structure and function of the digestive tract of the grasscarp

cover photograph: Pharyngeal teeth of the grasscarp.
Usually, a well developed pharyngeal masticatory apparatus is present in stomachless fish. The food is fragmented between the ventral teeth (photograph) and a dorsal masticatory plate. Scanning electron.micrograph x 45.

Promotor: dr. L.P.M. Timmermans, hoogleraar in de algemene dierkunde

Co-Promotor: dr. J.W.M. Osse , hoogleraar in de algemene dierkunde

HENRI W.J. STROBAND

STRUCTURE AND FUNCTION OF THE DIGESTIVE TRACT OF THE GRASSCARP

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr.H.C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op woensdag 10 september 1980
des namiddags te half drie in de aula
van de Landbouwhogeschool te Wageningen

CONTENTS/INHOUD

STELLINGEN

1. Er zijn geen argumenten voor de veronderstelling dat bij juveniele en adulte maagloze vissen de eiwitvertering in het darmlumen minder effektief zou zijn dan bij maaghoudende soorten.

Dit proefschrift.

2. De opvatting van Trevisan, dat vooral het caudale deel van de graskarperdarm betrokken is bij de resorptie van nutriënten, is onjuist en komt voort uit het trekken van voorbarige konklusies uit alleen morfologische informatie.

Trevisan, P. : Anat. Anz. *145*, 237-248 (1979).

3. Het voorstel van Green & Phillips, graanmutanten te kweken met verminderde feedbackinhibitie van aspartaatkinase door lysine en threonine, om zo het gehalte aan één of meer aminozuren uit de aspartaat-familie te vergroten, verdient stellig waardering maar lijkt geen praktische bijdrage tot de oplossing van het wereldvoedselvraagstuk te kunnen leveren.

Green, C.E. & Phillips, R.L. : Crop Science *14*, 827-829 (1974).

4. De circadische fluktuaties in gevoeligheid van diverse weefsels voor bepaalde hormonen suggereert dat hormoonreceptoren een korte levensduur hebben.

Meier, A.H. ; John, T.M. & Joseph, M.M. : Comp. Biochem. Physiol. *40A*, 459-465 (1971).
Meier, A.H. ; Trobec, T.N. ; Joseph, M.M. & John, T.M. : Proc. Soc. Exp. Biol. and Med. *37*, 408-415 (1971).

5. De isolatie van zowel één glycoproteinerijk- als één glycoproteinearm gonadotroop hormoon uit hypofyses van beenvissen ondersteunt de opvatting dat "globulaire" en "vesiculaire" P.A.S.- positieve gonadotrope cellen tot hetzelfde celtype behoren, maar sluit niet uit dat er een tweede gonadotroop celtype, mogelijk niet P.A.S.- positief, aanwezig is.

Ng, T.B. & Idler, D.R. : Gen. Comp. Endocrinol. *38*, 410-420 (1979).
Peute, J. ; Goos, H.J.Th. ; de Bruin, M.G.A. & van Oordt, P.G.W.J. : Ann. Biol. Anim. Bioch. Biophys. *18*, 905-910 (1978).
Ueda, H. : Bull. Fac. Fish. Hokkaido Univ. *31*, 1-15 (1980).

6. Het zou van groot belang voor de wereldvrede kunnen zijn, onderzoek te verrichten naar de oorzaken van het verschijnsel dat achteruitgang van de levensstandaard veelal leidt tot toename van onredelijk vijand-denken en stijgende wapenproduktie, en in dit verband onder andere aandacht te schenken aan de mogelijke rol van het "Militair Industrieëel Komplex" .

7. De boycot van de Olympische Spelen in Moskou, waarvoor de verschillende redenen op zichzelf terecht werden aangevoerd, doet vermoeden dat wij, westerlingen, het restant van de boterberg die aan de Sovjetunie is verkocht op ons hoofd hebben gesmeerd in plaats van ook onszelf boter op het brood te geven.

8. De "vertrossing" van de omroepen leidt er toe dat de afleiding die wordt gebracht de bevolking afleidt van de meest wezenlijke problemen van onze samenleving.

9. Het feit, dat de vervoersorganisaties bezwaar maken tegen het gebruik van "hun" belastinggelden ten behoeve van de aanleg van fietspaden betekent dat de wegvervoerders te weinig oog hebben voor de fietsende medemens en onderstreept derhalve de noodzaak van genoemde wielerpaden.

10. De "brede maatschappelijke discussie" over kernenergie zal, wanneer het kabinet zich niet onthoudt van voorbarige uitspraken, vergelijkbaar blijken met een golfstroom : oeverloos en met voorspelbare bestemming.

Proefschrift van H.W.J. Stroband
Structure and function of the digestive tract of the grasscarp.
Wageningen, 10 september 1980.

of protein absorption during the transport of food in the intestine of the grasscarp, *Ctenopharyngodon idella* (Val.) Submitted to J. Fish Biol .....

E. Stroband, H.W.J. & Annet G. Kroon. The development of the stomach in *Clarias lazera* and the absorption of protein macromolecules. Submitted to Cell Tiss.Res.....

URRICULUM VITAE

IJLAGE :

Stroband , H.W.J., J.H.W.M. Rombout & J.H.M. Davina. Maagloze vissen; Bouw en functie van het darmkanaal. Natuur en Techniek *48*, 38-55 (1980)

Voorwoord

De natuur heeft op mij altijd een grote aan trekkingskracht uitgeoefend. Nieuwsgierigheid en verwon dering hebben daarbij altijd een hoofdrol gespeeld. He bewerken van een onderzoek in een natuurwetenschapp lijke richting biedt een gelegenheid om met enige diep gang bezig te zijn met een klein facet van het Grote Gebeuren dat de natuur stellig is. Wat mij betreft heeft dit geleid tot een toenemende eerbied voor datgene wa voor mij de Schepping is, op welke wijze deze ook tot stand mag zijn gekomen.

Mijn kennismaking met wetenschappelijk onderzoek heeft me heel sterk de relativiteit van de menselijke ken nis doen ervaren en heeft me in veel opzichten teruggewor pen tot het nivo van zien, horen, voelen, ruiken en verba zen. Gevoelens van verwondering, eerbied en dankbaar heid treden op de voorgrond en het zijn deze gevoelens waaraan ik in de eerste plaats uitdrukking wil geven in dit voorwoord, dat vooral een dankwoord is.

Graag wil ik van de mij nu geboden gelegen - heid gebruik maken om iedereen te bedanken die, direc of indirect, een rol heeft gespeeld bij mijn vorming, studie, of het tot stand komen van dit werkje. Lang niet alle namen kan ik in dit bestek noemen, maar ik hoop dat zij die het aangaat zich aangesproken we ten.

In de eerste plaats ben ik mijn ouders dank verschuldigd, vooral ook omdat ze mij van jongs af aan een grote vrijheid van denken en handelen ga ven, waardoor mijn kans op ontplooiing sterk werd ver groot. Verder heeft hun directe steun in woord en middelen mij ook later gemotiveerd en mijn studie m gelijk gemaakt.

Evenals mijn ouders wil ik mijn schoonouders danken voor de bijzondere wijze waarop zij altijd inge

sprongen zijn om mij, ondanks het leven in een erg jong gezin, de gelegenheid te geven optimaal te studeren.

Van de vele vrienden met wie ik waardevolle en duurzame kontakten heb mogen hebben wil ik hier de familie Zeelenberg noemen die, door haar gastvrijheid en discussie-zin ongetwijfeld grote invloed op mijn ontwikkeling heeft gehad. Wim Feyten, onze gesprekken waren en zijn voor mij van grote betekenis.

Ten aanzien van het hier beschreven onderzoek ben ik in de eerste plaats dank verschuldigd aan mijn promotor, Lucy Timmermans, en co-promotor, Jan Osse. Zij hebben er met grote inzet voor geijverd een vakgroep te formeren die, mede door de inbreng van onderzoekers op individu-, weefsel- en cel nivo, een plaats kan zijn waar ook een onderzoek als het onderhavige zinvol kan worden uitgevoerd. Verder dank ik hen voor de grote mate van vrijheid die mij t.a.v. het onderzoek gelaten werd, en voor de zeer konstruktieve wijze waarop we van gedachten hebben kunnen wisselen over de inhoud van de verschillende manuskripten.

De kollega's van de vakgroep Experimentele Diermorfologie en Celbiologie, die door hun kritische zin een positieve bijdrage hebben geleverd aan het onderzoek, wil ik hier als groep bedanken. Een enkele uitzondering maak ik voor Jan Rombout: wij kunnen terugzien op zeven jaar naar ik meen fijne en vruchtbare samenwerking.

Anja Thiele en Hans van der Meer moet ik noemen in verband met de belangrijke bijdragen die ze door hun kwalitatief hoogstaande technische hulp hebben kunnen leveren. Felix Debets, Han Schipper, Gertjan de Graaf, Annet Kroon en Erik van der Veen hebben als doctoraal studenten de voortgang van het onderzoek in belangrijke mate mede bepaald. Dit laatste geldt ook voor de stage-analisten" die voor korte tijd aan het onder-

zoek hebben meegewerkt. Overigens zou één en ander niet mogelijk geweest zijn zonder de voortreffelijke verzorging die onze biotechnikus Sietze Leenstra de proefdieren heeft gegeven.

Van de collega's buiten de vakgroep die bijdroegen tot het onderzoek wil ik noemen de heren J. van den Hoek (Fysiologie der Dieren), die "zijn" isotopenlaboratorium gastvrij voor ons openstelde; E.A. Huisman die als medewerker van de Organisatie ter Verbetering van de Binnenvisserij de meeste graskarpers leverde waaraan het werk is verricht; Vonk (C.A.B.O.) die, evenals de heer A.W.S.M. van Egeraat (microbiologie) tot onze grote dankbaarheid bereid was de vele aminozuuranalyses uit te voeren en tenslotte de heer C.J.J. Richter (Visteelt en Visserij) die ons de Clarias leverde voor onderzoek naar de ontwikkeling van de maag.

Het vele werk dat Wim Valen verrichtte ten behoeve van de illustraties in dit proefschrift waardeer ik bijzonder. De heer H.G. Eleri (T.F.D.L.) dank ik voor de verzorging van het E.M. fotomateriaal.

Dank ben ik ook verschuldigd aan hen die het vele typewerk voor hun rekening namen: Willy Tapilaha, Hanny Vertregt, Gerda van Malsen, Nel van Cappelle en mejouw P. Treffers (Tekstverwerking).

De inspanningen van de heer L. Boomgaart ter korrektie van mijn (school-) "engels" heb ik bijzonder op prijs gesteld.

Marijke, naast jouw rol als steun en toeverlaat heb je de barensweeën, gepaard gaande met het tot stand komen van dit proefschrift, in hoge mate beperkt door jouw bereidheid de eerste paar versies van de verschillende manuskripten uit te tikken. Je wilt niet dat ik je daarvoor bedank, maar wij spreken elkaar nog wel nader.

Henri

GENERAL INTRODUCTION

A : KADERSTELLING

Het in dit proefschrift beschreven onderzoek werd gestart in 1973 binnen de toenmalige afdeling Dierkunde van de Landbouwhogeschool waarvan de leiding berustte bij prof. dr. J.W.M. Osse en mevr. dr. L.P.M. Timmermans. In die periode werd een aantal jonge biologen aangetrokken in verband met de in 1970 gestarte studierichting Biologie aan de Landbouwhogeschool. Het is dan ook niet verwonderlijk dat het onderzoek van meet af aan beïnvloed werd door drie faktoren:

1. Het moest toegankelijk zijn voor doctoraal studenten, niet alleen in de biologie maar ook in bijvoorbeeld voeding en (vee)teeltwetenschappen. Daarom diende het voldoende breed te worden opgezet.
2. Het diende te passen binnen het kader van het onderzoek van de Landbouwhogeschool, dat wil zeggen dat "landbouwkundige" aspekten o.i. gewenst waren en het onderzoek daarom niet al te zeer zuiver wetenschappelijk van karakter moest zijn.
3. De vele op te zetten prê doctorale onderwijs elementen eisten relatief veel tijd op, in de eerste jaren zelfs praktisch 100%. zodat het onderzoek een trage start ondervond.

De derde faktor leidde er toe dat er gedurende een lange periode kon worden nagedacht over de invulling van de onderzoekstaak van de afdeling, de eerste twee faktoren bepaalden mede de richting waarin gedacht werd.

Bij enkele van de betrokken medewerkers bestond ervaring in het onderzoek met vissen. De wetenschap dat vissen een belangrijke voedselbron (kunnen gaan) vormen in vele landen naast het feit dat fundamenteel onderzoek aan deze lagere gewervelde dieren aan de Landbouwhogeschool ontbrak hebben er toe geleid dat van meet af aan vissen als proefdieren het meest in aanmerking leken te komen.

Tenslotte werd gekozen voor o.a. onderzoek naar de voedselopname en -verwerking bij vissen, waarbij morfologisch onderzoek t.a.v. de voedsel verwerving werd uitgevoerd binnen de sectie Functionele Morfologie terwijl binnen de sectie Histologie/Ontwikkelingsbiologie onderzoek werd verricht naar bouw en funktie van het darmkanaal. Dit laatste onderzoek bestond uit een tweetal

projecten, waarin respectievelijk vorm en functie van het darmepitheel en aspecten van endocriene regulatie, met name structuur en funktie van hormoon producerende cellen,de aandacht hadden (Rombout).

Van de diverse takken van onderzoek die zich later (met name na de splitsing van de afdeling Dierkunde in een voorlopige vakgroep Experimentele Diermorfologie en Celbiologie en een voorlopige vakgroep Dierkunde) binnen de vakgroep E.D.C. ontwikkeld hebben is er één zeer duidelijk aan het darmonderzoek gerelateerd, namelijk het onderzoek naar de ontwikkeling van het immuun-apparaat bij vissen (v. Muiswinkel). De relatie met dit binnen de sectie Celbiologie bewerkte projekt ligt vooral in de waarschijnlijk niet onaanzienlijke rol die afweercellen in het darmepitheel spelen bij het voorkomen van infekties via het (maagloze) spijsverteringskanaal van de door ons onderzochte proefdieren.

B : FORM AND FUNCTION OF THE DIGESTIVE TRACT IN TELEOSTS.

1. Morphology

a. Mouth cavity, pharynx and esophagus.

The digestive system of teleosts is not basically different from that of other vertebrates including mammals. In a number of respects, however, its structure is a simplified one. Digestive and absorptive functions seem to be carried out by a lesser diversified morphological system.

Mouth cavity and pharynx in aquatic vertebrates are for continuous respiratory and discontinuous feeding functions, more so than in terrestrial vertebrates. Gills and associated structures occupy nearly the whole pharynx (fig. 3). Taste buds in the pharyngeal epithelium are numerous in many fish species (Curry, 1939; Mc Vay & Kaan, 1940; Girgis, 1952; Kapoor et al., 1975[a]; Sinha, 1976[a]; Sinha & Moitra, 1975[a]; fig. 1, 2). No salivary glands have been found (Fahrenholz, 1937). The position of the mouth, the presence or absence of teeth on the jaws, vomer, palatines and pharyngeal bones, the morphology of the gill rakers, and other characteristics are closely related to the feeding habits. Reviews on this subject have been presented by Suyehiro (1942) and Kapoor et al. (1975[b]).

The esophagus is usually lined with a squamous epithelium, just as the pharynx (fig. 2). Its surface shows concentric microridges, just as epithelial cells in the skin of teleosts (Merrilees, 1974; Reutter et al., 1974). In the epithelium, mucous cells are abundant and tastebuds may be present (Chitray, 1965; Sinha, 1976[b]; Sinha & Moitra, 1975[a]; Verigina, 1976; Moitra & Ray, 1977). Esophageal multicellular glands are not common (Kapoor, 1975[b]).

b. Stomach.

In about 85% of all teleost species the esophagus leads into the stomach. The other 15% of the bony fishes do not have a stomach (Jacobshagen, 1937); the esophagus enters the intestine directly. The same applies to the larval stages of most species of fish (Balon, 1975), when they take exogenous food although their stomach has not yet developed.


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The walls of the stomach and the intestine consist of similar layers of tissue as found in higher vertebrates ,but the intestinal mucosa lacks a muscularis mucosae (Ciullo, 1975; Korovina, 1976).

The stomach usually shows two distinct sections: a corpus part with a lining of mucous-producing cells with underlying gastric glands, and a pyloric part without gastric glands (Mohsin, 1962; Kapoor et al., 1975[b]; Moitra & Ray, 1977). The glands are formed by only one type of cell; no distinction can be made between pepsin producing cells and oxyntic cells. It has been shown that corpus gland cells produce pepsin as well as hydrochloric acid in bony fishes (Blake, 1936; Barrington, 1957; Bucke, 1970; Verma & Tyagi, 1974; Moitra & Ray, 1977; Noaillac Depeyre & Gas, 1978). The same applies to all nonmammalian Vertebrates (Smit, 1968).

c. Pyloric caecae

Pyloric caecae are found in many teleosts with a stomach. The histology is not different from that of the intestine and this suggests that their primary role is to enlarge the intestinal area (Moitra & Ray, 1977).

d. Intestine.

In stomachless fish and in fish larvae, the first part of the intestine is a widened tube, called intestinal bulb. It is assumed to have a storage function (Babkin & Bowie, 1928; Mc Vay & Kaan, 1940; Berry & Low, 1970; Verigina, 1978[b]). In the bigmouth buffalo, Verigina (1976) found an intestinal bulb with a very thick partly striated muscularis. This may be related to the mechanical processing of food, and might be seen as an adaptation to the poorly developed pharyngeal teeth and the absence of a pharyngeal plate in this species.

The bile and pancreatic ducts, generally located closely together,join the intestinal bulb at a short distance from the entrance of the esophagus (Rogick, 1931; Curry, 1939; Girgis, 1952; Noaillac-Depeyre & Gas, 1976; Verigina, 1978[b]).

Fig. 1. Scanning electron micrograph of the rostral part of the buccal cavity of a 6-months old grasscarp (x 105). L = dorsal lip; V = valve prohibiting outflow of water during the expiratory phase of respiration. R = roof of the buccal cavity with concentrations of taste buds (arrows), also present in the area directly behind the lip.

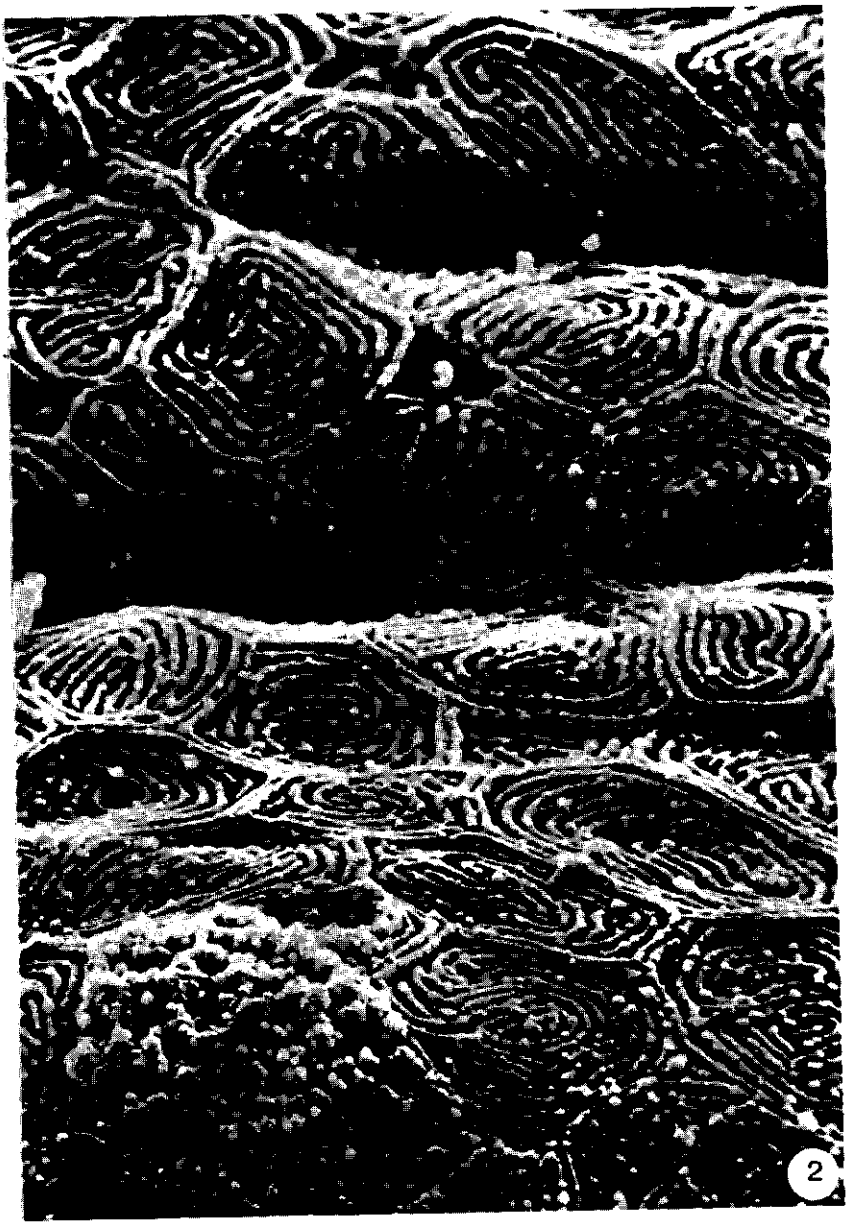


fig. 2. Scanning electron micrograph of part of the roof of the pharynx. Note the presence of concentric microridges on epithelial cells, possibly facilitating gas exchange of the cells and/or holding mucous at the cell surface (Reutter et al., 1974). In the left lower corner, a taste bud (x 5250).

Morphologically, no clear regional differentiation was made in the intestine of teleosts with a stomach. Some species have a rectum, set apart from the rest of gut by some kind of valve or folds (Jacobshagen, 1937; Ciullo, 1975).

e. Histology of the intestinal mucosa

The mucosa of the teleost intestine shows a more or less complex pattern of folds (fig. 4). Villi have never been observed, multicellular glands and crypts of Lieberkühn, present within the wall of parts of the mammalian intestine, are absent in teleosts, except in the family *Gadidae* (Jacobshagen, 1937; Klust, 1940; Bishop & Odense, 1966). The epithelium is a simple columnar one and contains three epithelial cell types. Absorptive enterocytes bear microvilli (fig. 6) and cilia have been found in a few species (Barrington, 1957; Iwai, 1967[a, b]; Bucke, 1970; Verigina, 1978[a]). Furthermore the epithelium contains mucous goblet cells (fig. 3, 4) and enteroendocrine cells.

Between the three epithelial cell types, migrating cells may be present (Rogick, 1931; Girgis, 1952; Bullock, 1963; Hale, 1965; Smit, 1968; Bucke, 1970; Krementz & Chapman, 1974; Weinberg, 1975; Davina et al. 1980). These are probably lymphocytes, macrophages and granular leucocytes. The presence of socalled "pear-shaped cells" or "rodlet cells" in the epithelium has been reported for many teleost species. Some authors are of the opinion that these cells are in fact protozoan parasites (*Rhabdospora thelohani*) (Bannister, 1966; Iwai, 1968). Al Hussaini (1949[b], 1964) mentioned the possibility of rodlet cells being developing mucous cells, but this is not correct (Hirji & Courtney, 1979). Catton (1957) suggested that the rodlet cells are granulocytic leucocytes, but concentrations in bloodcell-producing organs were never found. Many recent authors consider the rodlet cells as unicellular glands (Leino, 1974; Grünberg & Hager, 1978; Mattey et al., 1979).

f. Cytology of absorptive cells.

The general morphology of the absorptive enterocytes of bony fishes shows a striking resemblance with that of higher vertebrates. Similar organelles are found. Minor differences are the lack of complex interdigitations

fig. 3. Lateral part of the pharynx floor, with two branchial arches, gi
filaments (F) and gill lamellae (L). On the branchial arches the
gill rakers (R) (x 100). Inset: Taste buds (arrows) on the gill
rakers (x 700).

of the lateral plasma membranes of adjacent cells, and the presence of lamellar infoldings of the plasma membrane in the basal part of the cells in teleosts (Yamamoto, 1966). These infoldings appear to be similar to those described for the "basal labyrinth" of proximal tubule cells of the kidney in mammals. A function in osmoregulation has been suggested (Yamamoto, 1966; Noaillac-Depeyre & Gas, 1973[b]).

In stomachless fish three segments can be distinguished, on the strength of the morphology of the absorptive cells, and this morphology is directly related to the absorption of food. Therefore, some information about the digestive enzymes and absorption in teleosts should be discussed before dealing with the regional differentiation.

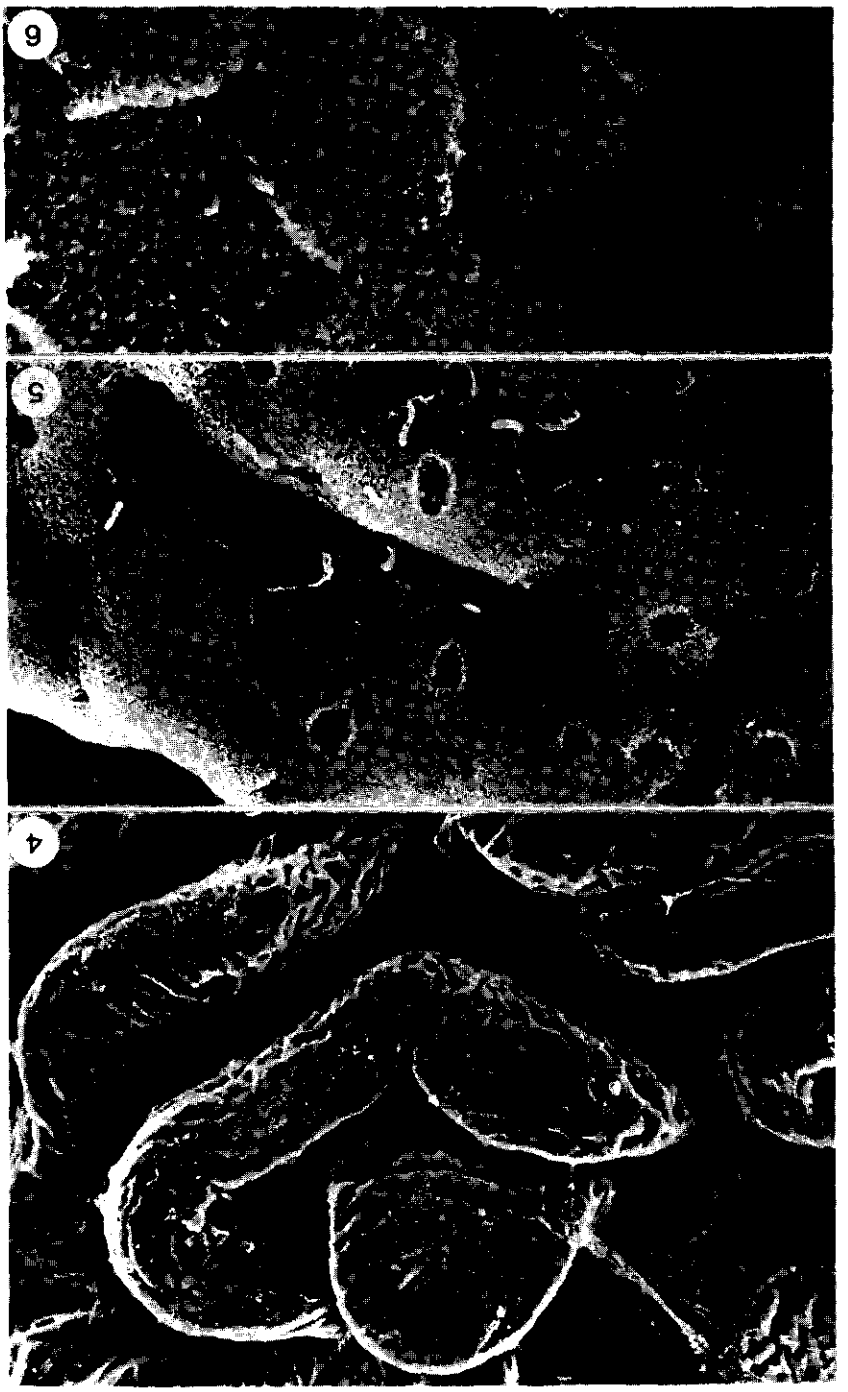
2. *Physiology*

a) Digestive enzymes

The enzymes in the intestinal lumen of bony fishes are essentially similar to those found in mammals. In theory they are produced in the pancreas, the gastric mucosa or the intestinal mucosa (including pyloric caecae). Production by the intestine is doubtful (Kenyon, 1925; Jany, 1976), although Kapoor et al., (1975[b]) are of the opinion that the main protein-, carbohydrate- and fat digesting enzymes are also produced by the pyloric caecae and intestinal mucosa. It is more likely, however, that enzyme molecules, derived from the pancreas, have the tendency to accumulate in the glycocalix of the enterocytes (Fänge & Grove, 1979). This may also explain the presence of proteinases, lipase and amylase in extracts from carp intestine (Al Hussaini, 1949). Only an enterokinase and probably an amino peptidase are produced by the mucosa of the fish gut (Creach, 1963; Ishida, 1936; Bondi & Spandorf, 1954).

In fish with a stomach the corpus glands produce hydrochloric acid and pepsinogen. When activated the enzyme shows a optimum pH of about 2.5, which is common in vertebrates. Since more than one pH optimum has often been found in acid protease activity (Alliot et al., 1974; Creach, 1963), a second proteolytic enzyme is likely to be present, probably cathepsin with an optimum pH of 3-3.5. This has the same quantitative proteolytic activity as pepsin in pike and trout (Buchs, 1954).

There are no important differences in the production of enzymes by the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pancreas for fish with or without a stomach. In both cases the pH in the intestine is neutral to slightly alkaline (Shcherbina & Kazlauskene, 1971; Creach, 1963; Alliot et al., 1974). Consequently there is no peptic activity in the digestive tract of stomachless fish (Kenyon, 1925; Babkin & Bowie, 1928; Smit, 1968; Ishida, 1936; Kawai & Ikeda, 1972; Jany, 1976). The proteolytic enzymes produced by the pancreas are trypsin, chymotrypsin and carboxy peptidases (Creach, 1963; Alliot et al., 1974; Fänge & Grove, 1979). Among the other enzymes in the intestinal lumen of fish are the carbohydrases. Amylase, maltase, glycogenase, sucrase and saccharase activities have been found in some stomachless teleosts by Ishida (1936), Sarbahi (1951), and Kawai & Ikeda (1971), invertase by Ishida (1936) and Dhaliwal (1975). Cellulase could not be detected in the teleost intestine (Ishida, 1936; Migita & Hashimoto, 1949). The pancreatic juice and the intestinal lumen of most of the studied teleosts contain also lipase (Babkin & Bowie, 1928; Agrawal et al., 1975; Goel, 1974; Sastry, 1974[a,b]; Kapoor et al., 1975[b]; Falge & Shpannkhof,1976). Patton et al.1975, suggested the presence of another fat-hydrolysing enzyme in fish that may compete effectively with lipase as a major fat digesting enzyme.

Apart from endogenous enzymes, exogenous substances might be of importance for the digestion of food. The possible role of microorganisms in digestion has been studied for carp, grasscarp and tench by Jankevicius and colleagues (Syvokiene et al., 1974; Syvokiene & Jankevicius, 1976, 1977; Lubyanskiene et al., 1977). Other studies were made by Paris et al. (1977) and Sacquet et al. (1979)for carp, grasscarp and trout. The results show that microorganisms may play a role in the fermentation of carbohydrates and the digestion of proteins, but only the latter may be physiologically relevant in stomachless fish. According to Dabrowski & Glogowski (1977), autolytic enzymes in food might play an important role in the digestion of proteins in fish larvae.

Other enzymes, probably produced by intestinal cells, and located in the membranes of the microvillous border of the absorptive enterocytes might be of

Fig. 4. Scanning electron micrograph of some mucosal folds in the intestinal bulb. Note the many mucous goblet cells (arrows) (x 150).

Fig. 5. As fig. 4, x 1800. B = bacteria.

Fig. 6. As fig. 4, x 8750. Note the presence of many microvilli on the epithelial cells.

significance at least in mammals (Ugolev, 1971; Gossrau, 1975). Only a few studies on this subject have been made for teleosts. Of interest are the findings of Fänge & Grove (1979) in white grunt. A dipeptidase activity was noticed, especially in the epithelium of the anterior intestine. In mammals proteins are likely to be broken down to oligopeptides, and these are absorbed (Smyth, 1971; Crampton, 1972).

The presence of a dipeptidase, especially in the anterior gut musoca is in accordance with Babkin & Bowie (1928), Hickling (1966), Alliot et al., (1974) and Cockson & Bourn (1973), who found maximum proteolytic activity in the anterior intestine of the studied fish species. Similar results were obtained for lipase and amylase activities (Hickling, 1966; Al Hussaini, 1949) but Cockson & Bourn (1973) found similar amylase activity in anterior and posterior intestine of *Barbus paludinosus*.

b. Absorption of nutrients.

The expectation that the localization of most digestive enzyme activities in the anterior part of the intestine might lead to a proximal to distal gradient in absorption of nutrients has not yet been confirmed. There are a number of studies, however, that suggest this to be true for many fish. Alkaline phosphatase activity is higher in the anterior than in the posterior part of the gut of several teleosts (Al Hussaini, 1949 ; Arvey, 1960; Sastry, 1975; Srivastava, 1966). Khalilov (1969) found an increase in the size of the Golgi apparatus and in alkaline phosphatase activity in the anterior intestine of tench after a fatty meal. The same was found after feeding starch. Broussy & Serfaty (1958), Sivadas (1964), Iwai (1968, 1969), Tanaka (1972), Gauthier & Landis (1972), and Noaillac-Depeyre & Gas (1974, 1976) by applying morphological techniques found that most of the lipid was absorbed in the anterior intestine of carp, goldfish, *Tilapia*, and in a number of fish larvae. This was confirmed by physiological experiments (Shcherbina, 1973, concerning lipids, Shcherbina & Sorvatchev, 1969 and Shcherbina et al., 1976, for proteins; Farmanfarmaian et al., 1972, Sastry en Garg, 1976 and Shcherbina et al., 1977 for absorption of sugar). Sastry en Garg (1976),however, found absorption of lipids all over the intestine of *Ophiocephalus* and *Heteropneustus* by the application of histochemical techniques.

3. Regional differentiation of the intestine.

In stomachless teleosts and in fish larvae two intestinal segments can be often distinguished: an anterior segment with enterocytes loaded with lipid particles after a fatty diet, and a posterior segment with many pinocytotic vesicles in the apical part of the cells (Yamamoto, 1966; Iwai, 1969; Gauthier & Landis, 1972; Tanaka, 1971; Noaillac Depeyre & Gas, 1973[a], 1974, 1976). In some cases a third segment (rectum) has been described. The caudal segment contains many lamellar infoldings, which according to Noaillac-Depeyre & Gas (1973[b]) might have a specialized function in osmoregulation.

Orally administered horseradish peroxidase was absorbed by cells of the second segment in adult stomachless fish (Gauthier & Landis, 1972; Noaillac-Depeyre & Gas, 1973[a]). This capacity to absorb protein macromolecules is also known for suckling mammals, in which the digestive system is still not fully developed (Clark, 1959; Leissring et al., 1962; Kraehenbuhl & Campiche 1969; Staley et al., 1972). Some authors suggested that the presence of a "second segment" is to be correlated to the lack of a stomach and peptic digestion in order to account for the inefficiënt protein digestion in the gut lumen (Yamamoto, 1966; Gauthier & Landis, 1972; Noaillac-Depeyre & Gas, 1976). It is of interest that Shcherbina & Sorvatchev (1969) found a protein absorption of 78 % in carp. In fish with a stomach, even higher percentages of ingested protein may be absorbed (Kapoor et al., 1975[b]). It should be realized, however, that protein digestibility can be correlated to the same extend with the composition of the diet (Inaba et al., 1962, 1963). Kitamikado & Morishita (1965) found a protein digestibility of 70% if trout were fed with soybeans. Aoe et al. (1974) noticed that for carp hydrolysates of casein (amino acids and peptides) are far inferior in nutritive value to intact protein. Some native proteins seem difficult to be digestid by carp (Jany, 1976).

4. Relations between food preference and digestive tract

Many authors tried to correlate the morphology and physiological characteristics of the digestive tract to the feeding habits of certain species. Reviews were presented by Suyehiro (1942) and Kapoor et al. (1975[b]). The possible relationships seem to be very complex. This applies in particular

to the lack of a stomach in a number of teleosts: stomachless fish are supposed to be descendant from fish with a stomach and many of them are believed to have adapted their diets, as a result of which there are herbivorous, omnivorous and carnivorous stomachless fishes (Rogick, 1931; Klust, 1940; Girgis, 1952; Kapoor et al., 1975[b]; Kafuku, 1977).
Many ecological studies indicate an apparent preference for one or several types of food, but others point to the opportunistic feeding behaviour of fish in periods of food scarcity. Carnivorous, herbivorous, etc. only indicate general tendencies in feeding habits, and not characteristic habits.

Only the length of the intestine seems to be clearly associated with the feeding habits of a particular species. Some herbivorous and microphagous stomachless fish have a relatively long gut: 7 x to 24 x standard body length (Rogick, 1931; Girgis, 1952; Das & Nath, 1965; Sinha & Moitra, 1975 [a, b]; Kafuku, 1977). The gut length of omnivorous species like *Cyprinus carpio* and *Barbus conchonius* is 2-3 times the body length (Curry, 1939; Das & Nath, 1965). Carnivorous species have the shortest gut lengths, approximately 0,5 - 0,8 times the body length (Klust, 1940; Das & Nath, 1965). Khanna & Mehrotra(1971) found a relatively short gut in 5 species of carnivorous teleosts. A large variability may be found within a given species, for example in the cyprinid *Carassius auratus* (Vickers, 1962). This seems to be related to the kind of food administered during the ontogeny. The same is known from some cyprinodonts (Hykes & Moravek, 1933). Therefore, the variability is evidently less pronounced in stenophags (Kapoor et al., 1975[b]). An additional problem is that seasonal factors (quantity of food?) may affect the gut length (Ciborowska, unpublished results).

In some studies a relation is described between the diet and the activity of several digestive enzymes. According to Sarbahi (1951) and Kitamikado & Tachino (1961), carbohydrases are more abundant in the stomachless goldfish than in the largemouth bass. Carbohydrases show the highest activities in herbivorous fish (Vonk, 1927; Al Hussaini, 1949 ; Agrawal et al., 1975). Turpayev (1941) found tryptic activity to be dominant in a carnivorous cyprinid *(Aspius)* , and in the herbivorous *Scardinus* the amylase activity was evident. Agrawal et al. (1974) noticed a higher peptidase activity in omnivorous than in herbivorous species. Kawai & Ikeda (1972) found a relation

of the activity of amylase and protease in carp to the composition of the food. According to Sinha (1978), protease and lipase are the major digestive enzymes in *Cirrhinus mrigala* larvae, which are zooplankton feeders. In juveniles (omnivorous) and adults (herbivorous) strong amylase and weak protease activities have been observed.

Of special interest is the development of the digestive tract and its functions during ontogeny, as most fish larvae are first carnivorous, feeding on zooplankton. The food regime of omnivorous and herbivorous species changes during ontogeny. This may be correlated with changes in the morphology and physiology of the digestive apparatus.

GENERAL INTRODUCTION

C : Objectives of our investigations.

From the start of this study we have realized that some fish change their feeding habits during development. Grasscarp larvae were supposed to be zooplankton feeders, just as most fish larvae, whereas the adults were called herbivorous (Gidumal, 1958; Aliyev & Bessmertnaya, 1968; Fisher, 1968). The grasscarp might be of special importance to agriculture because it feeds on and thus controls water weeds (Cross, 1969; Stott & Robson, 1970; Opuszynsky, 1972; Michewicz et al., 1972). The species might be also valuable for consumption, as it has a high growth rate (Hickling, 1960; Anderson, 1970). The selection of grasscarp for the present study was not based primarily on the absence of a stomach.

The first objective was to find morphological evidence for the adaptation of the intestine to the changing feeding habits during ontogeny. For this purpose the experiment described in paper A has been carried out. The results were not encouraging, but our attention was attracted to the distinct regional differentiation of the intestine in this stomachless species.

Because, just as in mammals, the intestinal epithelium in cyprinids is regularly renewed (Vickers, 1962; Hyodo, 1965; Gas & Noaillac-Depeyre, 1974), the species might be used for studying the differentiation of enterocytes within th successive segments. Paper B deals mainly with the renewal of the gut epitheliu and the morphology of absorptive and differentiating cells. Many interesting results have been obtained, but the unexpected discovery of functional cells in the proliferative area indicated that the intestinal epithelium is not the most useful tissue for studying the differentiation of absorptive cells.

It appeared that just as in other cyprinids a segment with the ability of pinocytosis is present in the caudal part of the gut, and this might be related to the lack of a stomach. First it had to be ascertained whether active absorption takes place in the rostral intestinal segment and absorption of macromolecules by pinocytosis may be restricted to the second part of the gut. In additi the absorptive activity along the mucosal folds had to be investigated. Therefo the experiments described in paper C have been carried out. To test whether the ability to absorp protein macromolecules is related to the lack of a stomach, t location of protein absorption in the grasscarp intestine had to be determined (paper D).

The development of the intestine in a teleost (*Clarias lazera*) in which a stomach is developed at the end of the larval stage is described in paper E. It was investigated whether the development of the stomach might be related to the disappearance of pinocytosis in the second gut segment.

GENERAL DISCUSSION AND SUMMARY

General morphology of the digestive tract

Structurally and functionally the digestive tract of fishes has much in common with that of other vertebrates, but in fishes the system is simplified. Salivary glands are absent, just as esophageal glands. Approximately 15 % of the species does not have a stomach. The intestine is less complex due to the absence of folds of Kerkring and real villi. Furthermore, most fishes lack the crypts of Lieberkühn, the glands of Brunner and a muscularis mucosae, there is no differentiation in small and large intestine, and in many species a rectum cannot be recognized.

For these reasons fishes appear to offer several advantages for studying morphological and physiological aspects of the digestive system.

Food preference and the need for animal protein

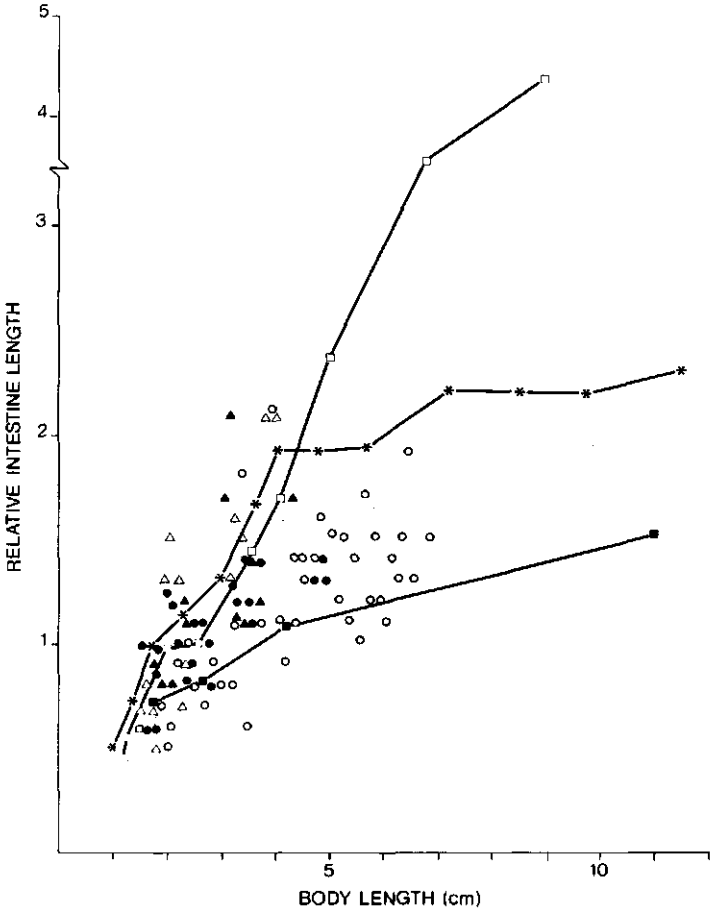
Another advantage is that many species change their food preference during their life. An example is the chinese grasscarp, *Ctenopharyngodon idella*, the animal used for our experiments. Paper A deals mainly with changes in the morphology of the intestine in relation to growth and changing diet of the young grasscarp.

Animal food was found to stimulate rapid growth, also after the animals becom capable of ingesting plant material. The composition of the food protein, especially as to the essential aminoacids may be the main factor determining the effectiveness of the food. Many plant materials are known to contain only small amounts of methionine and lysine and much vegetable material in the food might cause an improper amino acid balance (Shcherbina et al. 1964, quoted by Phillips 1969) and consequently a low growth rate. Grasscarp, kept in ponds for weed control, may form a serious threat to other species as a predator as well as a competitor for food.

Length and regional differentiation of the gut

The digestion and absorption of plant food in grasscarp is mainly facilitated by an increase of the relative length of the intestine from $\pm$ 0,7 to about twic the bodylength. The latter was found in 6 months old specimens and also in adult (paper D), and is low for a presumably herbivorous species.

In many other fish species also a change in feeding habits takes place during evelopment. This is always related to an increase in gut length from ± 0,5 x odylength in the carnivorous larvae to much higher values in omnivorous and erbivorous juveniles and adults. In fig.7 the gut lengths of several Cyprinids re given at a number of stages of development. In the herbivorous *Catla catla* he most rapid increase in relative gut length was found, while the grasscarp hows a slower increase in gut length than the mirror-carp, *Cyprinus carpio*, presumably omnivorous species.



g. 7. Graph showing the relative length of the gut of some Cyprinid larvae at several ages. From Stroband, H.W.J. & Dabrowski, K.R., in: "Nutrition des Poissons" C.N.E.R.N.A. Paris (in press).
○- common carp,●- grass carp *(Ctenopharyngodon idella)*,△- silver carp *(Hypophthalmichthys molitrix)*,▲- big head *(Aristichthys nobilis)*(after Ciborowska, unpublished data),✱- common carp (after Klust, 1940), ■- grass carp (after Stroband, 1977),□*Catla catla* (after Kafuku, 1977).

The morphology of the intestinal epithelium of the stomachless grasscarp is described in paper A and, in more detail, in paper B. The gut shows a similar regional differentiation as in other cyprinids (Yamamoto, 1966; Gauthier & Landis, 1972; Noaillac-Depeyre & Gas, 1974, 1976). The anterior segment shows the characteristics of lipid absorption; in the"second segment"enterocytes con tain many pinocytotic vesicles and one or more supranuclear vacuoles probably representing large secundary lysosomes. A third caudal segment contains enterc cytes with the characteristics of water or ion transport.

The length of the intestine is also affected by the diet during early life. Vegetable food causes a slight increase in gut length, which was also found ir other species. Our results indicate that the anterior segment is involved in t growth, and this might well be related to the main absorptive function of this part of the intestine (paper D).

The intestinal epithelium as a cell renewal system

The regional differentiation and the relatively less complex structure of t mucosa seem to make the fish intestine a useful model for studying the differe tiation of epithelial cells. In paper B the cell renewal system of the gut epi thelium of the grasscarp is described with light-microscopic radioautography, using ^{3}H thymidine. The system appeared to be comparable to that in the mamma- lian small intestine; proliferation takes place in the basal parts of the mucc sal folds in fishes, and in the crypts of Lieberkühn in the small intestine of mammals. The renewal time in the grasscarp is relatively long: 10-15 days at 20 ^{o}C. In paper B the ultrastructure of the intestinal epithelium is describec for starved and fed specimens.

Functional absorptive cells proved to be present in the proliferative area whereas undifferentiated cells could not be identified. This is a major diffe- rence in respect to mammals, in which undifferentiated proliferative cells in the crypts only become functional after migration towards the intestinal villu (Rijke, 1977).

A comparison of radioautographs and electronmicrographs for larvae of *Barbus conchonius* (Rombout et al., 1980) and *Clarias lazera* (paper E) shows that func tional absorptive cells are capable of proliferation. Similar results were fou in the larvae of the amphibian *Xenopus laevis* (Marshall & Dixon, 1978). The difference of the cell renewal system in mammals and fishes is also reflected the presence of alkaline phosphatase activity in the microvillous border of enterocytes in the proliferative area of the grasscarp (paper C), which also

ndicates the cells to be capable of absorption.

Intracellular factors, related to the state of differentiation, and extra-
ellular factors (chalones, hormones, stimuli from the food) are possibly in-
olved in determining the rate of proliferation of vertebrate cells. Inhibition
f proliferation by intracellular factors possibly develops only when the cells
ave reached a relatively high state of differentiation in fishes. In this
onnection it is interesting that in the colon of mammals functional cells and
ndifferentiated proliferative cells are intermingled (Lipkin, 1973). This im-
lies a major effect of the intracellular factors in blocking cell division in
ıe differentiated cells.

he digestion and absorption of protein and the function of the second gut
egment

An important factor is the regional differentiation of the grasscarp intestine,
ıd especially the presence of a "second segment" with pinocytotic activity.This
ıs also found after the ingestion of exogenous food in other stomachless fishes
ıd in fish larvae, which usually are initially stomachless (Tanaka, 1969;
ıper E).

Paper C deals with the activity of alkaline phosphatase and the uptake of
·ally administered horse radish peroxidase in the intestine of grasscarp larvae
ıd juveniles. A proximo-distal gradient in alkaline phosphatase activity can
e demonstrated. This suggests that the main active absorption takes place in
ıe anterior gut segment. Peroxidase was demonstrated histochemically in entero-
·tes of only the second segment. This implies that the enzyme must have been
)sorbed by pinocytosis. Similar results have been found in other Cyprinids
loaillac-Depeyre & Gas, 1973) and in *Clarias lazera* larvae (paper E).

The enterocytes in the second segment of the grasscarp have also the ability
) pinocytosis of large ferritin particles (paper D). Since pinocytosis of macro-
)lecules takes place also in the intestine of suckling mammals before the sto-
ich functions are fully developed, it is widely believed that the presence of
"second segment" in stomachless fishes is related to the lack of peptic di-
stion. Recently, however, a "second segment" was also found in the intestine
the perch, a fish with a stomach (Noaillac-Depeyre & Gas, 1979): this segment,
wever, is relatively small ($\pm$ 11 % of the gut length and $\pm$ 20 % in fish larvae
d stomachless fishes).

Shcherbina and Sorvatchev (1969) and Shcherbina et al. (1976), who used the
ert marker technique, discovered that most protein is absorbed in the anterior

60 % of the carp intestine, i.e. in the anterior gut segment. In their experiments Shcherbina et al. pooled the gut contents of 6 specimens. Therefore, the location of protein absorption in the grasscarp intestine has been determined also in individual test animals. The results are described in paper D. It will be proved that proteins are almost completely absorbed within the anterior 40-50 % of the gut i.e. in the rostral two third of the first segment. This implies adequate digestion of protein in the gut lumen, since no noticeable pinocytosis of macromolecules was found in the anterior segment. Our results also indicate that essential amino acids are preferentially absorbed in the grasscarp, just as in mammals (Orten, 1963; Ben-Ghedalia et al., 1974). The rapid disappearance of lysine and arginine from the chyme suggests a tryptic breakdown of proteins.

In their paper on the perch, Noaillac-Depeyre & Gas (1979) suggested that protein digestion in the gut lumen of fish might be less efficient than in mammals. They suggest that completion of hydrolysis takes place by intracellular enzymes in the second segment. Our results prove this to be incorrect proteins are digested and absorbed adequately within the anterior gut segment, but an exception should possibly be made for fish larvae, in which food reache the distal part of the intestine very shortly after feeding (Fänge & Grove, 1979).

Under physiological circumstances pinocytosis of a few macromolecules undoub ly takes place. This may be relevant in a qualitative sense. It may be compara to the situation in adult mammals, in which macromolecules are absorbed in sma quantities and may be antigenic or biologically active (Walker & Isselbacher, 1974; Warshaw et al., 1974).

Similarity between the anterior gut segment in fish and the small intestine in mammals

As to its absorptive functions, the anterior intestinal segment of fishes h much in common with the small intestine of mammals. Mammals also show a proxim distal gradient in alkaline phosphatase activity, and in both classes of verte brates sugars, lipids and protein are merely absorbed in the anterior part of the gut (Bell et al., 1972; Crampton, 1972; Farmanfarmaian et al., 1972; Shche bina et al., 1973; 1976; 1977; Ben Ghedalia et al., 1974; Noaillac-Depeyre & Gas, 1976; paper B). The morphological characteristics of lipid absorption are similar in mammals and fishes, and in both the transport of sugars and amino-acids is sodium-dependent (Cartier et al., 1979).

The development of the stomach in Clarias lazera

The digestive system of fish larvae resembles that of stomachless teleosts in many respects: a "second gut segment" may be recognized immediately after the beginning of exogenous feeding. This is also true for fish species that develop a stomach at the end of the larval period.

In paper E the results are described of another attempt to establish a possible relation between the presence of a "second segment" and the lack of a stomach. The development of the stomach in *Clarias lazera* is described, and its ultrastructure is compared to that of adult specimens. It is of interest that the stomach corpus glands contain only one cell type, which apparently is involved in producing hydrochloric acid and pepsinogen. From the ultrastructure of the glands, the ^{3}H-thymidine labeling index, and the pH indicator tests, it is concluded that a functional stomach is present from approximately 12 days after fertilization. However, in larvae as well as in juvenile *Cl. lazera* a "second segment" of approximately 20 % of gut length shows the ability to pinocytosis of horseradish peroxidase. The same may apply to adult specimens,since they also possess a "second segment" of 20 % of gut length, as proved with light- and electron-microscopic studies. Therefore, the conclusion seems justified, that the ability to pinocytosis of macromolecules is not positively correlated to the lack of a functional stomach. Our results also indicate that a regional differentiation of the intestine, including a gut segment with pinocytosis, is a general feature of the digestive tract in teleosts.

Function of the stomach in vertebrates

Since proteins are digested adequately in the intestine of stomachless fishes - although food passes through the gut in not more than about 7 h in the grass-carp (Hickling, 1966) - the question arises whether peptic digestion in an acid environment, as found in other vertebrates, is in fact of minor importance. According to Bertin (1958), most stomachless teleosts possess some kind of masticatory apparatus, like the pharyngeal teeth in Cyprinids. Fishes without this apparatus are supposed to need a stomach with a low pH to fractionate the food. Both are therefore believed to facilitate feeding with relatively large organisms. It is widely believed that the stomach evolved together with the jaws but primarily as a storage organ. Acid production may have followed, and acted in fractioning large food elements and possibly in protecting the stored food against bacterial digestion. The production of pepsin for the digestion of proteins in

the acid environment might have been the last important step in the evolution of the stomach (Barrington, 1957; Romer, 1962; Waterman et al., 1971). From phylogenetic studies it is generally believed that the absence of a stomach in teleosts is a secundary feature (Barrington, 1942).

CONCLUSIONS

1. The grasscarp is a stomachless teleost. The intestine does not contain multicellular glands.
2. The relative length of the grasscarp intestine increases from 0,7 x body length in young larvae to 2 x body length in adults. The gut length is the only morphological characteristic to change when the grasscarp turns from carnivorous to herbivorous feeding.
3. After feeding the grasscarp with vegetable food, the increase in gut length is higher than in fishes fed with animal food.
4. Animal food stimulates rapid growth in the grasscarp, also after they are able to ingest plant material. Grasscarp may represent a serious threat to other species as a predator as well as a competitor for food.
5. The intestine of the grasscarp shows a similar regional differentiation as found in other Cyprinids,with a proximo-distal gradient in alkaline phosphatase activity.
6. The anterior gut segment is involved in the absorption of lipids and proteins (and probably also of sugars), which are absorbed in enterocytes after hydrolysis in the lumen. Also in regard to the morphological characteristics of lipid absorption, the epithelium shows resemblance with the epithelium in the small intestine of mammals.
7. A"second gut segment",running from $\pm$ 60-85 % of gut length, is characterized by enterocytes with a high pinocytotic activity. These cells are capable of absorbing protein macromolecules like horseradish peroxidase and ferritin.
8. Protein digestion is efficient despite the absence of a stomach. Quantitatively relevant amounts of protein are not absorbed by the"second segment".
9. The "second segment" is not related to the lack of peptic activity in stomachless fishes, since it is also present in *Clarias lazera* after the stomach has developed and has become functionally active.
10. The stomach of *Clarias lazera* contains only one type of corpus glandular cells with the morphological characteristics of chief- as well as parietal cells.

1. The intestinal epithelium of the grasscarp represents a cell renewal system and is completely renewed within 10-15 days.
2. In contrast to mammals the proliferative pool of cells consists of functional cells in grasscarp larvae and juveniles and in *Clarias lazera* larvae.

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SAMENVATTING

De bouw van het spijsverteringskanaal van vissen is in het algemeen minder complex dan bij hogere vertebraten het geval is. Zo ontbreken speekselklieren en gewoonlijk ook slokdarmklieren. Ongeveer 15% der vissoorten, waaronder de karperachtigen, is maagloos. Wat de darm betreft valt vooral het ontbreken van plooien van Kerkring, villi met crypten en meercellige klieren in de darmwand op. Bovendien kan er geen onderscheid worden gemaakt in dunne- en dikke darm. Eén en ander heeft er toe geleid dat vissen een steeds belangrijker plaats innemen in het fundamenteel onderzoek naar vorm en funktie van elementen van het spijsverteringsstelsel.

De graskarper is als larve carnivoor en voedt zich met zoöplankton. Tijdens zijn ontwikkeling, waarschijnlijk al gedurende de eerste maanden, verandert het dieet en aangenomen wordt dat de volwassen graskarper herbivoor is. Deze verandering lijkt deze vissoort bijzonder geschikt te maken voor onderzoek naar de relatie tussen de struktuur van de darm en het dieet.

Uit artikel A blijkt echter dat graskarpers, ook lang nadat ze voor het eerst in staat zijn plantaardig voedsel te nuttigen, het snelst groeien wanneer ze met dierlijk materiaal worden gevoed. De enige morfologische verandering die werd waargenomen gedurende de periode waarin het dieet zich zou wijzigen was een toename van de relatieve darmlengte van 0,7 tot 2 maal de lichaamslengte. Dit is tamelijk kort voor een vis die verondersteld wordt herbivoor te zijn, en wijst als zodanig meer op een omnivore levenswijze. Dit kan betekenen dat graskarpers, wanneer ze massaal worden uitgezet ter bestrijding van waterplanten, een bedreiging worden voor andere soorten, zowel als predator alsook als konkurrent wat het voedsel betreft.

In publikatie A, en meer gedetailleerd in artikel B, wordt de morfologie van het darm epitheel beschreven. Er zijn 3 darmsegmenten te onderscheiden. Het meest rostrale segment, dat ± 60% van de darm beslaat, bezit resorberende cellen die dezelfde morfologische kenmerken van vetopname vertonen die ook in enterocyten in de dunne darm van zoogdieren worden aangetroffen. In het middelste of tweede segment (± 25% van de darmlengte) komen resorberende cellen voor die veel pinocytotische aktiviteit vertonen. Het caudale of 3e segment (± 15%) speelt waarschijnlijk een rol bij de osmoregulatie.

Wegens deze vormverschillen, die ook een regionale differentiatie t.a.v. resorptie van bepaalde voedingsstoffen lijken in te houden (zo kunnen eiwitten in het tweede segment worden gepinocyteerd), werd de graskarper een geschikt object geacht om de differentiatie van verschillende typen resorptie cellen te bestuderen.

In artikel B wordt de vernieuwing van het darm epitheel beschreven. Deze blijkt relatief traag te verlopen: 10 à 15 dagen bij 20°C tegenover 2 à 3 dagen bij de meeste zoogdieren. In de dunne darm van zoogdieren is de proliferererende "pool" van cellen gelokaliseerd in de crypten van Lieberkühn. Die bestaat uit ongedifferentieerde cellen, die zich differentieren tijdens de migratie naar de villus, alwaar zich funktionele cellen bevinden. De resultaten bij de graskarper wijken in belangrijke mate af van dit beeld. Daar crypten ontbreken, bevinden zich de prolifererende cellen aan de basis van de darmplooien, zoals blijkt uit de ^{3}H-thymidine labeling van epitheelcellen, die alleen in dit gebied optreedt. Er worden hier echter alleen funktionele cellen aangetroffen. In publikatie E wordt aangetoond dat ook bij *Clarias lazera* larven de proliferative cel "pool" in het darm epitheel uit funktionele cellen bestaat. Bovendien blijken bij deze vissoort funktionele cellen uit het maag epitheel in staat te zijn zich te delen. Terwijl zoogdiercellen al vroeg in het differentiatie proces, lang vóórdat ze funktioneel worden, hun delingsvermogen verliezen, lijkt de blokkering van de mogelijkheid tot proliferatie bij vissen pas in een veel later stadium van de differentiatie op te treden. Eén en ander houdt in dat de graskarper zich minder goed leent voor het bestuderen van morfologische aspecten van het differentiatie proces van darm epitheel cellen.

In artikel C wordt aangetoond dat min of meer komplete proteine macromoleculen (mierikswortel peroxidase) in het tweede darmsegment kunnen worden gepinocyteerd. Terwijl in het eerste segment de microvilli der resorptie cellen een hoge alkalische fosfatase aktiviteit vertonen, ontbreekt deze vrijwel geheel in het tweede segment. Dit wijst er op dat aktief transport door de apicale celmembraan vooral plaatsvindt in het eerste segment.

Omdat ook pasgeboren zoogdieren de mogelijkheid tot pinocytose van macromoleculen bezitten - vóórdat alle maagfunkties tot ontwikkeling gekomen zijn - wordt wel aangenomen dat deze mogelijkheid in verband staat met het ontbreken van een maag en vooral van peptische eiwit vertering. Deze hypothese maakte het wenselijk de eiwitvertering en opname nader te onderzoeken. Hiertoe werden de experimenten zoals beschreven in publikatie D uitgevoerd.

Bij de graskarper blijkt, dat de 75% van het voedsel eiwit die worden opgenomen vrijwel volledig door de rostrale 40% van de darm worden geresorbeerd. Een kwantitatief belangrijke rol kan dus, wat de eiwit opname betreft, niet aan het 2e segment worden toegeschreven. Uit de experimenten blijkt tevens dat er een preferentie bestaat voor essentiële aminozuren, zoals dat ook bij zoogdieren het geval is. De relatief snelle resorptie van arginine en lysine wijst op het belang van vooral tryptische vertering bij de graskarper.

Een tweede manier om na te gaan of de aanwezigheid van een tweede segment met pinocytotische aktiviteit in relatie staat tot het ontbreken van een maag is het bestuderen van de ontwikkeling van het spijsverteringskanaal van een maaghoudende vis. Bij vislarven is de maag nog niet aangelegd, en de darm vertoont dezelfde regionale differentiatie als die van maagloze vissen. In artikel E wordt de vorming van de maag beschreven bij de tropische meerval *Clarias lazera*.

Een bijzonderheid ten aanzien van de struktuur van de maag van vissen is, dat - in tegenstelling tot zoogdieren - slechts één type kliercel voorkomt waarin zowel pepsinogeen als HCl wordt geproduceerd. Het tweede segment blijkt na de vorming en het funktioneel worden van de maag te blijven bestaan. Bij larven én bij adulte specimen van *Clarias lazera* beslaat het ± 23% van de darmlengte. Er bestaat dus geen relatie tussen de mogelijkheid macromoleculen op te nemen en het ontbreken van een maag. Uit artikel D blijkt bovendien dat de afwezigheid van een maag niet of nauwelijks van invloed is op de efficientie van de eiwit vertering.

De funktie van het 2e segment blijft dan ook onduidelijk. Bij vislarven, waarbij het voedsel zéér snel het achterste deel van de darm bereikt, kan het een kwantitatief belangrijke rol spelen bij de eiwitopname, maar bij oudere vissen heeft de pinocytose van macromoleculen vermoedelijk alleen kwalitatieve betekenis. Ook bij zoogdieren worden kleine hoeveelheden eiwit als macromoleculen opgenomen. Deze hebben geen betekenis voor de voeding, maar zijn wellicht op één of andere wijze biologisch aktief (bijvoorbeeld als antigeen).

Uit het feit dat bij de graskarper ondanks het ontbreken van een maag en een vrij snelle passage van het voedsel door de darm (± 7 uur)toch een zeer efficiënte eiwitvertering plaatsvindt valt af te leiden dat de maag met betrekking tot de eiwitvertering slechts van secundaire betekenis is, of althans dat de productie van pepsine en/of zuur geen voorwaarde vormt voor een adequate vertering van voedseleiwitten.

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Growth and diet dependant structural adaptations of the digestive tract in juvenile grass carp (*Ctenopharyngodon idella*, Val.)

H. W. J. STROBAND

Agricultural University, Department of Experimental Animal Morphology and Cell Biology, Hollandseweg 13, Wageningen The Netherlands

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The morphology of the intestinal epithelium is described and compared with published data. In the gut of this stomachless fish, three zones can be distinguished based on light and electron microscopic observations of absorptive cells: a rostral zone with numerous fat droplets, a second zone with large P.A.S.-positive supranuclear vacuoles and pinocytotic vesicles, and a third zone without these characteristics. In these zones deep invaginations of the cell membrane is a feature of the basal part of the cells. The presence of pinocytotic vesicles in the second zone is considered evidence of the uptake of undigested protein-like substances.

Juvenile grass carp from 15 to 335 days, fed on either animal or vegetable food, were measured. The growth rates indicate that animal food stimulates rapid growth. The data also show a vegetable diet causes a slight increase in relative gut length, mainly in the length of the first zone.

I. INTRODUCTION

The adult White Amur or grass carp (*Ctenopharyngodon idella* Val.) eats large quantities of aquatic plants and as a result is effective in weed control (Lin, 1935; Penzes & Tolg, 1966; Fischer, 1968; Cross, 1969; Stott & Robson, 1970). The fish also has a high growth rate (Hickling, 1960; Anderson, 1970) which increases its economic significance, and consequently the grass carp has been the subject of many investigations.

Several authors have described the histology of the alimentary tract in Cyprinids (Rogick, 1931; Curry, 1939; Klust, 1940; McVay & Kaan, 1940; Al Hussaini, 1949*a*, *b*; Girgis, 1952). It appears to be difficult to relate histological and morphological adaptations to the types of food. According to Nikolsky (1956, cited by Hickling, 1966) the young grass carp is carnivorous until reaching a length of 2·5 cm; when its feeding habits change rapidly, and it reaches the herbivorous stage at a length of 3 cm.

In several Cyprinids, fat and protein appear to be absorbed in different parts of the gut (Yamamoto, 1966; Iwai, 1968, 1969; Gauthier & Landis, 1972; Noaillac Depeyre & Gas, 1973). The location, relative length and histological features of these parts were the subject of our investigation.

II. MATERIALS AND METHODS

A number of grass carp fry (15 days after hatching) were provided by the ' Organisatie ter Verbetering van de Binnenvisserij ', Holland in February 1975. On arrival the fishes were

divided into three groups, all were fed on *Tubificidae* (their own bodyweight a day). Group 2 received additional plant material (young darnel). From the 28th day after hatching (when the darnel was consumed for the first time) the fish in group 2 received tubifex and an excessive amount of darnel and the third group was fed an excessive amount of darnel only. After 50 days the darnel was replaced by grass pellets and the daily amounts of tubifex were reduced to 100 g a day per 30 animals. Each group of 30 animals was kept in a 200 l tank at 23 °C±1° C, with light for 14 h a day. Standard length and weight were regularly measured. For histological examination, the animals were sacrificed at 15, 27, 48 days, 3 and 7 months, each time 2–4 h after feeding. The whole fish (up to 2 months old; the ventral body wall removed) or small pieces of tissue (fish 2 months or older) were fixed in Flemmings osmium tetroxide fixative (Romeis, 1968). The tissues were embedded in paraffin wax and cut at 1–4 μm.

A zonal differentiation of the gut was made histologically. To determine the length of the subdivisions, the number of histological sections of each zone was counted. In fish larger than 50 mm, the length of the total alimentary tract was measured before fixation, and parts of the gut were examined histologically to locate the transition of each zone.

For electron microscopical examination, the tissues were prefixed in a mixture of 1% osmium-tetroxide and 2% glutaraldehyde buffered with 0·1 M sodium cacolylate buffer pH 7·2 for 15 min, and postfixed for 45 min in the same mixture to which 1% potassium bichromate was added. Fixation was carried out at 0° C. The tissues were embedded in epoxy resin. Ultrathin sections were cut on a LKB Huxley or LKB III Ultramicrotome and photographs were made on a Philips E.M. 300 electron microscope.

TABLE I. Growth rate of grass carp fed on animal or vegetable food

	Standard length (mm)		
Age (days)	Group 1 (animal food)	Group 2 (control animals)	Group 3 (plant food)
15	—	16·3±1·4 (6)*	—
28	—	25·7±2·8 (9)	—
48	43±4·7 (8)*	42·2±3·9 (5)	31·9±3·2 (8)*
57	49±7 (3)	—	45±4 (3)
70	61±5·6 (2)	55±7 (2)	56±5·4 (4)
110	95±7 (2)	88±5 (2)	66·7±5·2 (4)
206	113±9·4 (10)	—	76±8·1 (10)
335	120±8 (10)	110±13 (10)	75±6 (10)

* The number of fish examined is indicated between brackets.

III. GROWTH RATE

A difference in growth rate was observed between grass carp fed on tubifex (group 1) and those fed on grass (group 3) (see Table I). Between the groups 1 (animal food) and 2 (animal+plant food) no significant differences were found. Tubifex-fed fish grew about 0·8 mm/day to an age of 110 days; then the growth diminished. A reduced growth rate for plant fed animals was noticed from the start of the experiment. The reduced growth of tubifex fed fish between 110 and 335 days may be partly due to crowding.

The mean weight of specimens from the two groups is also different: at 30 days this was 370 mg, at 7 months the mean weight of plant fed animals was 7·3±2·6 g, and that of tubifex fed specimens was 24·7±7 g (for ten animals per group).

IV. MORPHOLOGY AND REGIONAL DIFFERENTIATION OF THE GUT

In Cyprinids, the stomach is absent. The oesophagus opens directly into the intestine a few mm anterior to the entrance of the ductus choledochus. The first limb or intestinal bulb (intestinal swelling) is wide. Its rostral part has complex branching mucosal folds; at the end of the intestinal swelling, these folds are at right angles to the long axis of the gut. From this area the diameter of the intestine decreases towards the anus.

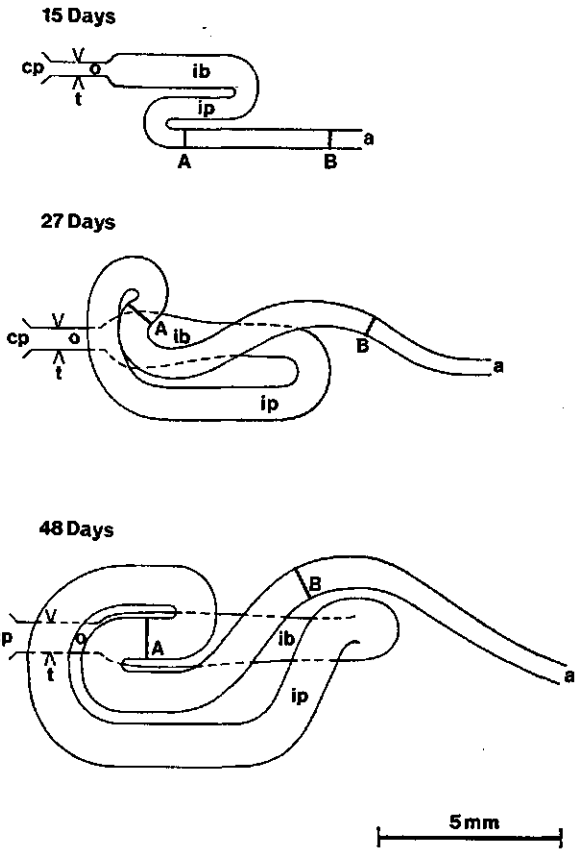

FIG. 1. A schematic drawing of the digestive tract of grass carp at three stages of development. Ventral view: cp, caudal pharynx; t, position of pharyngeal teeth; o, oesophagus; ib, intestinal bulb; ip, intestine proper; a, anus. A and B, boundaries of the second zone of the intestine.

Based on histological features the intestine can be divided into three zones (Fig. 1). Table II shows the relative length of the total alimentary canal, the intestine and its three zones in specimens of different ages and fed on different kinds of food. The relative length of the gut increases from approximately 1 × standard body length in 15 days to 2 × body length in 210 days. This increase was mainly due to growth of the first zone. The relative length of the gut is larger in grass fed animals than in those fed on tubifex. The difference can be attributed to growth of the first zone of the intestine.

TABLE II. Ratio of standard body length to length of the gut and its subdivisions

Age (days)	Total canal	Intestine			
		Total	Zone 1	Zone 2	Zone 3
15	1·0	0·7	0·4	0·3	0·05
27	1·1	0·8	0·4	0·3	0·1
48 (1)*	1·4	1·1	0·6	0·3	0·2
48 (3)	1·4	1·1	0·6	0·3	0·2
210 (1)	1·8	1·5	0·9	0·3	0·3
210 (1)	1·8	1·5	0·9	0·4	0·2
210 (1)	1·7	1·5	0·9	0·3	0·3
210 (1)	1·9	1·6	0·9	0·4	0·3
210 (3)	2·4	2·0	1·3	0·4	0·3
210 (3)	2·2	1·9	1·2	0·4	0·3
210 (3)	2·0	1·7	1·0	0·7	
210 (3)	1·9	1·6	0·9	0·4	0·3
210 (3)	2·0	1·7	0·9	0·4	0·3

* (1) = group 1 fed on animal food.
(3) = group 3 fed on plant food.

V. HISTOLOGY

HISTOLOGY OF THE INTESTINAL EPITHELIUM

The gut does not possess villiform projections, but the surface contains mucosal folds which gradually decrease in height from the anterior to the posterior parts of the intestine. The mucosa is lined with a columnar epithelium consisting of two types of cells: absorptive cells with microvilli and, less frequently, glandular cells of the goblet type with P.A.S. positive contents. The latter cells are more abundant in the caudal part of the gut. Other cell types found between the epithelial cells include: lymphocytes (Plate I), cells with rod-like inclusions [probably *Rhabdospora thelohani* (Laguesse)], and a few acidophilic granular cells. At the base of the folds, mitotic stages can be observed (Plate I). Special attention was paid to the appearance of the absorptive cells throughout the intestinal canal. These cells are high columnar with nuclei situated in the lower third, the nuclei have clear nucleoli, the cytoplasm is granular and has a subapical basophilic band and a basal basophilic area.

The intestine was divided into three distinct zones on the basis of the appearance of the absorptive cells. In the first zone (including the intestinal bulb and the rostral part of the intestine proper), many cells in the apical region of the mucosal folds showed a number of clear vacuoles between nucleus and the subapical basophilic cytoplasm (Plate I). The vacuoles after Bouins fixation appeared empty but showed an osmiophilic substance after fixation in Flemmings fluid (Plate II). The nuclei in this zone become larger and more rounded towards the apical region of the mucosal folds. The cells contained small P.A.S. positive granules in the perinuclear cytoplasm.

The second zone of the gut is characterized by the presence of large ' supra-nuclear bodies ' and vacuoles with basophilic and P.A.S. positive contents (Plate III). Cells with vacuoles were observed all over the mucosal folds. The volume of the vacuoles

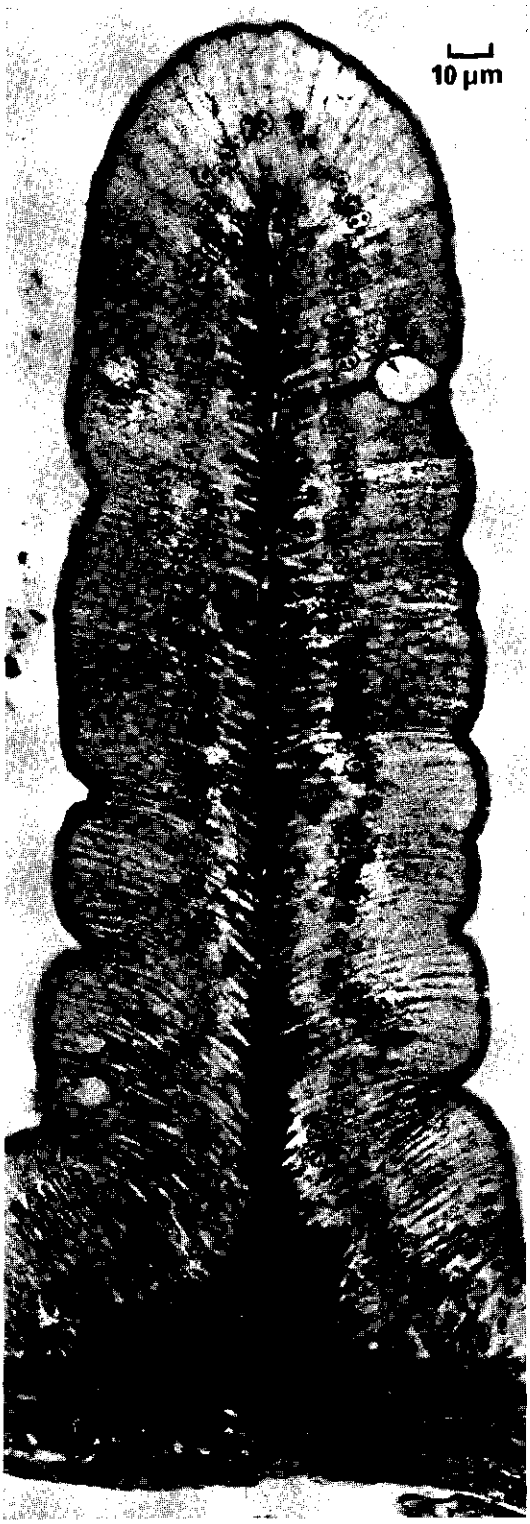

PLATE I. Cross section through mucosal fold in the first zone of intestine of an animal of 48 days, fed on tubifex and plant material. Note the presence of clear vacuoles in absorptive cells at the apex of the fold. Mitotic figures can be seen at the base of the fold. A few goblet cells are present (arrows). Bouin fixative, Azan stain.

increased from rostral to caudal in the second zone. The apical basophilic area of the cells is distinct in this zone (Plate III). Finally, a third zone is found near the anus. In this zone the absorptive cells do not contain vacuoles.

This description is based on control and tubifex fed animals, between which no difference could be detected. The epithelium of plant fed fish is nearly similar to that of animal fed specimens, the absorptive cells of the first zone of the gut do not, however, contain vacuoles, and the cells in all three zones are shorter.

ULTRASTRUCTURAL FEATURES OF ABSORPTIVE CELLS* OF CONTROL ANIMALS

The absorptive cells in the first zone of the intestine are typically columnar and contain well-developed microvilli (Plate IV). In the terminal web area beneath the microvillous border, cellular organelles are absent and the cytoplasmic matrix consists of a dense fibrillar meshwork. Between the terminal web and the nucleus (which is situated at one third of cell height) the cytoplasm is packed with vesicles varying in diameter. They are less numerous in the cytoplasm between nucleus and basal membrane. The vesicles contain small particles with a diameter of approximately 70 nm (Plate IV). Similar particles were found in the numerous intercellular spaces. Mitochondria and polyribosomes are present throughout the cell, the former are more numerous in the basal region (Plates IV, V). In some cells strands of rough endoplasmatic reticulum (RER) have been detected. In the basal part of the cells and occasionally in apical positions lamellar structures are present, generally running parallel to the long axis of the cells (Plate V). Only cells at the apices of the mucosal folds contain large vacuoles with electron lucent contents (Plate IV).

In the second zone of the gut the absorptive cells show invaginations of the apical cell membrane into the terminal web area (Plate VI). One or more large supranuclear vacuoles, mostly surrounded by mitochondria, contain an electron lucent mass and numerous fibrillar structures (Plate VI). A distinct Golgi apparatus, a number of mitochondria and scattered strands of RER are present between these vacuoles and the nucleus. Many mitochondria, RER and lamellar structures are present in the basal cytoplasm.

The cells of the third zone of the intestine are characterized by the irregular arrangement of the microvilli, which are short and few in number (Plate VII). The cells contain many mitochondria, especially in apical and basal position. A perinuclear Golgi apparatus is always present. Many vesicles and tubules of smooth endoplasmatic reticulum can be observed in the cytoplasm. Lamellar structures similar to those described for first zone cells are also present near the basal membrane.

VI. DISCUSSION

The observation that the grass carp juveniles grow faster on animal than vegetable food suggests the fishes are not herbivorous in the first 7 months after birth. This is in accordance with Fischer (1972) who stressed the need for animal proteins for normal growth of *Ctenopharyngodon idella*. The length of the intestinal canal of 7 month old specimens does not indicate a herbivorous way of life since other presumed herbivorous Cyprinids have an intestine of six times the body length (*Campostoma* sp. Rogick,

* Details of ultrastructure are given as far as they underline the characteristics of the absorptive cells in the three zones, and to compare our results with experimental ultrastructural data in the literature. A detailed description of the intestinal ultrastructure will be published elsewhere.

1931) or even 15 times the body length (*Labeo horie*, Girgis, 1952). Our results do not allow conclusions as to the time interval in which the change from a carnivorous to a strictly herbivorous way of life takes place. Older juveniles and even adult grass carp have the same relative gut length as our 7 months old fish (Inaba & Nomura, 1956; Berry & Low, 1970); consequently the suggestion of Inaba & Nomura (1956) that grass carp gradually acquires omnivorous habits after the first month and actually never becomes real herbivorous seems plausible. Hickling (1960) reported very rapid growth of grass carp on Napier-grass. However the fish were kept in large ponds and the stomach contents were not analysed, it cannot be excluded therefore that small amounts of animal food were swallowed with the grass.

McVay & Kaan (1940) were the first authors to describe a regional differentiation of the gut of a Cyprinid. According to these authors, the anterior half of the goldfish *Carassius auratus*, intestine is characterized by the presence of absorptive cells without a single perinuclear vacuole, which was invariably found in cells of the posterior intestine. This is supported by our results in plant fed animals. In tubifex fed grass carp, however, osmiophilic droplets were found in cells situated in the apical part of the mucosal folds in the first zone of the intestine. This suggests that absorption of fat takes place in these cells. Gauthier & Landis (1972) found absorbed lipids appearing first in the apical cells and later in more lateral regions of folds in the anterior gut of the goldfish. Our ultrastructural examination indicates the presence of lipid droplets, possibly chylomicrons, in vesicles of different sizes and in intercellular spaces of only the first zone of the intestine of the grass carp. The large vacuoles found at the apex of the mucosal folds possibly represent stored lipids, since absorption seems most active in this area. The absence of pinocytotic invaginations in anterior intestine absorptive cells was also observed by Yamamoto (1966) and Gauthier & Landis (1972) in goldfish and by Iwai (1969) in juvenile carp. Therefore, fat is probably hydrolysed in the gut lumen and absorbed and resynthesized in cells of the first zone of the gut. This is in accordance with Al Hussaini (1949*b*), Sarbahi (1951) and Hickling (1966), who stated that lypolytic activity is located mainly in the anterior intestine of stomachless teleosts. This mechanism of fat absorption closely resembles that of the intestinal triglyceride absorption in the rat (Cardell *et al.*, 1967).

In the grass carp, the large supranuclear vacuoles in the second zone of the gut contain a P.A.S.-positive substance. This is consistent with the results of Gauthier & Landis (1972) and Noaillac-Depeyre & Gas (1973*a*), obtained in goldfish and carp respectively. The latter authors proved that digestion by amylase did not affect this affinity for P.A.S. On the basis of this observation and of the staining reaction of the substance in the vacuoles with toluidine blue, they concluded the substance is a neutral or slightly anionic glycoprotein. The same authors showed that proteins are absorbed as intact molecules in the epithelial cells of the posterior intestine of goldfish and carp; in these cells they found indications of pinocytotic activity. Yamamoto (1966) concluded that pinocytosis takes place in absorptive cells of the posterior half of the gut in the goldfish. Our results indicate that protein absorption in the grass carp takes place by pinocytosis in the second zone of the intestine, as in other cyprinids. Studies of the activities of several digestive enzymes made it clear that peptic digestion is absent in these stomachless teleosts (Sarbahi, 1951; Ishida, 1936). Tryptic activity, however, was found in the posterior half of the intestine in grass carp (Hickling, 1965), goldfish (Sarbahi, 1951), carp, roach *Rutilus rutilus* and gudgeon *Gobio gobio* (Al Hussaini, 1949*b*). The incomplete digestion of proteins by the absence of a

stomach is probably compensated by pinocytosis. A regional differentiation as in the gut of adult Cyprinids was observed in some teleost larvae (Iwai, 1968*a*, *b*, 1969; Iwai & Tanaka, 1968*a*, *b*).

Most authors do not distinguish different zones in what they call the posterior intestine of fishes. In the grass carp a caudal third zone can be clearly distinguished histologically from the second zone. The presence of relatively few short microvilli suggests that absorption is not as important as in other zones of the gut. The presence of many lamellar structures in the basal part of the absorptive cells is in accordance with the results of Noaillac-Depeyre & Gas (1973*b*) for the ' rectum ' of the goldfish. These authors suggested that this part of the gut is specialized in ion transport. Yamamoto (1966) did not pay special attention to the most posterior part of the intestine of the goldfish, but described lamellar structures in the absorptive cells of the whole intestine. If these structures are related to ion transport, our results indicate that in the grass carp the first and the third zone of the gut are best adapted to this function.

Our results indicate the more rapid increase in relative length of the intestine of plant fed grass carp compared to that in tubifex fed specimens is merely a relative increase in length of the lipid absorbing zone of the gut. This zone may be also responsible for carbohydrate absorption, as a P.A.S. positive granulation was found in the absorptive cells, and amylase is found chiefly in the anterior intestine of a number of Cyprinids (Al Hussaini, 1949*b*, Sarbahi, 1951; Hickling, 1966). The cause of the enlargement of the gut and its physiological significance needs further investigation.

The distribution of absorptive cells in the intestine and mucosal folds differs from that in mammals. The distinct regional differentiation in the carp intestine is considered useful for investigating the differentiation of epithelial cells. The cyprinid is a useful model for examining fat and protein absorption pathways, the kinetics of the epithelial cells in different zones of the gut, and the influence of different kinds of food on the latter.

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The Ultrastructure and Renewal of the Intestinal Epithelium of the Juvenile Grasscarp, *Ctenopharyngodon idella* (Val.)

H.W.J. Stroband and F.M.H. Debets*

Agricultural University, Department of Experimental Animal Morphology and Cell Biology, Zodiac, Wageningen, The Netherlands

Summary. The intestinal absorptive epithelium of starved and fed fish has been studied electron microscopically. After feeding, cells of the proximal segment of the intestine show morphological characteristics of lipid absorption. Absorptive cells in the middle segment contain many pinocytotic vesicles in both fasted and fed specimens. Absorption of protein macromolecules is supposed to be one of the main functions of this part of the gut. In the most caudal part of the intestine, absorptive cells carry relatively few and short microvilli. The proximal and distal segments show structural indications of a function in osmoregulation.

The renewal of the epithelium has been studied with light microscopic autoradiography, using tritiated thymidine. The intestinal mucosal fold epithelium represents a cell renewal system. The cells proliferate at the base of the fold and migrate towards the apex in 10–15 days at 20° C. The functional absorptive cells proved to be generally present in the intestinal epithelium, including the proliferative area. Undifferentiated cells have not been identified.

The results will be compared with data on absorption of lipid and protein macromolecules in teleostean and mammalian intestines and with descriptions of the cell renewal system in the mammalian intestine.

Key words: Intestine – Teleost – Epithelium – Renewal – Ultrastructure.

Introduction

A differentiation of the intestinal epithelium was found in a number of Cyprinids (which do not have a stomach) (Yamamoto, 1966 and Gauthier and Landis, 1972 in the goldfish; Iwai, 1969 in the carp; Noaillac-Depeyre and Gas, 1976 in the tench). The rostral part of the gut, including the intestinal bulb, was shown to absorb lipids,

Send offprint requests to: Dr. H.W.J. Stroband, Agricultural University, Department of Experimental Animal Morphology and Cell Biology, Zodiac, Prinses Marijkeweg 40, Wageningen, Niederlande

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and the posterior part of the intestine absorbs protein by pinocytosis. Noaillac-Depeyre and Gas (1973b) demonstrated that ion transport takes place in the most caudal part of the gut of the goldfish. In a recent morphological study, the grasscarp intestine was shown to consist of three segments (Stroband, 1977).

In the goldfish (Vickers, 1962; Hyodo, 1965) and the carp (Gas and Noaillac-Depeyre, 1974), the intestinal epithelium represents a cell renewal system. Cell proliferation takes place in the basal part of the mucosal folds, and the cells migrate to the top of the folds where they are probably discarded.

The present study deals mainly with the renewal of the intestinal epithelium of the grasscarp. Autoradiography was applied after injecting ^{3}H-thymidine, to locate the supposedly present proliferative pool of cells and to trace the renewal of the epithelium. The ultrastructure of absorptive cells has been studied in three areas of the mucosal fold: the base, the central part and the apex. The purpose was to obtain general information on the ultrastructure of absorptive cells and on the morphology of possibly differentiating cells, migrating from the proliferative pool to the apex of the folds. To identify functional absorptive cells, the differences have been studied between these cells in fed fish and in starved specimens.

Materials and Methods

Autoradiographic studies were made on 21 one year old juvenile grasscarp (*Ctenopharyngodon idella*, Val.) provided by O.V.B. Lelystad, Holland. These were injected intraperitoneally with methyl-^{3}H-thymidine (1 µCi/gram body-weight; spec. activity 18 Ci/mmol; Radiochemical Centre, Amersham, England). Before and during the experiment the fish were fed with Tubificidae. The water temperature was 20°C±1°C. Three animals were killed with $\pm 0.3^0/_{00}$ MS 222 (Sandoz, Basel, Switzerland) after 3 h, 24 h, 2 days, 5, 10, 15 and 21 days. Tissues were fixed in Bouin's fixative and embedded in paraffin. Sections of 3 µm were stained with haemalum and eosin. Radioautograms were made with slides prepared in Kodak NT B 2 emulsion (Eastman-Kodak, Rochester, New York), diluted 1 : 1 with distilled water. The slides were exposed for three weeks.

For electron microscopic purposes, 7–9 months old grasscarp were starved for 15 days (2 animals) or 40 days (4 animals). In addition, two specimens were regularly fed. Three of the starvelings and the two regularly fed fishes were given tubifex approximately 3 h before being killed. All these animals were anaesthetized in $0.1^0/_{00}$ MS 222, and small fragments of intestinal tissue were excised from the locations shown in Fig. 1. For electron microscopical examination, tissues were prefixed for 15 min in a mixture of 1% osmium tetroxide and 2% glutaraldehyde buffered with 0.05 M Na-cacodylate (pH 7.2) at 0° C. Postfixation was made in a similar mixture to which 1% potassium bichromate was added. The tissues were sectioned on a LKB III ultramicrotome. The sections were contrasted with uranyl acetate, followed by lead citrate according to Reynolds (1963), and photographs were made on a Philips 300 electron microscope.

Results

1. The Renewal of the Intestinal Epithelium

3 h after injection of ^{3}H-thymidine, labelled epithelial cells can be noticed in the three segments of the gut. Most of these cells are found in the central segment (Table 1), and they are concentrated in the basal third of the folds (Figs. 2, 4; Table 1).

Table 1. Percentage of labelled cells in a given area of the mucosal folds in two specimens (I and II), 3 h after ^{3}H-thymidine injection

Area (in % of total fold length) 0% = base of the fold	Percentage of labelled cells in a given area measured in 5–8 folds/segment					
	proximal segment		middle segment		distal segment	
	I	II	I	II	I	II
0–10	3.3±0.5	6.5±1.0	8.2±1.8	10.2±2.6	3.3±0.9	3.9±1.1
10–20	1.1±0.4	2.9±1.6	4.6±1.1	2.3±1.7	3.7±1.1	1.5±0.4
20–30	1.3±0.7	–	2.5±0.8	–	0.7±0.5	0.6±0.3
30–40	–	–	2.0±1.0	–	1.7±1.3	–
40–50	–	–	0.9±0.4	–	–	–
50–60	–	–	0.7±0.4	–	–	–

Figure 4 shows that labelled cells migrate to the apex of the mucosal folds. The result after 10–15 days are shown in Figure 3. The height in the folds reached by labelled cells 48 h after ^{3}H-thymidine injection and the large standard deviation in this column suggest a considerable individual variation in the proliferative activity of the intestinal epithelium.

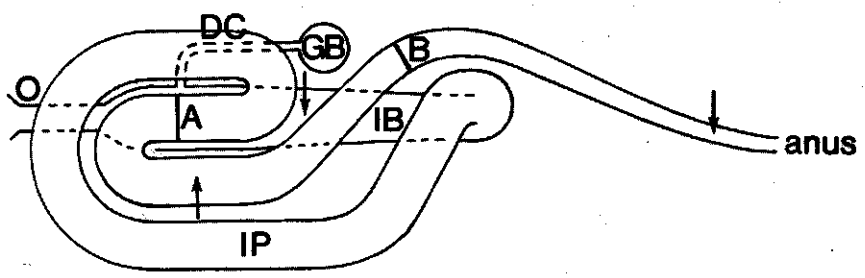
Three hours after injection labelling in the intestinal epithelium may be quite different from place to place; this indicated that proliferation varies in the folds with time. The well-labelled areas have been used for quantification.

2. The Ultrastructure of the Epithelium of the Proximal Segment

a) In Fed Fish. Most of the cells of the (columnar) epithelium consist of absorptive cells. Goblet cells are present too, and also some endocrine cells (Fig. 18; Rombout, 1977) and migrating cells (lymphocytes, granulocytes and macrophages).

The apical part of the absorptive cell bears numerous microvilli (Fig. 5) with an asymmetric cell membrane (Fig. 8). The part of the cell adjacent to the lumen is covered with a thin fuzzy coating or glycocalyx. The cytoplasm of the microvilli contains a number of longitudinal fibrillar structures, penetrating the terminal web. The tips of the microvilli have a dense cap (Fig. 5), named *capitulum* by Krementz and Chapman (1974).

The lateral plasma membranes of adjacent cells run closely together from the microvillous border to the basement membrane without complex interdigitations. Desmosomes are frequently encountered, most of them lying in the terminal web area where a junctional complex is present (Fig. 9). In the basal half of the cells, the lateral plasma membranes form long lamellar infoldings parallel to the long axis of the cells. These infoldings can be recognized by the regular distance between the membranes (±250 Å) and by the presence of lumen material with a relatively high electron density. The membranes are relatively thick and asymmetrical, just as in the microvilli (Fig. 6). The same type of infoldings originates in the basal plasma membrane (Fig. 7). Similar structures were described by Noaillac-Depeyre and Gas



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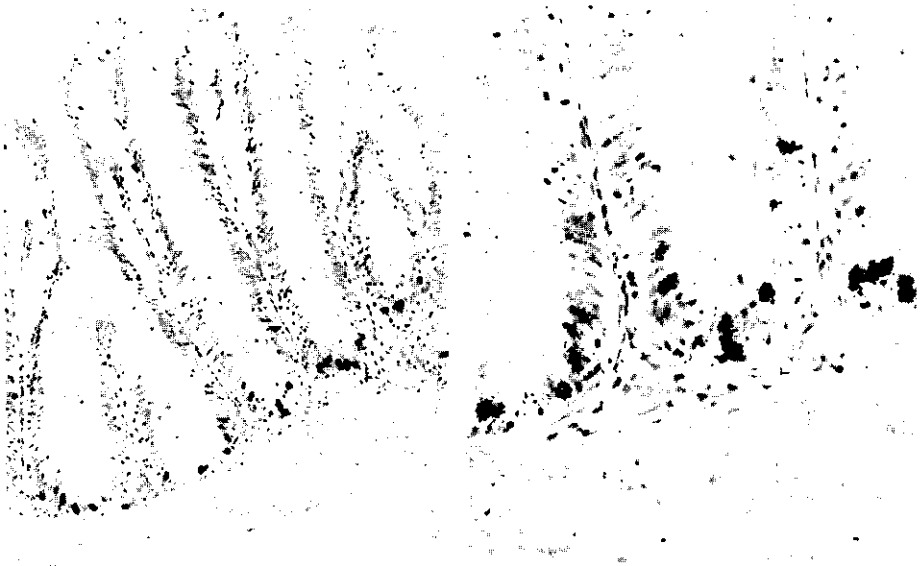

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3

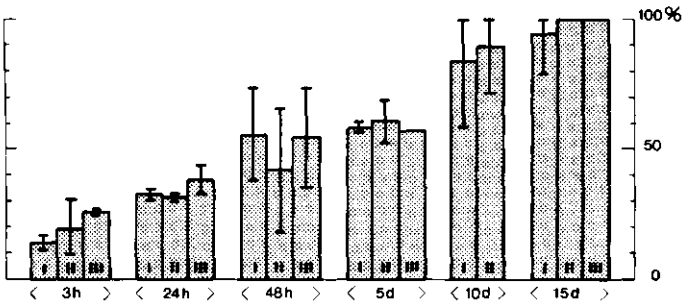


Fig. 4. Areas of the mucosal folds containing labelled cells. Each value represents the mean of 3 animals; 5–8 folds have been measured per fish. Ordinate: % of fold height. Abscissa: time after injection

(1973b) in cells of the "rectum" of the carp, and it was suggested that they may play a role in ion transport.

Between the microvilli, pinocytotic vesicles may be present (Fig. 9), which do not contain fat droplets, most membranes of which have a coated appearance.

A terminal web is present below the microvillous border (Fig. 5). In this area cytoplasmic organelles are scarce, and ribosomes and some vesicles may be present also. Mitochondria, located in the basal parts of the cells (Fig. 13), are generally closely connected with the lamellar structures.

The nucleus of the absorptive cell is oval and located in the basal part of the cell at about one third of the height. A few ribosomes may be attached to the outer membrane of the nuclear envelope, which is generally smooth (Fig. 10).

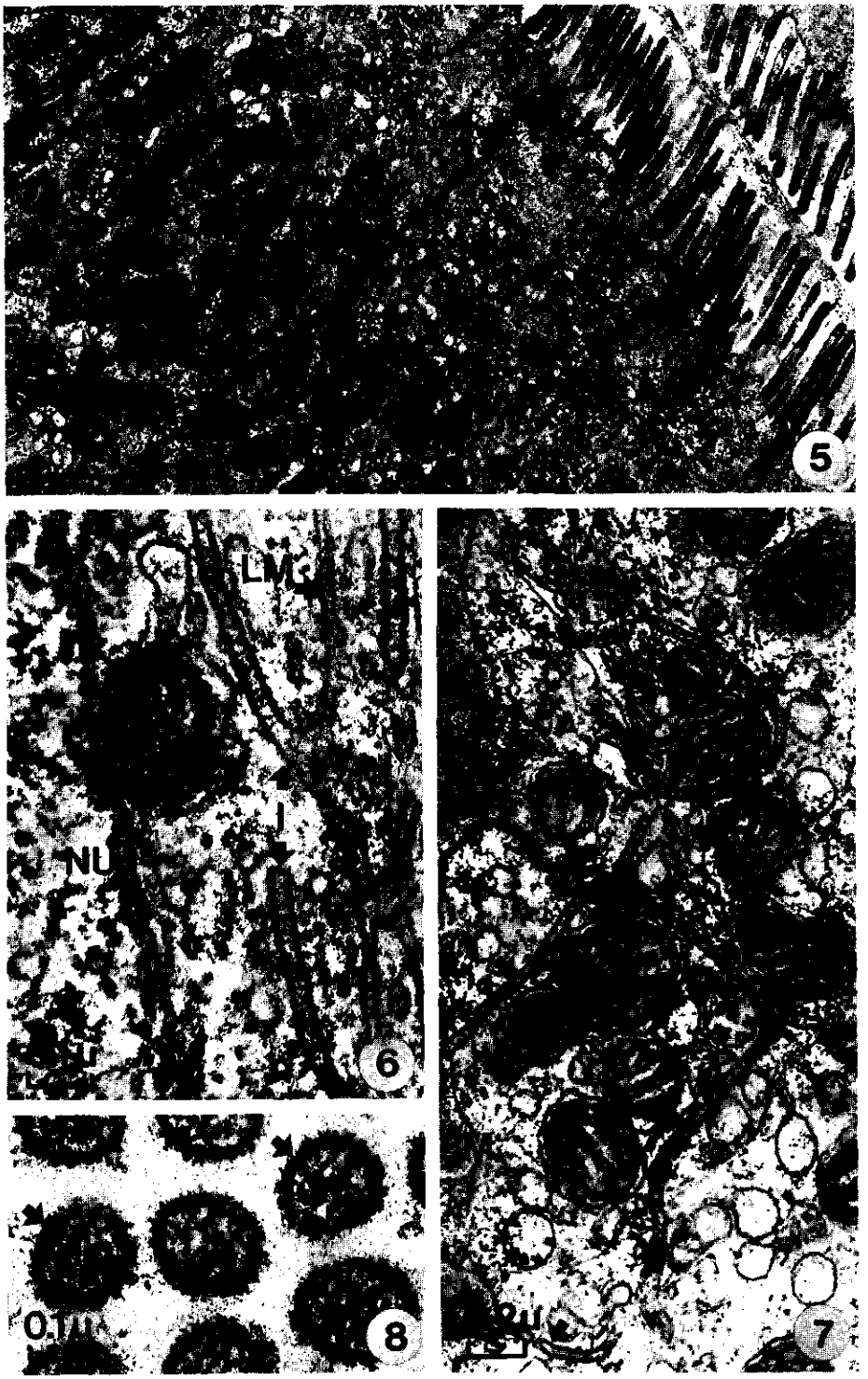
Rough-(RER) and smooth-(SER) endoplasmic reticulum are overall present, except in the terminal web area. In the upper part of the cells, the SER is predominant. Many small fat particles, approximately 700 Å in diameter, are present in the SER, RER, Golgi apparatus (which is located in the perinuclear cytoplasm) and intercellular spaces (Figs. 9, 10, 11, 12). Large droplets of fat are present too (Fig. 10), especially in the cytoplasm above the nucleus, and these are surrounded by a membrane.

b) In Fasted Fish. The most striking change in the absorptive cells after fasting is the absence of fat particles and fat droplets in the cells and in the intercellular spaces.

Fig. 1. Scheme of the position of the grasscarp intestine. The oesophagus (*O*) opens directly into the gut. In the wide first limb, or intestinal bulb (*IB*), the ductus choledochus (*DC*) opens a few mm from the oesophagus (*O*). From the end of the first limb the intestine proper (*IP*) narrows and leads to the anus. The arrows indicate the locations of the tissues used in this study. *A* and *B* represent the boundaries of the middle segment. *GB* gallbladder

Fig. 2a and b. Radioautograph of the middle segment of the intestine 3 h after injection with ^{3}H-thymidine. The DNA precursor is present in nuclei in cells of the basal part of the mucosal folds. × 125. **b** As Fig. 2a. × 250

Fig. 3. Radioautograph of the middle segment 10 days after injection. Labelled cells have reached the apex of the mucosal folds. × 125



Figs. 5–8. Absorptive cells in the proximal segment

Mitochondria were not only found in large numbers in the basal part of the cells, as in fed fish, but they are concentrated in the upper third of the absorptive cells also. This suggests that several of the mitochondria have migrated to more apical parts of the cells during the fasting period. This type of apical concentration of mitochondria is not noticed three hours after feeding, following a long period of fasting. The matrix of mitochondria of fasted specimens (Fig. 5) contains dense granules, which disappear after feeding.

c) Cells at Different Locations of the Mucosal Folds. All cell organelles appear to be well developed, even in the most basal part of the folds. A distinct indication of cells at the base of the folds being less differentiated is their narrow shape compared to the shape in other areas of the fold (the average number of microvilli per cell is 15 in sections at the base of the folds and 30 in sections at the top of the mucosal folds in the proximal segment) and the great number of free polyribosomes (Fig. 15). One type of columnar cell is restricted to the base of the folds. This cell contains many large mitochondria with electron dense granules intermingled with ER and free ribosomes (Fig. 17). This type may be found in groups of two or three cells. Lymphocytes are present in the basal part of the mucosal folds, generally in large numbers, most of which are located near the basement membrane.

After feeding, the cells in the basal part of the folds are similar to cells at other locations. The ER (Fig. 16) and Golgi vesicles are filled with lipid particles, and most mitochondria are located in the basal part of the cells, but this is less distinct than at other locations of the fold. After fasting neither lipid particles nor lipid droplets have been observed.

After feeding the cells at the top of the folds are different from those at other locations. Next to structures mentioned above, several large lipid droplets (up to 4 μ) are present, most of them in the supranuclear cytoplasm (Fig. 14). The extracellular spaces are very large in this area and the different location of the mitochondria in fed and fasted fish is most conspicuous in these parts.

Fig. 5. The apical part of some cells in the middle area of a mucosal fold in a fasted fish. Many vesicles of SER without lipid particles below the terminal web (*TW*). Note the abundant presence of mitochondria (*M*) in this part of the cells, containing dense granules ×16,600

Fig. 6. The lateral plasma membranes (*LM*) of adjacent cells in the nuclear (*NU*) area. The membranes form infoldings (*I*) containing an electron dense material. The membranes are asymmetric and thicker than the lateral plasma membrane. ×81,500

Fig. 7. Basal portion of a cell with infoldings of the basal plasma membrane (arrows), closely connected with mitochondria (*M*). Between the basal cell membrane and the basement membrane (*BM*) a narrow space can be noticed. ×41,000

Fig. 8. Transverse section through microvilli. Many fibrils form the core of the microvilli. The unit membrane is asymmetric. The microvilli are covered with a thin fuzzy coating. ×104,000

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Histochemistry

Regional Functional Differentiation in the Gut of the Grasscarp, *Ctenopharyngodon idella* (Val.)

H.W.J. Stroband, H. v.d. Meer, and Lucy P.M. Timmermans

Agricultural University, Department Experimental Animal Morphology and Cell Biology, Zodiac, Marijkeweg 40, Wageningen, The Netherlands

Summary. A regional differentiation – reflecting structural differences – of the intestine of larval and juvenile grasscarps can be illustrated by studying the activity of alkaline phosphatase and the uptake of orally administered horseradish peroxidase.

Pinocytosis of peroxidase takes place in a welldefined area of about 23% of the length of the gut (segment II). Neither the rostral ±68% (segment I) nor the caudal ±9% (segment III) shows absorption of the enzyme.

Alkaline phosphatase activity, mainly localized at the microvilli of the enterocytes is high in the first segment of the gut and low in the second segment. In larvae, the activity decreases sharply at the transition from segment I to segment II. The activity is weak or absent in the caudal third segment. Quantitative histochemical data are confirmed by biochemical analyses.

Alkaline phosphatase activity is found all over the mucosal folds of the first segment, with relatively weak activity at the base and at the tip of the folds. This may be related to a renewal of the epithelium.

Our results suggest that active absorption of digested food takes place mainly in the rostral first segment, while the uptake of macromolecules by pinocytosis is a function of the second segment. Comparison of the results with information available in literature leads to a rejection of the hypothesis that the uptake of protein macromolecules in Cyprinids is to be attributed to the absence of a stomach and therefore to an inefficient digestion of proteins.

Introduction

An understanding of absorption processes and related morphological and physiological characteristics in larval, juvenile and adult stomachless teleosts is valuable for fish culture. This is especially true because most teleosts pass through a larval period (Balon, 1975) in which exogenous food is used when a stomach

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LOCALIZATION OF PROTEIN ABSORPTION DURING TRANSPORT OF FOOD IN THE INTESTINE OF THE GRASSCARP, *CTENOPHARYNGODON IDELLA* (VAL.)

H.W.J. Stroband and F.H. van der Veen,

Agricultural University, Department of Experimental Animal Morphology and Cell Biology, Zodiac, Marijkeweg 40, Wageningen, The Netherlands

SUMMARY

The uptake of protein and aminoacids in the intestine of young (18 months old) and adult grasscarp has been studied with the inert marker (Cr_2O_3) method. The absorption of protein takes place in the anterior 40-50% of the gut.

The second intestinal segment (± 25% of gut length) has the ability to absorb macromolecules of protein. Ferritin (MW 300.000) was found in pinocytotic vesicles in enterocytes of this second segment. On the basis of morphological and histochemical evidence, it has previously been suggested that the pinocytotic uptake of whole protein molecules might be related to the lack of peptic digestion in stomachless fishes. A major quantitative function in protein absorption however cannot be attributed to the second gut segment, and proteins appear to be digested adequately in these stomachless animals. The function of this part of the gut and the role of the stomach in protein digestion in other vertebrates remain to be explained.

The absorption of individual aminoacids in the intestine of the grasscarp suggests that essential aminoacids are preferentially absorbed. The rapid disappearance of lysine and arginine from the chyme points to a tryptic breakdown of proteins.

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THE DEVELOPMENT OF THE STOMACH IN *CLARIAS LAZERA* AND THE INTESTINAL ABSORPTION OF PROTEIN MACROMOLECULES

H.W.J. Stroband and Annet G. Kroon

Agriculture University, Department of Experimental Animal Morphology and Cell Biology, Wageningen, The Netherlands.

SUMMARY

The development of the stomach of the teleost *Clarias lazera* is described for the early posthatching period. A comparison is made to the stomach of adult *Clarias*.

The stomach develops in two distinct parts: the corpus, which is the earliest differentiated part, and the pylorus. The corpus contains a mucous surface epithelium, arranged in folds, and a tubular gland system containing only one type of gland cell, to which the secretion of pepsinogen and HCl is attributed. The pyloric region does not contain tubular glands.

From the ultrastructure of the gland cells, the ^{3}H thymidine labeling index, and the onset of acid production (as determined with pH indicators) it is concluded that a functional stomach is present in juveniles with a standard length of $\pm$ 11 mm. (approximately 12 days after fertilization at 23-24^{o}C).

In addition to the stomach, the ultrastructure of the intestinal epithelium has been studied. The gut shows three segments, similar as described for stomachless teleosts and a number of fish larvae. In larvae as well as in juveniles, the enterocytes of the second segment show pinocytosis of horse radish peroxidase, although in the juveniles the stomach has already developed. This second segment has the same relative length in all studied larvae and juveniles and is also present in adult *Clarias*.

Therefore it must be concluded that the ability of absorbing protein macromolecules is not definitely related to the absence of a functional stomach in this teleost species.

Key words: Stomach - Intestine - Epithelium - Teleost - larvae.

INTRODUCTION

In stomachless teleosts the intestine shows a regional differentiation. The anterior segment has the morphological characteristics of lipid absorption, while the shorter second segment has the ability to absorb protein macromolecules by

pinocytosis (Yamamoto, 1966; Iwai, 1969; Gauthier & Landis, 1972; Noaillac-Depeyre & Gas, 1973[a], 1976; Stroband, 1977; Stroband & Debets, 1978; Stroband et al., 1979). The third segment is probably involved in ion and water transport (Noaillac-Depeyre & Gas, 1973[b]; Stroband & Debets, 1978).

It was postulated by the above mentioned authors that the absorption of macromolecules might be related to the lack of a stomach and to an inadequate protein digestion. This is also known in suckling mammals, in which pinocytosis of protein macromolecules takes place in the small intestine before the stomach is fully functional (Cornell & Padykula, 1965; Kraehenbühl & Campiche, 1969; Krause et al., 1977). In our view there are two ways for testing the mentioned hypothesis. If no appreciable quantities of protein are absorbed by the second gut segment in stomachless fish, the hypothesis would be without any foundation. Experiments are in progress and the first results will be published elsewhere (Stroband & Van der Veen, in preparation). The second way is to study the post hatching development of the digestive tract of a teleost with a stomach. Usually teleost larvae lack a functional stomach until several weeks after the start of exogenous feeding (Tanaka, 1972). During this period the intestine shows similar morphological characteristics as found in the stomachless fishes. Consequently, the second segment of the intestine of teleost larvae is likely to absorb protein macromolecules also.

The principal objective of the present study is to ascertain whether changes occur in the form and function of the intestine, especially in the second gut segment, when the stomach becomes functional. Therefore, the development of the digestive tract has been studied with light- and electron microscopical methods.

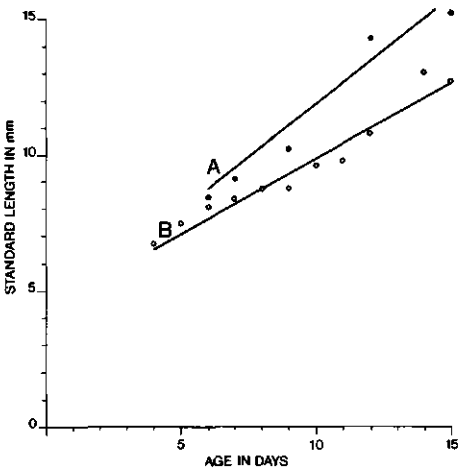
The period of development of the stomach was also determined with ^{3}H thymidine as a marker of cell proliferation. Moreover it was expected that the labeling index decreases when cells become functionally active.

pH Indicators have been used to find out when acidity reaches pH 5 - 4, as cathepsin as well as pepsin has an optimum pH below 5. The uptake of horse radish peroxidase served as a marker for macromolecule absorption.

MATERIAL AND METHODS

Two batches of new-hatched *Clarias lazera* (Cuv. and Val.) larvae and 6 adult specimen were kindly provided by the Department of Fish Culture and Inland Fisheries of the Agricultural University. Although the larvae were reared in exactly the same way, the two batches showed somewhat different growthrates (fig. 1).

g. 1. Growth rates of the two batches of *Clarias* larvae, (a) and (b).



The larvae were reared at 23-24°C. At 5, 6, 8, 9, 12 and 15 days after fer-
lization a number of fishes of bath (a) were injected with ± 30-40 nl. of a
l thymidine solution (1 Ci/L, spec. act. 24 Ci/mmol, Radiochemical Centre,
ersham, England). After 3-4 h incubation some specimens were fixed in Bouin's
xative and subsequently embedded in paraffin. 4 µ Sections were stained with
emalum and eosin or with Alcian Blue and Schiff's reagens (Romeis, 1968).
dioautographs were made with Kodak NTB-2 liquid emulsion (Eastman-Kodak,
chester, New York), diluted 1 : 1. The slides were exposed for two weeks. For
e experiments described below, the second batch (b) of Clarias larvae was used.
ecimens of 4, 7, 9, 10, 11, 12 and 15 days old were prepared for electron
croscopy: they were fixed in a mixture containing 2% osmium tetroxide, 2% glu-
raldehyde and 1% potassium bichromate, buffered with 0,05 M Na - cacodylate
H 7.2) at 0°C. 1 µ Sections were prepared for radioautography. Ultrathin sec-
ons were made on a Reichert Ultratome IV microtome, contrasted with uranyl
etate and lead citrate according to Reynolds (1963), and photographed with a
ilips 300 electron microscope.

Furthermore, specimens of 4 to 21 days were fed with *Artemia salina* in a
horse radish peroxidase solution (Sigma, St. Louis; type II, mol. Wt. ±
.000). They swallowed the enzyme together with the food. After 2 h the fishes
re fixed for 2 h in 3% glutaraldehyde buffered with 0,05 M Na-cacodylate (pH
2), rinsed with water for 3 h, frozen in liquid nitrogen and freeze dried for
h. After embedding in paraffin, 5 µ sections were prepared and incubated at
om temperature for 10-30 minutes in a medium after van Duyn (Pearse, 1972).

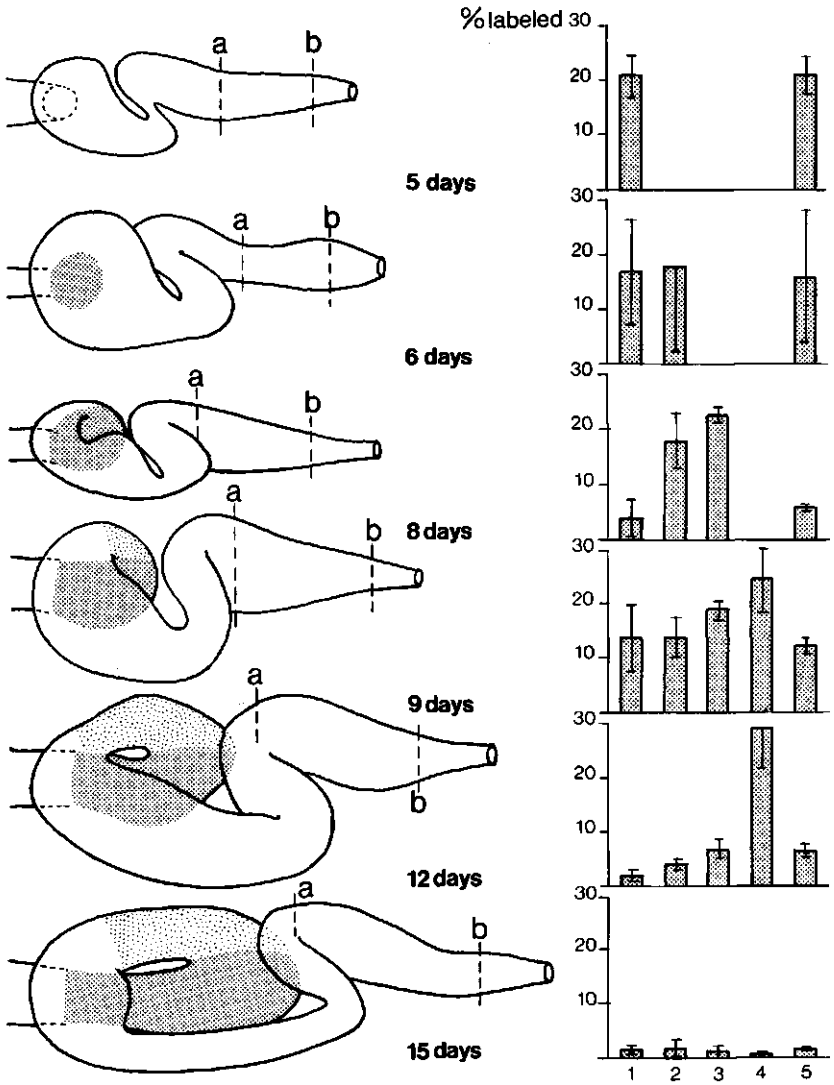


Fig. 2. a. schematic drawings of the digestive tract of *Clarias* (batch a) at 6 stages of development. Dorsal view: o = oesophagus; s = stomach; i = intestine a = anus. A and B represent the boundaries of the second gut segment
▒ = wall of the corpus of the stomach.
▒ = wall of the pyloric part of the stomach.

b. ordinate: Mean percentages of ^{3}H thymidine labeled epithelial cells three fishes at the same 6 stages of development as fig. 2 a. abscissa: 1, 2, 4 and 5 represent the caudal oesophagus, the most rostral, mid and caudal (pyloric) part of the stomach, and the intestine respectively. For the stomach 2 slides have been counted per location in each fish. In the intestine the percentage of labelled cells was counted at 7 locations over the entire length.

During the experiments, every day some specimens of bath (b) were anaesthe-
ized with 0,1 °/oo MS 222 immediately after feeding. A small dose of methyl-red
r congo-red was injected into the stomach through a glass capillary.

Parts of the digestive tracts of adult *Clarias lazera* were fixed and pre-
ared for light and electron microscopy as described above.

ESULTS

. *The morphology and regional differentation of the digestive tract.*

Fig. 2a shows the morphology of the digestive tract as reconstructed from
ght microscopic preparations. Before day 5, an "Anlage" of the future stomach
uld not be detected. The intestine showed the three segments as described for
omachless fish. The esophagus is directly followed by the first segment, and
e bile duct enters this first segment very close to the esophagus. As from day
, the most caudal part of the "esophagus" gradually develops into the stomach,
ich is easily recognized histologically by the glandular tissue in the thickened
ll (figs. 3, 4, 5). Between day 9 and day 15 the pyloric part of the stomach is
veloped.

After this stage no important changes seem to take place in the morphology
the digestive tract, apart from a proportional growth of the various parts.
e relative length of the gut (as compared to body length) and the relative
ngths of the intestinal segments remain the same (table 1). In adults the third
gment is relatively short, but the second segment has the same length as in
rvae.

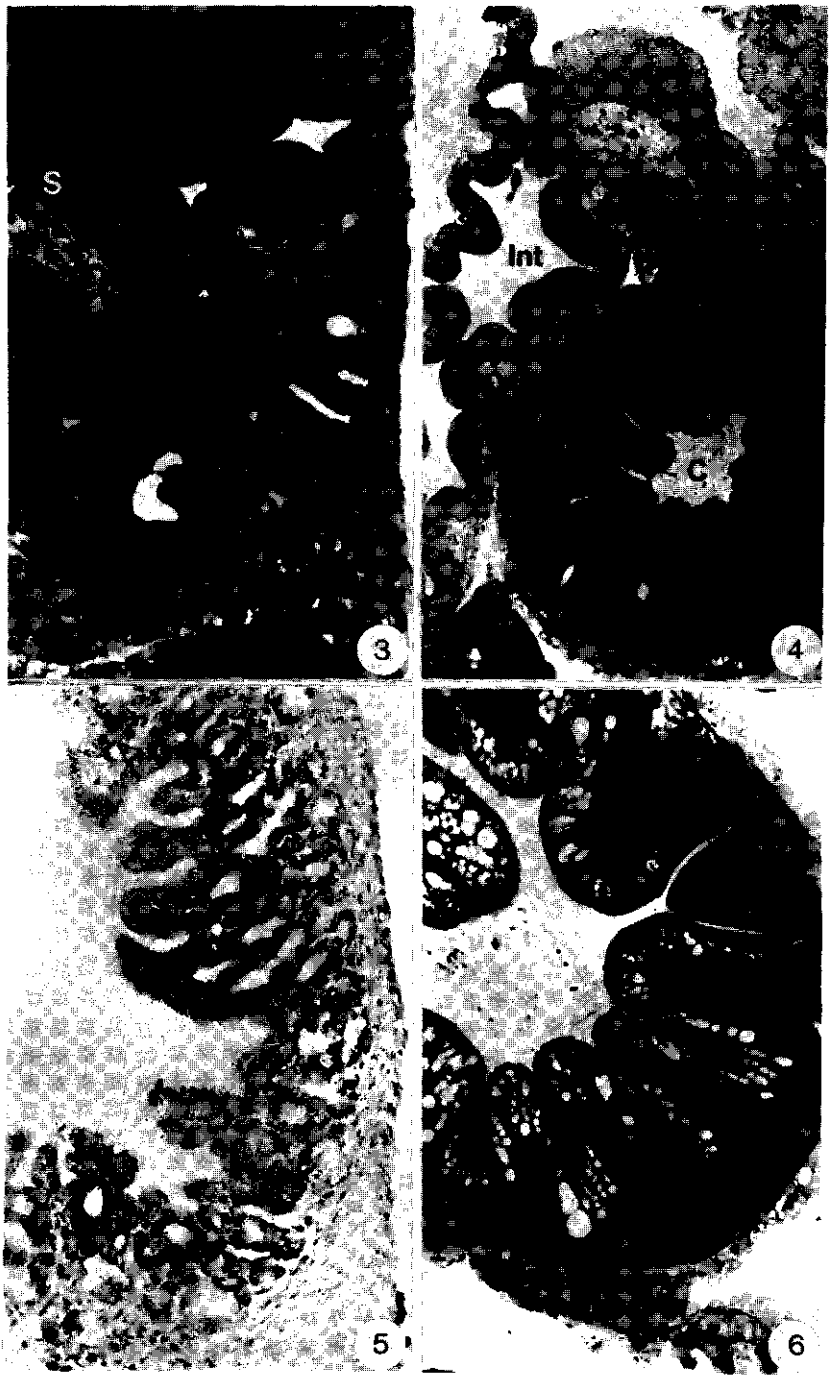

S
3
Int
C
4
5
6

Table 1. Ratio of the length of the intestine to the standard bodylength, and the relative length of the three gut segments as determined for 3 specimens of each age (batch a).

age (days)	standard length (mm)	length intestine / body length	relative length of gut segments (%) I	II	III
5	8,0*	0,36	68,3 ± 1,6	22,0 ± 1,6	9,8 ± 2,4
6	8,4 ± 0,5	0,44	73,0 ± 4,0	16,7 ± 3,8	10,3 ± 1,5
8	10,4*	0,34	67,7 ± 3,5	20,0 ± 2,6	12,3 ± 1,5
9	11,9 ± 0,5	0,29	69,7 ± 2,1	22,0 ± 1,0	8,3 ± 1,5
12	14,3 ± 0,6	0,35	64,3 ± 5,5	20,7 ± 4,0	12,0 ± 1,0
15	15,2 ± 0,8	0,37	66,0 ± 1,0	23,7 ± 0,6	10,1 ± 1,5
adult	257,8 ± 15,7	0,45	70,8 ± 3,1	23,9 ± 2,4	5,2 ± 0,5

* : extrapolations from fig. 1.

2. *The differentiation of the digestive tract epithelium as determined by its ^{3}H-thymidine labeling index.*

The percentage of ^{3}H-thymidine labeled cells in several parts of the digestive tract and at different stages of development are presented in fig. 2b. In larvae of 5 days, 21 to 23% of the epithelial cells were labeled over the entire length of the intestine, but large variations were noticed in each specimen. Labeled cells were found all over the epithelium, but most are present in the lower parts of the mucosal folds (fig. 6). During development the percentage of labeled cells in the gut gradually dimished to ± 3% on day 15, when labeled cells

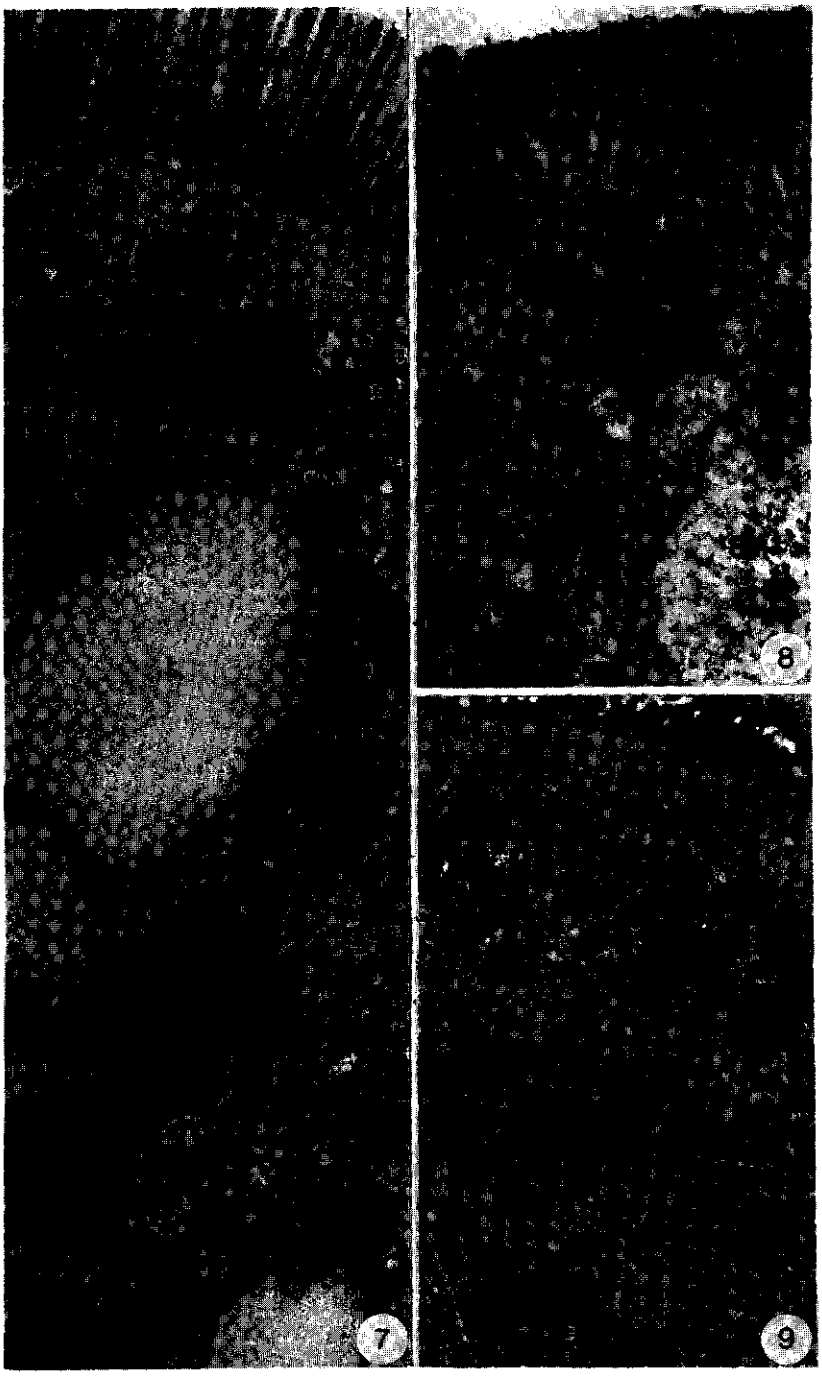
Fig. 3-6: Incorporation of ^{3}H thymidine in the gut of *Clarias lazera*.

Fig. 3. Larva, 4 days old. Light microscopic radioautograph of a 1 µ epon section stained with toluidine blue. Note the high labeling index of the tissues. S = surface epithelium; Gl = undifferentiated glandular cells. (x 500)

Fig. 4. 1 µ epon section through the corpus (C) pylorus (P) and rostral intestine (Int) of a 7 days old *Clarias*. Note the relatively high labeling index in the pyloric area and the very low percentages of labeled cells in the gut (x 250)

Fig. 5. Developing stomach of a 12 days old larva. A few labeled cells in the anterior corpus of the stomach (C) and a high labeling index in the posterior pyloric area (P). x 250

Fig. 6. Mucosal fold of the second intestinal segment of a 4 days old larva. Many supranuclear vacuoles in the enterocytes and most labeled cells at the base of the folds. x 500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ıre restricted to the basal part of the mucosal folds. In the area of the develo-
›ing stomach, the labeling index diminishes later, first in the anterior part and
ater more caudally (figs. 2-5). As from day 9 the pyloric area can be recognized.
ts labeling index of ± 30% decreases between day 12 and day 15 to similar values
s found in other areas of the digestive tract. As in the intestinal epithelium,
roliferative surface mucus cells in corpus and pylorus are merely found in the
asal part of the folds after the drop of the labeling index. Glandular cells
emain labeled at random in all stages.

For the proliferation of the epithelium of the successive parts of the di-
estive tract, the following is of interest. A comparison of radioautographs of
μ sections with ultrathin sections did not show any distinct differences between
he cytology of ^{3}H-thymidine labeled cells and other cells, neither in the surface
pithelium (fig. 21) and glandular epithelium of the stomach nor in the epithelium
f the intestine of the studied fishes (upto 10 days old). Functional cells appa-
ently have the facility to synthesize DNA; consequently they are able to divide.

. *Ultrastructure and regional differentiation of the intestine.*

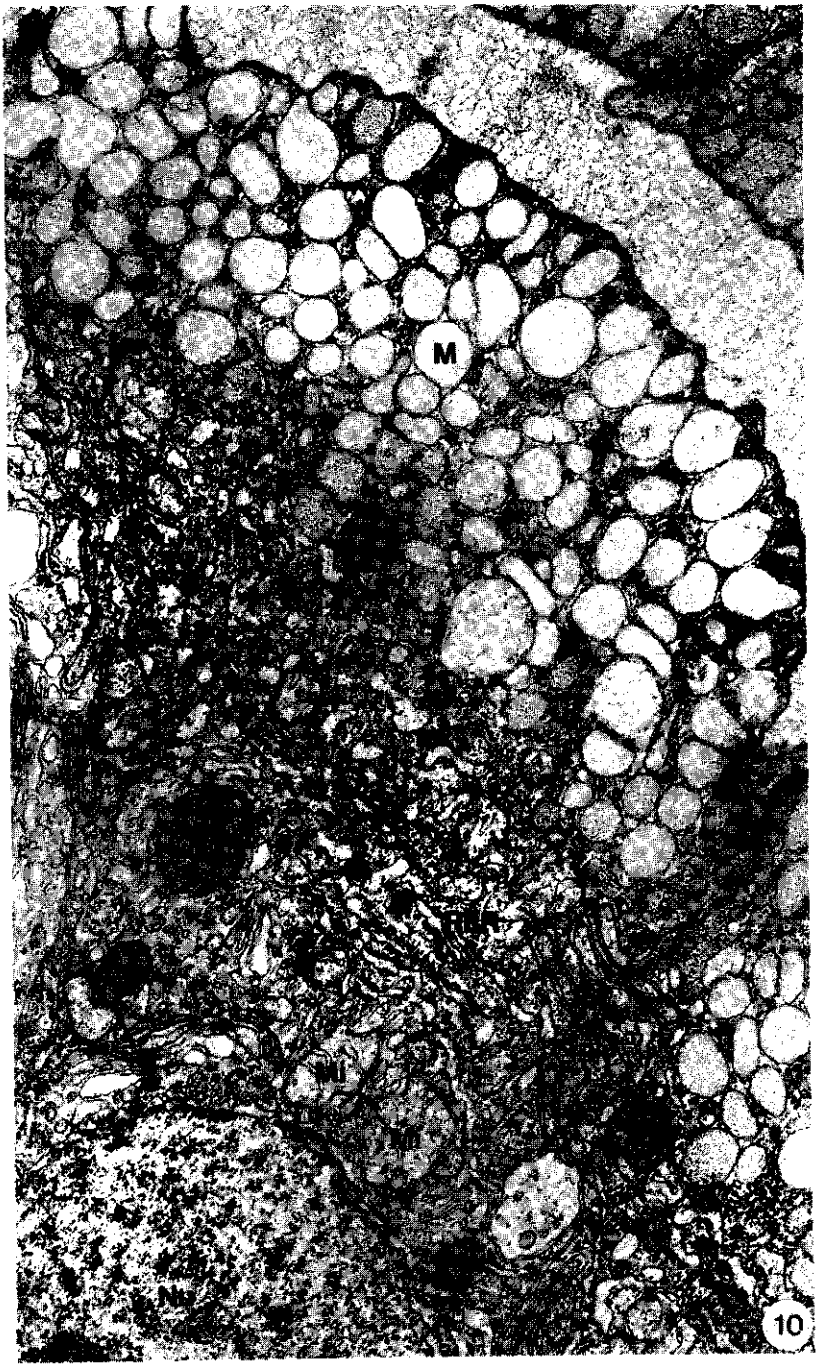
In larvae of 4 days the esophagus, mainly lined with mucous cells, enters
irectly into the first segment. This first segment is involved in lipid absorp-
ion. Absorptive enterocytes, recognized by the apical border of microvilli, con-
ain chylomicrons in their endoplasmatic reticulum (ER) and Golgi apparatus.
arge lipid droplets are also present (fig. 7). Chylomicrons are also found in
ıe intercellular spaces between the enterocytes. Pinocytotic vesicles are scarce
r absent. Mitochondria and strands of ER are present throughout the cytoplasm

igs. 7-9: Electron micrographs of gut absorptive cells in *Clarias lazera*.

ig. 7. Apical part of an absorptive cell in the caudal part of the first
ıtestinal segment of a 4 days old larva. MV = microvilli; TW = terminal web;
i = mitochondria; L = lipid droplet; Ch = chylomicrons; G = Golgi apparatus
ith chylomicrons. x 17.000

ig. 8. Some absorptive cells in the second intestinal segment. Many pinocytotic
esicles (arrows) are present beneath the microvillous border (MV), Supranuclear
acuoles (SV), probably originating from fusing pinocytotic vesicles and lyso-
›mes. 4 Days old larva. x 26.000

.g. 9. Enterocytes in the third segment of the gut of a 7 days old larva. Many
ımellar infoldings of the plasma membrane in stacks (arrows) and a few small
.crovilli (MV). x 13.000



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except in that of the terminal web. Lamellar infoldings of the basal and lateral plasma membranes occur, just as in enterocytes of other teleosts.

Absorptive enterocytes of the second segment show heavy pinocytosis beneath their microvillous border (fig. 8). Moreover many pinocytotic vesicles and one or more large supranuclear vacuoles may be present (figs. 6, 8). Chylomicrons have not been found in the ER, in the Golgi apparatus, and in the intercellular spaces but some fat droplets may be present in the cytoplasm.

The most caudal segment does not have the characteristics of the first and second segments; it lacks lipid droplets, chylomicrons, pinocytotic vesicles, and supranuclear vacuoles in the enterocytes. The microvilli are relatively short and few in number. As in other teleosts, food absorption does not seem to be an important function of these cells. Lamellar infoldings, however, are found in large numbers, generally concentrated in stacks (fig. 9).

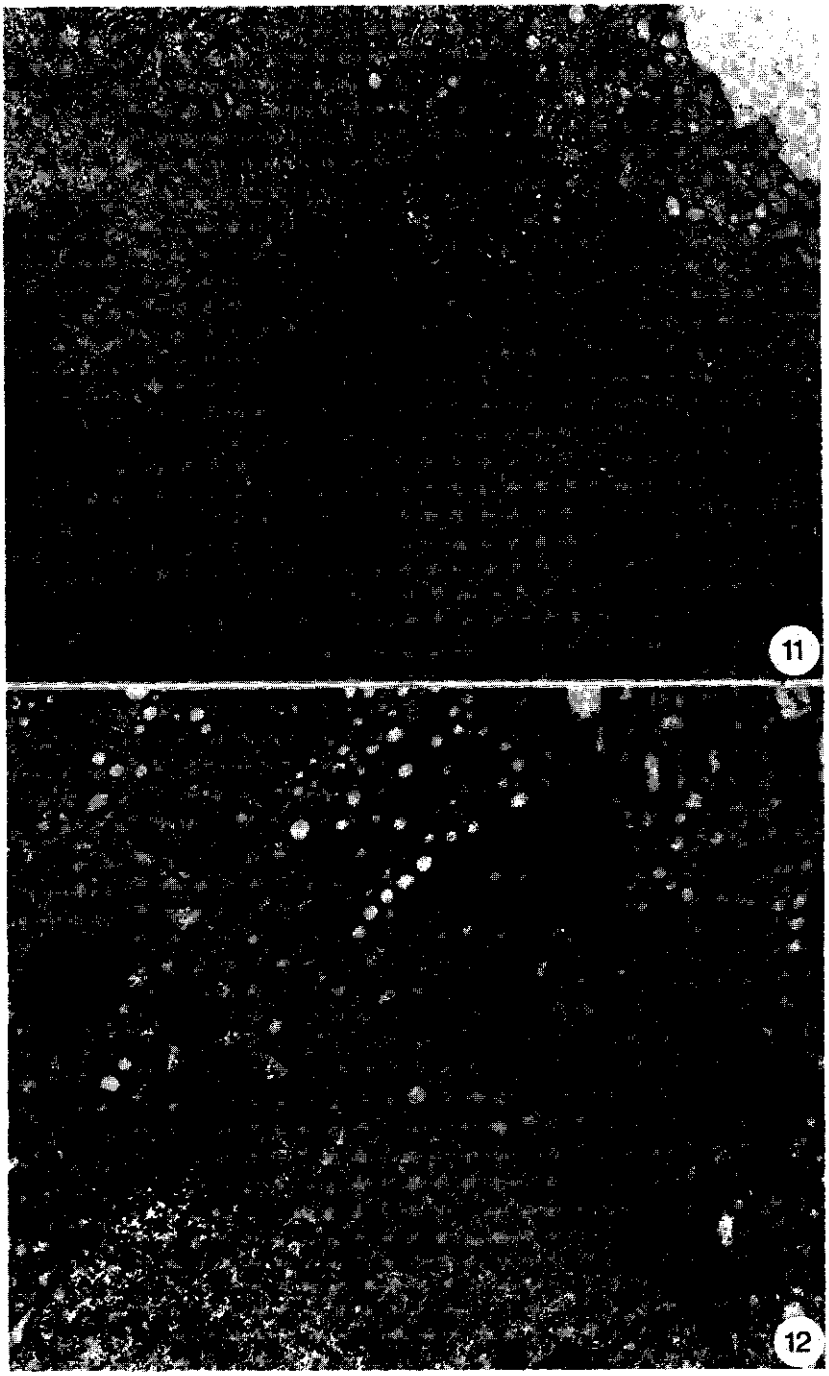
During development no important changes can be noticed in the morphology of the absorptive enterocytes. It is noteworthy that the second segment remains of the same length in all stages. In adult *Clarias* this segment has the same characteristics as in larvae (table 1).

4. *Ultrastructure of the developing stomach.*

The first "Anlage" of the stomach can be recognized on day 4 with the electron microscope by the presence of a few lobules of future glandular cells, forme by invaginations of the surface epithelium of what seems to be the most caudal part of the esophagus.

Epithelial cells (fig. 10) are elongate, and the nucleus (with large nucleolus) is situated in the basal half of the cells. Neighbouring cells have complex interdigitations of the lateral cell membranes. A distinct Golgi apparatus is present and many strands of RER are found throughout the cells. Mucoid granules may be found in the cytoplasm, but are accumulated in the apex of the cell. The round to ovoid granules are membrane bound (fig. 10). They proved to be P.A.S. positive after Alcian-blue-P.A.S. reaction on paraffin material; therefore these granules probably contain neutral mucopolysaccharides. The cytology of the epithelium lining of the lumen does not change much in later stages of development, but

Fig. 10. Epithelial cells from the stomach of an 11 days old larva.
NU = nucleus; Int. = complex interdigitations of the lateral plasma membranes; G = Golgi apparatus; M = Mucoid granules; Mi = mitochondria; RER = rough endoplasmatic reticulum. x 28.000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12

more granules and strands of endoplasmic reticulum and fewer free ribosomes are found in older specimens. A few very short microvilli may be present on the surface of the epithelium lining. In adult specimens the secretion granules are flatter and more numerous (fig. 19); they are more electron dense than in larvae.

The transition from surface epithelial layer to tubular gland epithelium is very abrupt, without a transition or "neck" zone.

The glandular cells can be easily distinguished from the epithelium lining cells by the lack of mucous granules and the presence of a number of smooth vesicles in the apical area (figs. 11-16). The cells are pyramidal and show complex interdigitations of the lateral cell membranes. The nuclei contain prominent nucleoli. Many mitochondria are present in the cytoplasm. A welldeveloped Golgi apparatus is found near the nucleus. At 4 days only a few strands of RER have been noticed. At that age the cytoplasm is filled with free ribosomes (fig. 11).

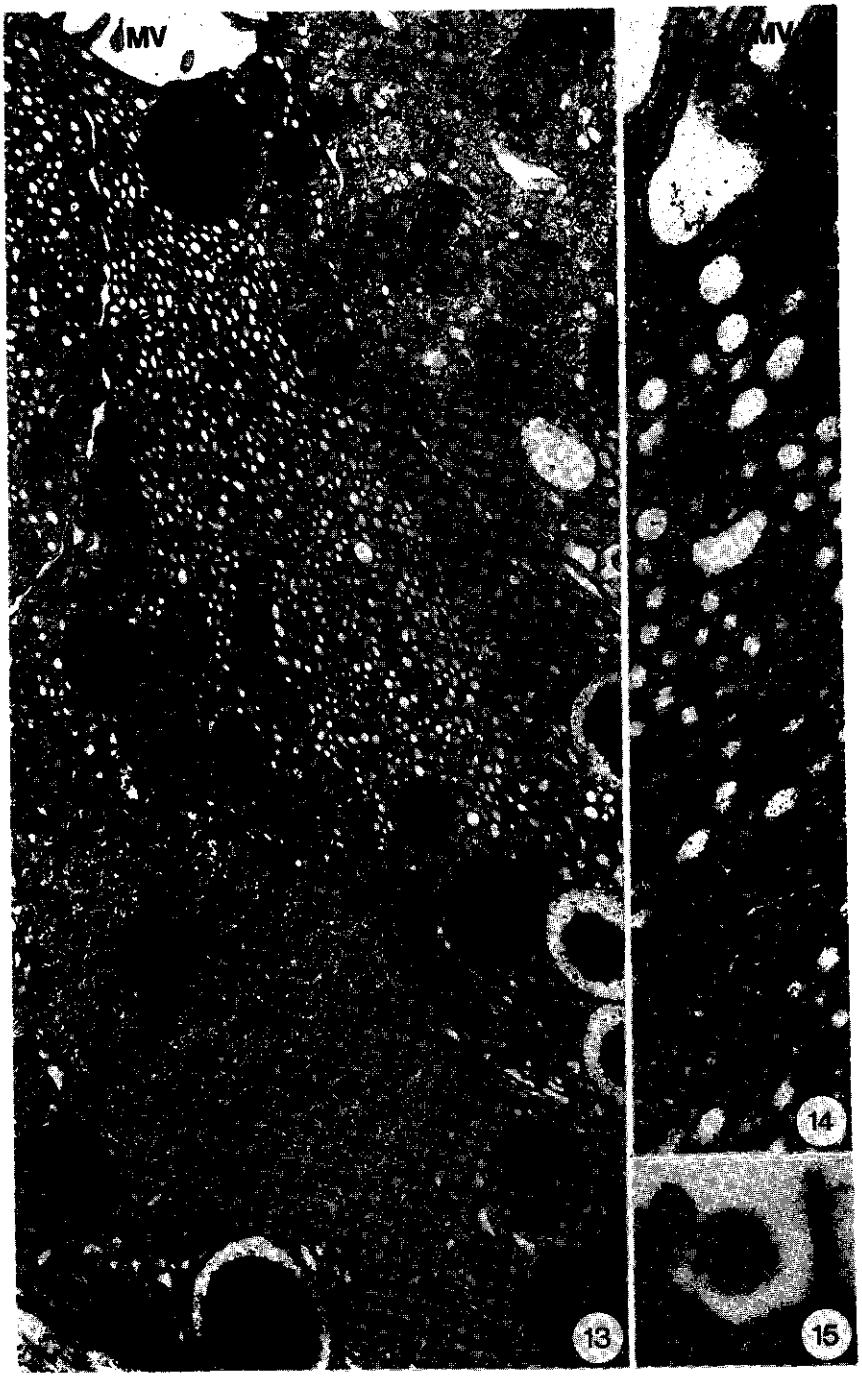
Apart from the lining epithelium only one type of stomach glandular cell was found in the various stages. The apical tubular system, in early stages consisting of a number of vesicles, develops rapidly (fig. 13-14) and fills up the greater part of the cells in specimens of 11 days. Since the membranes of the vesicles strongly resemble the apical plasma membrane (fig. 14), these membranes may have the same origin. Important changes have been noticed in the morphology of the tubular system during later ontogeny. In larvae from 11 days old the vesicles were more elongated, possibly because of mutual fusion. In adult specimens the more elongate tubules are merely extending in longitudinal direction (fig. 16).

As from the age of 7 days small secretion granules appear in the cytoplasm of the glandular cells (fig. 12). In the following days large zymogen granules are formed (figs. 4, 13) and on day 11 the first indications of exocytosis can be observed (fig. 15).

In adult specimens the RER fills up the basal part of the glandular cells (fig. 17). A difference in the morphology of glandular cells of young animals and adults is the presence of many microfilaments in adults, running from the junctional complex into the cytoplasm (fig. 18). Glandular cells are absent beneath the epithelium lining in the pyloric area of the stomach, which is also

Fig. 11. Corpus gland cells of a 4 days old larva. T = apical tubular system; Nu = nucleus; Mi = mitochondria; G = Golgi apparatus x 22.000

Fig. 12. Corpus gland cells in a 7 days old larva. Some small secretion granules (arrows). Legends as fig. 11. x 22.000


MV
MV
13
14
15

ecognized by a relatively well developed muscular wall. The cells of the epi-
helial lining are similar to those in the corpus region.

. *pH indicator test.*

The pH indicator methyl-red (colour change from yellow to red between pH
nd 4,4) only shows a red colour when injected into the stomach of 13 days an
lder larvae. However, after injecting a large dose of indicator this colour
hange was not noticed at 13 days. In larvae of 14 days and older the colour
hange was distinctly visible, even with larger quantities of indicator. Congo
ed (pH 5,2 to 3,3) changes to blue in larvae of 15 days. During the next few
ays the reaction was even more distinct. This shows that the acidity of the s
ach contents after feeding is higher than pH 5 in young larvae of up to 11,5
tandard length. During the next few days the pH drops to values of 5,2 - 3,3.

A number of older larvae survived the treatment with indicator. The day a
he experiment the dyes were found in the gut lumen. Their colours were yellow
nd red (methyl-red, resp. Congo-red), which indicates that the pH in the gut
umen is higher than 5.

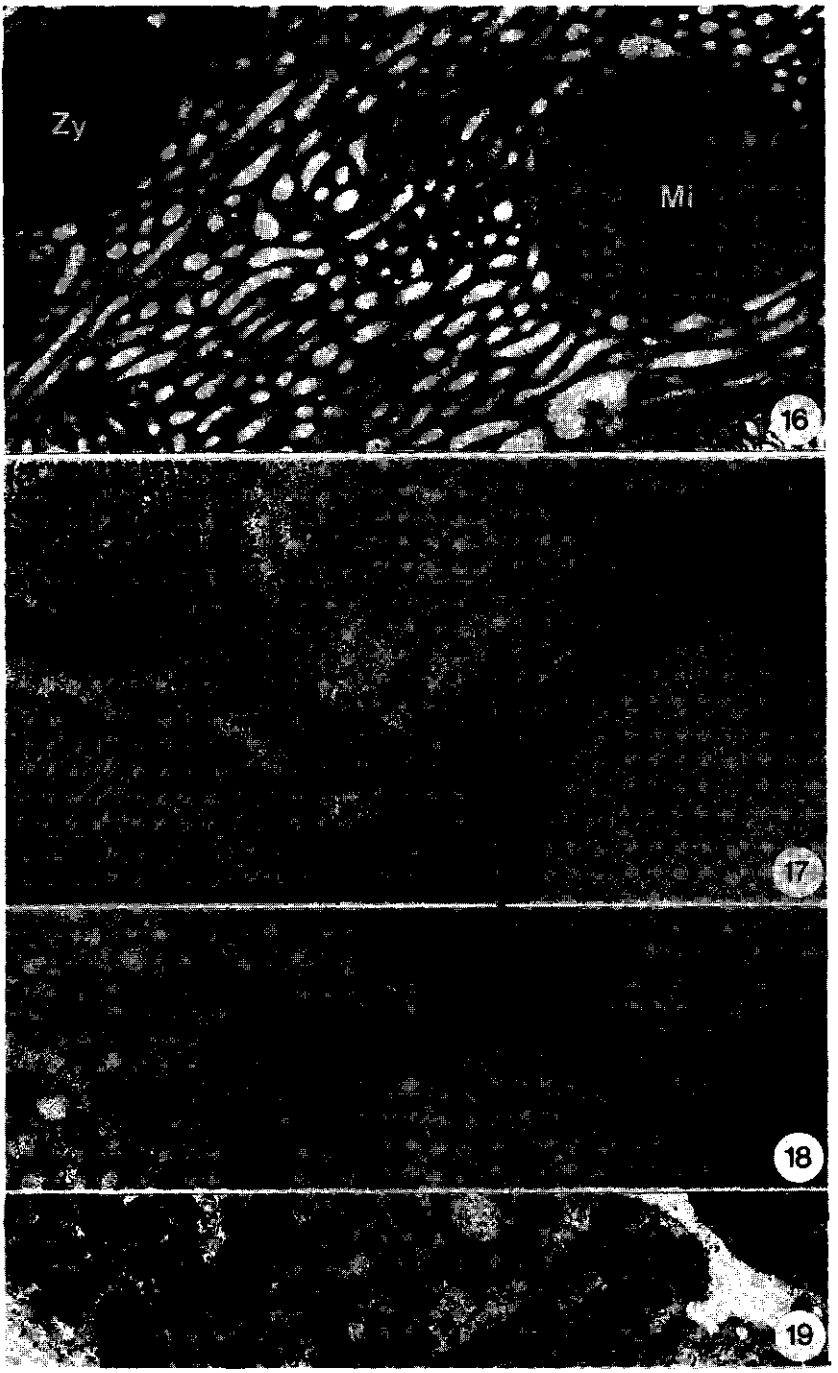
. *Uptake of horse radish peroxidase by enterocytes.*

After the ingestion of horse radish peroxidase, enzyme activity is notice
n enterocytes of the second segment only (fig. 20). A positive reaction was f
n all larvae and juveniles we studied (4 to 15 days). The localization of the
eroxidase activity suggests that the enzyme is present in the supranuclear pa
f the absorptive cells, evidently within the pinocytotic vesicles and the lar
upranuclear vacuoles. The length of the segment with peroxidase absorption an
s location correspond to the second segment as determined histologically.

g. 13. Corpus glandular cells in a 12 days old larva. Many large zymogen gra
les (Zy) especially in the basal part of the cells. The apical part is fille
th tubules. Legends as fig. 11. x 15.000

g. 14. The apical portion of a corpus gland cell of a 12 days old larva. The
ructure of the apical plasma membrane is similar to that of the membranes of
e many apical tubules, since both are asymmetrical. x 52.000

g. 15. Apical part of a corpus gland cell of a 12 days old larva, showing
ocytosis of a secretion granule. x 26.000


Zy
Mi
16
17
18
19

DISCUSSION

1. *Regional differentiation of the intestine.*

In the intestine of larval *Clarias lazera* a regional differentiation in three segments can be made, similar to the one in stomachless fish. In *Clarias* larvae the enterocytes in the different segments have the same histological characteristics as in stomachless fish (Yamamoto, 1966; Iwai, 1969; Gauthier & Landis, 1972; Noaillac Depeyre & Gas, 1974, 1976; Stroband & Debets, 1978). Similar features, including the presence of a second segment with pinocytosis of macromolecules, have been described for the intestine of other initially stomachless larvae of fish species which possess a stomach in the juvenile and adult stages (Iwai & Tanaka, 1968; Tanaka, 1972).

2. *General morphology of the epithelium of the stomach.*

For the morphology of the stomach epithelium, the results are similar to those obtained by others in ultrastructural studies (Ling & Tan, 1975 in *Chelmon rostratus*; Huebner & Chee, 1978 in *Hoplosternum* sp.; Gas & Noaillac-Depeyre, 1978 in *Ameiurus* sp. and Noaillac-Depeyre & Gas, 1978, in perch). Also in many light-microscopical studies on fishes, only one type of glandular cell was found in the corpus, apart from the surface mucous cells (Al Hussaini, 1946; Weinreb & Bilstad, 1955; Verma & Tyagi, 1974; Moitra & Ray, 1977; Reifel & Travill, 1978; Weisel, 1973). The differentiation of the glandular cells into two types, one of which produces pepsinogen and the other hydrochloric acid, is only known for the mammalian stomach (Smit, 1968). Special neck cells, as found in *Mulloides* sp. (Al Hussaini, 1946), stickleback (Hale, 1965) and perch (Gas & Noaillac-Depeyre, 1978), have not been found in *Clarias lazera*, just as in the sea robin (Blake, 1936) in *Colisa* sp. (Moitra & Ray, 1977), and in a number of other teleost species (Reifel & Travill, 1978).

Figs. 16-18. Stomach gland cells of adult *Clarias lazera*.

Fig. 16. Apical tubular system. Mi = mitochondrium; Zy = zymogen granule. x 23.000

Fig. 17. RER in the basal part of a cell. x 40.000

Fig. 18. Desmosomes and many microfibrills in the apical part of a glandular cell. x 40.000

Fig. 19. Adult *Cl. lazera*;secretion granules located in the apex of a surface mucous cell. x 18.000

Fig. 20. Light micrograph of part of the gut after horse radish peroxidase ingestion. Black deposits of reaction product indicate the presence of enzyme activity. 1 = segment 1; 3 = segment 3; A = anus.

Fig. 21. Functional cell of the epithelial lining of the stomach. x 10.000 The inset shows a light microscopical radioautogram. The arrow points to the ^{3}H-thymidine labeled nucleus of the cell in the electron micrograph. x 400

The cells of the epithelial lining have the same appearance in all studied sh species. Usually these cells show PAS positivity in their apices after cian Blue-PAS staining, and this suggests that they contain neutral mucopoly-ccharides. In pike, however, Reifel & Travill (1978) found acid carbohydrates these cells.

Development of stomach functions.

It is difficult to determine when the stomach becomes actually "functional". e volume increases rapidly as from day 8, and one of the important stomach nctions, the storage of food may begin as of that day. The digestive function the stomach clearly develops later, between the formation of secretion granules the corpus gland cells and the production of as much hydrochloric acid as re-ired for reaching low pH values in the stomach lumen.

From the morphological evidence it can be concluded that in *Clarias lazera* e corpus of the stomach is formed between day 4 when the first glandular cells e found, and day 12 when exocytosis of secretion granules is first observed.

An acid environment in the stomach as from day 13 allows catheptic or peptic gestion. From day 12 the apical vesicular system shows a tendency to develop bules, which are distinct in adults. These vesicles and tubules possess mem-anes with a coating at the inner side. This coating, also present on the mem-anes of the microvilli, consist of glycoproteins in *Ameiurus nebulosus* (Gas & aillac-Depeyre, 1978) and in *Perca fluviatilis* (Noaillac-Depeyre & Gas, 1978). similar tubulo-vesicular system is also present in stomach gland cells of her non-mammalian vertebrates and apparently plays a certain role in hydrochlo-c-acid production (Sedar, 1961). In mammals the volume of the tubulo-vesicular stem, as only found in parietal cells, is in inverse ratio to the volume of e socalled intracellular canaliculi, which are maximally developed when much drochloric acid is produced (Padykula, 1977). Intracellular canaliculi, however, ve neither been found in *Clarias lazera*, nor in *A. nebulosus*, *P. fluviatilis*, d *Chelmon rostratus* (Ling & Tan, 1975).

After day 13 the acidity increases. The pyloric sphincter is present as m day 12. These results are in general accordance with the radioautographic sults; the labeling index decreased sharply in the stomach corpus glands between y 9 and day 12 and in the pyloric area between day 12 and 15. Differentiation the epithelium must take place first in the intestine, then in the rostral rt of the stomach, and finally in the pylorus.

Grizzle & Curd (1978) found a larval period of approximately 35 days in logperch (*Percina caprodes*), reared at 22°C. The first gastric glands were fou on day 27, whereas the pyloric sphincter developed some days later. This is o interest, as Tanaka (1971) concluded for a large number of teleost species th gastric glands develop after about 3/4 of the total larval stage. The stomach develops very early in *Clarias lazera* and the larval stage is evidently very short when the fish are reared at 23-24°C. The latter is beginning when, at ± 30 h after fertilization, exogenous feeding starts. The completion of the sto is usually considered to coincide with the end of the larval stage (Tanaka, 1

4. *Physiological relevance of the second gut segment.*

The morphology of the intestine does not change after the time when the mach becomes functional. As the second segment with the facility for absorbin protein macromolecules remains to be present beyond this period, this facilit cannot be related to the lack of a stomach in *Clarias lazera*. This is underli by the fact that the length of the segment remains the same and also by the p sence of pinocytotic enterocytes in the posterior intestine of adult *Clarias*. last argument is in according with the results of Krementz & Chapman (1974), Bergot (1976) and Noaillac-Depeyre & Gas (1979), who found pinocytosis in the posterior part of the gut of adult catfish (*Ictalurus punctatus*), in 15 cm lo trout, and in adult perch, respectively. However, supranuclear vacuoles have been found in adult *Ictalurus*. This might be attributed to the long time inte between feeding and fixing of the tissues; starvation causes the disappearanc supranuclear vacuoles but not of pinocytosis, as was found in *Coregonus* larva (Stroband & Dabrowski, in press). In the adult perch the second segment seeme relatively short (± 11% of gut length, but ± 20% in *Clarias lazera* larvae, ju niles and adults). Tanaka (1972) studied 15 fish species. In 5 of these he no ced at the transitional stage from larva to juvenile, when the gastric glands become functional, the gradual disappearance of acidophylic granules (i.e. su nuclear vacuoles) in the posterior intestine. This was not observed in the ot 10 species. In his experiments some fixations were possibly carried out long after feeding, and ultrastructural studies might show pinocytosis in the seco segment of the gut.

The facility of absorbing macromolecules is probably a general feature o the teleostean intestine, but it might be more common in larvae and stomachle species than in specimens with a functional stomach. The physiological role o

"second segment" in fish larvae might be different from that in adults. In
arvae the food reaches the caudal intestine in a very short time, and absorption
macromolecules might be of quantitative importance (Iwai, 1968). In adult spe-
mens, including stomachless fish, many hours go by before the food reaches the
sterior gut, and digestion of protein then may be in an advanced stage. That
sorption of undigested food in the second gut segment plays a role in fish lar-
e is also indicated by the absence of supranuclear vacuoles in starved *Coregonus*
arvae (O.C.). The observation of Iwai (1969) that after feeding the number of
sosomes in carp larvae increases together with the supranuclear vacuoles points
intracellular digestion of absorbed material.

In adults, even in stomachless fish, absorption of protein takes place main-
in the anterior part of the intestine (Shcherbina & Sorvatchev, 1969; Stroband
Van der Veen, in press; studying carp and grasscarp respectively). Therefore it
ems likely that adult stomachless fish are able to digest proteins efficiently
though a pepsin-hydrochloricacid system is absent (Creach, 1963; Jany, 1976).
fferences have not yet been found in the part of food protein absorbed by fishes
th or without a stomach. The question is why other species developed a stomach
d a pepsin-HCl system. Other stomach functions, e.g. storage of food, parti-
oning of food items, or defence against microorganisms may have to be considered
this respect.

Renewal of the epithelium.

The random distribution of ^{3}H-thymidine labeled cells in the digestive tract
ithelium in the early stages of development is in accordance with the results
Rombout et al. (in press) obtained in *Barbus conchonius* larvae, in which the
beling index sharply declined before the first exogenous feeding. In *Clarias*
zera the decrease was noticed two days later. In both species, the location of
beled cells during this decrease changes towards the basal parts of the intes-
nal mucosal folds. The presence of thymidine label in nuclei of functional
lls (glandular and surface stomach cells as well as absorptive cells in
arias lazera larvae) was observed also by Rombout et al. and by Stroband &
bets (1978) in the intestinal absorptive enterocytes of *Barbus* larvae and
venile grasscarp, respectively. The ability to proliferate was also found in
nctional small-intestinal cells in *Xenopus* laevis larvae by Marshall & Dixon
978).

It is interesting that notwithstanding the decreasing labeling index, whe the tissues have differentiated and become functionally active, only functiona proliferating cells appear to be present. This in contrast to the mammalian sr intestine, where only undifferentiated crypt cells are able to proliferate (Lipkip, 1973). The results indicate that fish enterocytes and stomach cells loose their proliferative activity in a relatively late stage of differentiat

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CURRICULUM VITAE

Het levenslicht werd door mij aanschouwd in Zeist op 31 december 1946. Hoewel naar ik meen nooit door mijn ouders opgejaagd, voelde ik, als ieder kind, al snel dat ik geboren was in een samenleving waarin "het presteren" van grote betekenis wordt geacht. Groot was dan ook mijn frustratie toen ik na een vliegende start (ik werd pas eind februari 1947 verwacht) al op de openbare kleuterschool "Griffensteijn" in Zeist mijn eerste doubleren te verwerken kreeg. Achteraf heeft 3 jaar fröbelen stellig in positieve zin aan mijn vorming bijdragen.

Later kon ik aan de maatschappelijke verwachtingen voldoen door het slagen voor het toelatingsexamen, na daarop uitstekend voorbereid te zijn op de jongensschool der Evangelische Broedergemeente.

Het voorbereidend wetenschappelijk onderwijs werd aangevangen in 1959 aan het Ie Christelijk Lyceum te Zeist. Een poging tot het volgen van het Gymnasium was tot mislukken gedoemd, maar in 1965 werd het einddiploma H.B.S.-B verworven.

Na een korte periode van arbeid bij Philips telecommunicatie industrie te Hilversum volgde een oproep voor militaire dienst. Deze werd vervuld van januari 1966 tot juli 1967, grotendeels bij een infanterie bataljon te Ermelo. Hoewel strepen voor mij niet waren weggelegd, behaalde ik het diploma "militaire lichamelijke vaardigheid" in 1967, iets waarop ik vanzelfsprekend nóg trots ben.

In september 1967 ben ik, na enig "voorwerk" in diensttijd (aula-pockets e.d.) gestart met de studie Biologie aan de Rijksuniversiteit te Utrecht. Het kandidaatsexamen werd, tot niet alleen mijn genoegen, behaald in mei 1970 en in december 1972 studeerde ik "met lof" af met als hoofdvak Vergelijkende Endocrinologie (prof. v. Oordt) en als bijvakken Plantenfysiologie (Prof. v. Die) en Didaktiek der Biologie (Drs. Saaltink).

Vanaf januari 1973 ben ik werkzaam bij de afdeling Dierkunde, later vakgroep Experimentele Diermorfologie en Celbiologie aan de Landbouwhogeschool te Wageningen. Naast vele andere aktiviteiten zoals onderwijs aan pre- en postkandidaten van verschillende studierichtingen en het behalen van het diploma "C-deskundige" aan het ITAL te Wageningen in 1978, bleef er tijd beschikbaar voor het doen van het onderzoek waarvan de resultaten in dit proefschrift zijn neergelegd.