plexus of *Lampetra japonica* and *L. planeri*, which presumably mediate inhibition. Nonadrenergic nerves in the gut wall of hagfish and lampreys are likely to be cholinergic and possibly also purinergic, coordinating peristalsis, and appear to be innervated by fibers of vagal origin.

More recently, Baumgarten *et al.* (1973) have carried out an extensive study of monoaminergic neurons in the gut wall of *Lampetra fluviatilis* using the fluorescent histochemical technique together with microspectrofluorimetry, chromatography, and electron microscopy. Green fluorescent nerve cells were detected in the submucosa of the fore- and midgut which may inhibit the intestine, and which contain primary catecholamines such as dopamine or noradrenaline. These authors detected an extensive submucosal plexus of tryptaminergic axons and cell bodies which connected with a subserous plexus containing nerve terminals. They propose that the 5-hydroxytryptamine transmitter released by these endings elicits peristalsis but point out that similar intramural neurons have not been described in the gut wall of true vertebrates.

C. Elasmobranchs

In comparison with actinopterygii, relatively little work has been done on elasmobranch gastrointestinal motility. The early reports of Lutz (1931), Nicholls (1933), and Dreyer (1949) state that all parts of the gut are contracted by acetylcholine and pilocarpine. von Euler and Östlund (1957) confirmed the observation of Nicholls that atropine prevents the action of acetylcholine. Adrenaline sometimes causes contraction of the gut (Babkin *et al.*, 1935a,b; Dreyer, 1949; Young, 1933) but according to Nicholls (1933) this is only at lower doses. Inhibitory effects of adrenaline have also been recorded on the dogfish rectum (Lutz, 1931) and ray intestine (von Euler and Östlund, 1957). Without more detailed studies it is impossible to conclude whether adrenaline has different actions in different parts of the gut of different species. Recently Moore and Hiatt (1967) reinvestigated the problem of the action of adrenaline in elasmobranchs. They elected to work with *Squalus acanthias*, using intragastric balloons *in vivo* to record contractions. Injection of adrenaline produced a clear contraction, which they believed would be due to occupation either of α- or β-adrenoceptors. However, they found that neither α (phenoxybenzamine) nor β (propranolol) adrenoceptor blocking agents pre-

vented the action of adrenaline. To extend their study, they tested other sympathomimetic amines which have differential potencies on α- and β-receptors in other vertebrates. Surprisingly, isoprenaline, dopamine, or noradrenaline failed to contract the stomach in control animals. Phenylephrine was effective, but phenoxybenzamine, which in most vertebrates is a powerful phenylephrine antagonist at α-adrenoceptors, failed to antagonize the response. In further studies, aminophylline was found to abolish the adrenaline-induced contraction and the effect of adrenaline decreased rapidly with successive tests in control animals. They concluded that adrenaline had no inhibitory effect on *Squalus* gastrointestinal smooth muscle. The stimulatory action of a first test dose of adrenaline depends not on the usual cell membrane adrenoceptors but on increased glycogenolysis presumably following an increased intracellular build-up of cyclic AMP. However, once under way, this intracellular activity hyperpolarizes the muscle membranes, thus suppressing responses to subsequent adrenaline treatment. Clearly this abnormal action of adrenaline should be investigated in other species. It should not be concluded that adrenoceptors are necessarily aberrant in elasmobranchs since Nilsson *et al.* (1975) have shown α-adrenoceptors in spleen and artery preparations of *Squalus* and *Scyliorhinus* with properties similar to those in teleosts and higher vertebrates.

Gzgzyan and Kuzina (1973) investigated spontaneous peristaltic contractions of the stomach of the ray, *Dasyatis pastinaca*. This species does not exhibit alternation of periods of activity and rest typical of empty mammalian stomachs. The contractions were enhanced when cholinomimetics (carbachol, pilocarpine) were injected but atropine was not a powerful inhibitor of the spontaneous contractions. There is evidence here that cholinergic nerves may play a coordinating role in gastrointestinal peristalsis but, in common with teleosts (see later), myogenic contractions may play a significant part in rhythmic contractions of the gut wall. Myenteric and submucosal plexuses have been described in the stomach and intestine of elasmobranchs (Nicol, 1952).

The digestive canal of elasmobranchs is supplied by vagal and splanchnic nerves. The vagal fibers are believed to be adrenergic (Fänge and Hanson, 1973). Campbell and Burnstock (1968) examined the published records from earlier workers of the effect of vagal stimulation on stomach activity. They concluded that the influence of the vagus is limited to the stomach and proximal intestine and that it is inhibitory. Earlier conclusions that the vagus is excitatory were explained by the long latency of the contraction, reminiscent of the "re-

bound contractions" described earlier. Campbell (1975) found that the regular spontaneous activity of *Scyliorhinus canicula* stomach, recorded by intragastric balloon, was abolished when the intracranial roots of the vagus nerve were stimulated electrically. He also observed a stimulatory effect of the vagus on esophageal striped muscles. By inference from other vertebrates, Burnstock (1969) suggests that at least part of the inhibitory response mediated by the vagus depends on the presence of inhibitory, nonadrenergic neurons in the stomach wall. The role of sympathetic nerves on gastrointestinal motility is as yet unclear. Burnstock (1969) concluded that the records in earlier publications show evidence of both cholinergic excitatory and adrenergic inhibitory fibers supplying both stomach and intestine in elasmobranchs.

D. Teleosts

Gastrointestinal motility, and its control, has been more extensively studied in teleosts. The sympathetic system, like that of higher vertebrates, consists of two ganglionated chains which extend into the head as far as the trigeminal nerve (V). The esophagus and stomach have a rich vagal innervation, while in many stomachless fish including the cyprinidae the vagal influence extends to the intestine. In the stomachless pleuronectids *Rhombosolea* and *Ammotretis*, however, the vagal influence does not extend beyond the esophagus (Grove and Campbell, unpublished observations). The stomach and intestine are also innervated by fibers from the splanchnic nerves which originate from sympathetic ganglia in the first few spinal segments. The gut of the tench (*Tinca*) contains striated and smooth muscles innervated by the vagus nerve (Ohnesorge and Rehberg, 1963). Only the smooth muscle elements participate in the peristaltic movements. Results of pharmacological analysis indicate that the peristalsis is due to a reflex involving intramural neurons (Ohnesorge and Rauch, 1968). A posterior sympathetic nerve to the rectum has been described in the trout (*Salmo trutta*) by Burnstock (1958a,b). The ramus intestinalis of the vagus nerve probably receives sympathetic fibers from the ganglionated chains and may therefore more properly be termed a vagosympathetic trunk (Young, 1931).

1. Analysis of Gastrointestinal Receptors

Few studies have been made to estimate the affinity for specific receptors on gastrointestinal muscle and nerve cells of agonist

molecules by dose–response curve analysis. Table VIII summarizes much of the recently published data for a number of teleost fish. In general, cholinesters and 5-hydroxytryptamine (5-HT) contract the muscle coats whereas sympathomimetic amines relax the gut or inhibit spontaneous activity. The problems facing the researcher lie mainly in the complexity of the gut wall, since the site of action of a drug must be determined, and the specificity of the drugs, which have usually been assumed to act in fish as in mammals.

Acetylcholine is probably a direct stimulant of muscarinic receptors on many parts of the gut musculature since atropine is the most potent inhibitor of its action (*Salmo trutta*, Burnstock, 1958a,b; *Gadus morhua*, S. Nilsson and Fänge, 1969; *Carassius auratus*, Ito and Kuriyama, 1971; Saito, 1973; *Pleuronectes platessa*, Edwards, 1972b; Goddard, 1975; *Blennius, Myoxocephalus*, Grove *et al.*, 1974). Acetylcholine also exerts effects on nicotinic receptors of a variety of neurons in the enteric plexus, and the high, potentially nonselective doses of atropine used in the *Gadus* and *Carassius* studies may block this action as well. Where dose–response analysis has been undertaken, the pA_2 of atropine has been found to lie between 8.2 and 9.4. Concentrations of atropine at ca. 10^{-7} g/ml or less should be more than adequate to abolish the muscarinic action of acetylcholine without significant side effects (Edwards, 1972b; Goddard, 1975; Grove *et al.*, 1974). There is no doubt that acetylcholine effects in *Carassius* involve the intramural ganglion cells. Saito (1973) believed that all mechanical effects exerted by the cholinester (10^{-6} g/ml) on the intestine were indirect since tetrodotoxin (10^{-8} g/ml), which prevents axonal conduction by blocking sodium channels in the neuronal membrane, abolished all its actions. He recognized a brief contraction of striated muscle fibers when the drug was applied, followed by a slower smooth muscle contraction. After the administration of atropine (10^{-6} g/ml) to impair the response of cholinergic excitatory cells, he recorded relaxation of the preparation. Since this effect is tetrodotoxin-sensitive, inhibitory neurons activated by preganglionic cholinergic fibers are also present in the intestine. Dimethylphenylpiperazinium (DMPP) (10^{-6} g/ml) which also briefly excites ganglion cells in mammals mimicked acetylcholine. In contrast, the earlier study by Ito and Kuriyama (1971) on silver carp showed that acetylcholine excitation persisted after tetrodotoxin. They proposed that at least part of the stimulus must be directly on the muscle cells. *d*-Tubocurarine (10^{-6} g/ml) abolishes the effect of acetylcholine on striated muscle in *Carassius* (Saito, 1973) and in *Tinca* (Mahn, 1898). Hexamethonium, which in mammals is a relatively potent nicotinic

Table VIII

Effects of Agonist Drugs on Resting Gastrointestinal Muscle Preparations of Teleosts[a]

Species and organ	Cholinergic stimulants		5-Hydroxytryptamine		Sympathomimetic amines		Purines	
Salmo trutta								
a. Stomach	a,b,c. ACh + 10^{-8}–10^{-4}	(1)	a,b,c. + 10^{-9}	(1)	a. NA + 10^{-6}	(1,2)		
b. Intestine	Pil + 10^{-5}	(1)			A + 10^{-6}	(1,2)		
c. Rectum					ISO + 2×10^{-5}	(1,2)		
					b,c. A − 10^{-8}–10^{-5}	(1)		
Carassius auratus								
Intestine	ACh (1st) + 10^{-6}	(3,4)			NA − 10^{-6}–10^{-5}	(3,5)	ATP + 10^{-5} *M*	(5)
(*Cholinesters*	ACh (2nd) + 10^{-6}	(3,4)			A − 10^{-6}	(3)		
cause	ACh (3rd) − 10^{-6}	(3,4)			ISO − 10^{-6}	(3)		
multiple	DMPP (1st) + 10^{-5}	(3)						
contractions)	DMPP (2nd) + 10^{-5}	(3)						
Tinca tinca								
Intestine	ACh (1st) + 5×10^{-8}–10^{-5}	(6,7)	+ 10^{-8}	(8)	A − 10^{-8}–10^{-7}	(7)		
(Cholinesters	ACh (2nd) + 5×10^{-8}–10^{-5}	(6,7)			NA − 10^{-8}–10^{-7}	(7)		
cause	DMPP + 10^{-8}–10^{-6}	(7)						
multiple								
contractions)								
Anguilla anguilla								
a. Esophagus	a,b,c. ACh + 10^{-5}	(9)			a,c. A − 10^{-5}	(9)		
b. Stomach					b. A + then − 10^{-5}	(9)		
c. Intestine					Tyr + then − 10^{-5}	(9)		
					ISO + then − 10^{-5}	(9)		

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Species and organ	Cholinergic stimulants		5-Hydroxytryptamine		Sympathomimetic amines		Purines	
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b. Intestine	Pil + 10^{-5}	(1)			A + 10^{-6}	(1,2)		
c. Rectum					ISO + 2×10^{-5}	(1,2)		
					b,c. A – 10^{-8}–10^{-5}	(1)		
Carassius auratus								
Intestine	ACh (1st) + 10^{-6}	(3,4)			NA – 10^{-6}–10^{-5}	(3,5)	ATP + 10^{-5} *M*	(5)
(*Cholinesters*	ACh (2nd) + 10^{-6}	(3,4)			A – 10^{-6}	(3)		
cause	ACh (3rd) – 10^{-6}	(3,4)			ISO – 10^{-6}	(3)		
multiple	DMPP (1st) + 10^{-5}	(3)						
contractions)	DMPP (2nd) + 10^{-5}	(3)						
Tinca tinca								
Intestine	ACh (1st) + 5×10^{-8}–10^{-5}	(6,7)	+ 10^{-8}	(8)	A – 10^{-8}–10^{-7}	(7)		
(Cholinesters	ACh (2nd) + 5×10^{-8}–10^{-5}	(6,7)			NA – 10^{-8}–10^{-7}	(7)		
cause multiple contractions)	DMPP + 10^{-8}–10^{-6}	(7)						
Anguilla anguilla								
a. Esophagus	a,b,c. ACh + 10^{-5}	(9)			a,c. A – 10^{-5}	(9)		
b. Stomach					b. A + then – 10^{-5}	(9)		
c. Intestine					Tyr + then – 10^{-5}	(9)		
					ISO + then – 10^{-5}	(9)		

blocker, has less specificity in fish although its use has been extensive. Edwards (1972b) found that it noncompetitively antagonized acetylcholine and other cholinesters ($pD_2' = 3.1–4.4$) and also 5-HT ($pD_2' = 3.9$) stimulation of the plaice stomach. Recently Stevenson and Grove (1977) have found that, after tetrodotoxin (10^{-7} g/ml) the direct muscarinic actions of cholinesters are blocked by hexamethonium (10^{-4}–10^{-3} g/ml) but not by mecamylamine (10^{-3} g/ml). It is unfortunate that hexamethonium has been the drug of choice for many analyses of teleost gut function. In some of the studies on peristalsis and extrinsic nerves to be reviewed below, it is not unusual to find doses of hexamethonium as high as 10^{-3} g/ml being used as a "selective" nicotinic atagonist. At present the bulk of the evidence suggests that exogenous acetylcholine acts at smooth muscle muscarinic sites (*Salmo, Gadus, Pleuronectes, Blennius, Myoxocephalus*) whereas in the stomachless *Tinca* and *Carassius* much of the action involves ganglion cells of the enteric plexus.

The actions of adrenoceptor stimulants are equally complex. α-Receptors, mediating inhibition of the gut by adrenaline and noradrenaline, have been postulated in *Carassius* intestine (Saito, 1973) and *Salmo* intestine (Burnstock, 1958a,b). The inhibition was antagonized by phentolamine, phenoxybenzamine, or dihydroergotamine in relatively high concentrations (ca. 10^{-5} g/ml). In teleost and elasmobranch cardiovascular systems (S. Nilsson and Grove, 1974; S. Nilsson *et al.*, 1975) these drugs have pA_2 values of the order 6.5–7.5, suggesting that lower doses than used by Saito and Burnstock are sufficient to block α-receptors without side effects. Young (1931) found that ergotoxine (10^{-6} g/ml) was sufficient to block the inhibitory action of adrenaline. β-Adrenoceptors mediating inhibition have been demonstrated in the intestine of *Carassius* (Saito, 1973) and *Pleuronectes* stomach and intestine (Goddard, 1975; Stevenson and Grove, unpublished observations). In *Pleuronectes* the inhibition is reversibly blocked by butoxamine whereas propranolol at doses of 10^{-5} g/ml and above had nonspecific depressant effects on spontaneous activity and acetylcholine responses. In *Rhombosolea* or *Ammotretis*, however, the inhibitory actions of adrenaline, which are mediated by β-adrenoceptors, are competitively antagonized by propranolol ($pA_2 = 7.9$; Grove and Campbell, unpublished observations). Several anomalous effects of adrenergic agonists have been detected. In *Anguilla* gastric cecum (S. Nilsson and Fänge, 1967), *Gadus* stomach (S. Nilsson and Fänge, 1969), *Salmo* stomach (Burnstock, 1958b; Campbell and Gannon, 1976; and occasionally in *Pleuronectes* stomach (Edwards, 1972b) and intestine (Goddard, 1975) and *Blen-*

nius intestine (Grove *et al.*, 1976), treatment with catecholamines other than isoprenaline causes excitation. In *Gadus* these were antagonized by high doses (10^{-5} g/ml) of α-adrenoceptor antagonists but in *Pleuronectes* were resistant to both α and β-blockade. These effects recall those described for the stomach of elasmobranchs (Section VII,C) and remain similarly unexplained.

5-Hydroxytryptamine is a powerful stimulant of the gastrointestinal tract of various fish (Table VIII) (Fänge, 1962). Few studies have been made to determine the site of action of this amine. Edwards (1972b) showed that atropine ($pD_2' = 7.5$) and hexamethonium ($pD_2' = 3.9$) were noncompetitive antagonists of 5-HT and suggested that it acted in fish, as in mammals (Gershon, 1967), on cholinergic enteric neurons. Grove *et al.* (1974) extended the study and found that the excitatory action persisted after tetrodotoxin, morphine, ergotamine, methysergide, or 2-bromo-lysergic acid diethylamide (each at 10^{-6} and 10^{-5} g/ml). Since hemicholinium impaired the effect, they concluded that much of the action of 5-hydroxytryptamine is indirect, displacing acetylcholine from cholinergic terminals at a site peripheral to that blocked by tetrodotoxin or morphine. In the relaxed *Pleuronectes* intestine, 5-HT stimulates the longitudinal, but not the circular, muscle layer ($pD_2 = 5.2$) and is not affected by attempted blockade with morphine or 2-bromo-LSD. In the active isolated preparation however, the amine inhibits peristalsis when applied to the serosal (but not the mucosal) surface (Goddard, 1975).

Burnstock *et al.* (1970, 1972) have proposed that a new class of neurons releasing a purine as neurotransmitter exists in vertebrates. Adenosine triphosphate has been found to stimulate goldfish intestine (Burnstock *et al.*, 1972) and *Pleuronectes* stomach (Stevenson and Grove, unpublished observations) but to relax *Pleuronectes* intestine (Goddard, 1975).

2. Effects of Drugs on Active Preparations of the Teleost Gut

Despite the inadequate knowledge of the sites of action of the agonist and antagonist drugs described above, many workers have used them in attempts to analyze the role of the enteric plexus (Kirtisinghe, 1940) in coordinating peristalsis in the isolated gut. Furthermore, it has become clear that myogenic contractions as well as neurogenic peristalsis may develop when an isolated segment is distended. True peristalsis was described by Burnstock (1958a,b) in the isolated trout gut after distension. At 8°–14°C, a powerful longitudinal

contraction precedes each peristaltic wave which then moves anally at ca. 2 cm/min, such waves originating in the cardia, antrum, or about one-third of the way along the intestine. Similar preparations have been made *in vitro* for *Tinca* intestine (Ohnesorge and Rauch, 1968), *Pleuronectes* stomach (Stevenson and Grove, unpublished observations) and intestine (Goddard, 1975), and *in vivo* using intragastric balloons for *Scorpaena* (Gzgzyan *et al.*, 1973), *Salmo* and *Conger* (Campbell, 1975). Typically, an increase in intraluminal pressure of 3–5 cm H_2O initiates rhythmic contractions of the gut preparation. The effects of drugs and the doses used on this activity in various fish are listed in Table IX.

There is general agreement that cholinesters stimulate spontaneous contractions when the gut is distended whereas atropine inhibits them. Eserine has been found to increase peristaltic rate in *Salmo* and *Tinca*. On the assumption that these effects depend on the activity of ganglion cells in the enteric nerve plexus, drugs which block the effect of acetylcholine on ganglion cells (nicotine, DMPP, mecamylamine, or hexamethonium) or which prevent axonal conduction (tetrodotoxin, procaine) have been tested and generally found to diminish or abolish peristalsis. Frequently, the reports have noted that after blockade with nicotinic and muscarinic blocking drugs, or with tetrodotoxin, the pattern of rhythmic contraction changes but is not abolished. Ito and Kuriyama (1971) recorded the mechanical activity of *Carassius* intestine using a strain gauge while simultaneously recording the electrical activity with the sucrose-gap technique. They detected contractions after neuronal blockade which were abolished by exposure to manganous ions, and concluded that these were myogenic contractions based on calcium fluxes across the muscle membranes. Similar activity independent of the enteric nervous system has been described for the gut of *Salmo* (Burnstock, 1958a,b), intestine of *Tinca* (Ohnesorge and Rauch, 1968), and stomach and intestine of *Pleuronectes* (Goddard, 1975; Stevenson and Grove, 1977). The question arises of the role of myogenic contractions in teleost "peristalsis." A major difference exists in the onset of rhythmic activity in isolated, distended fish gut segments when compared with mammalian preparations. In *Pleuronectes*, a delay of several minutes follows distension of the stomach or intestine before peristalsis begins and rings of contraction pass anally (Fig. 9A). In *Tinca* and *Pleuronectes* the longitudinal and circular muscle coats contract synchronously, there being little sign of the "preparatory" and "expulsion" phases typical of mammals (Ohnesorge and Rauch, 1968; Goddard, 1975). The enteric nerve cells appear essential for the initiation of peristalsis, since pretreatment of

Table IX

Effects of Drugs on Active Preparations of Teleost Gastrointestinal Muscles[a]

Drug	*Dose* (g/ml)	Source	*Salmo trutta* Stomach and intestine (a.) (b.)	*Carassius auratus* intestine	*Gadus* morhua Stomach	*Tinca* tinca Intestine	*Pleuronectes platessa* Stomach and intestine (a.) (b.)	*Scorpaena porcus* Stomach
ACh	10^{-8}–10^{-4}	(1,3,4,6,7,10,13,14,15)	+(M)	+	+	+	+	+
CCH	10^{-8}–10^{-5}	(10,13,14)			+		+	
Eserine or vag	10^{-6}–10^{-5}	(1,7)	+			+		
Atropine	10^{-7}–10^{-5}	(1,3,4,7,10,13,14,15)	−(M)	−(M)	−	−	0/(−)	−
dTc	10^{-7}–10^{-4}	(7,14)				−	−	
DMPP	10^{-8}–10^{-6}	(7)				+/−(M)		
Nicotine	10^{-7}–10^{-4}	(7)	+			+/−(M)		
Mecamylamine	10^{-6}–10^{-4}	(13,14)					a. 0 b. −	
Hexamethonium	10^{-7}–10^{-3}	(1,7,13,14)	−(M)			0(M)	a. 0 b. 0/−	
NA	10^{-7}–10^{-5}	(3,4,7,10,13,14)		−	+	−	a. +/− b. −	
A	10^{-10}–10^{-5}	(1,3,4,7,10,13,14)	a. +/− b. −	−	+	−		
Pheny	10^{-7}–10^{-5}	(10,13,14)		−	+		−	
ISO	10^{-8}–10^{-5}	(3,10,13,14)			−		−	
5-HT	10^{-9}–10^{-5}	(13,14)	+				a. + b. −	
ATP	10^{-6}–10^{-3}	(5,13,14)		+			a. + b. −	
Tetrodotoxin	10^{-7}–10^{-6}	(3,4,13,14)		−(M)			−(M)	
Procaine	10^{-6}–10^{-4}	(7,13,14)				−	−(M)	

[a] Drug abbreviations and sources (numbers in parentheses) are given in footnote to Table VIII. Key: +, enhancement of peristalsis; 0, no effect; −, inhibition; (M), unmasking of myogenic rhythms; /, followed by (e.g., +/−, excitation followed by inhibition).

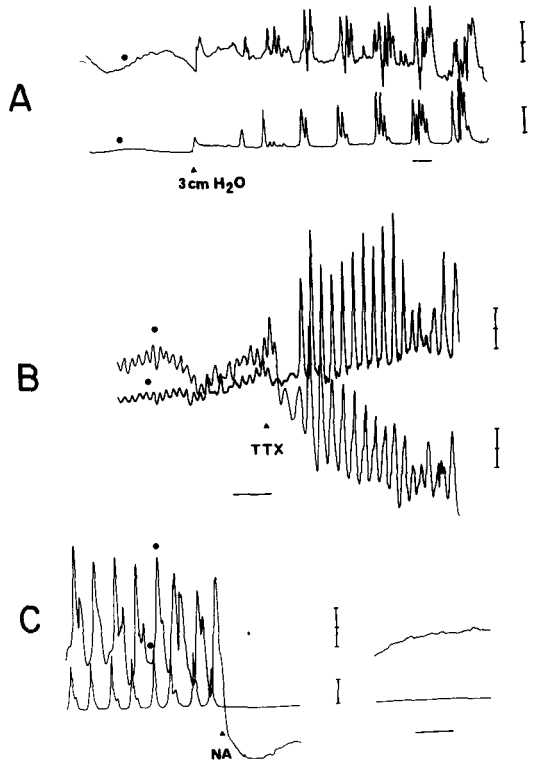


Fig. 9. Trendelenburg preparations of *Pleuronectes platessa* intestine. (A) Peristalsis induced by distension with 3 cm H_2O intraluminal pressure. (B) Change from peristaltic to myogenic activity following 7×10^{-7} g/ml tetrodotoxin (TTX). (C) Inhibition of myogenic activity, in the presence of TTX, by noradrenaline (NA) (10^{-5} g/ml). Upper trace in each figure is longitudinal muscle tension (scale = 1 g) and the lower is intramural pressure (scale = 5 cm H_2O). Time scale = 1 min. (From Goddard, 1975.)

the stomach or intestine of *Pleuronectes* with 10^{-7} g/ml tetrodotoxin prevents the onset of activity. Atropine, even in high concentrations (up to 10^{-5} g/ml) does not prevent the onset, suggesting that non-cholinergic excitatory neurons are involved. However, once activity has been generated, attempts to impair the neurogenic control of activity unmask the myogenic rhythms of many fish preparations as shown in Fig. 9B and Table IX. The amplitude, but not the frequency, of the myogenic activity of *Pleuronectes* and *Limanda* stomach increases with distension (Grove *et al.*, 1976) thereby suggesting a mechanism whereby the digestion rate of larger meals (Section VI) can exceed the predicted exponent of meal size. Ohnesorge and Rauch (1968) discuss

the possibility that myogenic activity is controlled by acetylcholine released from muscle cells themselves when stretched, as described in mammals (Ikeda *et al.*, 1958). On this basis, the potentiation by cholinesters or eserine and the depression by atropine, need not involve cholinergic neurons in the maintenance of fish peristalsis.

There is also general agreement that the intestine, and usually the stomach, is inhibited by sympathomimetic amines (Fig. 9C), although in *Salmo* and *Gadus* a paradoxical stimulation of gastric activity is well established. In *Gadus* the excitation is prevented by high doses (10^{-5} g/ml) of α-adrenoceptor blockers (S. Nilsson and Fänge, 1969). Stevenson and Grove (unpublished observations) examined the inhibition of gastric peristalsis in Trendelenburg preparations by sympathomimetic amines. It is likely that the receptors are of the beta variety since the order of potency of agonists is isoprenaline, adrenaline, phenylephrine (pD_2 = 7.8, 7.3, and 5, respectively). Of a wide variety of α- and β-adrenoceptor antagonists tested, only the β_2 blocker butoxamine (10^{-5} g/ml) clearly antagonized inhibition by these amines. The β receptors are likely to reside on the smooth muscle cells rather than enteric neurons, since in tetrodotoxin-treated stomachs exhibiting myogenic activity adrenaline abolished activity.

Other effective agents on active gut preparations include 5-hydroxytryptamine and adenosine triphosphate. 5-HT stimulates *Pleuronectes* stomach smooth muscle and the intestinal longitudinal coat. When applied to a nonactive stomach, whether before or after tetrodotoxin, activity is initiated apparently by a direct stimulation of muscle cells. 5-HT also enhances ongoing gastric peristalsis but inhibits intestinal peristalsis when presented from the serosal side only (Goddard, 1975). Adenosine triphosphate excites *Pleuronectes* stomach (Stevenson and Grove, unpublished observations) and *Carassius* intestine (Burnstock *et al.*, 1972) but inhibits intestinal peristalsis in *Pleuronectes* (Goddard, 1975).

3. Studies on Intrinsic and Extrinsic Nervous Control of the Teleost Gut

A number of workers have stimulated the gut wall electrically to analyze the role of enteric neurons in the control of activity. Saito (1973) and Ito and Kuriyama (1971) described a polyphasic reaction to transmural stimulation in *Carassius*. Saito observed a preliminary twitch (blocked by tubocurarine or atropine), a subsequent relaxation (unaffected by adrenergic or cholinergic blocking agents), and a final slow contraction (partly blocked by atropine but possibly involving a

"rebound contraction"). All phases were abolished by tetrodotoxin (10^{-8} g/ml) and presumably represent the activity of several types of nerve cells. Ito and Kuriyama found that field stimulation induced:

1. A fast twitch at higher frequencies (ca. 30 Hz), blocked by tetrodotoxin or *d*-tubocurarine and apparently caused by striated muscle fibers
2. A subsequent relaxation (3 Hz and above), blocked by tetrodotoxin (10^{-6} g/ml) but not affected by combined α- and β-adrenoceptor blockade with phentolamine and propranolol, each at 10^{-5} g/ml
3. A slow phasic contraction following phase 2
4. A delayed contraction following phase 3 and often lasting several minutes

Phases 3 and 4 were prevented by pretreatment with tetrodotoxin (10^{-6} g/ml) or atropine (10^{-6} g/ml) leading these authors to believe that they depend on cholinergic neurons and are not "rebound" contractions, following activity in the nonadrenergic inhibitory fibers responsible for phase 2.

Edwards (1972a) and Grove *et al.* (1974) found that transmural excitation of *Pleuronectes* stomach at 20–30 Hz caused a large contraction within a few seconds of the onset of stimulation and which is readily blocked by low doses of atropine (10^{-8} g/ml). Treatment with tetrodotoxin (10^{-7} g/ml) abolished this action, which is believed to represent the action of cholinergic motor nerves on longitudinal muscle. In contrast, when the stomach is prepared for recording from the circular coat as well (Trendelenburg preparation) a triphasic pattern of mechanical activity is recorded (Fig. 10A). A brief twitch (a) caused by striated muscle elements is followed by an atropine-sensitive primary contraction (b, cf. Fig. 10B) and a subsequent rebound contraction (c). The rebound is closely related to the cessation of stimulation when the period of excitation is varied between 5 and 60 secs, and is resistant to α and β-adrenoceptor blockers or to atropine, but is abolished by tetrodotoxin. Stimulation of the plaice vagus nerve (Edwards, 1972a) causes a powerful contraction of the longitudinal muscles within the period of stimulation which is strongly antagonized by atropine (10^{-9} g/ml) and less effectively by hexamethonium (10^{-6} g/ml) which has proven muscarinic blocking actions on this organ. A brief relaxation followed the primary contraction. When responses were recorded *in situ* using an intragastric balloon, the biphasic contractions of the smooth muscle coats (Fig. 11A) persisted when stimulation of the vagal roots was carried out intracranially. Lesions in the spinal cord

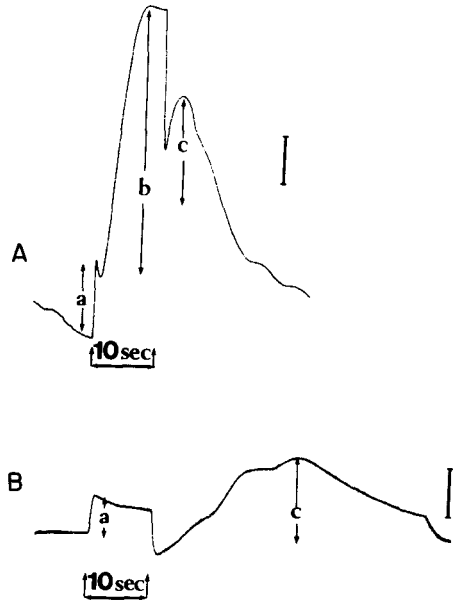


Fig. 10. Trendelenberg preparation of *Pleuronectes platessa* stomach. The fish was injected with reserpine (2 × 3 mg/kg, 72 and 24 hr previously) to abolish adrenergic nerve function. (A) Vagal stimulation at 6 V, 50 Hz, 1 msec pulse-width for 10 sec. (B) The same after 10^{-5} *M* atropine added to the bath (final conc.). Muscarinic blockade abolishes the "primary" cholinergic contraction (b), but leaves the striated muscle twitch (a) and rebound contraction (c) unimpaired. Vertical scale: 2 cm H_2O pressure; temp., 8°C. (From Stevenson and Grove, 1977, *Comp. Biochem. Physiol. C* **58**, 143–151.)

and medulla showed that this mixed innervation derives from the medulla, and not by way of a spinal/sympathetic pathway. Injection of the fish with atropine (1 mg/kg) (Fig. 11A), or bathing the vago-sympathetic ganglion with mecamylamine or hexamethonium (10^{-4} g/ml) abolished the primary contraction often unmasking an inhibitory phase. The plaice vagus apparently carries both excitatory and inhibitory tracts directly from the medulla. In the intact fish, the nerve exerts some inhibitory tone since Edwards (1973) found an acceleration in gastric emptying after vagotomy. Decrease in vagal tone may underly the change in gastric emptying rate when fish are fed low calorie diets (Section VI).

A separate extrinsic nerve supply has been found controlling plaice gastric peristalsis (Stevenson and Grove, 1978). In Trendelenburg preparations, stimulation of the splanchnic nerve at 15–30 Hz produces a biphasic response (Fig. 11B). The excitatory phase is

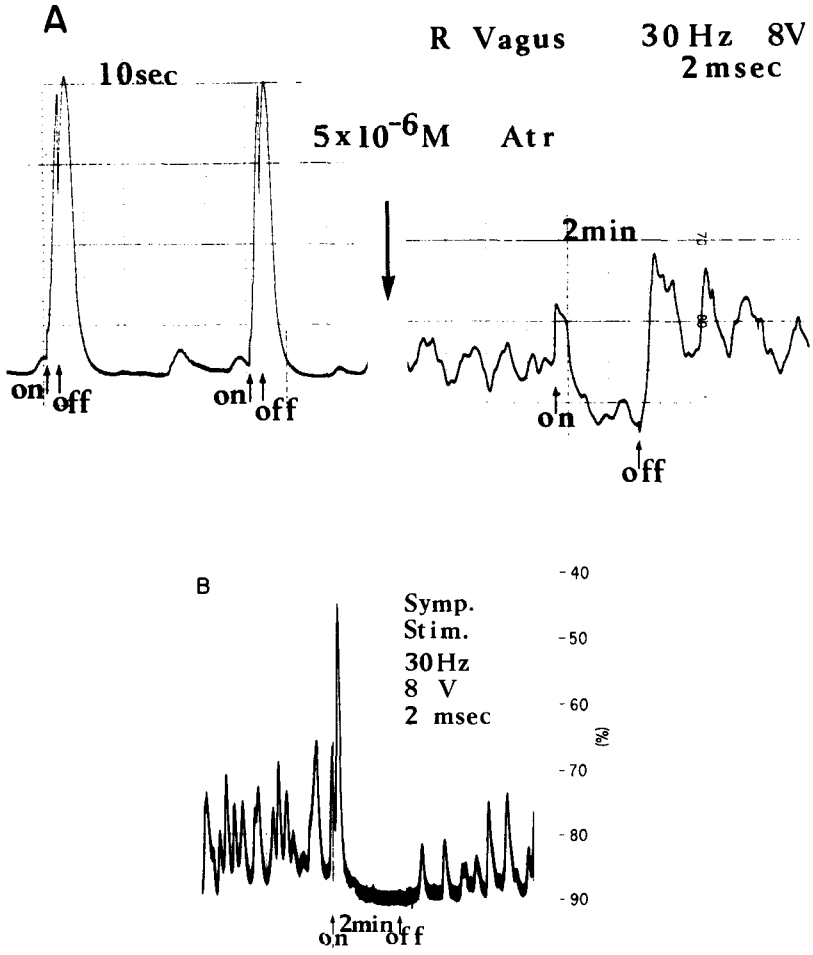
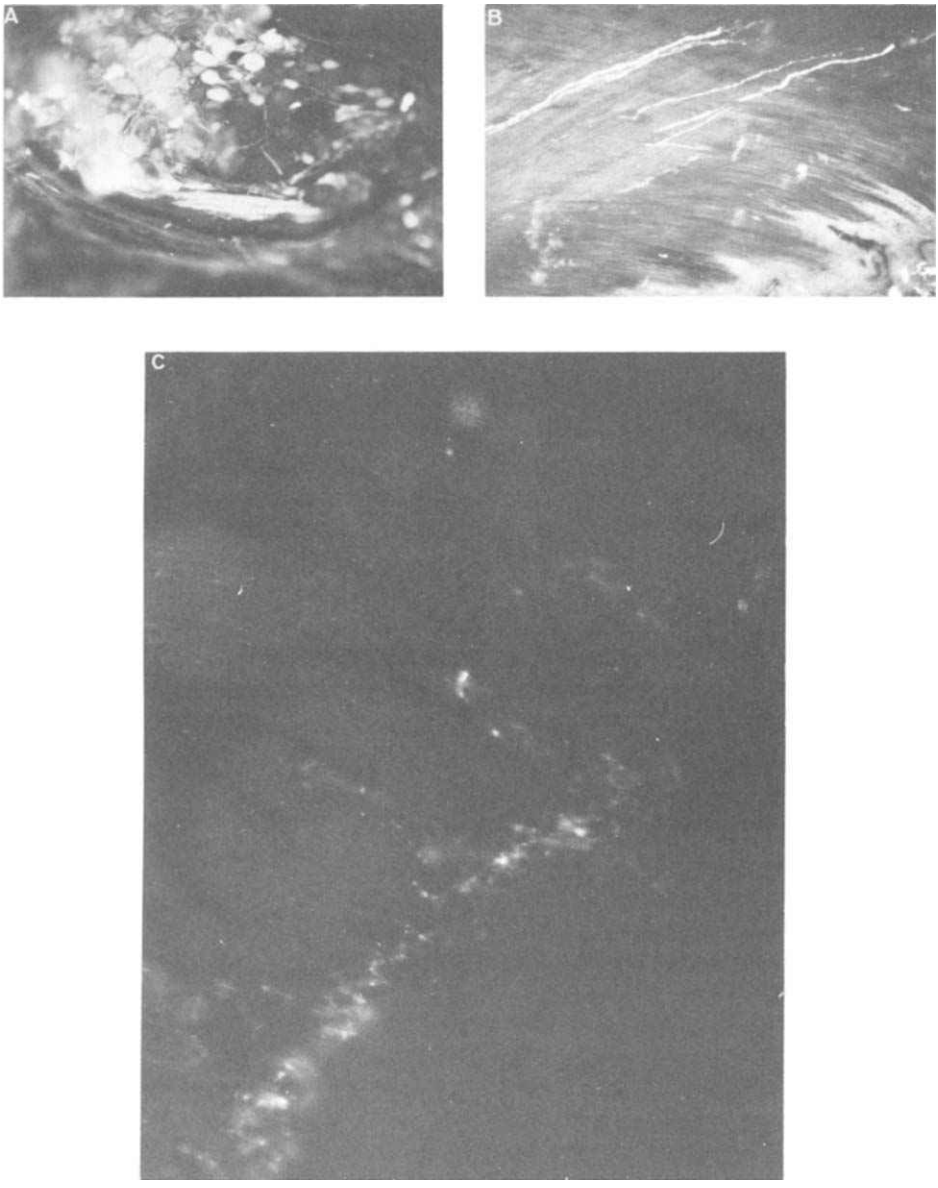


Fig. 11. Effects of stimulation of the extrinsic nerves on *Pleuronectes platessa* stomach activity, recorded by intragastric balloon. (A) Biphasic contraction after stimulation of the vagus nerve. Both primary and secondary ("rebound") contractions are seen. After atropine, excitation is abolished and vagal stimulation causes inhibition. (B) Biphasic response to stimulation of the splanchnic nerve, which carries both cholinergic excitatory and adrenergic inhibitory fibers. (From Stevenson and Grove, 1977, 1978.)

abolished by atropine (10^{-7} g/ml) and the inhibitory phase by reserpine pretreatment (5 mg/kg) or in the presence of butoxamine (3×10^{-5} g/ml). Adrenergic nerve terminals are present in the flatfish enteric plexus and circular muscle coat (Fig. 12) and extracts of the plaice stomach contain 0.05 μg/g adrenaline and 0.04 μg/g noradrenaline when measured by the method of Häggendal (1963) (Grove and S.



abolished by atropine (10^{-7} g/ml) and the inhibitory phase by reserpine pretreatment (5 mg/kg) or in the presence of butoxamine (3×10^{-5} g/ml). Adrenergic nerve terminals are present in the flatfish enteric plexus and circular muscle coat (Fig. 12) and extracts of the plaice stomach contain 0.05 μg/g adrenaline and 0.04 μg/g noradrenaline when measured by the method of Häggendal (1963) (Grove and S.

Nilsson, unpublished observations). Extensions of the splanchnic nerves supply the plaice intestine and mediate inhibition (Goddard, 1975). The effect is mimicked by adrenaline (10^{-10} g/ml) and other catecholamines. The intestine contains 0.06 μg/g adrenaline and 0.02 μg/g noradrenaline. However Goddard found a further nonadrenergic inhibitory system in the wall of the plaice intestine. Transmural stimulation relaxes the intestine (Fig. 13) but atropine, ganglion blockers, or α- and β-blockers failed to antagonize the response. Adenosine triphosphate (10^{-6} g/ml) both relaxes the intestine and desensitizes the receptors to transmural stimulation. The response is also blocked by tetrodotoxin, but not reserpine pretreatment, suggesting that intramural inhibitory purinergic neurons are involved.

As a measure of the complexity of the nervous coordination of gastrointestinal activity of teleosts, a comparison with *Salmo trutta* and *S. gairdneri* is revealing. In this more primitive teleost, Burnstock (1969) reinterpreted his early results on vagal stimulation, concluding that the vagus sends cholinergic excitatory nerves to the esophageal striated muscle but inhibits the stomach. The latter response is often accompanied by a strong "rebound" contraction, is unaffected by at-

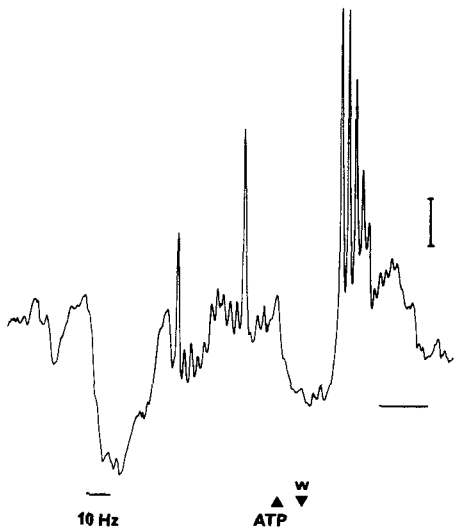


Fig. 13. Magnus preparation of *Pleuronectes platessa* intestine. The inhibitory effects of transmural stimulation (10 V, 10 Hz, 1 msec pulses for 60 sec) are compared with the response to 10^{-6} g/ml adenosine triphosphate. The time course of each relaxation is similar and each is followed by spontaneous "rebound" contractions. Vertical scale, 2 g; horizontal scale, 120 sec. (From Goddard, 1975.)

ropine at high doses (10^{-5} g/ml) and is only impaired by high doses of nicotine (10^{-4} g/ml) and hexamethonium (5×10^{-3} g/ml) which are probably nonselective. His claim that the response is dependent on preganglionic cholinergic fibers depends on the specificity of the above concentrations of ganglion blocking agents. Campbell (1975) showed that spontaneous stomach contractions in *Salmo gairdnerii* were inhibited by intracranial stimulation of the vagal roots (0.5–8 Hz), often accompanied by a large "rebound" contraction at the cessation of stimulation. More recently, Campbell and Gannon (1976) have shown that stimulation of the splanchnic nerve at 5–10 Hz contracts *Salmo* stomach, confirming the studies of Burnstock (1958a). The response was not affected by hyoscine (10^{-7} g/ml) but was abolished by bretylium ($1–3 \times 10^{-6}$ g/ml) and reduced by *d*-tubocurarine (3×10^{-5} g/ml). Adrenergic fibers were detected histochemically in the splanchnic nerve which proceed to form a plexus in the stomach wall and, in view of the excitatory effect of catecholamines described earlier, are believed to mediate the excitation. Excitation of the posterior splanchnic nerve (Burnstock, 1958a) inhibits or excites the rectum depending on the frequency of stimulation. He proposed that both cholinergic and adrenergic nerves are involved, and the latter have been detected histochemically (Read and Burnstock, 1968a,b, 1969).

Clearly the roles of the extrinsic nerves to the stomach and intestine of *Pleuronectes* and *Salmo* are very different. It is not yet possible to distinguish the roles of intramural neurons which may be linked with, or independent of, extrinsic nerve tracts. The control of peristalsis in *Tinca* is also different in that the vagus in this stomachless fish extends its influence to the intestine (Mahn, 1898) and the gut wall consists of the usual smooth muscle coats together with two layers of striped muscle (circular and longitudinal) with several nerve cell plexuses (Baumgarten, 1965, 1967). In addition to the left and right *rami vagi intestinales,* which exert cholinergic excitatory influences on all muscle coats, a splanchnic nerve originating from the right sympathetic chain innervates the intestine. Baumgarten (1967) also showed that this sympathetic supply carries fluorescent nerves which innervate the muscle layers and which apparently contain dopamine, as judged by the thionylchloride test of Corrodi and Jonsson (1967), and which inhibit the intestine. He also obtained fluorescent histochemical evidence for the presence of 5-hydroxytryptamine in some nerves and pointed out that this amine contracts smooth muscle of the tench intestine. Saito (1973), using the fluorescent technique in *Carassius* intestine, also detected adrenergic nerves which he proposed inhibit spontaneous activity.

4. *In Vivo* STUDIES

Very few attempts have been made to analyze the control of peristalsis in living, feeding fish. Burnstock (1957) observed *in situ* peristalsis of *Salmo trutta* by implanting an abdominal window. Edwards (1973) observed that carbachol dramatically accelerated the gastric emptying of a 1% meal of *Arenicola* by *Pleuronectes* whereas atropine delayed gastric emptying. Goddard (1974) found that intestinal transit time was also shortened by carbachol and extended by atropine when these were injected at the appropriate time, but that this region of the gut was less sensitive than the stomach to these drugs. In a separate study, Goddard (1973) demonstrated similar actions of these agents in the stomachless *Blennius pholis*. After 7 days food deprivation, *Pleuronectes* (300 g at 15°C) did not show impaired efficiency in transporting food through the gastrointestinal tract but during the subsequent weeks the transit time slowly increased as the physiological effects of starvation developed.

Gzgzyan *et al.* (1973) examined *in vivo* gastric contractions in *Scorpaena porcus* using a balloon to record activity. In addition to concluding that the stomach was under excitatory cholinergic control, they showed that extracts of the pituitary gland of *Scorpaena* contained a powerful gastric inhibitor, reminiscent of the inhibitory action of oxytocin in mammals. In the dog, it has been found that injected insulin at first depresses gastric activity but then, as hypoglycemia develops, vagal centers in the medulla activate gastric activity. In *Scorpaena*, insulin injections depressed stomach activity but no compensatory increase accompanied the ensuing hypoglycemia.

E. Summary

Figure 14 represents a generalized account of the mechanisms controlling teleost gastrointestinal motility. Longitudinal and circular coats of smooth muscle fibers develop spontaneous, myogenic activity when the gut is distended. In *Pleuronectes*, noncholinergic excitatory nerves are necessary to induce this response. Peristalsis depends on intramural neurons (cholinergic and noncholinergic excitatory; noncholinergic/nonadrenergic inhibitory) in synaptic connection with sensory and other neurons in Auerbach's plexus. In flatfish, but to a lesser extent in salmonids and cyprinids, peristalsis in isolated preparations is mainly myogenic, similar to that described by Yung *et al.* (1965) for the tortoise *Amyda japonica*. Localized release of 5-hydroxytryptamine generally enhances activity. Cranial vagal fibers

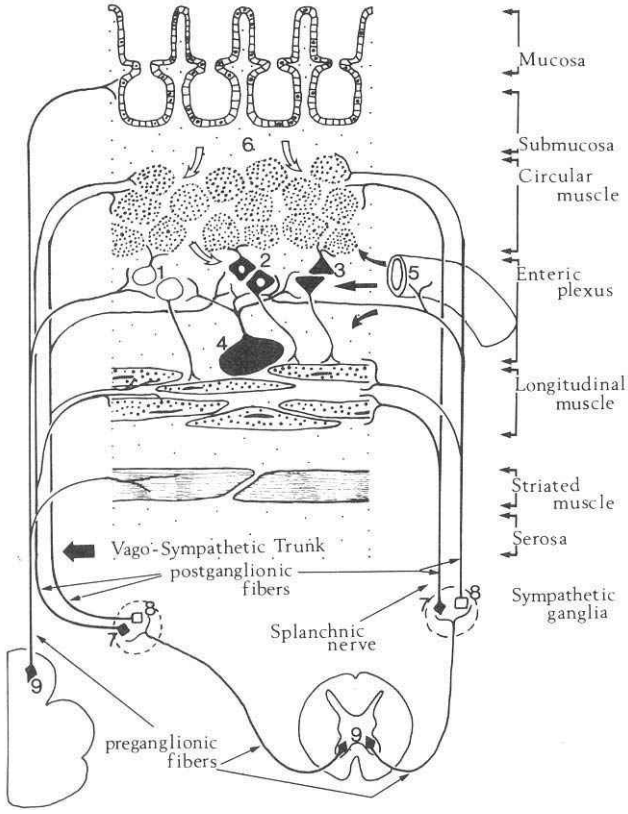


Fig. 14. Diagram of the intrinsic and extrinsic nerve fibers believed to control the muscularis of the teleost stomach, together with sites of action of circulating catecholamines. (1) Nonadrenergic, noncholinergic inhibitory neurons; (2) cholinergic excitatory neurons; (3) nonadrenergic, noncholinergic excitatory neurons; (4) stretch receptors; (5) circulating adrenaline; (6) endogenous 5-hydroxytryptamine; (7) cholinergic excitatory neurons of postganglionic, sympathetic origin; (8) adrenergic inhibitory neurons of postganglionic, sympathetic origin; (9) preganglionic cholinergic neurons from central nervous system. Notes: (a) striated muscle is not always present in the stomach and intestine; (b) in *Salmo*, element 8 to the stomach is excitatory; (c) in *Pleuronectes* an additional element 9 in the vagus synapses with excitatory cholinergic neurons in the jugular ganglion; (d) a vagal element is suggested which controls gastric gland secretion. The intestine is controlled by elements 1–8 but, in stomachless fish, vagal influences may extend to this region.

in fish primarily activate noncholinergic intramural inhibitory fibers. Postganglionic cholinergic excitatory fibers reach the stomach in the splanchnic nerve and, in some acanthopterygians, in the vagal pathway to innervate muscle cells directly. Adrenergic neurons reach the

stomach and intestine through the splanchnic route and enmesh ganglion cells in Auerbach's plexus as well as entering the muscularis. Catecholamines from the head kidney (e.g., Grove *et al.*, 1972; Nilsson *et al.*, 1976) generally inhibit activity, even in denervated preparations, but the clear gastric excitation which occurs in elasmobranchs and some teleosts requires further analysis.

It will be obvious from the account given in this section that the present knowledge of gastrointestinal motility and its control in fish is sparse. The onus is on the comparative physiologist to undertake better designed studies if the complexity of the system is to be unraveled. The present authors believe that, for a variety of fish species, careful characterization of tissue receptors and the affinity of agonists and antagonists is long overdue. The doses of antagonists which are *selective* against candidate neurotransmitter substances must be determined. Only then can drugs be used as tools in the study of coordinated peristalsis. The researcher must recognize that both neurogenic and myogenic rhythmic contractions may be involved in the transport of ingested food. Mechanical recordings should be accompanied by electrical measurements of the activity of elements in the gut wall. Detailed study of the ganglion cells of the enteric plexus, including their neurotransmitters, and the origin and distribution of extrinsic nerves should be undertaken. No mention has been made here of the sensory connections of the gut to the central nervous system, although it appears from Section VI that such nerves play a significant part in appetite of fish. Windell and Norris (1969a,b) proposed that the production of enterogastrone in response to high energy food may delay the gastric emptying phase and yet the available data on this and other local gastrointestinal hormones is almost nonexistent. Although motor nerves of various origins impinge on the gastrointestinal tract, and hormones of the adrenal medulla, pituitary, and other endocrines are also likely to affect motility and secretion, very few attempts to investigate their significance to the fish by *in vivo* experiments have been undertaken. Kerkut (1976) has mentioned some of the important contributions to physiology made by comparative pharmacologists; a fuller understanding of the processing of food by the gastrointestinal tract is likely to contribute significantly to the husbandry of fish.

VIII. PERSPECTIVES

In Section VI it is apparent that digestion rate is related to the natural diet. The return of appetite following a meal is to a large extent

controlled by the rate of stomach or foregut emptying (Table V). The physiological data presented in Section VII suggest that, when a fish in culture is presented with artificial foodstuffs, the rate of gastrointestinal activity can be changed by including a stimulant or inhibiting drug in the diet. In this way a faster or more efficient rate of food utilization could be obtained, supplemented by further additives (such as steroids) which promote anabolism. Appropriate balance of nutrients, such as amino acids or oligosaccharides, which compete for the active transport sites in the gastrointestinal epithelium, may increase assimilation efficiency. Incorporation in the artificial diets of exogenous enzymes which become active when the gastrointestinal juices are absorbed may allow the fish to overcome the "surface area" limitation to digestion rate. Environmental conditions which minimize the circulation of adrenaline or discharge of the adrenergic nerves ("stress") will allow peristaltic rate and visceral blood flow to be at a maximum. There is a case for incorporating anti-adrenaline agents or sedatives to the diet during "thinning-out" operations on the fish farm, to allow rapid adjustment to the new environment and early return of feeding. This latter suggestion may be most appropriate for marine fish which frequently tolerate crowding to a lesser extent than freshwater fish. These, and similar factors which may emerge, will be additional to improvements in fish production brought about by genetic selection, control of disease, diet formulation, and control of environmental variables such as temperature and photoperiod.

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