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THE GAS BLADDER AS A HYDROSTATIC ORGAN IN *THYMALLUS THYMALLUS* L., *OSMERUS EPERLANUS* L. AND *MALLOTUS VILLOSUS* MÜLL.

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INTRODUCTION

The suborder Salmonoidei within the order Clupeiformes (BERG 1940) has typically a physostomous gas bladder. The suborder comprises several families, some of which contain only deep-sea species. In these the gas bladder either is absent — as in the family Bathylagidae (MARSHALL 1960) — or has become modified to a type suited to the high hydrostatic pressure of great depths; it has lost the pneumatic duct and developed *retia mirabilia* and a posterior resorbent chamber (COHEN 1958, FÄNGE 1958, MARSHALL 1960, FAHLÉN 1965). Of those families which have retained a physostomous gas bladder, only a few are represented in Scandinavian waters. There are several species of the family Thymallidae in N. America and Asia, but only one in Scandinavia, *Thymallus thymallus* (L.). The family Osmeridae comprises three genera, two of which, *Osmerus* and *Mallotus*, are each represented by one species in Scandinavia, *Osmerus eperlanus* (L.) and *Mallotus villosus* (MÜLL.). The other Salmonoid families, apart from the family Salmonidae, which is wide-spread in the Northern hemisphere, are found principally in eastern Asia, S. America and Australia.

The gas bladders of the three species mentioned have been subjected to very little attention. However, their general morphology was early described by CUVIER and VALENCIENNES (1848). BEAUFORT (1909) mentioned the bladders of these species very briefly, though without adding anything to the description given by CUVIER and VALENCIENNES (1848). More detailed investigations on the morphology of the Salmonoid gas bladder dealt exclusively with species belonging to the genera *Salmo* (CORNING 1888, EISSELE 1922, JASINSKI 1963) and *Coregonus* (JASINSKI 1963, FAHLÉN 1967b), as did also physiological works (SUNDNES, ENNS and SCHOLANDER 1958, WITTENBERG 1958, SUNDNES 1963). SAUNDERS (1953) is the only one who carried out experiments on one of the three species mentioned. He analyzed the contents of the gas bladder of *Osmerus eperlanus* and measured the flotation pressure of the fish.

The present investigation is an attempt to reveal those morphological structures which are necessary for a possible function of the gas bladder as a hydrostatic organ. The mechanism by which gases are introduced

into the Salmonoid gas bladder is unknown. The formerly prevailing opinion, that all fishes with a physostomous gas bladder fill it only by swallowing air at the surface, has lately been strongly debated. WITTENBERG (1958) stated that *Salmo* species are able to fill their gas bladders, though very slowly, even if they are denied access to the surface. And as early as 1892 HÜFNER pointed out that deep water Coregonids have to introduce gas into the bladder against considerable pressure gradients. SAUNDERS (1953) stated the same for *Osmerus* and *Leucichthys*. The secretion of gas into the bladder of *Coregonus* has been assumed to be of the same nature as the gas secretion of the physoclistous gas bladder (SUNDNES 1963). Though the physiological experiments are very few (SAUNDERS 1953), it has been assumed that some sort of secretion also may occur in the gas bladders of *Osmerus*, *Thymallus* and *Mallotus*.

The principle of gas secretion in the Salmonoid gas bladder is not clear. SUNDNES (1963) suggests that the mechanism might be the same as that of the physoclist bladder. KUHN, MARTI, KUHN and RAMEL (1963) and STEEN (1963), however, show that the counter current capillary system, present in all physoclist gas bladders and also in the eel, is necessary for building up a partial pressure of the gases in the blood, which makes it possible for the gases to pass into the gas bladder. Among fishes with a physostomous gas bladder, the families Cyprinidae and Esocidae are able to secrete gases into the bladder (JACOBS 1934), and they possess counter current systems (RAUTHER 1923). The Clupeid gas bladder has no *retia mirabilia* and no gas secretion (FAHLÉN 1967a). In *Coregonus*, a counter current system is present (FAHLÉN 1967b), and there is a secretion of the same gases as in the physoclist bladder, which is reflected in the composition of the gas (FÄNGE 1953, SUNDNES 1963).

MATERIAL

Thymallus thymallus: 6 specimens, caught in Lilla Lule River, Northern Sweden.

Osmerus eperlanus: 1 specimen, caught in the Baltic in nets.

Mallotus villosus: About 40 specimens, caught in the Barent Sea by means of trawling.

METHODS

The gas analyses of *Mallotus villosus* were made in a Krogh microgas analyzer (KROGH 1926) with KOH and pyrogalloll as absorbents for carbon dioxide and oxygen respectively, on the Norwegian research vessel 'G. O. SARS'.

The sole specimen of *Osmerus eperlanus* was fixed in BOUIN's fluid.

The gross morphology of the gas bladder of the other two species was studied on fresh specimens and on specimens fixed in 4% formaldehyde. Injections of the vascular system were made with Indian ink.

Fixation for histological and histochemical investigations were made with BOUIN's fluid or its modification B 15 containing also chromic acid and urea (ALLEN)*, in uranylacetate-sublimate, potassiumdichromate (SCHILLER)* or in osmiumtetroxyde-chromic acid fixation (CHAMPY).* For electron microscopic investigations fixation was carried out in buffered osmiumtetroxyde (PALADE)* or in osmiumtetroxyde-chromic acid (DALTON).* As imbedding media paraffin or polyester wax was used for histological and histochemical studies and metachrylate for the electron microscopic investigations. Cutting for the last mentioned purpose was carried out on an LKB Ultrotome ultramicrotome, and the electron microscope used was an Akashi TRS 50.

Paraffin- and wax-imbedded material was stained according to different hematoxyline methods (HEIDENHAIN, EHRLICH, WEIGERT)* with counterstains and azocarmine with counterstains. Silver-impregnation for nerves and cell nuclei was carried out according to the BODIAN* method. For histochemical purposes the periodic acid-Schiff technique according to HOTCHKISS (PARS)* was used for carbohydrate-containing proteins, the paraldehyde-fuchsin method (GABE 1953) for mucopolysaccharides, the alcian blue method for acid mucopolysaccharides and the carmine method for glycogen (BEST)*.

In order to induce gas secretion, drugs were injected into a number of speciemens of *Mallotus villosus*. Pilocarpine is a secretion-stimulating drug and is reported to induce gas secretion in *Esox* (DRESER 1892). Yohimbine and dibenzyline are sympathicolythic substances. The former has been shown to induce secretion in *Coregonus* (FAHLÉN 1967b), the latter causes inhibition of gas loss from the gas bladder (HARVEY, in press).

RESULTS

THE GAS BLADDER OF THYMALLUS THYMALLUS

Topography.

CUVIER and VALENCIENNES (1848) describe the gas bladder of the grayling, *Thymallus thymallus*, as extremely big and communicating with the oesophagus by an unusually short pneumatic duct. BEAUFORT (1909) has nothing to add to this description. According to the present investigation the organ comprises 5—6% of the total volume of the animal, which

* Descriptions of histological and histochemical methods, referred to by name only, are found in ROMEIS (1948) and PEARSE (1960).

is normal for Salmonoid fishes. It is of the ordinary Salmonoid fusiform shape, stretches from the supracardial region to the anus and the hindmost part of the abdominal cavity. As is usual for Salmonoid gas bladders, it is only loosely connected with the surrounding organs and tissues. The bladder is ventrally covered by the mesothelium of the peritoneal cavity. The posterior part ends blindly in a rounded tip immediately above the anus, and anteriorly the bladder tapers towards the mouth of the pneumatic duct. This originates at the anterior end and immediately bends beckwards. Thus there is a relatively sharp boundary between the gas bladder proper and the pneumatic duct. In a gas bladder of about 60 mm length it first runs backwards about 5 mm, then sharply bends forwards and after about 5 mm it ends in the ventral wall of the oesophagus. The diameter of the duct is practically constant. Only where it leaves the gas bladder it is somewhat wider than in the rest of its course. The debouch of the pneumatic duct into the gas bladder is normally wide open and seems never to be closed by a sphincter. At the other end, however, the opening in the wall of the oesophagus is often tightly closed.

Vascularization.

The vessels reaching the gas bladder are two branches of the coeliacomesenteric artery, running along the pneumatic duct. They give off only few vessels to this part of the organ, and these penetrate the submuscularis and the muscularis as arterioles and venules and form in the lamina propria a capillary network, which lies close to the base of the inner epithelium. Then the vessels run laterally along the gas bladder backwards. These longitudinal arteries give off branches which again branch repeatedly and dichotomously. The drainage of the gas bladder is carried out by branches of the hepatic portal vein, which accompany the arteries to the gas bladder. The veins branch in the same way as the arteries do, and flat bundles are formed which consist of three or more vessels, with alternating arterial and venous capillaries (Figs. 1, 3). The number of vessels forming the bundles is about 100 in a transversal section of a gas bladder of 60 mm length. There seems to be no difference in frequency of the vascular bundles in the different parts of the gas bladder. The total length of all vessels running in bundles in one and the same gas bladder is estimated at about 6 meters.

The vascular bundles all run in the submuscular connective tissue layer. At irregular intervals they cross the muscularis and enter the subepithelial layer of connective tissue, the lamina propria. Here the vessels form a capillary network in direct contact with the base of the

epithelium. The vessels sometimes run some millimeters together after they have reached the lamina propria and split into capillaries, but vascular bundles of the same type as in the submuscularis are formed only occasionally. The capillaries form a network, which is somewhat drawn out in the longitudinal plane of the gas bladder. The average distance between the longitudinal capillaries is about 20 microns. The diameter of the capillaries is about 8 microns.

Histology.

The layers known from other physostomous gas bladders are present also in that of the grayling. As usual, the organ is ventrally covered by the peritoneal epithelium, which here is cubical. Beneath the epithelium ventrally, and at the outermost layer dorsally, the tunica externa forms a tough sheath round the organ (Fig. 1). However, the tunica externa

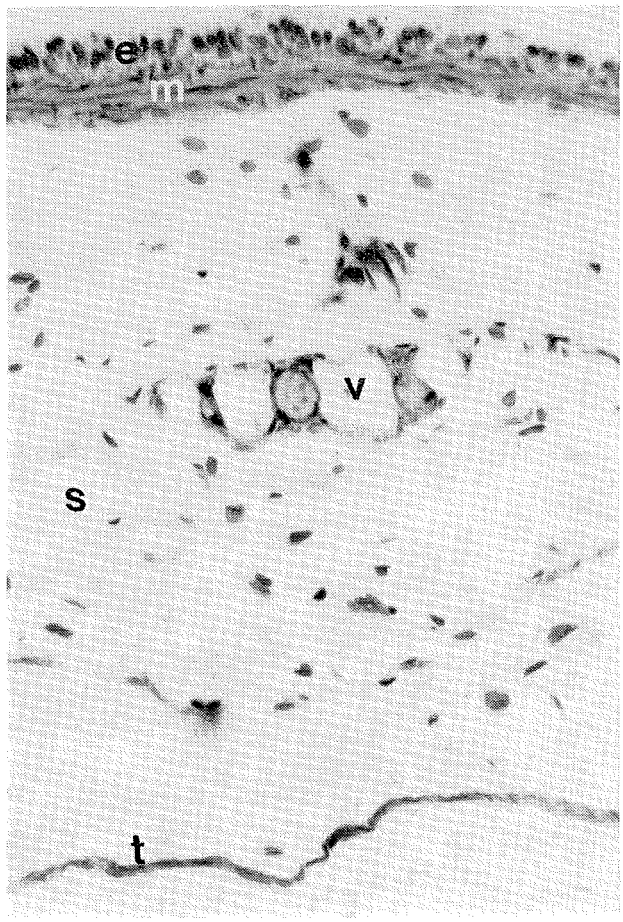

Fig. 1. *Thymallus thymallus.*
Transverse section of gas bladder wall.
e = epithelium,
m = muscularis,
s = submuscularis,
t = tunica externa,
v = vascular bundle.
400x.

in the gas bladder of the grayling is considerably thinner than in other Salmonoids investigated (FAHLÉN 1967b, FAHLÉN, unpublished observations). It consists of a network of connective tissue fibers, some of them elastic but mostly collagenous. Smooth muscle fibers occur very rarely in this layer. The submuscularis is the predominating layer of the grayling gas bladder, and it is built up by somewhat irregularly but mainly circularly arranged fibers of mostly collagenous connective tissue. Single elastic fibers are also present. This layer lodges the vascular bundles described above (Figs. 1, 3). — The muscularis in a relaxed gas bladder is thinner than in any other physostomous gas bladder investigated. Its thickness is only about 10 microns and it consists mainly of circular fibers. The outermost part of the muscularis contains longitudinal muscle fibers. Intermingled with these, longitudinal nerve bundles run from the perikarya situated in the wall of the pneumatic duct, and from these bundles nerve fibers run to the separate muscle fibers of the muscularis. The muscle layer is crossed by the vessels which come from the vascular bundles of the submuscularis. As stated before, they often also cross the muscularis together in bundles.

The outer layers of the wall of the pneumatic duct are built up in the same way as those of the gas bladder proper, but the proportional thickness of different layers varies (Fig. 4). An outer layer of connective tissue, continuous with the submuscularis of the bladder, is predominant close to this but becomes gradually thinner nearer to the oesophagus. When the duct enters the wall of the oesophagus, the thin submuscularis runs over into the very thin outer layer of connective tissue of the alimentary canal. The muscularis of the duct does not undergo the same changes as the submuscularis. Continuous with the muscularis of the gas bladder, it is thicker than this. It is built up almost entirely by circular smooth muscle fibers, though the outer of these have a tendency to run diagonally. However, no separate outer muscle layer is present. When the pneumatic duct enters the wall of the oesophagus, the muscularis unites with the thick muscle layer of the oesophagus. This consists entirely of striated muscle fibers. The muscularis of the pneumatic duct is thinner at the debouch into the oesophagus, and at the entrance into the bladder its thickness does not exceed that of the bladder muscularis. The striated muscle fibers of the oesophagus, which are mainly circularly arranged, surround the debouch of the duct and may act as a sphincter when contracted. In intact gas bladders this opening is often seen to be closed. — The lamina propria of the gas bladder proper is extremely thin. It consists of collagenous fibers and is only 2—3 microns thick, except where the capillaries of the subepithelial plexus run. No elastic fibers could be found in the lamina propria.

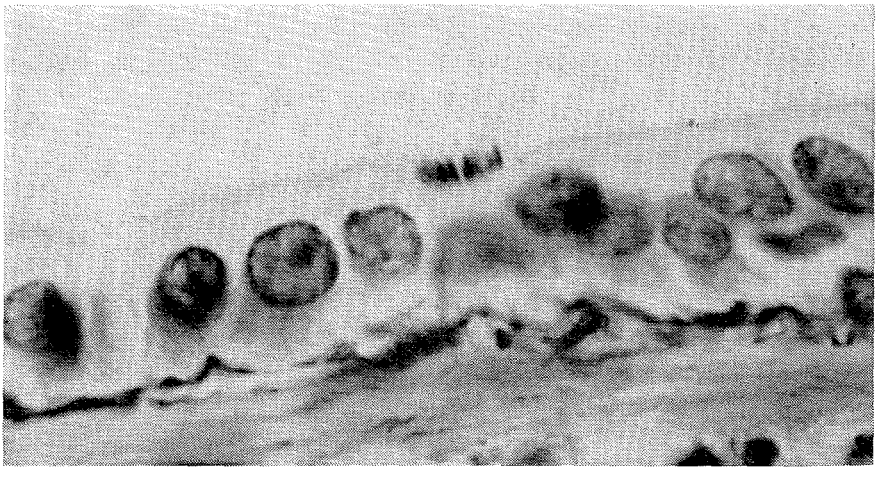

Fig. 2. *Thymallus thymallus.* Section of gas bladder epithelium. Chief cells and one ciliated cell. 1500x.

The epithelium, which is only light-microscopically investigated, is cubical, sometimes even flattened (Fig. 2). Three cell types are present in the epithelium, one of which are no real epithelial cells but lymphocytes, invading the epithelium from beneath. The lymphocytes are found between the epithelial cells, mostly in a basal position. The main part of the epithelium is built up of cells of cubical type. The chief cells are of the size 10 × 10 microns. The nucleus is rounded, rather large and situated centrally or somewhat basally. The cytoplasm is devoid of visible inclusions and stains rather weakly with all sorts of colours. The distal part, however, shows a weak basophilia. The part of the cytoplasm which

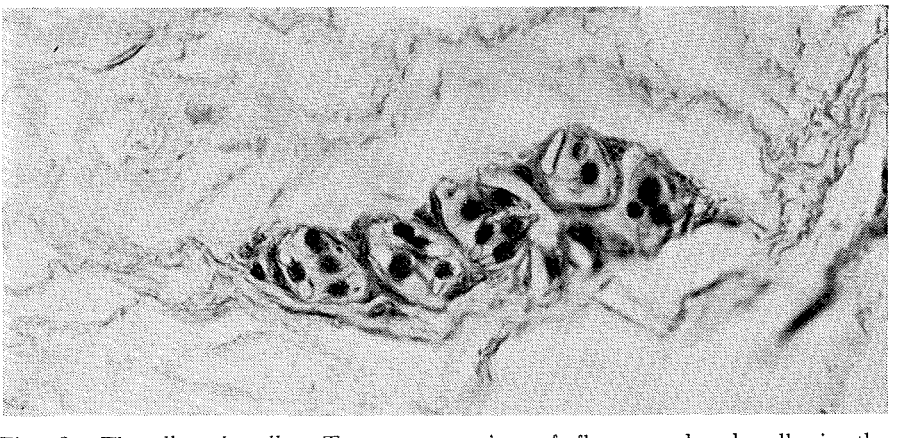

Fig. 3. *Thymallus thymallus.* Transverse section of flat vascular bundle in the submuscularis. 800x.

lies close to the subepithelial vessels never shows any differentiation. The cell membranes are quite uncomplicated laterally and basally, but the distal cell membrane has a brush border, probably consisting of microvilli. The other type of epithelial cell differs from the chief cell mainly in that it has no brush border but instead a tuft of cilia, which stain with silver and have distinct basal bodies. The ciliated cells are rather unfrequent — about 1 ciliated cell to 25 chief cells in the gas bladder proper.

Histochemical tests show that the nucleodistal part of the epithelial cells shows a very weak positive reaction to PARS and paraldehyde-fuchsin and reacts negatively to the BEST carmine method. The alcian blue method gives a weak but definite reaction in the distal border of the cell. This seems to be limited to the distal cell membrane and the brush border. It could not be observed with certainty if this positive reaction comprises not only the chief cells but also the ciliated cells.

The two inner layers of the wall in the pneumatic duct (Figs. 4, 5), the lamina propria and the epithelium, are strongly folded even when the muscularis is practically relaxed. The lamina propria consists entirely of collagenous connective tissue. It lodges the subepithelial capillary layer. The folds of the inner epithelium fill up a large part of the lumen

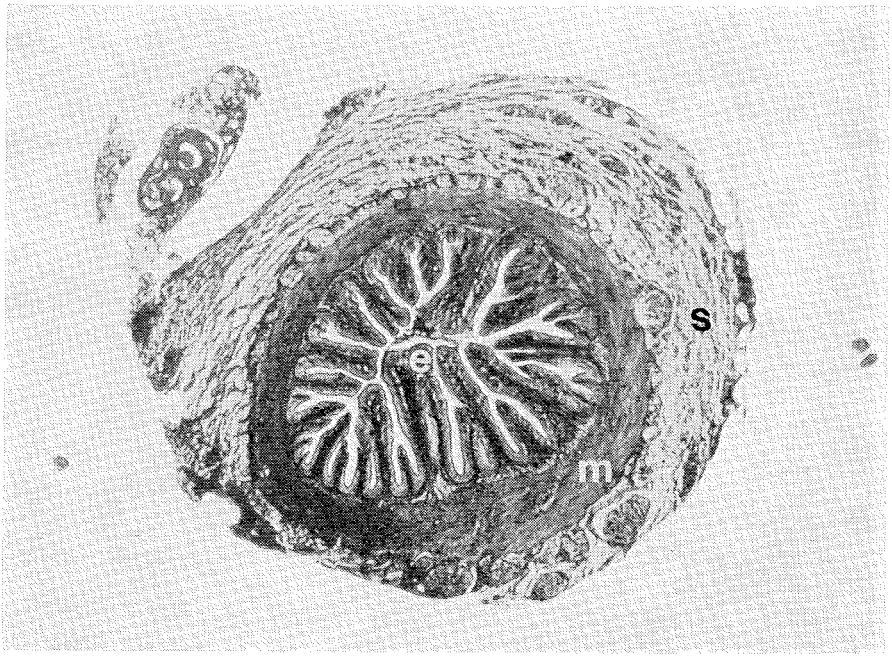

Fig. 4. *Thymallus thymallus*. Transverse section of the pneumatic duct. e = epithelium, m = muscularis, s = submuscularis. Note the almost disappeared lumen, due to sphincter effect of the muscularis. 80x.

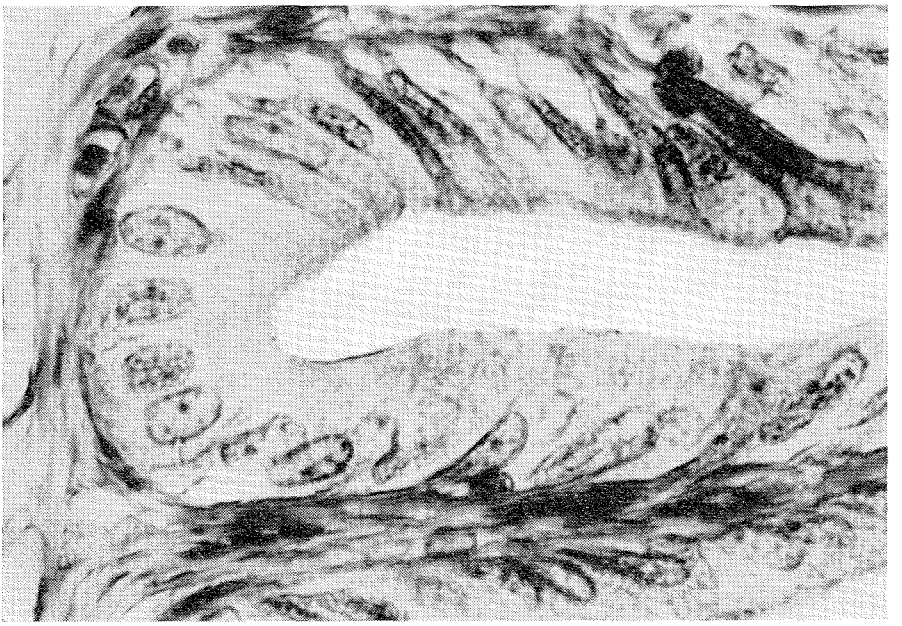

Fig. 5. *Thymallus thymallus*. Epithelium of the pneumatic duct. 1280x.

of the duct. The folds are sometimes so deep that real crypts are formed. These are in contact with the central lumen only by narrow canals. Here too the epithelium is invaded by lymphocytes. It consists of columnar cells, 15—20 microns high and 5 microns broad. They are of the the two types known from the gas bladder proper. The chief cell type has a nucleus, situated in the basal half of the cell. The apical cytoplasm is weakly basophilic and in many cells is seen to differentiate into a cup, similar to that of goblet cells of the intestine. No differentiations could be observed light-microscopically in the basal cytoplasm or in any other part of the cell. The cell membranes seem to be unfolded. The ciliated cell type is found also in the pneumatic duct, where it is more common than in the gas bladder proper — one of five cells is of this type and about the same ratio applies along the whole duct. Structurally they are similar to the chief cells except that the distal cytoplasm shows still weaker basophilia in the ciliated cells.

THE GAS BLADDER OF OSMERUS EPERLANUS

Topography.

The gas bladder of the european smelt, *Osmerus eperlanus*, as usual in Salmonoid gas bladders, is an elongated sac, situated dorsally to the peritoneal cavity and connected with the oesophagus by an open canal,

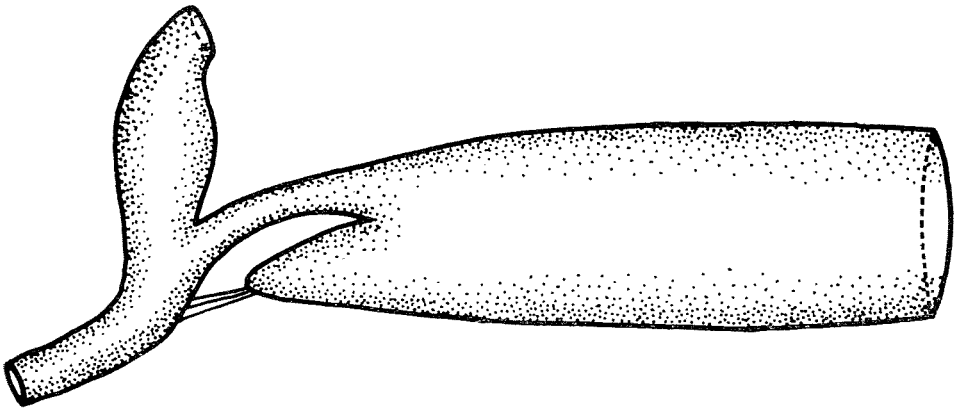

Fig. 6. *Osmerus eperlanus*. Anterior part of gas bladder and oesophagus. Note the blind sac, at the anterior part of which vessels and nerves reach the bladder.

the pneumatic duct. So it was described by CUVIER and VALENCIENNES (1848). At the side of the pneumatic duct a blind sac from the gas bladder proper protrudes about one mm (Fig. 6). The posterior end of the gas bladder ends blindly above the anus. The organ lies ventrally to the kidneys and is ventrally covered by the cubical, ciliated serous epithelium.

Vascularization.

The arterial supply of the gas bladder is mainly furnished by a branch of the coeliaco-mesenteric artery. It divides on the oesophagus into several branches. Some of the small ones accompany the pneumatic duct and apparently their principal task is to supply only the duct with blood. Other arteries, bigger than those along the pneumatic duct, run in a ligament, together with some of the nerves to the bladder, to the tip of the blind sac protruding at one side of the duct (Fig. 6). There they enter the wall of the gas bladder and run along the organ to its posterior end. It seems that these vessels are the only arterial supply of the gas bladder proper. No branches of the gonadal or intercostal arteries or from the other arteries to the alimentary canal are given off for the gas bladder.

The drainage of the gas bladder is effected exclusively by veins belonging to the hepatic portal system. Venous vessels accompany all the arteries described above and fuse on the oesophagus to a common vein which empties into the hepatic portal vein.

The longitudinal arteries and veins run parallel in the submuscular connective tissue layer of the bladder wall. Irregularly they give off branches in such a way that one artery and one vein always go together.

These pairs of vessels cross the muscularis and enter the lamina propria which is a rather thick layer of connective tissue (Fig. 7). Here the vessels branch into arterioles and venules, and the paired arrangement of the vessels is lost. The arterioles and venules split further, and a network of capillaries is formed in the lamina propria.

Histology.

The layers of the wall are the same as in other physostomous gas bladders (Fig. 7). The terminology suggested for this type of gas bladder (FAHLÉN 1967a) is used here. The ventral side of the bladder is covered with the peritoneal epithelium, which consists of cubical, ciliated cells. The outermost layer of the gas bladder wall proper is the tunica externa, which contains smooth muscle fibers. In the posterior part of the bladder the tunica externa is thick and very rich in muscle fibers. Inside this layer follows the submuscularis, a layer of circularly arranged bundles of connective tissue, collagenous and elastic mixed. The muscular layer consists of smooth muscle fibers which form a coat around the bladder. Breaks in this coat, due to the passage of vessels, do occur but are not

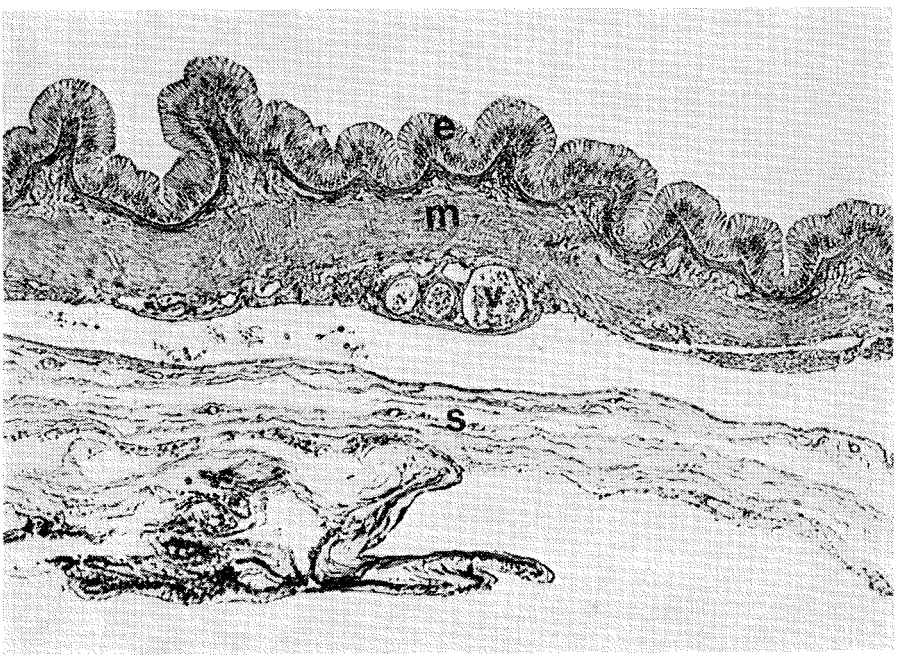

Fig. 7. *Osmerus eperlanus*. Transverse section of the gas bladder wall. e = epithelium, l = lamina propria, m = muscularis, s = submuscularis, v = blood vessels. 160x.

Fig. 8. *Osmerus eperlanus*. Section of gas bladder epithelium. Chief cells with the apical cup visible in most of them. 900x.

frequent. In the posterior part of the gas bladder, the muscularis consists almost wholly of circular fibers, which form a 20—30 microns thick layer. Longitudinal muscle fibers are rare here and are arranged in bundles outside the circular coat, mainly along the big vessels. In the middle of the bladder, the circular muscle coat is somewhat thicker, and the longitudinal fibers have increased in number and form on the ventral side an almost complete layer. On the dorsal side they are still single bundles. In the anterior part, the circular layer has not increased in thickness, but the longitudinal fibers now form an almost continuous layer outside the circular one and about as thick as this. In the blind sac, the muscle coats lose their limitations and become mixed up with one another. In the pneumatic duct the muscularis first becomes thinner, but when the duct enters the wall of the oesophagus it is surrounded by a muscle coat, 100—150 microns thick and consisting mainly of circular smooth muscle fibers (Fig. 13). Furthermore, striated muscle fibers of the muscularis externa of the oesophagus are arranged circularly around the mouth of the pneumatic duct.

The lamina propria in the gas bladder of the smelt has a considerable thickness, about the same as the muscularis. It consists of connective tissue, mainly collagenous fibers, irregularly arranged. Intermingled

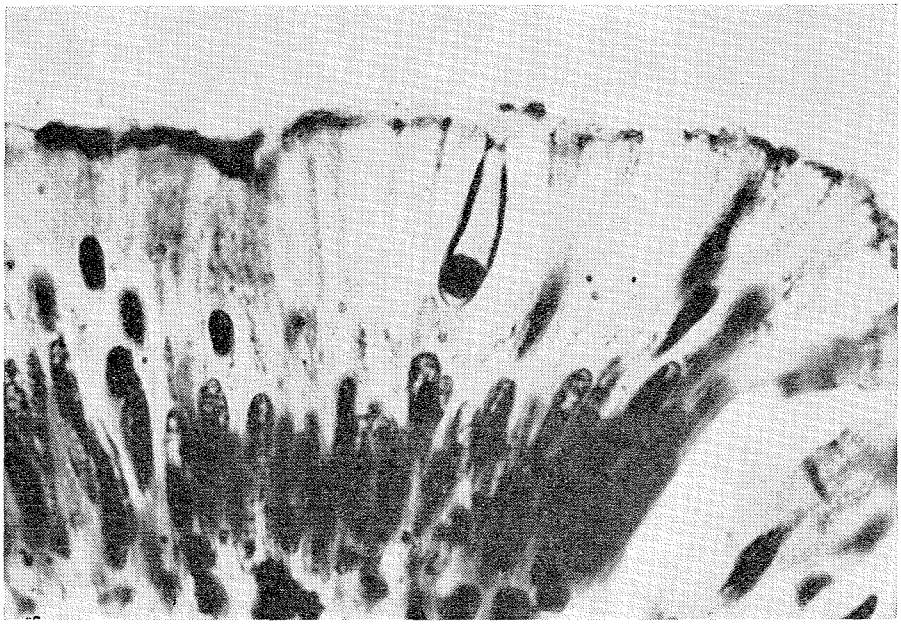

Fig. 9. *Osmerus eperlanus*. Section of gas bladder epithelium. A single, 'pearshaped' cell visible. 1280x.

with these, single elastic fibers are found. In the lamina propria a network of capillaries is lodged. It is of moderate density and the diameter of the capillaries is 10—15 microns. They run on different levels in the connective tissue and are not always in immediate contact with the epithelium. In the epithelium, three kinds of cells can be observed. The majority of cells, which may be called chief cells, are high columnar cells with a somewhat basally situated nucleus (Fig. 8). The nucleus often possesses several nucleoles. The cytoplasm is basally neutrophilic, but the distal part of the cytoplasm is differentiated into a cup, which in standard staining seems almost empty. In staining with paraldehyde-fuchsin for mucopolysaccharides (Fig. 10) and in using the PARS method for polysaccharides (Fig. 11), a moderate reaction occurs in the cuplike distal part of the cytoplasm. Also alcian blue gives a positive reaction in the same part of the cells (Fig. 12). A positive reaction also to the Best carmine method, which is specific for glycogen, is obtained in the distal half of the cytoplasm of the epithelial chief cells in the gas bladder proper as well as in the pneumatic duct (Fig. 15). No reactions are visible in the basal cytoplasm. Low power EM investigation shows that the cell membranes of the chief cell are not folded. The apical membrane is smooth but sometimes slightly domed. The lateral walls are rather un-

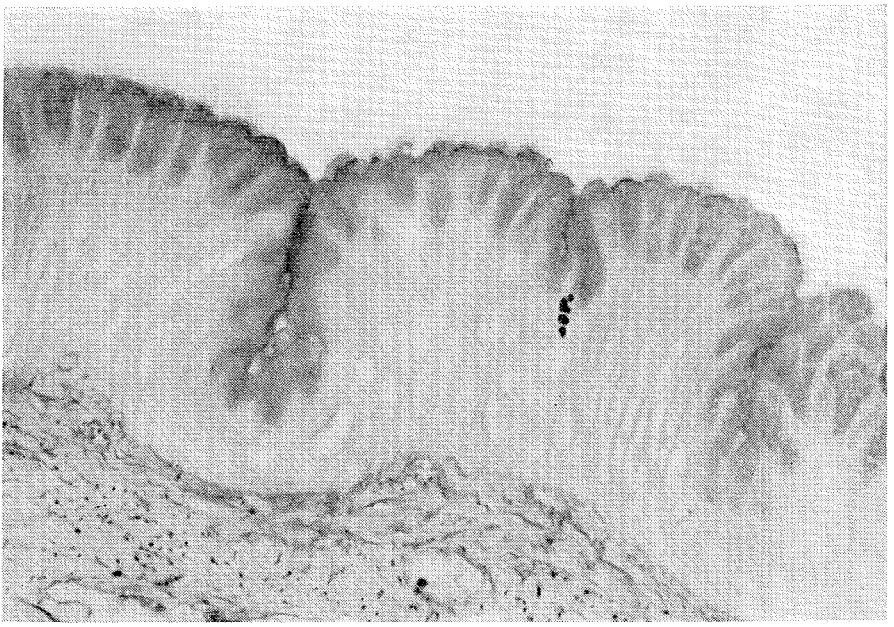

Fig. 10. *Osmerus eperlanus*. Section of gas bladder epithelium. Gabe's paraldehydefuchsin. Positive reaction in the cup of the chief cells. 900x.

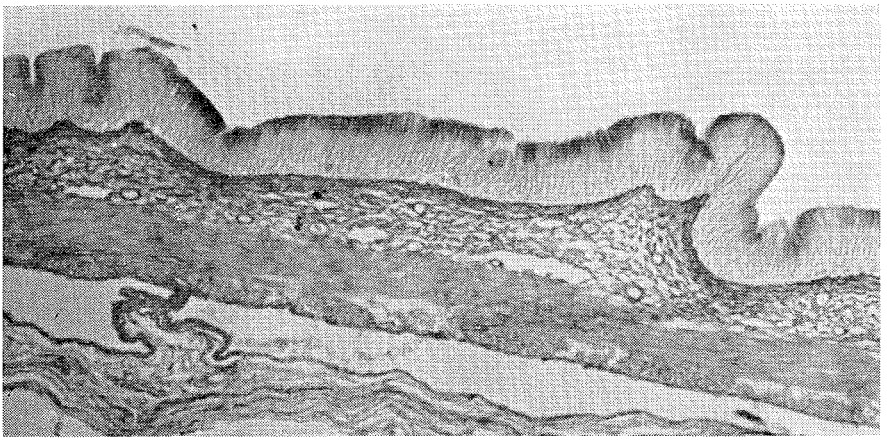

Fig. 11. *Osmerus eperlanus*. Section of gas bladder wall. PARS reaction, positive in the distal part of the epithelium. 250x.

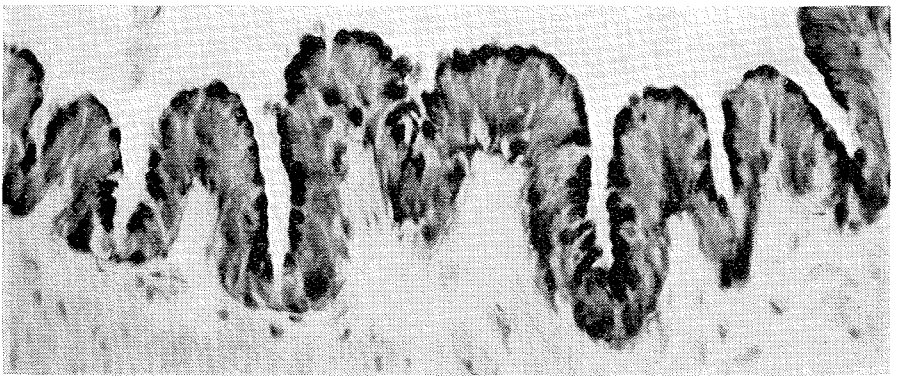

Fig. 12. *Osmerus eperlanus*. Section of gas bladder epithelium. Alcian blue. Positive reaction in the most apical part of the epithelial cells. 410x.

complicated but a very distinct desmosome is present on the boundary between all cells. The basal cell membrane is quite unfolded and there are no infoldings as reported in the eel (DORN 1961). Cells of another type, the frequency of which is very low, are situated between the chief cells (Fig. 9). They are ovoid, do not reach the basal membrane of the epithelium but lie always adjacent to the lumen of the gas bladder. They

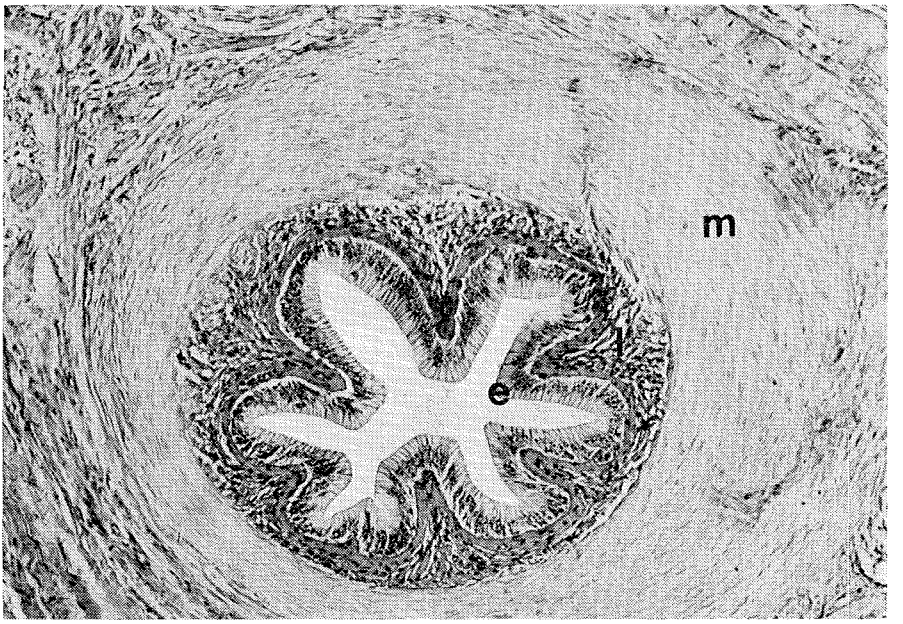

Fig. 13. *Osmerus eperlanus*. Transverse section of pneumatic duct with muscular sphincter. e = epithelium, l = lamina propria, m = muscularis. 180x.

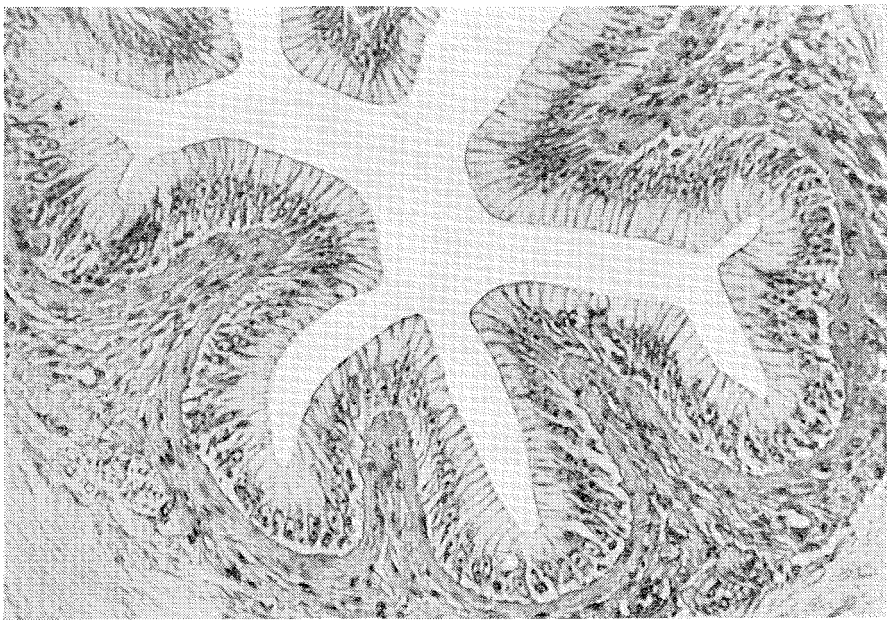

Fig. 14. *Osmerus eperlanus*. Section of epithelium of pneumatic duct. 410x.

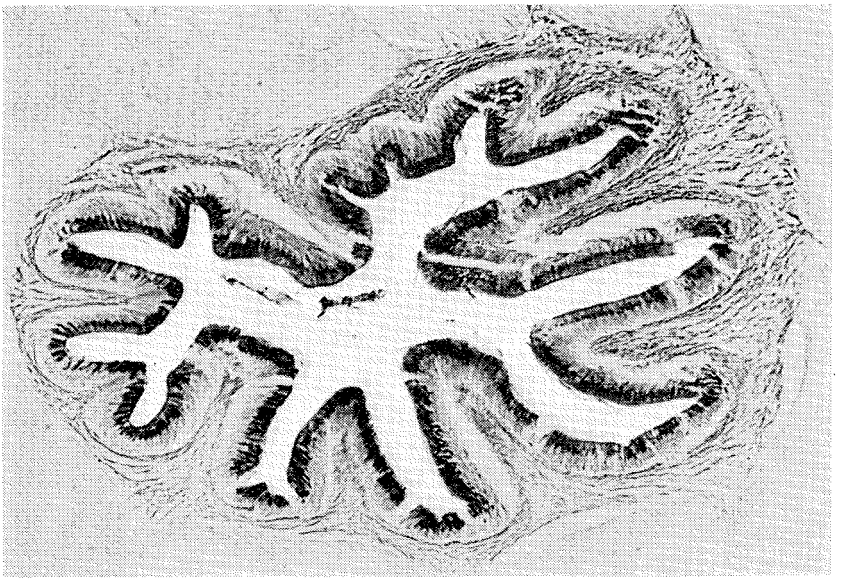

Fig. 15. *Osmerus eperlanus*. Transverse section of the pneumatic duct. BEST's carmin. Positive reaction in the distal cytoplasm of the epithelium.

have a basal nucleus, which is smaller and denser than that of the chief cell. The cytoplasm, all of which is situated distally to the nucleus, is acidophilic and contains granules, which are strongly stained with paraldehyde-fuchsin (Fig. 10). They are reminiscent of the pear-shaped cells of the gas bladder of the herring (FAHLÉN 1967a). — Finally, the epithelium contains a lot of lymphocytes, mainly situated between the basal parts of the chief cells. They are also found, though less frequent, between the distal parts of the cells.

The epithelial cells of the different parts of the gas bladder do not differ very much. The same types are found in the pneumatic duct as well as in the gas bladder proper (Fig. 14). The only change from the posterior end forwards, is that the distal cup of the chief cells gradually increases in depth and basophilia. In the pneumatic duct the cup comprises about half the cytoplasm of the cell, and at the opening of the duct in the oesophagus no sharp boundary concerning the epithelial cells is visible. The histochemical reactions are the same as in the epithelium of the gas bladder proper.

THE GAS BLADDER OF MALLOTUS VILLOSUS

Gas analyses.

The composition of the contents of the gas bladder undergoes relatively small changes under different experimental conditions (Table 1). The mean oxygen percentages do not deviate from those of the control group by more than 2,3%, though the individual values vary. However,

Table 1. *Mallotus villosus*. Analyses of gas bladder contents after injection of drugs.

Drug	Number of animals	Carbon dioxide		Oxygen	
		%	range	%	range
Pilocarpine 0,5 mgm, analyses after 1 hr	2	0	—	11,2	9—14
Yohimbine 0,5 mgm, analyses after 2 hrs	4	0,5	0—1	8,0	8—8
Dibenzyline, 4 × 0,5 mgm, 1 hour's interval, analyses 3 hrs after first injection	6	1,0	0—2	10,0	0—12
NaCl-solution (control), analyses after 5 hrs	7	0,5	0—1	10,3	6—18

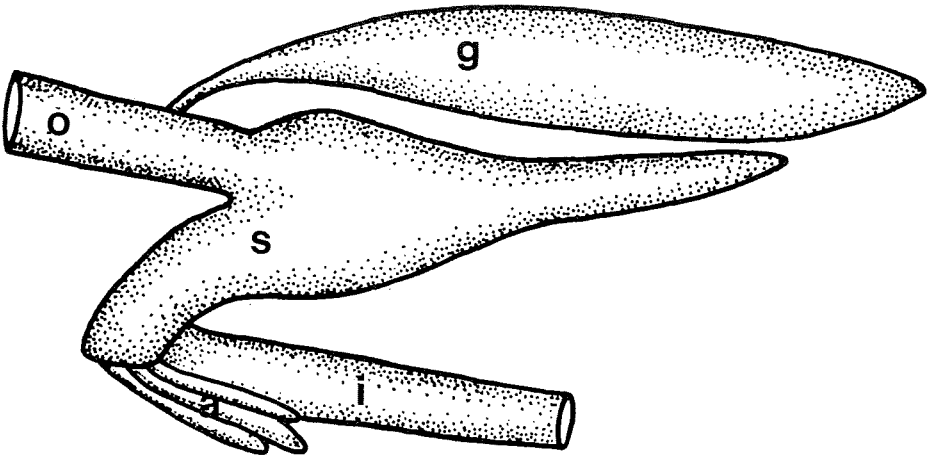

Fig. 16. *Mallotus villosus*. Gas bladder and its relation to the intestinal canal. g = gas bladder, o = oesophagus, s = stomach, a = appendices pyloricae, i = intestine.

this is also the case in the control group. The oxygen percentage never exceeds that of atmospheric air (21%). The carbon dioxide percentage is also very constant and never exceeds 2%.

Topography.

CUVIER and VALENCIENNES (1848) described the gas bladder of *Mallotus villosus* thus: 'La vessie natatoire communique avec l'oesophage, elle est simple, ses parois sont argentées.' BEAUFORT (1909) did not add anything to this description. The gas bladder of the capelin is of the usual Salmonoid shape. As usual it ends blindly at the posterior end of the peritoneal cavity. Anteriorly, it tapers gradually, and there is no distinct border between the gas bladder proper and the pneumatic duct. This opens into the oesophagus further back than in other Salmonoids, only some millimeters in front of the constriction, which is the border between the oesophagus and the coecal part of the stomach (Fig. 16). It is thin-walled, and the silvery appearance attributed to this organ by CUVIER and VALENCIENNES (1848) can not be verified. As is usual for Salmonoid gas bladders, the organ is very loosely attached to surrounding organs.

Vascularization.

As in most Salmonoid species, the blood supply to the gas bladder comes only via a branch of the coeliaco-mesenteric artery, which reaches the organ via the pneumatic duct. Along this, the gas bladder artery

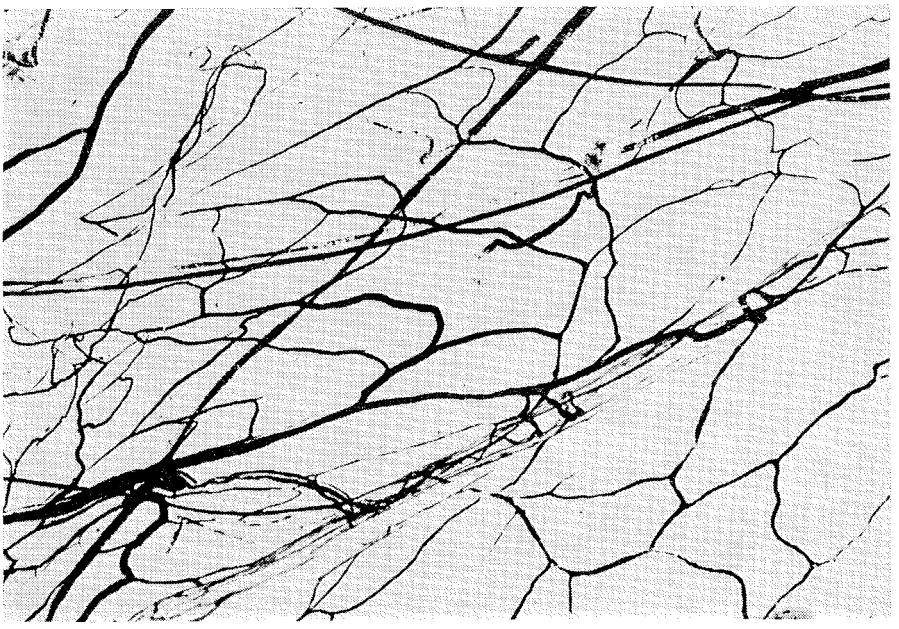

Fig. 17. *Mallotus villosus*. Vascular plexus of the gas bladder wall. Indian ink injection, total preparation. 55x.

gives off several small vessels to the musculature and the base of the epithelium. When the duct runs over into the gas bladder proper, the vesical artery divides into several branches, which run backwards on the surface of the bladder. Penetrating the submuscularis, branches from these arteries reach the outer layer of the muscularis. From these vessels, arterioles cross the muscularis and after further division form a capillary plexus in the lamina propria (Fig. 17). The arteries and arterioles are usually coupled with venous vessels belonging to the hepatic portal system. No vascular bundles with more than two, or occasionally three, vessels are formed.

Histology.

The layers of the gas bladder wall are the usual for the physostomous type (Fig. 18). The total thickness of the wall is 120—150 microns and it varies little in the different parts of the organ, except the pneumatic duct. The outer, denser layer of the submuscularis, the tunica externa, is a very thin layer of collagenous and elastic fibers and single smooth muscle cells. The submuscularis has the usual structure with circular fibers of connective tissue, arranged in layers which are easily detachable

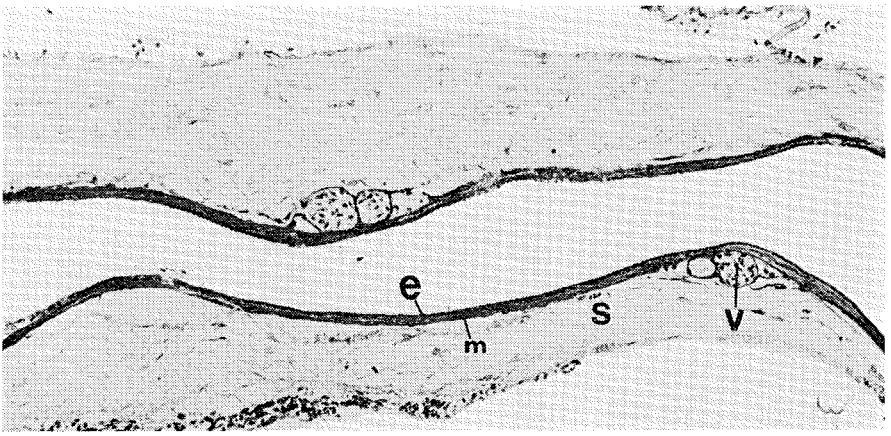

Fig. 18. *Mallotus villosus*. Transverse section of gas bladder wall. e = epithelium, m = muscularis, s = submuscularis, v = blood vessels. 160x.

from each other. It lodges the principal part of the vessels, which run singly or in simple bundles, at different levels. The muscularis, in the main part of the gas bladder about 15 microns thick, consists of an outer thin layer of longitudinal muscle fibers and the inner, principal part of circular musculature. It is vascularized by single capillaries from the submuscular plexus. The muscularis is also penetrated in places by arterioles and venules from this plexus, which in the about 10 microns thick lamina propria splits into a capillary plexus in close contact with the base of the epithelial cells. In the pneumatic duct, the submuscularis soon is changed into a very thin sheet of connective tissue outside the muscularis. The latter gradually becomes thicker, but reaches a constant thickness of about 25 microns, which it retains along the whole duct. At the debouch into the oesophagus, the muscularis of the pneumatic duct is continuous with the striated muscularis externa of the oesophagus. Some of the oesophageal muscle bundles are arranged circularly around the opening of the pneumatic duct.

The epithelium is composed of two kinds of cells. The chief cells (Fig. 19) are cubical, 8—10 microns high. In the pneumatic duct, however, they are of cylindrical shape. The cell membrane is quite devoid of folds both laterally and basally, where it is delimited from the lamina propria by a thin basement membrane. Apically, the cell membrane has small, irregular microvilli, about 5000 Å high. The nucleus is relatively large, occupies a great part of the basal region of the cell and has a regular periphery. The cytoplasm, the fine structure of which could not be satsfactorily investigated, contains several kinds of organelles and inclusions. Small mitochondria are present in a moderate number both

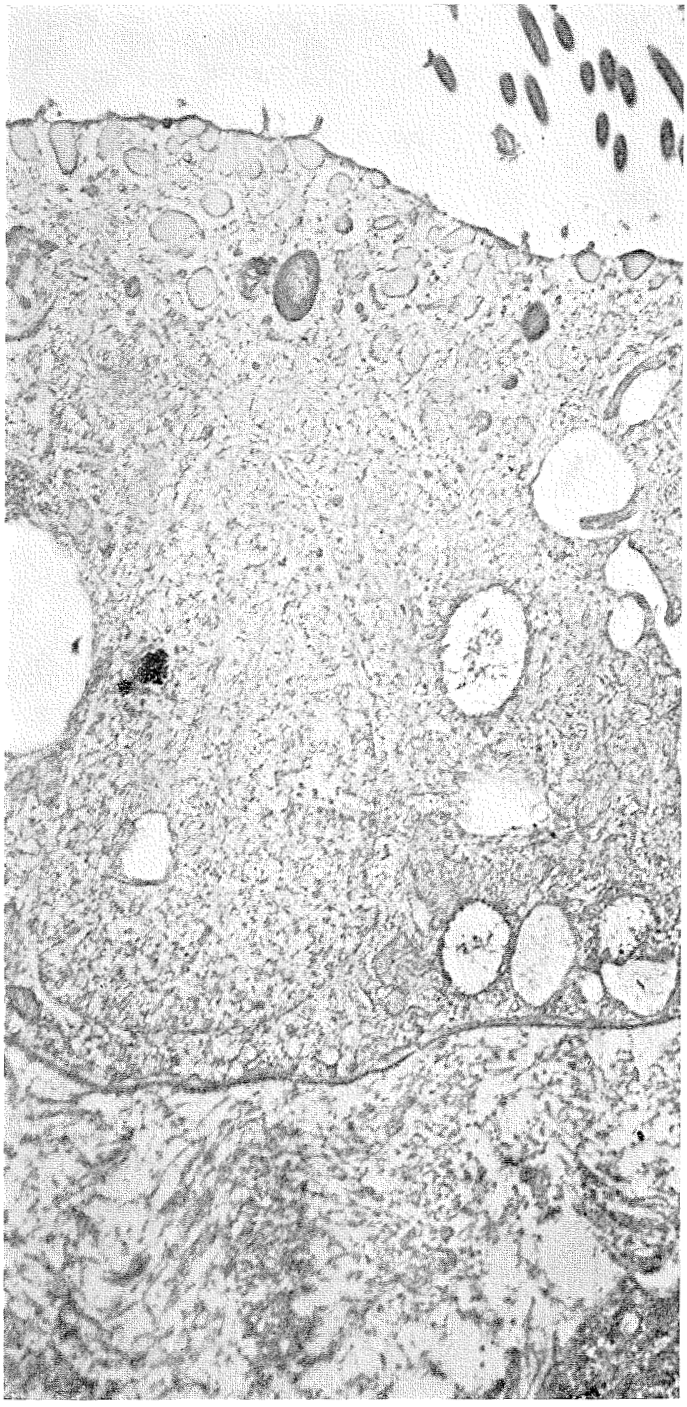

Fig. 19. *Mallotus villosus*. Electron micrograph of the gas bladder epithelium. Cilia from an adjacent ciliated cell visible in the lumen. 11.500x.

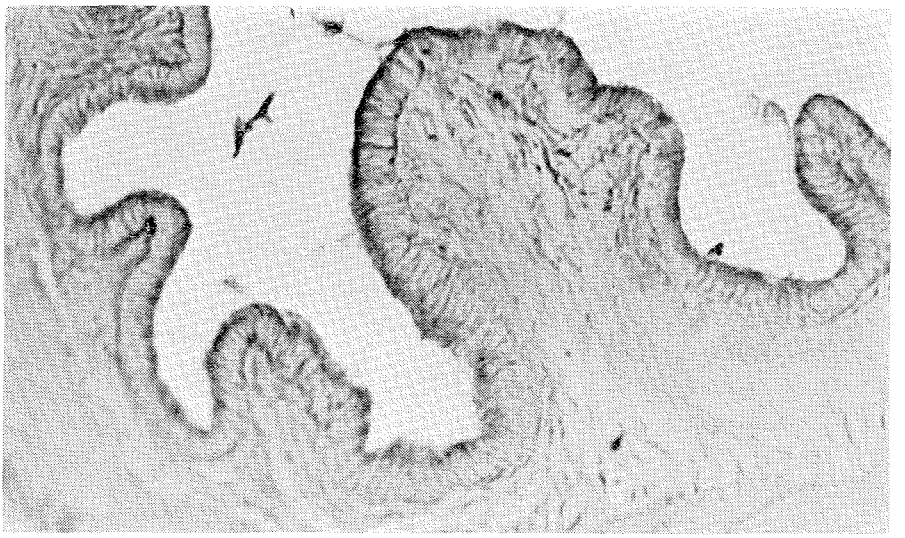

Fig. 20. *Mallotus villosus*. Section of gas bladder wall. PARS reaction, positive in the distal cytoplasm. 460x.

basally and apically. Vacuoles, the limitations and contents of which could not be sufficiently well defined because of the unfavourable result of fixation and imbedding, are present, mainly in the basal and nucleolateral regions. Distally, very distinct inclusions are accumulated near the apical cell membrane. They are moderately electron-dense, 1000—5000 Å large, and are often seen to cause the cell membrane to bulge out.

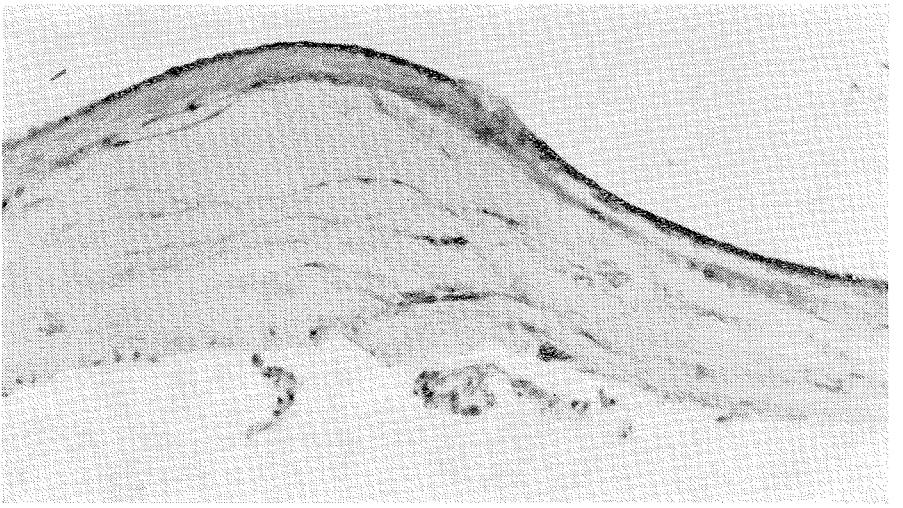

Fig. 21. *Mallotus villosus*. Section of gas bladder wall. Alcian blue. Positive reaction in the distal cytoplasm of the epithelial cells. 410x.

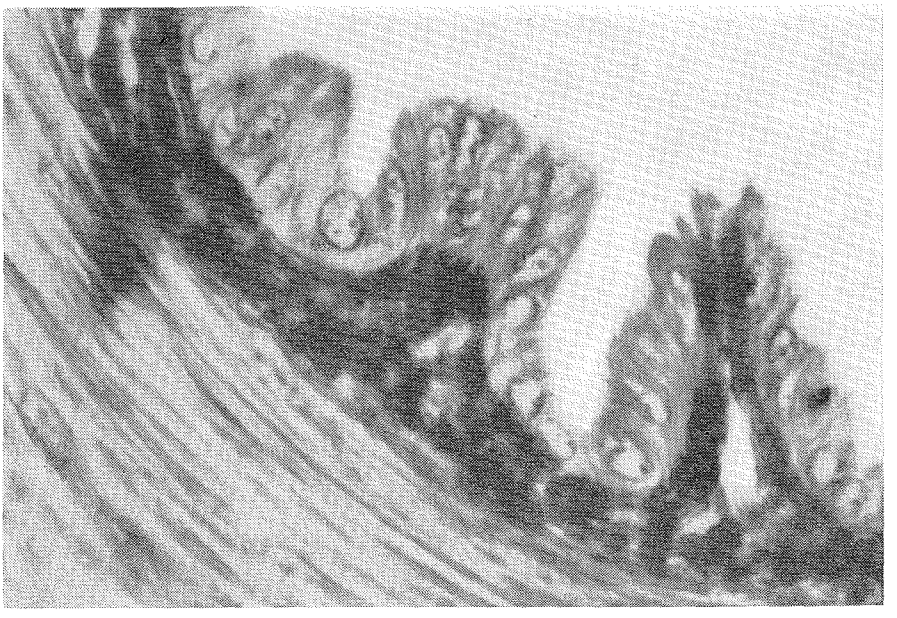

Fig. 22. *Mallotus villosus*. Section of part of the wall of the pneumatic duct. e = epithelium, l = lamina propria, m = muscularis. 1820x.

Besides the chief cells, there is a second type of epthelial cell, the frequency of which, however, is very low. This cell type is ciliated (Fig. 19). Along the apical cell membrane no vacuoles are visible, though this region is relatively rich in mitochondria. Histochemical tests on the gas bladder of the capelin gave different results. The paraldehyde fuchsin reaction was negative in all parts of the gas bladder. The PARS reaction was positive for the epithelial chief cells (Fig. 20). The reaction was partly very strong and as such strictly localized to the apical border, including the region where the moderately electron-dense vacuoles were concentrated. A weak, hardly significant reaction was observable also in the rest of the cytoplasm. In the pneumatic duct (Fig 22), the likewise strong PARS reaction enclosed a greater part of the distal cytoplasm (Fig. 23) than in the gas bladder proper (Fig. 20). In sections tested with alcian blue for acid mucopolysaccharides, a positive reaction was observed in the apical cell membrane of the gas bladder proper as well as of the pneumatic duct (Figs. 31, 24). None of these reactions occurred in the ciliated cells. The carmine reaction for glycogen (BEST) was negative in all parts of the gas bladder.

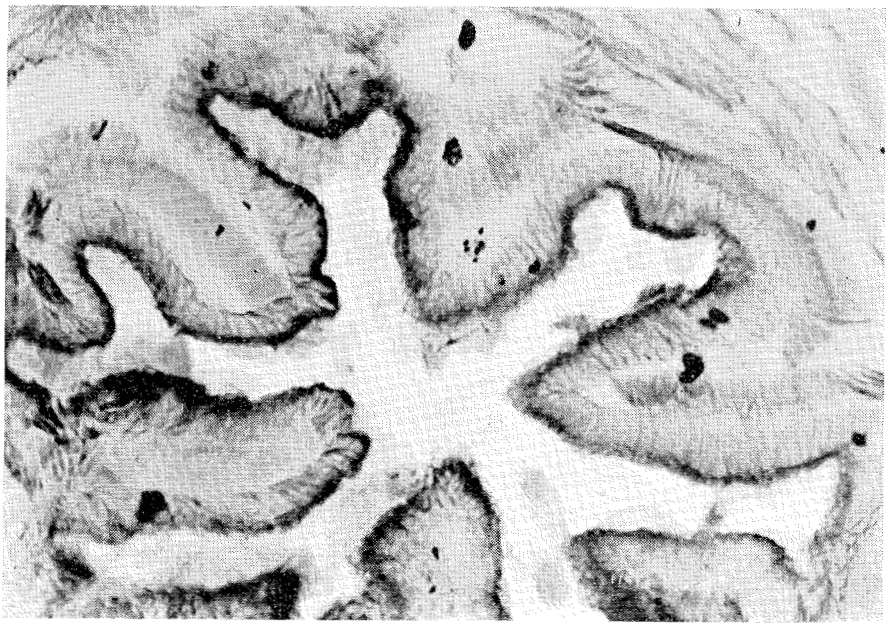

Fig. 23. *Mallotus villosus*. Transverse section of the pneumatic duct. PARS reaction, positive in the distal cytoplasm of the epithelial cells. 410x.

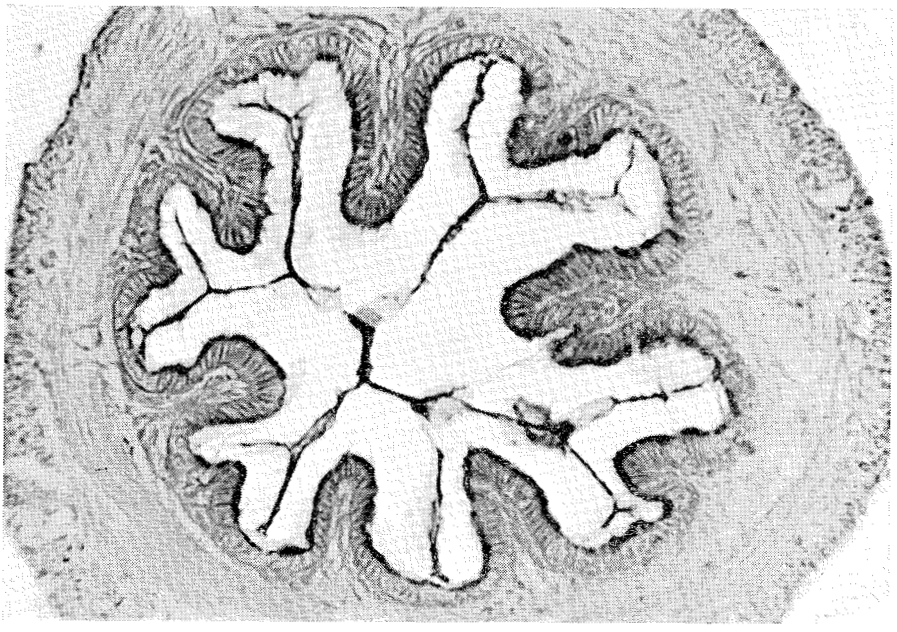

Fig. 24. *Mallotus villosus*. Transverse section of the pneumatic duct. Alcian blue. Positive reaction in the distal cytoplasm of the epithelial cells. 410x.

CONCLUSIONS AND DISCUSSION

Among the gas bladders of the three species described above, two types are distinguishable. One type is represented by the gas bladder of *Thymallus th.*, which has several similatities with the corresponding organ of *Coregonus lavaretus* (FAHLÉN 1967b). Conspicuous is the relative abundance of vessels, which in the grayling are arranged in bundles of the same type as the counter current bundles of the whitefish gas bladder. Their frequency, however, is much lower in the grayling, but no arterial vessel reaches the base of the epithelium without having passed a considerable way in contact with one or two venous vessels leaving the epithelium. Thus, there is a prerequisite condition for a counter current exchange in *Thymallus* as well as in *Argentina*, which has a closed gas bladder and a counter current system built up of *microretia* with 3—10 vessels in each (FÄNGE 1958). A counter current system of the same type is present also in *Coregonus lavaretus* and *C. acronius* (FAHLÉN 1967b). However, the capacity of the counter current system of *Thymallus th.* must be considerably lower than that of *Argentina* and *Coregonus*. The lining epithelium of the grayling gas bladder also has similarities with that of the whitefish bladder. It is cubical to low columnar and in its morphological details and histochemical reactions has only few characteristics in common with the secretory cells known from the gas bladders of the physoclistous type (FÄNGE 1953, JASINSKI and KILARSKI 1964) and the eel (DORN 1961). Possibly, it has no secretory function at all, but the similarity with the epithelium of the whitefish gas bladder, which is reported to secrete oxygen and carbon dioxide (SUNDNES 1963, FAHLÉN 1967b) does not exclude a secretory function of the same type as in the gas bladder of *Coregonus lavaretus*.

The vessels of the gas bladders of *Osmerus eperlanus* and *Mallotus villosus* are arranged quite differently. Though the vascularization in these species too is rather rich, and a capillary bed beneath the epithelium is present, the vessels of the gas bladder wall form bundles only occasionally. Most of them are running as arterioles and venules, single or in pairs, to and from the subepithelial vascular plexus. No real counter current system is thus present in these gas bladders. The lining epithlium of the gas bladders of *Osmerus* and *Mallotus* also have some similarities. They apparently secrete substances directly to the lumen of the gas bladder, but the secretory products are not quite the same in the two species. The positive PARS-reaction of both species indicates the presence and probable secretion of a neutral polysaccharide. In *Osmerus*, this is partly a mucopolysaccharide, partly glycogen. The question as to whether or not mucopolysaccharides may give rise to gaseous products must

be left open until the possibility of such a transformation is clarified. Concerning the glycogen in the cells, this substance is present during the inactive phase of gas gland cells in physoclistous gas bladders (FÄNGE 1953). There, however, it participates in the gas secretion mechanism mostly in an indirect way, by releasing acid substances to the blood vessels (FÄNGE 1953, STEEN 1963). But in the breakdown of glycogen free gases may be released, e. g. carbon dioxide. This substance may give rise to some of the gases filling the gas bladder during secretion. A secretion in the smelt has been demonstrated by SAUNDERS (1953). He showed by measuring the flotation pressure that captured american smelt (*Osmerus mordax*) had been in buoyancy with the hydrostatic pressure at the depth where the fish had been captured. WITTENBERG (1958) showed that *Salmo gairdneri* and *S. trutta* were able to fill their gas bladders in about 12 days without access to the surface. The histology of the organ seems to be about the same in *Osmerus* and *Salmo* (WEINREB and BILSTAD 1955), though glycogen has not been demonstrated to be present in the *Salmo* species.

In *Mallotus*, the histochemical tests reveal that the epithelium contains acid and neutral polysaccharides. These substances are present in rather small amounts in the epithelial chief cells. The histochemical localization is the apical zone of the cytoplasm, which lodges vacuoles with a probable secretory content, and probably the polysaccharides are lodged in these vacuoles. The strict localization of these vacuoles to the apical part of the cell and their often close relation to the apical cell membrane leads to the conclusion that their contents are given off directly to the lumen of the bladder. Concerning their chemical composition it can only be stated that they are not of mucoid nature and not glyogen. The absence of the last polysaccharide shows that the possible secretory activity is not the same as in the euphysoclist gas bladder, where this substance during secretion breaks down to carbon dioxide and lactic acid. The gas analyses have not shown any increase in the percentages of neither carbon dioxide nor oxygen. The latter is the principal gas secreted in the euphysoclist gas bladder. The relative amounts of these two gases are comparable with those of the herring gas bladder (FAHLÉN 1967a). Besides the apical secretory vacuoles, the gas bladder epithelium of the capelin has great similarities with that of the herring, where no structural signs of a secretory function are present in the gas bladder proper. As the capelin lives at about the same moderate depths as the herring (MØLLER and OLSEN 1962) gas secretion must not necessarily take place to keep the animal in buoyancy with the hydrostatic pressure at the actual depth.

The muscle layer of the gas bladder wall in the three species described

above, as well as in physoclistous and other physostomous gas bladders, is intimately related to the epithelium and the lamina propria. Functionally it therefore corresponds to the muscularis mucosae of the alimentary canal, and in full consequence with this aspect FÄNGE (1953) suggests the same name for the muscle layer of the euphysoclistous gas bladder. In all the species described in this paper, the layers of the wall of the gas bladder can be followed along the pneumatic duct. When this emerges into the oesophagus, the loose connective tissue of the gas bladder proper (submuscularis) is reduced to a thin sheet outside the muscularis externa of the oesophagus, and in all cases the muscle layer of the bladder (muscularis) is clearly continuous with the muscularis externa of the alimentary canal. This condition is also found in *Clupea* (FAHLÉN 1967a) and in *Coregonus* (FAHLÉN 1967b). Therefore it must, from the view of homology, be more correct not to use the names of the layers of the alimentary canal. The name muscularis externa for the muscle layer of the gas bladder is, however, not suitable, as this layer is not an external one. Thus, there are strong arguments for a more neutral name on the muscle layer of the gas bladder wall, such as muscularis, as suggested by FAHLÉN (1967a). As the continuity of the muscularis of the gas bladder with the muscularis externa of the alimentary canal is a fact in all species so far investigated, it seems probable that also the muscularis mucosae of the physoclistous gas bladder (FÄNGE 1953) is homologous with the muscularis externa of the alimentary tract. This can be confirmed only by investigations in embryos, where the connexion between the gas bladder and the oesophagus still exists (TRACY 1911).

The muscularis of the pneumatic duct does not show any sphincter-like organization where it leaves the gas bladder in any of the three species. At the debouch into the oesophagus, however, the muscularis has often its greatest thickness and is there also surrounded by striated muscle fibers of the muscularis externa of the oesophagus. Apparently, this part of the muscle coat may function as a sphincter, which is in accordance with observations on *Salmo* species by FÄNGE (1953). For complete understanding of the intimate function of the pneumatic sphincter of Salmonoidei, further investigation is necessary.

SUMMARY

1. The gas bladder of *Thymallus thymallus* is investigated concerning the topography, vascularization, histology and histochemistry. Simple *micro-retia mirabilia* are found, forming a counter-current vascular system of probably very low exchange capacity. The epithelium, lining the lumen,

is probably one of very low activity. If a secretion of gases into the bladder occurs, it is probably very slow.

2. The gas bladder of *Osmerus eperlanus* is investigated as described above. The blood vessels are not arranged as a counter-current system, though capillaries reach the base of the epithelium. The epithelium, which is also EM investigated, contains apically considerable amounts of mucopolysaccharides and glycogen. A secretion, directly to the lumen, of gases or of a matter which can be transformed into gases, is proposed.

3. The gas bladder of *Mallotus villosus* is investigated as described under point 1. Besides, low power electron micrographs has been studied, and analyses of the gaseous content of the bladder were made after administration of pilocarpine, dibenzyline and yohimbine. No counter current vascular system is present but capillaries reach the base of the epithelium. The epithelial cells contain and probably secrete polysaccharides (though not glycogen) directly to the lumen. The gas is composed of 0—2% carbon dioxide, 0—18% oxygen and 80—100% inert gases. The administration of drugs did not in any case increase the percentage of oxygen or carbon dioxide, compared with the control animals. It is proposed that no gas secretion occurs.

4. The muscle layer of the gas bladder is in all the three species continuous with the muscularis externa of the oesophagus. This argues for a neutral terminology concerning the layers of the gas bladder wall.

5. A muscular sphincter is morphologically documented at the debouch of the pneumatic duct into the oesophagus.

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