A CINERADIOGRAPHIC STUDY OF RESPIRATION IN *MYXINE GLUTINOSA* L.

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(With Plates 11 and 12)

The anatomy of the respiratory organs, as well as the mechanism of breathing in myxinoids, is distinctly different from that in all other fish-type craniotes even including the petromyzonts. In *Myxine* the branchial gill lamellae are enclosed in rounded sac-like pouches, with ducts conveying the water from the oesophagus to the gills from which it travels through efferent ducts uniting on each side, finally leaving the animal through paired branchial apertures. In addition to the paired gill pouches there exists an oesophago-cutaneous duct connecting the pharynx to the exterior behind the left, last gill sac. The exterior opening is confluent with the left gill pore. Regarding the course of the respiratory current in this system contradictory explanations have been presented. More recent authors seem, however, to agree upon the concept that the water current is solely dependent upon the pulsating activity of the velum and is not influenced actively by either the gill pouches or their ducts (Gustafson, 1935; Strahan, 1958; Marinelli, 1956). The velum and its connected muscles, which is a unique structure in myxinoids, was already anatomically described by Müller in his *Myxine* monograph (1835) and later by Fürbringer (1875) and Cole (1907). The first two authors, however, did not contemplate the velum as being of any functional value for respiration. Cole, on the other hand, suggested a role for this structure in the maintenance of the respiratory current. Goodrich (1930) has advocated that breathing in *Myxine* takes place by expansion and contraction of the muscular elements in the gill sacs. That the gill sacs in *Myxine* contain distinctly striated muscular elements has been described by Cole (1912) and later by Goodrich (1930) and Hofbauer (1937). It seems obvious, upon reviewing the literature, that the physiological interpretations of this problem in earlier works are mostly founded on anatomical studies with little or no physiological experimentation. The excellent works of Gustafson (1935) and Strahan (1958) base their functional interpretations entirely upon external observations of the living animal or upon dropping coloured solution in the respiratory water and following its course into the nostril and out of the branchial apertures. The present study attempts to analyse the mechanics of respiration in *Myxine* by utilizing modern

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cineradiographic equipment which allows fluoroscopic examination of all events inside the respiratory passages; these may be recorded on a camera at 26 frames per second. The method has been used earlier for the study of the circulation in snakes (Johansen & Hol, 1960) and of the central circulation in *Myxine* (Hol & Johansen, 1960).

MATERIAL AND METHODS

The specimens of *M. glutinosa* used in this study were trapped near the Dröbak Biological Station in the Oslofjord in Norway. The animals were never anaesthetized but were immobilized in a lucite box filled with cold oxygenated sea water during the experiments. Two different contrast media were used: barium suspension with a particle size of less than 40 μ and the water-soluble Hypaque 45%. These media were introduced into the animal through a plastic cone fitting into the mouth or a polyethylene tube inserted into the nostril.

RESULTS

Experiments with barium suspension

Most of these experiments were accomplished by introducing the contrast into the pharynx through the mouthpiece. Following contrast filling of pharynx and part of the oesophagus it turned out that no noticeable filling of the gill ducts and gill sacs was achieved. Several different movements could be observed in the branchial region at this time. The afferent gill ducts, scantily filled with barium suspension, could be seen to empty their contents back into the oesophagus (Pl. 11, fig. 1). This could occur simultaneously with increased filling of the oesophagus. By an attempt to overdistend the oesophagus in order to force the barium through the gills, the contrast was ejected forcefully through the oesophago-cutaneous channel (Fig. 2). These experiments are by no means claimed to reproduce normally occurring physiological conditions but were performed in order to elucidate the puzzling problem as to how myxinoids can discriminate the sizes of particles in their respiratory water entering the oesophagus and thereby avoid filling the delicate gill structures with smothering particles.

On one occasion a forceful antiperistaltic movement of the lower oesophagus was observed. By this act both the afferent gill ducts and the lower oesophagus emptied their contents in cephalad direction (Fig. 3). Fig. 4 illustrates another situation during which the oesophagus in the branchial region is contracted, forcing the contrast caudally through the oesophago-cutaneous channel and cranially through the mouth. Fig. 4 also shows a single gill body and its ducts filled with barium suspension which is seen to be squeezed out in peripheral direction. This seemed to be accomplished by contraction of the gill body and its ducts, occurring simultaneously with contraction of the musculature in the oesophagus and the body wall.

Experiments with water-soluble Hypaque 45%

The water-soluble contrast was injected through a polyethylene tube inserted into the mouth or the nostril of the animal. This contrast medium easily filled the

gill ducts and gills without the slightest overdistension of the pharynx and oesophagus. In Fig. 5 the gills and gill ducts are shown well outlined in a somewhat oblique prone position. Following this filling, the various phases of the contrast passage through the gills could be observed. There were no signs of discomfort or unnatural behaviour in the animals during these experiments. The oesophagus and afferent and efferent gill ducts as well as the gills themselves seemed to take active part in the forward propulsion of contrast. In what follows a more detailed description is given as to how each part of the gill system participates.

The afferent gill ducts. During contraction of the gill bodies we observed a narrowing and closing of the afferent gill ducts by sphincter-like mechanisms. The closure took place both at the oesophageal end and at the gill end of the ducts. The former we have named the juxta-oesophageal gill-duct sphincter and the latter the afferent gill-duct sphincter (Fig. 5). The juxta-oesophageal gill-duct sphincter was also seen to contract during the barium injections, when it seemed to be the primary obstacle to the entrance of the suspension (Fig. 2). In the resting phase the juxta-oesophageal gill-duct sphincter was sometimes slightly contracted (Fig. 5 A) while the afferent gill-duct sphincter was open and relaxed. In the early contraction phase the whole branchial region was shortened, most probably brought about by contraction of the m. constr. branch. At this time both the described sphincters were open (Fig. 5 B, C). They contracted and closed the duct lumen during the progressive contraction of the gill bodies (Fig. 5 D) while the juxta-oesophageal sphincter relaxed and opened lumen again during maximal contraction of the gill bodies and the muscles which shorten the branchial region (Fig. 5 E).

The gill bodies. During the initial shortening of the branchial region some further filling of the gills occurred. When the gills started contraction we observed a considerable reduction in their size and they were almost emptied of contrast (Fig. 5 D, E). Often a denser streak of contrast was observed centrally in the gills connecting the afferent and the efferent ducts (Fig. 5 A, D).

The efferent gill ducts. During the described muscular activity definite changes were also observed in the efferent gill ducts close to the exit from the gill bodies. These ducts seemed to be in a slightly contracted state during the resting phase. Immediately before the start of a gill contraction they were wide open, contracting again toward the maximal gill contraction. Thus there exists a third gill-duct sphincter, the efferent gill-duct sphincter (Fig. 5 B, D). The remainder of the efferent gill ducts which unite posteriorly and form a common collecting channel (on the left side also common with the oesophago-cutaneous duct) showed muscular activity of a peristaltic type actively forwarding the respiratory current. This contraction started at the gill end of the ducts and was propagated distally simultaneously with the gill contractions. As previously mentioned, the oesophago-cutaneous channel was observed being used for ejection of superfluous content in the lower oesophagus. These expulsions started and ended very abruptly, pointing to a closure mechanism on this duct also.

Experiments designed to study the activity of the velum

What we could call the upper respiratory tract or the velum region in *Myxine* could also be studied with the technique, although the study of these delicate structures lies on the borderline of what can be well depicted on the films. Through a catheter inserted into the nostril we injected the contrast (water-soluble Hypaque 45%) very slowly. Thereby we achieved a seemingly passive filling of the nasal passages, the velum chamber and the upper pharynx (Fig. 6). Experiments were made both in the antero-posterior and in lateral view. In the former position the movements in the lateral direction were best discernible, while in the lateral view we could better observe the more rapid upward movements of the velum.

The antero-posterior projection. According to Strahan (1958) the velum has a high position in the resting or dead animal. From this position it unrolls ventrally and laterally. By this movement the amount of water in the velum chamber is reduced whereas water flows in between the right and left velar scrolls. These movements were detectable on the films. During the entry of water between the two parts of the velum, the velum chamber and the upper part of the pharynx were slightly reduced in size.

The lateral projection. In the lateral projection the resting phase and the phase of lateral velar movement were difficult to detect. The rapid upward movement of the velum was accompanied by a straightening of the dorsal and ventral contour of the chamber which consequently was reduced in size. Furthermore, the pharynx was wider open. Our observations justify the assumption that the lateral velar movements contribute to the propulsion of water into the oesophagus. On one occasion we followed a combination of movements resulting in a vomiting as shown in Fig. 7. A contraction of the pharynx could be seen to be propagated to the velar chamber and buccal cavity. The position of the velum cannot be distinguished initially but at the end of the contraction the upward maximum or resting position is reached.

DISCUSSION

It is difficult to make definite statements as to the physiological significance of the phenomena described in this paper. The contrast media applied are both foreign and unfamiliar materials to the animals. They have, however, a low toxicity (Hypaque) or none at all (barium). The water-soluble Hypaque was passively injected without causing the slightest discomfort to the animals. There is no doubt that under these circumstances the movements found and described can be easily provoked and give clues to the mechanics of breathing during strictly physiological conditions.

Cunningham (1887) was the first to describe the unusual pattern of respiration in myxinoids, although without giving any explanation as to how the water current is maintained. In 1935, Gustafson made the definite statement that the current of water is driven solely by the velum. Marinelli (1956) confirmed Gustafson's statements and added that the posterior part of the nasopharyngeal duct also

pulsates and thus promotes the water current. Recently Strahan (1958), in line with the conclusions of Gustafson, found it untenable that the gill pouches or their ducts participate actively in the propulsion of the respiratory water.

Taking into account the well-developed muscular elements in the gill pouches it seems precipitate definitely to exclude them from an active role in the maintenance of the water current. The results presented in this study leave no doubt that the contractions in the wall of the oesophagus and other branchial muscles, as well as in the gills and gill ducts, may actively assist the velum in the maintenance of the respiratory current. Control of the inflow of water and of its direction may be excited by the system of sphincters in the gill ducts, described above. Our studies also revealed periods when the water flowed rather passively through the relaxed gills but even at this time opening and closing of the gill-duct sphincters was observed, contemporary with a flow of water following central pathways through the gills. When this situation is interrupted by the contraction of the muscular elements in the gill bodies the more tiny water channels inside these gill bodies, constituting the active respiratory area, are inclined to be emptied and refilled, causing an effective exchange of respiratory gases.

Our cineradiographic data from the velum region confirm the statements of Strahan (1958) with one important exception: the velum chamber is not rigid but can reduce its dimensions by contraction. These contractions closely accompany the velar movements and most probably support the backward propulsion of water. We further presume that the phase of lateral velar movements also represents an active stage in the propulsion of water.

An animal like *Myxine* living mostly in muddy regions is permanently exposed to the danger of inhaling particles with a consequent smothering and impairing effect on respiration. According to Strahan (1958) inhalation of large particles evokes a violent expulsion of water through the nostril. This act he has termed sneezing. The mechanism behind this, he states, remains obscure and he suggests as a possible explanation that it resides in the pharyngeal constrictor muscle which encircles the posterior part of the velar chamber. Following contraction of this muscle, water and possibly particles would be driven out through the naso-pharyngeal duct. Occasionally during our experiments we observed this sneezing and recorded it on our films (Fig. 7). The results confirm Strahan's suggestions regarding the influence of the pharyngeal constrictor muscle. In addition, we also noticed contractions of the velar chamber during this sneezing or vomiting. Furthermore, we found it of considerable interest to observe the astonishing ability of the gill-duct sphincters to control the inflow to the gills. The experiments with the barium suspension (particle size less than 40 μ) clearly demonstrated this, and undoubtedly it has great physiological significance to an animal in which the respiratory and alimentary channels have extensive common ducts. The control system to avoid smothering of the delicate respiratory surfaces seems to have a third site of operation in the central channel inside the gill bodies. These channels may represent more open pathways for the water and yield passage to smaller particles which accidentally have passed the afferent ducts.

SUMMARY

1. Cineradiography has been applied to study the respiratory current in *Myxine glutinosa* L.

2. Our observations reveal that contractions in the wall of the oesophagus and other branchial muscles, as well as in the gills and gill ducts, actively assist the velum in the maintenance of the respiratory current.

3. A system of sphincters on the gill ducts has been described. These sphincters function as delicate filters removing particles from the water passing through the gills, and thus preventing smothering of the active respiratory area inside the gill bodies. The gill-duct sphincters take also active part in the directional propulsion of the respiratory current.

4. Additional information has been gained on the function of the velum. Thus the velar chamber is not rigid, but can reduce its dimensions by contraction. These contractions closely accompany the velar movements and probably support the backward propulsion of water. It is further suggested that the lateral velar movements also represent an active stage in the propulsion of water.

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EXPLANATION OF PLATES

PLATE 11

Fig. 1. A. Afferent gill ducts partly filled with barium from the distended oesophagus. B. The barium contrast is seen rejected from the gill ducts. Time between A and B, $\frac{19}{44}$ sec.

Fig. 2. A, B. Progressive ejection of barium contrast through the oesophago-cutaneous channel. Time between A and B, $\frac{8}{44}$ sec.

Fig. 3. A–C. Antiperistalsis in the lower oesophagus together with rejection of contrast from the gill ducts.

Fig. 4. A. The gills are seen partly filled after overloading and distension of the oesophagus. B. Maximal contraction of the gill region is seen to empty both the lower oesophagus and the gill systems.

PLATE 12

Fig. 5. The gill system studied by injection of Hypaque 45 % in water solution. A. Resting phase. The juxta-oesophageal sphincter is partly closed. B. The juxta-oesophageal sphincter opened. C. The efferent gill-duct sphincter opened. Slight twisting of the animal. D. Gill region contraction. The juxta-oesophageal sphincter and the afferent and efferent gill-duct sphincters are seen contracted. The whole gill region is shortened and the gill bodies are contracted with a consequent emptying. There is a noticeable reduction in the size of the gill bodies. E. Maximal contraction of the gill bodies with further emptying. The juxta-oesophageal sphincter is opened again. Time between C and E, $\frac{11}{44}$ sec. F. Schematic drawing of the gill system in *Myxine glutinosa*.

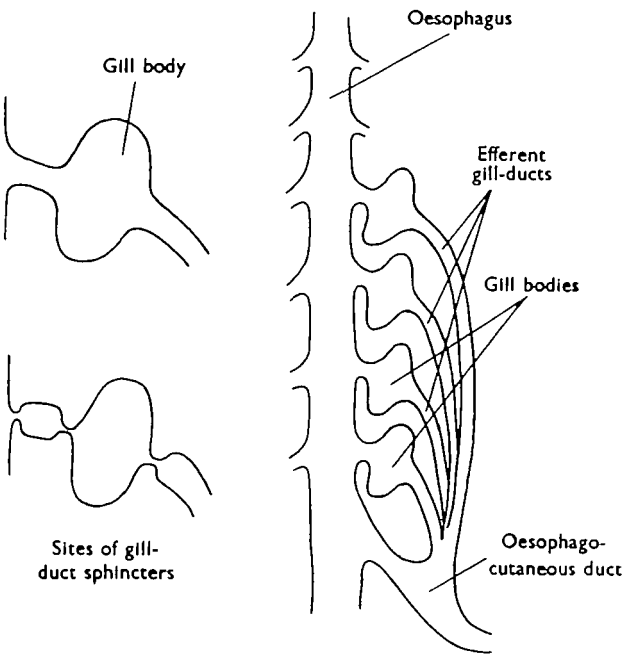

Fig. 5 F

Fig. 6. Filling of the velum region of *Myxine glutinosa*. The contrast medium is injected through a catheter inserted into the nostril. Antero-posterior aspect. A. Good filling of the nasopharyngeal duct and the left part of the velar chamber, early filling of the right part. B. Complete filling of the velar chamber. C, D. Filling of pharynx and oesophagus.

Fig. 7. Sneezing or 'vomiting' in *Myxine glutinosa*. Lateral view. A progressive contraction of the pharynx, velar chamber and buccal cavity drive out the contrast medium through the mouth. A minor amount is expelled through the nostril.

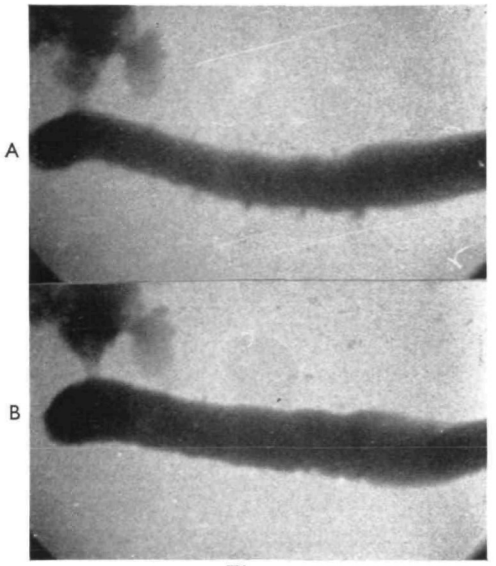

Fig. 1

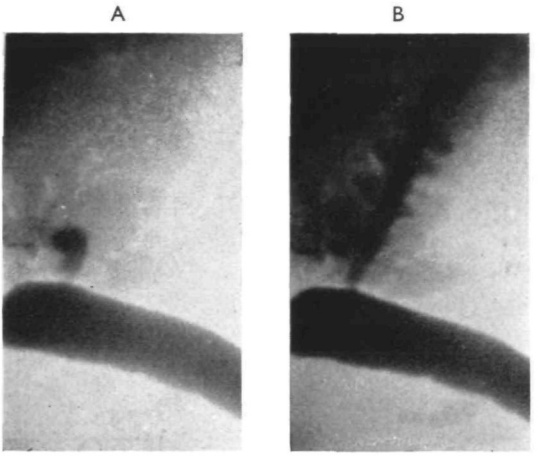

Fig. 2

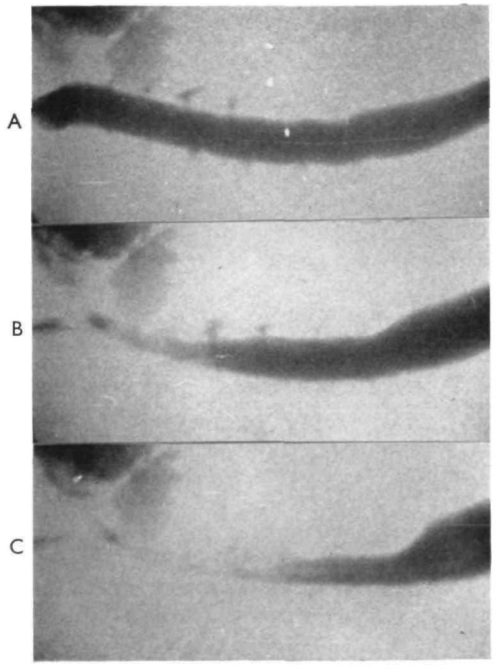

Fig. 3

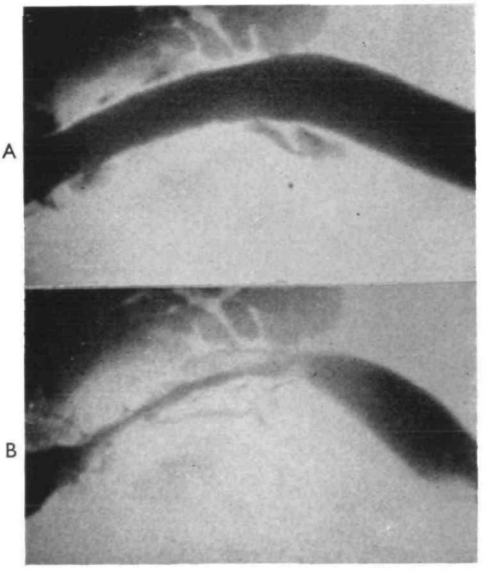

Fig. 4

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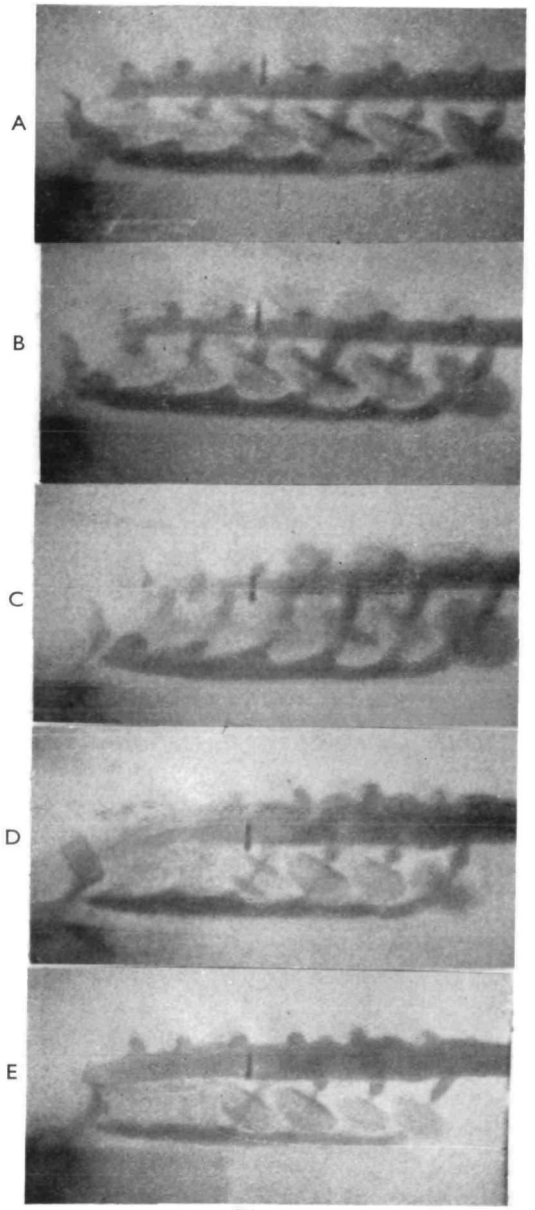

Fig. 5

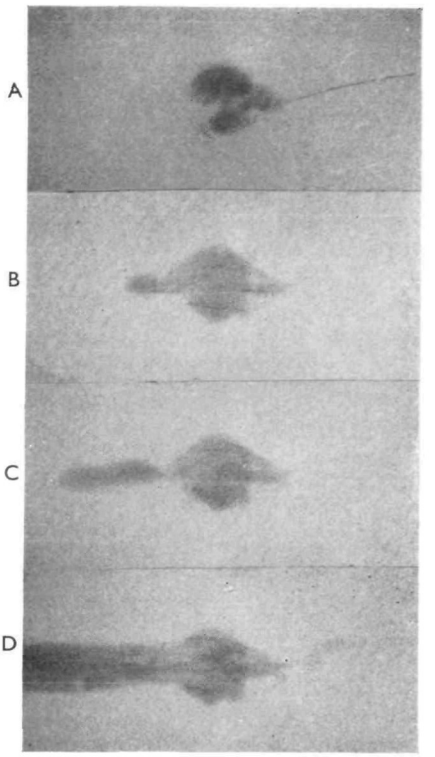

Fig. 6

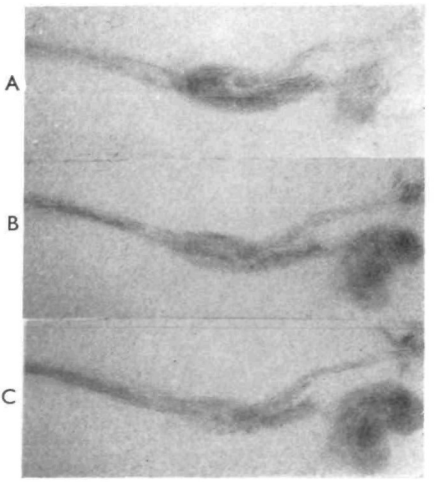

Fig. 7