

ACTIVITY OF THE BODY WALL MUSCULATURE OF THE AFRICAN CLAWED TOAD, *XENOPUS LAEVIS* (DAUDIN), DURING DIVING AND RESPIRATION

by

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With three text-figures and one plate

INTRODUCTION

Mechanisms for regulating the specific weight of the body are well known in fishes. In other aquatic vertebrates the lungs might have a function similar to that of the swimbladder or the lungs in fishes. In anuran tadpoles the lungs may serve as hydrostatic balancers, but this has not been verified experimentally. Active regulation of lung volume, and thus of body volume and specific weight, during diving is unknown in adult anurans, although Parker (1936) expressed the opinion that in male *Trichobatrachus robustus* the lungs, which possess a posterior diverticulum surrounded by specialized musculature, serve as a hydrostatic apparatus.

If there is no active regulation of lung volume during diving, the volume of the lungs will depend on such factors as pulmonary pressure initially reached while breathing at the surface, compliance of lung wall and body wall, and hydrostatic pressure acting on the body wall. If the volume of the other contents of the body cavity remains unchanged, the lung volume may be expected to adapt by contraction or expansion to the depth at which the animal is located. This expectation is based on the assumption that due to the absence of ribs anurans are unable to expand the body wall except by means of the buccal force pump.

If, on the other hand, the musculature of the body wall or of the lungs should act to compress the body cavity or the lungs, pulmonary pressure might be raised to a higher level. This would lead to a decrease in lung volume, an increase in specific weight and facilitation of diving. Relaxation of the muscles would then result in an upward movement of the animal.

In this paper the electromyographic activity is described of some muscles of the body wall which have a direct or indirect effect on the volume and the gas pressure in the lung of the aquatic anuran *Xenopus laevis* (Daudin).

MATERIAL AND TECHNIQUES

The electromyographic apparatus used in this investigation consists of 8 Princeton Applied Research Model 113 differential amplifiers, whose output was recorded on a CEC VR 3360 14-track tape recorder, on a Siemens Oscillomink B ink-writing chart recorder, and on 35 mm film through a Tektronix model 565 oscilloscope with a Cossor Mark II B camera. Frequency bandwidth was 30-30,000 Hz.

Bipolar electromyographic electrodes consisted of insulated 0.05 mm copper wires with hooked bared ends, which were introduced into the muscles by means of hypodermic needles through small skin incisions, which were subsequently sutured or closed with surgical glue. This technique is a modification of that developed by Basmajian & Stecko (1962). Electrode placement was checked by dissection after the experiments.

Analysis of the animals' movements during the experiments was performed by means of a Telford 16 mm cine camera, operated at a speed of 24 frames per second. Films were exposed by synchronized stroboscopic flash units; the flashes were registered by a photo cell and recorded along with the other data (Gans, 1966). The films were analyzed on an Analector variable speed projector.

Pulmonary pressures were recorded by means of a carrier amplifier and a Statham PM 5 gas pressure transducer, which was connected to one lung of the specimen by means of a polyethylene catheter (internal diameter 1.0 mm). The catheter was sutured into the lung wall. In order to prevent clogging of the catheter an end portion of slightly larger diameter was attached to it; this portion, which was perforated, was pushed into the central canal of the lung. Pressure leads were weighted with small strips of lead to compensate for their buoyancy. During the experiments the animals were placed in an aquarium (60 × 30 × 30 cm) at a water temperature of 20° C. All experiments were conducted in a shielded room.

The approximately 20 adult specimens of *Xenopus laevis* used in the experiments were obtained from a commercial source in The Netherlands. Weight of the specimens was 45-177 gr., snout-vent length 68-124 mm.

MORPHOLOGY

The lungs are relatively narrow and elongated, reaching beyond the sacroiliac joint to the posterior end of the body cavity (fig. 1). They have a narrow neck connecting them to the laryngotracheal chamber, and a slightly narrowed posterior tip. Their anterior portions are curved laterally.

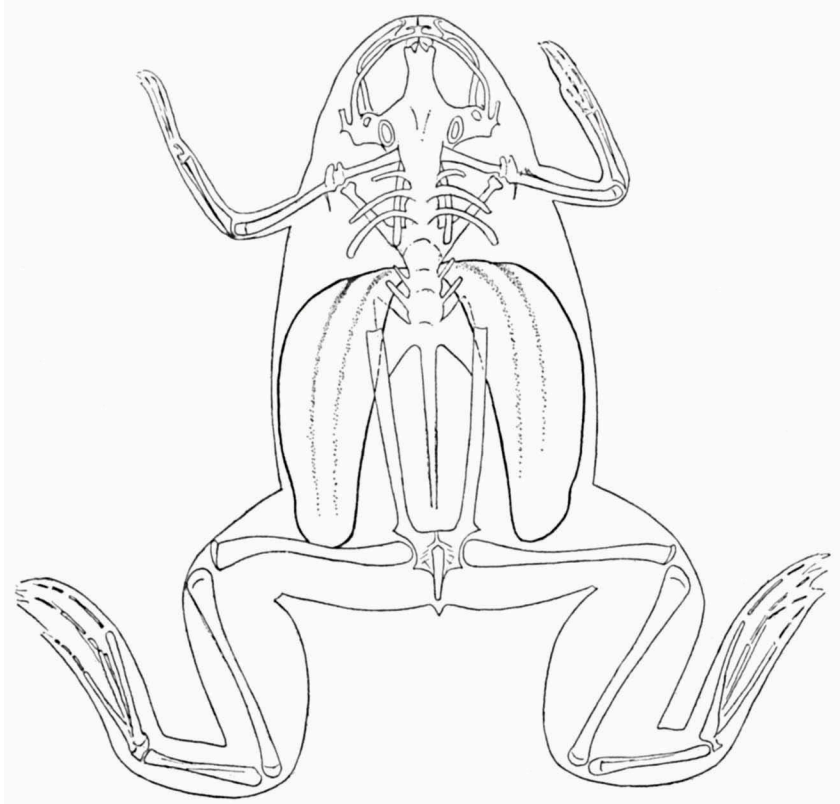


Fig. 1. Sketch of *Xenopus laevis*, redrawn from an X-ray picture, to show the size, shape and position of the lungs. The alveolar structure of the pulmonary walls is sufficiently dense to allow observation of the central canal of the lung.

The lung walls are highly alveolar. The alveoli of the first order have thickened free edges; these edges border a central canal of the lung, which extends almost to the posterior tip. Very small alveoli of the second, third and higher orders form a spongy layer of several millimetres thickness (fig. 2). Total lung volume, estimated from X-ray pictures, ranged from 3.5 to 12 cc.

Only anteriorly the lung is attached to the wall of the body cavity. The attachment continues as a small anterolateral ligament, which connects the lung to the body wall in the axillary region. The ligament consists of a double layer of pleuroperitoneal membrane, which encloses some muscle fibres; the ligament lies in a frontal plane.

The musculature of the body wall has been described by Grobbelaar (1924), and more recently by Ryke (1953), who also studied its ontogenetic

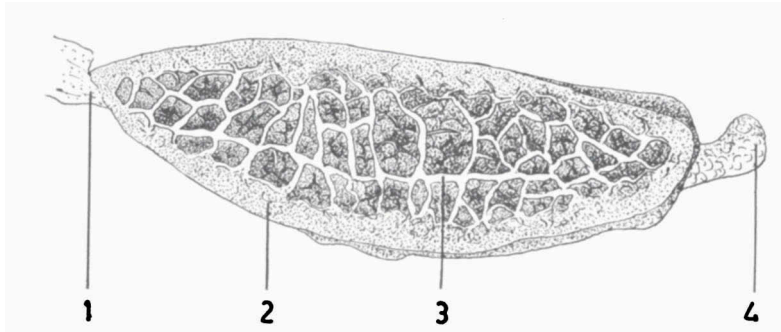


Fig. 2. Longitudinal section of the lung of *Xenopus laevis*. 1, neck of the lung; 2, alveolar layer of the wall; 3, free edge of a primary alveolus; 4, tip of the lung. In the fixed preparation the lung tip has contracted.

development. Beddard (1895) noted that some slips of the body wall musculature are associated with the lungs.

The lateral musculature of the body wall consists of the musculus transversus and the m. obliquus externus, but the latter is covered almost entirely by the m. latissimus dorsi and the portio abdominalis of the m. pectoralis. These muscles are connected to the surface of the m. obliquus externus by loose connective tissue; their lateral borders are connected to each other along the axillary line.

The musculus obliquus externus is a flat, parallel-fibred sheet, which covers the dorsolateral, lateral and ventrolateral body wall. Dorsally it is attached to the fascia dorsalis along a line corresponding with the lateral border of the dorsal trunk musculature and with the iliac wing. Ventrally it is attached to the ventral sheath of the m. rectus abdominis, along an irregularly curved line extending from the coracoid to the pubic region. The muscle fibres course ventrally and slightly posteriorly of the dorsal attachment.

The m. transversus is considerably thinner than the m. obliquus externus. It is closely associated with the internal serous lining of the body cavity. It consists of two portions. The posterior portion is attached to the dorsal fascia along the iliac wing; its fibres run anteroventrally, at an angle of approximately 40° with those of the m. obliquus externus. Anteriorly it is continuous with the posterolateral fibres of the anterior portion, which originates from the lateral surface of the posterior half of the iliac wing. Its fibres diverge anteriorly and laterally, surrounding the anterior and anterodorsal parts of the body cavity. The fibres curve ventrad around the anterior portion of the lung (musculus pulmonum proprius of Beddard, 1895) and attach to the rectus sheath, the pericardium and the oesophagus.

Most of the fibres of the posterior portion of the m. transversus are attached to the rectus sheath ventrally, but a few anterior fibres extend into the anterior pulmonary ligament.

The musculus rectus abdominis is very wide; it covers the entire ventral abdominal wall. It has a strong median raphe and four tendinous intersections. Anteriorly most of its fibres pass along the dorsal surface of the sternum and shoulder girdle, being attached to the hyoid, but a small superficial fibre group is attached to the xiphoid process and coracoids. Posteriorly the muscle fibres converge slightly. Medially they are attached to the anterior edge and the dorsal surface of the epipubic cartilage; a strong lateral fibre group possesses an aponeurosis which connects the muscle with the anterior musculature of the thigh (m. sartorius, m. adductor longus, m. adductor magnus).

PULMONARY PRESSURE AND MUSCLE ACTIVITY

When kept in a tank, the animals usually rest quietly on the bottom, becoming active at intervals of 3-10 minutes to swim to the water surface for respiration. The ascent is usually slow, and pulmonary pressure decreases slowly. Uneven speed of ascent, or short pauses, may be reflected in the pressure curve. When the snout reaches the surface the nostrils open; the animal continues to ascend until the entire head is above the surface, and then drops back slightly. The lung pressure continues to decrease for approximately 0.2 sec. after the nostrils have been opened, then rises quickly, remains stationary for a variable but as a rule very short period, and increases steeply as the animal dives. At the surface, the animal is generally in an almost vertical position, with the hind legs extended. During the initial phase of the dive it maintains this posture, but at a depth of 10-15 cm it protracts the stretched hind legs until the feet are at a level with the head, turns the body until the head points downwards, and quickly swims to the bottom. During the dive the pulmonary pressure usually increases more steeply than it decreased during the ascent. The pressure curve shows a slight overshoot and then returns to the initial level (fig. 3 and pl. 1). Quite often the animal emits an air bubble from the mouth after sitting on the bottom for a few seconds; this is always preceded by a slight rise and fall of the pulmonary pressure. More rarely a second and even a third air bubble may be released at short intervals.

When an animal is kept out of the water, the pulmonary pressure shows regular small variations at the rate of 1-3 per second, and larger decreases and increases at irregular intervals. These larger variations closely resemble the pulmonary pressure curve of *Rana catesbeiana* during ventilation cycles

(De Jongh & Gans, 1969), except for the fact that in *Xenopus* decrease and increase were always found to be equal. The small variations are not observed when the animal is under water; the large variations are similar to the lowest part of the pressure curve in an animal that surfaces after a dive. As in *Rana*, the pulmonary pressure is above atmospheric at all times.

Electromyographic records from some muscles associated with these events are shown in pl. 1. The results are summarized in fig. 3. The electromyograms provide information about the duration of muscle contractions; their amplitudes do not permit comparison of forces exerted by the muscles, except in the case of a series of recordings from a single pair of electrodes, when the relative force exerted by a single muscle at different moments may be estimated from EMG amplitude (Osse, 1969).

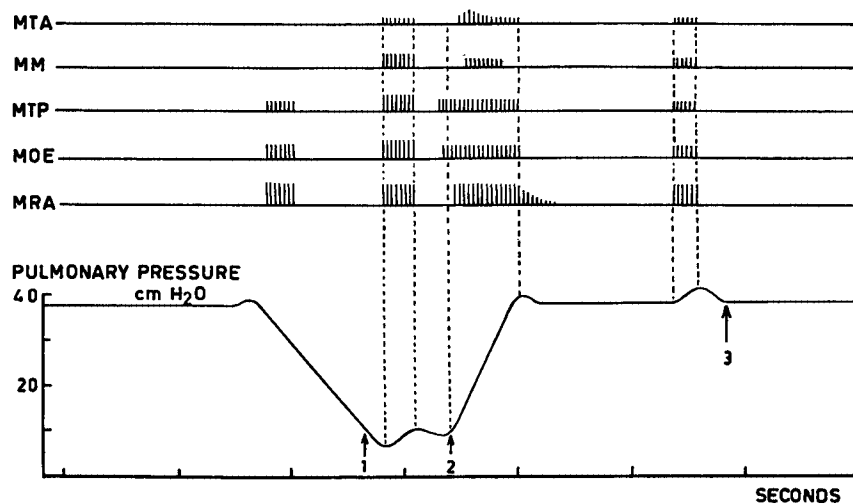


Fig. 3. Diagram showing the correlations between the activity of the body wall muscles during surfacing, respiration and diving, and the pulmonary pressure. For abbreviations see plate 1. The arrows indicate the moment that the nostrils reach the surface (1), the moment at which the animal starts to dive (2), and the moment the air bubble escapes from the mouth (3).

Low amplitude bursts of electrical activity are recorded from the m. rectus abdominis, m. obliquus externus and the posterior portion of the m. transversus during swimming. When animals move to the surface these muscles frequently, but not always, fire during the initial phase of the ascent. All the muscles which were investigated fire synchronously during the first rise of lung pressure after the animal has reached the surface. Thereafter the muscles remain silent until the animal dives. The m. obliquus

externus and the posterior portion of the m. transversus then start to fire simultaneously before the animal moves. The m. rectus abdominis starts to fire slightly later; it shows strong activity coinciding with the protraction of the hind legs. Activity of the muscle fibres of the pulmonary ligament was rarely recorded during the dive; when it occurs the amplitude is extremely low. All muscles fire simultaneously, but at relatively low amplitudes, during the pressure increase before the release of an air bubble.

DISCUSSION

From the pressure records it appears that the respiratory oscillations and inflation cycles described in *Rana catesbeiana* (De Jongh & Gans, 1969) do not occur in *Xenopus*. The only movements observed resemble the ventilation cycles of *Rana*. The small, regular pulmonary pressure variations occurring in animals sitting on land are due to the heartbeat; they are too small to be detected at the high pressure levels in the lungs of diving specimens.

The pulmonary ventilation cycle in *Rana* consists of an expiration followed by an inspiration. The cycle in *Xenopus* is similar, but in contrast with *Rana* the muscles of the body wall are actively involved in expiration, while they also contract during inspiration, repressurizing the pulmonary contents. The latter activity coincides with the descent of the animal in the water. Contraction of the body wall muscles in this phase increases pulmonary pressure to a level above hydrostatic pressure and limits the expansion of the lung which would otherwise result from filling by the buccal force pump. Expiration takes place almost immediately after the animal has surfaced. Exhalation is probably powered not only by body wall muscles, but also by hydrostatic pressure, pulmonary elasticity, and perhaps smooth muscle activity in the lung. With respect to participation of body wall muscles the respiratory mechanism of *Xenopus* differs from that of *Rana* (De Jongh & Gans, 1969), and possibly from those of *Protopterus* (McMahon, 1969) and *Lepisosteus* (Rahn et al., 1971). No respiratory activity of these muscles was observed in *Rana*; no EMG's are available for *Protopterus* and *Lepisosteus*. The respiratory mechanism of *Xenopus* needs further investigation, particularly in regard to structure and function of the buccal force pump.

The periods of activity of the body wall musculature in *Xenopus* correlate with ascent and descent in the water as well as with respiration. The initial activity of the m. rectus abdominis, m. obliquus externus, and the posterior portion of the m. transversus during the ascent to the water surface may counteract a too rapid expansion of the lungs resulting from decreasing

hydrostatic pressure; these muscles may thus control the speed of ascent to the surface.

All the muscles of the body wall are active during the descent, but they do not fire quite synchronously. During the first part of the descent the animals scarcely move their legs, and the movement of the body is a result of the contractions of the m. obliquus externus and the posterior portion of the m. transversus, which cause a decrease of the lung volume and thus increase of the specific weight. The m. rectus abdominis starts to fire a little later, adding to the increase of the lung pressure and simultaneously spreading and protracting the hind legs. In this phase the anterior portion of the m. transversus becomes active; this activity further accelerates the increase of the pulmonary pressure, but the correlation between the firing period of the muscle and the movements of the animal suggests that it also shifts the gas volume contained in the lungs backward, thus moving the centre of gravity in an anterior direction and causing the body to turn. The observation that the characteristic dorsolateral bulge of the body wall, which indicates the position of the lungs, increases in size at this time is in favour of this hypothesis.

The muscle fibres of the pulmonary ligament, belonging to the posterior portion of the m. transversus, seem to have a predominantly respiratory function.

After the dive, when the animal is resting on the bottom of the tank, muscle activity ceases. As a result the lungs will expand, and the muscles contract again, which causes the release of an air bubble from the lung via the buccal cavity and the mouth. The function of this pattern of activity seems to be the maintenance of pulmonary pressure and volume at a level determined by the requirements of specific weight and hydrostatic pressure, but which permits muscular relaxation.

In summary it may be stated that pressure exerted by the musculature of the body wall upon the contents of the body cavity in general and upon the lungs particularly influences the specific weight. It is a contributing factor in the control of ascent and it is the major factor which causes the increase of specific weight during the initial phase of the descent. The mechanism also adjusts the specific weight of an animal resting at a given water depth to a level determined by the hydrostatic forces acting on the body, so that no further expenditure of muscular energy is needed for the animal to maintain itself at that depth.

In *Xenopus* the lungs are relatively small and highly alveolar, and their volume changes seem to be kept within fairly narrow limits. The hydrostatic mechanism thus seems to be adapted to the requirements of animals living

in shallow water, since larger variations in pulmonary volume would be needed for diving to greater depths.

The evolutionary development of the hydrostatic apparatus of *Xenopus* apparently has not led to major structural modifications in comparison with other Salientia. Similar mechanisms might be present in other aquatic species, and perhaps also in Dipnoi.

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Plate 1

Electromyograms and pulmonary pressure records showing surfacing and diving sequences in *Xenopus laevis*. In experiment A a period of swimming along the bottom occurred between the dive and the moment of release of an air bubble (arrow). In experiment B no air was released. FS, film synchronization (24 frames/second); MTA, anterior portion of the m. transversus; MM, muscle fibres of the pulmonary ligament (posterior portion of m. transversus); MOE, m. obliquus externus; MTP, posterior portion of m. transversus; MRA, m. rectus abdominis. Both records were made at the same chart speed (50 mm/sec.).

