THE RESPIRATORY MOVEMENTS

OF THE LAMPREY

(Lampetra fluviatilis)

by

T.D.M. Roberts, B.Sc.(Lond.)

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Fig. 1. Parker's figure of the gill basket of Petromyzon marinus.
I INTRODUCTION.

The respiratory pumping movements of fishes are brought about by sets of muscles working on a system of articulated, stiff rods. The insertions of the muscles are so disposed that both increases and decreases in the volume of the respiratory cavity can be produced by muscular contraction.

In a lamprey there is no such system of jointed rods but only a continuous cartilaginous basket of which the classical diagram is that of Parker (1884) (fig.1.). The branchial basket is associated with a thin layer of muscle to which an expiratory function could readily be assigned. The present work is an attempt to answer the question as to how the inspiratory movement to refill the gill sacs is brought about. Dawson (1905) described the branchial musculature of the lamprey and concluded that "when the muscles relax, the elasticity of the branchial basket serves to elongate the gill sacs and thus to fill them again with water". Her descriptions of the muscles cannot, however, be reconciled with those of Tretjakoff (1926). In particular, her figure of the muscles of the gill pouch (fig.2) is difficult to interpret. Tretjakoff (1926) describes certain muscles but advances no theory
FIG. 8. Gill sac with its muscular pouch. The pouch is cut near its lateral end and reflected from the sac. 
a, gill sac; b, gill pouch; c, ental muscle; d, lines of the gill lamellæ showing through the gill sac; e, internal compressor muscle of the gill sac; f, deep compressor muscle of the gill pouch; g, external compressor of the gill sac.

Fig. 2. Dawson's figure of a gill sac.
Fig. 3. Tretjakoff's figure shewing the branchial musculature (bm) of the lamprey.
to account for the inspiratory movement. His representation of the branchial arches (fig.3) differs from Parker's (fig.1) sufficiently to justify a re-examination of the anatomy. Access to Balabai's 1935 paper was only obtained after the completion of the anatomical work to be reported here. His paper is in Ukrainian and it has not been possible to obtain a translation. From his figure it appears likely that his description of the gill muscles would not differ materially from the account contained in the present work. An anatomical study of the branchial region of the Lampetra fluviatilis has been made by dissection of fresh and formalin-fixed material under the binocular microscope. This has brought to light a new set of muscles and some hitherto unrecognised features of the branchial arches. (Both figured by Balabai).

Experiments were undertaken in which the respiratory movements were to be recorded on a kymograph at different intensities of the respiratory stimuli - temperature, dissolved CO₂, and O₂ lack. These experiments have for technical reasons proved rather unfruitful. The experiments were planned on the assumption that an inspiratory movement by muscular contraction can be distinguished from a movement caused
by elastic recoil, by studying the kymograph traces obtained at different respiratory rates. It was held that a movement by muscular contraction should be subject to the respiratory stimuli, and vary in a matter analogous to the variations produced in the expiratory movement and in the rate of respiration itself. An elastic recoil movement on the other hand should only be affected in so far as the elastic properties of the cartilage are altered by changing the temperature.

The response was to be assessed by measuring from the kymograph trace the time occupied by the inspiratory phase of the respiratory movement. It was expected that alterations in the amplitude attained by the expiratory movement would only have a small effect on the time of inspiration compared with the errors of measurement.

Special apparatus and technique were evolved for this experimental study but it was found that the kymographic method is not sufficiently sensitive to give the desired result. The movements of the body wall of the lamprey are too fast and too feeble for reliable recording.

The general question of respiratory control arose and was studied by counting the respiratory movements at various intensities of respiratory stimuli. A full set
of counts could, however, not be obtained owing to changes in the behaviour of the animals as the breeding season approached. A note on the seasonal behaviour of the lamprey is placed in an appendix.

The pressure changes within the gill sac of a lamprey have been recorded to show whether there is an active inspiratory phase.

The parts played by the different muscle groups have been worked out from extirpation and stimulation experiments.

The times at which the contractions occur in relation to the cycle of respiratory movements have been established from an oscillographic study of the action potentials of the muscles.
Fig. 4. A left branchial arch, shewing the re-entrant curves.
II MORPHOLOGY.

1. The gill bars.

The branchial arches forming the main bars of the branchial basket exhibit a marked regularity so that a description of one arch serves for all except the first and last. These have special modifications whose nature is sufficiently indicated by Parker's diagram (fig.1) and which will not be further considered.

Each gill bar arises from the parachordal sheath and curves round to meet its fellow of the opposite side in the ventral commissure. This ventral commissure connects the mid-ventral portions of all the gill bars and runs posteriorly into the cartilaginous pericardium.

Within the connective tissue of the parachordal sheath, each gill bar has processes extending forward and backwards. Some of these coalesce to form incomplete, paired, dorsal commissures which are not easily disentangled from the parachordal sheath. The course of each gill bar has been found to include three re-entrant curves (fig.4) so that the bar is thrown into six almost right-angled bends.

The lateral re-entrant curve at the level of the
Fig. 5. Two branchial arches of the left side to shew the positions of the processes and the relations to the lateral commissures.

branchiopore is indicated in Parker's diagram and described by Tretjakoff, but the dorsal and ventral re-entrant curves have not been previously described.

All the curves of the gill bar are approximately coplanar and the plane of a particular bar is inclined to that of its fellow of the opposite side at an angle of approximately $120^\circ$ to form a forward-facing wedge. (Parker's diagram shows the planes of the gill bars as directed forwards instead of backwards from the Ventral commissure.)

In addition to the dorsal and ventral commissures the gill bars are linked by two lateral commissures on each side, (fig.5). These run just above and just below the branchiopores and are accordingly named the "epitrematic" and "hypotrematic" commissures respectively. They run in a series of S-bends as mirror images of one another.

In front of the 1st branchiopore the epitrematic commissure turns ventrally and fuses with the hypotrematic commissure before meeting the most anterior gill bar, (fig.1, etc.).

II. 2 Nomenclature of the processes.

Each gill bar has six rather flattened processes which can be seen in the diagram (fig.5).
Fig. 6. The branchial constrictor muscle.

The terminology adopted is regarded as self-explanatory. Taking the processes of a single gill bar in order starting from the parachordal sheath, the suggested names of the processes are:

- dorso-caudal
- 1st dorso-rostral
- 2nd dorso-rostral
- (here are the lateral commissures)
- 2nd ventro-rostral
- 1st ventro-rostral
- ventro-caudal
- (here is the ventral commissure)

The epitrematic commissure has a latero-dorsal process, just in front of the gill bar, which together with the 2nd dorso-rostral process encloses an almost circular space.

A latero-ventral process of the hypotrematic commissure similarly encloses an almost circular space with the 2nd ventro-rostral process. The symmetry of the arrangement is most striking.

II. 3. The Branchial Constrictor Muscle.

The branchial constrictor is by far the most conspicuous muscle of the branchial apparatus. Its fibres run an almost semi-circular course from the parachordal sheath to the ventral commissure in planes at right angles to the long axis of the gill sac (fig.6). The fibres are grouped at the level of the branchiopores into a thick mass lying against the re-entrant curves of the next
Fig. 7. The branchial constrictor muscle seen from above the level of the branchiopores.

Int. Br. S., interbranchial septum; Br.Con., branchial constrictor muscle.
Fig. 8. The gill sac and associated structures.

Aff. Br., afferent branchial artery; Ao., ventral aorta; Br. Constr., cut end of branchial constrictor; Eff. Br., efferent branchial artery; Ext. Comp., external compressor muscle of the gill sac; G. Lam., gill lamellae seen through the wall of the gill sac; Int. Com., internal compressor muscle of the gill sac.
anterior gill bar. The mass thins out somewhat under
the lateral commissures to form a sheet thicker at its
anterior edge. Towards the dorsal and ventral insertions
the sheet spreads out a little, fanwise, and is here and
there slit to form ribbons. The most posterior ribbons
have their insertions in the 1st dorso-rostral and 1st ventro-
rostral processes instead of in the parachordal sheath and
ventral commissure respectively, (figs 6 & 7). The bran-
chial constrictor is not inserted in the lateral commissures,
nor has it any other insertions in the gill bars. The
inner surface of the branchial constrictor forms with the
inter-branchial septum the anterior and lateral walls of the
peribranchial sinus. There is no attachment here to the
gill sac.

II. 4 The Gill Sac.

Figure 8 indicates the position as seen after removal
of the branchial constrictor and inter-branchial septum.
The wall of the gill sac is thin and the roots of the gill
lamellae can be clearly seen through it.

The gill sac is supported anteriorly by the median
septum through which the internal branchiopore communicates
with the suboesophageal tube.

The anterior wall of the gill sac is attached to two
very delicate fans of muscle fibres which arise, one from the parachordal sheath and one from the ventral commissures, in the corners between the interbranchial and median septa, (fig. 8). These fans correspond in their relation to the gill sac to the "internal compressor muscle of the gill sac" of Dawson (fig. 2,e).

Another series of very delicate muscle fibres forms a band lying in the wall of the gill sac from the 2nd dorso-rostral process to the 2nd ventro-rostral process. This band of muscle corresponds approximately to Dawson's "external compressor of the gill sac" (fig. 2,g). Dawson's figure does not show either of these two last-mentioned muscle groups to be inserted in the branchial basket.

The gill sac is attached to the following four processes of the basket (fig. 8):

- the 2nd dorso-rostral process
- the latero-dorsal process
- the latero-ventral process
- the 2nd ventro-rostral process.

At the external branchiopore the gill sac is continuous with the skin of the general body surface to form a canal. This canal is surrounded by a small ring of cartilage with associated muscles whose function has not been considered in the present work. The muscles are not inserted in the
Fig. 9. The diagonal muscles.

branchial basket and the cartilage ring has no connection with the other branchial musculature. The posterior face of the gill sac is attached to the postero-median corners of the peribranchial sinus by the afferent branchial arteries serving the posterior gill lamellae.

II. 5 The diagonal muscles.

If the gill sac is removed to reveal the anterior face of the interbranchial septum, (fig. 9) two small muscles are seen which have not been previously described. The fibres of the dorsal diagonal muscle run a practically straight course in the interbranchial septum from their origins in the parachordal sheath to their insertions in the inner anterior face of the gill bar just under the epitrematic commissure.

The Ventral diagonal muscle runs from the Ventral commissure to the gill bar, reaching it just under the hypotrematic commissure.

These muscles thus span the dorsal and ventral re-entrant curves respectively and are attached to the ends of the lateral re-entrant curve.

II. 6 Muscles in the Septa.

The interbranchial septum contains some additional isolated muscle fibres running from the parachordal
sheath to the ventral commissure in arcs of various curvatures.

In the median septum a band of muscle fibres runs downwards and forwards along a diagonal of the rectangle formed by the inner edges of the two successive interbranchial septa. These fibres also run from the parachordal sheath to the ventral commissures.

II. 7 The Longitudinal Muscles of the body wall.

The longitudinal somatic musculature is not inserted in the branchial basket except near the mid-ventral line where there are insertions all along the ventral commissures. The fibres so inserted all run posteriorly from their insertions.

In some specimens fibres have been found inserted in a similar manner in the ventro-caudal processes.

II. 8 The problem restated in the light of the morphological findings.

If the inspiratory movement is produced by muscular contraction, the most likely muscles to be concerned are the diagonal muscles, their action on the cartilages of the basket not being self-evident. It is possible that the insertions might be so placed in relation to the re-entrant curves as to produce a movement of inspiratory type. If in fact they have such a function, then their
contraction should occur later in the respiratory cycle than the contraction of the branchial contrictor. The experiments using operative techniques were directed towards a solution of this question.
Fig. 10. Strait jacket shewing a lamprey in position.
III. APPARATUS AND METHODS.

1. Strait Jacket.

For the kymographic recording the animal was held in a simple strait jacket (fig.10). This consists of a glass tube in which the lamprey lies, together with a small glass bulb, about 2 cm. in diameter, arranged near one end of the tube in such a position that the lamprey can attach itself to the bulb by its oral sucker. The bulb and tube are held in a wooden jig. Once the lamprey is inside the tube and in contact with the sides for the greater part of its length, it usually comes to rest and can be persuaded to suck on to the glass bulb. At this stage the bulb is held near to the end of the tube. After the animal has sucked on, the bulb is moved away again, drawing the lamprey out until its gill apertures are clear of the end of the tube. Pieces of shaped glass rod are arranged as rests to fit round the head just in front of the eyes, so that if the sucker becomes detached it remains near the bulb ready for re-attachment. The whole assembly is then fixed in a retort clamp, with the lamprey fully immersed in water in a shallow trough.

In many cases the lamprey will remain in position for an hour or more.
Fig. 11. Experimental chamber with constant-flow circuit.
III. 2 Experimental Chamber.

The lampreys are placed for study in a trough, 16ins. long, 4ins. wide and 3ins. deep, which conveniently accommodates the strait jacket. Water circulation in the trough is maintained by a special piece of apparatus so that the gas content of the water, the temperature, and the rate of flow can be controlled.

The principle of the apparatus is as follows, (fig.11):- Water from a main reservoir flows through a temperature-control chamber to a first constant-level chamber A; from here it is siphoned into the experimental trough, another siphon providing an outlet to a second constant-level chamber, B. T-pieces on these siphon tubes provide convenient points at which to draw water samples for analysis. The overflows from the two constant level chambers drain into a lower reservoir from which the water is pumped through a gas exchanger, back to the main reservoir.

The main reservoir is a circular glass dish about 30 cms. diameter, so that the addition of a litre or so of water makes only 2 cms. difference to the water level. The rate of flow to the first constant-level chamber is controlled by a screw clip so that there is always some overflow.
The level in the trough always remains between the level of the overflow in the first constant-level chamber, A, and the opening in B of the outflow from the trough. The rate of flow through the trough depends on the difference between these two levels so that the level in the trough and the rate of flow are independently adjustable by moving the constant-level chambers bodily upwards or downwards.

The rate of flow is measured by collecting for a known time the water delivered by the special outflow of the second constant-level chamber. When this outflow is open the water-level in this chamber does not reach the overflow tube so that all the water passing through the trough leaves by the special outflow. A rate of flow of 100cc. per minute was usually employed.

Intermittent pumping from the lower reservoir was arranged by coupling the pump-motor switch to a float, as indicated in the diagram, (fig.11). The rapid delivery of the pump is accommodated by an emergency reservoir above the gas exchanger.

In the gas exchanger the water enters the side arm of a T-joint and flows under gravity, down the vertical arm. The rate of flow under gravity is arranged to be greater than the inflow in the side arm so that bubbles
The right-hand tube remains full of water even when the tank is drained. Water begins to leave by the right-hand tube as soon as the water-level rises above the inner end of this tube. If the inner end of the left-hand tube is covered the air pressure in this tube falls steadily by bubbles becoming trapped and removed in the water-stream below the T-joint. Water is thus drawn into the left-hand tube to fill it. When it is full the total rate of outflow by the siphon is greatly increased as it now depends on the height of the inner water-level above the outer end of the siphon instead of on the height above the T-joint.
are drawn into the water stream. The internal contour of the T-joint was manipulated in the flame until the volume of gas trapped was about equal to the volume of water passing. The "down tube" is about 1 metre long and dips into the water in the main reservoir, providing an efficient gas exchange. The upper "open" limb of the T-joint is brought down to bench level so that connection can be made at will to a gas reservoir. Contamination of the gas by the atmosphere, after all the water has run through from the emergency reservoir, is prevented by the small Y-trap shown.

Where it was suitable to use tap-water for the experiments it was supplied directly to the side arm of the gas exchanger. The overflows were directed into a sink and an approximately constant level in the main reservoir was maintained by the use of an overflow siphon of the type illustrated in figure 12.

The gas reservoir is a large rubber meteorological balloon which is usually only partly filled so that the walls of the balloon do not exert any pressure on the contained gas mixture.

On a change of gas mixture the routine followed is as follows: The temperature chamber is tipped and its contents, together with most of the water from the main reservoir, are poured into a large beaker. From here
the water is poured in portions into the lower reservoir from which the pump transfers it to the gas exchanger. The main reservoir now begins to receive water aerated with the new gas mixture. The first batch is poured off as before and transferred to the lower reservoir. Meanwhile most of the water from the experimental trough has also drained down to the lower reservoir. The temperature chamber is now replaced on its stand and the system now returns to equilibrium in a few minutes, all the water being now aerated with the new gas mixture.

III. 3. Sampling device.

The gas content of the water passing through the experimental trough is estimated from samples drawn from the inflow and outflow siphon tubes. A simple sampling device is used, consisting of a square-ended specimen tube, \( \frac{3}{4} \) in. diameter and 3 ins. long, supported in a wooden block. A side tube, sealed-in about \( \frac{1}{2} \) in. from the bottom of the tube, is connected to one of the appropriate T-joints in the constant-flow circuit. A spring clip regulates the flow into the specimen tube.

The use of the sampling device may be illustrated by the technique of drawing samples with a syringe.

The spring clip is opened to fill the specimen tube. The first two fillings are rejected and the sample is taken as the tube fills for the third time. The opening
Fig. 13. Gas analysis apparatus for dissolved gases
of the syringe is held opposite to the side tube of the sampling device and the spring clip is manipulated so that the water-level in the specimen tube rises continually as the syringe is being filled. Turbulent contact of the water actually sampled with the atmosphere is thus reduced to a minimum and it is believed that very little, if any, change in the gas content of the water occurs during the process of sampling.

III. 4 Gas analysis Apparatus for Dissolved Gases.

A new apparatus has been designed and constructed (fig.13) for the estimation of the concentration of dissolved carbon dioxide. In addition, the oxygen content of the water can be readily determined in the apparatus, using the same water sample for both determinations.

The water sample is subjected for a few seconds to a Torricellian vacuum and the gas which is evolved at this stage is collected and measured manometrically at constant volume. The carbon dioxide and oxygen are absorbed in turn by caustic soda and alkaline pyrogallol respectively, the amounts of each gas being determined by difference.

The design embodies features both of the Volumetric and of the manometric blood gas analysis apparatuses of
An evacuation chamber of about 50cc. capacity is fitted with taps above and below. The lower tap E is a 2-way tap connecting to a by-pass tube and a chamber of about 25cc. which acts as a trap as in the Volumetric apparatus of Van Slyke (1917). The trap and by-pass tube are connected together by a T-joint and communicate with a levelling reservoir R₂ through a length of pressure tubing.

From another T-joint in this tube, close to the evacuation unit, runs a short vertical tube closed at the top by a Tap F. This tube has another T-joint whose side-arm is directed slightly downward to communicate through a tap G with the bottom of a long vertical tube closed at the top by a tap H. This long vertical tube is fitted with a metre-scale in millimetres and acts as a pressure gauge as in the manometric Van Slyke apparatus.

Oscillations of the mercury in the manometer tube are avoided by closing the tap G to isolate the manometer from the rest of the apparatus except when a reading is to be taken. Water and gas bubbles carried in the mercury stream are prevented from entering the manometer as they collect below the tap F in the short vertical tube and can
be ejected as desired.

The tap D above the evacuation chamber is simple and leads to the measuring chamber which is closed at the top by a 3-way tap C. The measuring chamber bears two graduation marks corresponding to volumes of 0.5 cc and 5.0 cc measured from the tap C. The chamber has a "wasp waist" at the region of the 0.5 cc mark to increase the accuracy of measurement.

Above the three-way tap C is a funnel through which reagents may be introduced. The third tube of this 3-way tap is bent downwards to connect with a sampling unit consisting of two more 3-way taps and a bulb connected in series. The lower end of the bulb is continued into a short length of tubing bearing a graduation mark and to this tube is fitted a length of pressure-tubing communicating with a levelling reservoir R1.

The side tube of the first 3-way tap A of the sampling unit is used as an inlet for the water sample. A length of narrow-bore rubber tubing attached to this side-tube is dipped into the sampling device when samples are to be taken. The volume taken is adjusted by rejecting excess through the side-arm of the second 3-way tap B until the mercury piston reaches the graduation mark. A funnel arranged below the opening of this side-arm conveys the rejected fluid to a beaker.
placed behind the apparatus.

The tubes between A and B and between B and C are of capillary bore so that a water column can be positively driven along them by a mercury column. The sampling unit and evacuation unit are fused together, the whole being made of Pyrex glass and supported on a wooden frame. The frame also supports the manometer tube and has adjustable rests for the levelling reservoirs.

4.1. Calibration. - The positions of the graduation marks were fixed during the construction of the apparatus, before the measuring bulbs were fused to the tap D, yet after all the other necessary joints had been made.

The sampling unit was calibrated by delivering samples of 5% sulphuric acid through the measuring bulbs into a beaker, using a provisional mark and the procedure decided upon for taking water samples. The acid was washed through the measuring bulbs with distilled water from the funnel above C and titrated with alkali to brom-thymol blue. The position of the mark was adjusted until the titre for the acid delivered agreed with that for the delivery of a 10cc pipette.

The measuring bulbs were calibrated in an inverted position by introducing appropriate weighed amounts of
mercury. This procedure obviates corrections for
the meniscus as, in use, readings are taken with a water
surface at the mark.

4. 2. Use of the Apparatus.

After cleaning, the
apparatus is completely filled with mercury, the taps E,
F and G being secured with string before attempting to
expel air through H. To remove the small gas bubbles
which sometimes get trapped against the glass by the rising
mercury, a blank evacuation is usually performed and any gas
that collects is rejected through C.

The inlet tube attached to A is placed in position
in the sampling device, B is turned to ♣ and A to ♦
the
R₁ is lowered and when the mercury level has fallen to A this
is turned to ♣ . R₁ is now lowered further to draw
in water from the sampling device, the spring clip on which
is opened so that as water is drawn into the sampling bulb
it is continually replaced from the experimental chamber.

When the sampling bulb is full the spring clip is
closed, A is turned to ♣ and R₁ is raised to reject most
of this first batch of water through the side arm of B.
A is now turned to ♣ and R₁ is lowered again to draw in
another batch of water, which is rejected as before. After
thus rinsing two or three times, water is drawn in to fill
the sampling bulb well below the graduation. In manipulating the sampling device, the water-level is allowed to fluctuate as little as possible. The sample ultimately drawn is thus uncontaminated by gas exchange with the atmosphere.

A is now turned to $\odot$ B to $\odot$ and then A to $\odot$. $R_1$ is slowly raised and B cautiously turned towards $\odot$ to allow water to escape slowly. When the mercury meniscus comes to the mark, B is turned to $\odot$ and C to $\odot$. $R_1$ can now be slowly raised to pass the water sample over into the evacuation unit. A drop of mercury is allowed to follow the water sample through C, C is then turned to $\odot$, B to $\odot$ and $R_1$ is set aside on its rest.

$R_2$ is now lowered with $\odot$ pen and E at $\odot$ until the whole of the water sample has drained into the trap. E is turned to $\odot$ and $R_2$ is raised so that the mercury drives any gases which have been evolved up into the measuring chamber. When the mercury reaches D, this is closed and $R_2$ is lowered again to leave a Torricellian vacuum in the evacuation chamber. About 12 cm of mercury are left in this chamber, E is turned to $\odot$ and $R_2$ is raised slightly during the turning of the tap. The water sample spurts through the mercury layer into the evacuation chamber exposing a large surface to the vacuum. $R_2$ is now
lowered to set the water aside again in the trap; E is turned to and the evolved gas is passed into the measuring chamber. The time of exposure of the water sample to the vacuum is kept as short as possible.

The level is adjusted to the 0.5cc mark and G is opened. When the mercury has stopped oscillating G is closed again, R2 is replaced on its rest and the reading of the manometer is taken at leisure.

If the 0.5cc mark cannot readily be reached owing to the volume of gas present, the 5.0 mark is used with the appropriate modification to the calculations.

4.3 Measurement of the Carbon Dioxide

About 5cc of 5% NaOH is placed in the funnel above C. C is cautiously turned towards to admit about 2cc of the alkali and is then turned back to . About 1/2cc of mercury is placed in the funnel and C is manipulated so that mercury fills the core of the tap. A few droplets of mercury allowed to flow into the measuring chamber serve to detach the small quantity of Alkali which sometimes remains between the tap and the 0.5cc bulb.

R2 is now lowered and the alkali is drained into
the trap through E. The mercury level is allowed
to rise through the by-pass and when it reaches the mark
G is opened and closed, allowing time for the mercury levels
to come to rest at the mark. The new reading of the
manometer is taken.

The difference between the two pressure readings
is the pressure exerted by the CO₂ confined in 0.5 cc
(=a cms. Hg.) The volume which would be occupied by
this amount of CO₂ if it were measured at Standard Pressure
is

\[
\frac{0.5 \times a}{76} = a \times 0.00658 \text{ cc}
\]

so that
each cm. of pressure obtained from CO₂ at the 0.5 mark
corresponds to an evolution of 0.658 cc per litre from
the water sample.

4.4 Measurement of the Oxygen.

-A small quantity
of pyrogallol powder is added to the alkali in the funnel.
The mixture is gently stirred with a glass rod to allow
trapped air bubbles to rise, and about 2 cc of the alkaline
pyrogallol is admitted to the apparatus through C, follow-
owed by a few drops of mercury and leaving as before a little
mercury in the funnel to help to seal the tap.

After allowing the pyrogallol to run down the walls
of the apparatus, it is drained into the trap and a fresh
Fig. 14. Arrangement of aspirators for transferring a fixed volume of gas at atmospheric pressure.
pressure reading is taken as before.

The same conversion factor is applied to the pressure difference obtained to give the amount of $O_2$ evolved in cc per litre.

4. 5. Washing.

-The water and used reagents are rejected after the completion of an analysis through the side-arm of tap B, C being turned to $\bigcirc$ and B to $\bigcirc$. The funnel is filled with tap water, C is turned to $\bigcirc$ and R$_2$ is lowered so that water is drawn into the apparatus. When the water has passed down into the trap, R$_2$ is raised, C turned to $\bigcirc$ and the water is ejected.

Washing is repeated once with 1% nitric acid and then several times with distilled water until the washings are found to be neutral. The apparatus is then filled with mercury, gas bubbles are removed by a blank evacuation and all is ready for the next analysis.

III 5. Preparation of Gas Mixtures.

Gas mixtures for use in the experimental chamber were stored in a large rubber meteorological balloon. Two large aspirators of about 15 litres capacity, connected together by a wide-bore tube arranged as in the diagram (fig.14), are used as a means of transferring known amounts of gases to the balloon.
Sufficient water is introduced to fill one aspirator and the siphon. A stand is arranged so that one of the aspirators can be raised until the bottom of its siphon tube is just below the level of the bung of the other aspirator.

In this position water passes into the lower aspirator to fill it, yet the flow stops before the upper limb of the siphon is uncovered and before any water can leave the lower aspirator by its outlet tube. On exchanging the positions of the aspirators the water again flows into the lower. This apparatus can be used either to withdraw gas from the balloon - by connecting the balloon to the upper aspirator, or to introduce a fixed volume of gas - by connecting to the lower aspirator.

The balloon is first emptied, the connecting tube is closed with a spring clip and is detached from the aspirator. The lower (full) aspirator is connected to a gas cylinder, the positions of the aspirators are reversed and gas is cautiously admitted from the cylinder until the water-levels show a slight excess of pressure. The cylinder connection is now transferred to the lower aspirator while the upper aspirator is connected to the balloon. In making the latter connection a little gas
is allowed to escape so that the trapped gas comes to atmospheric pressure. The aspirator positions are now reversed and one volume of gas at atmospheric pressure passes to the balloon while another volume of gas is being drawn from the gas cylinder. By using cylinders of Oxygen, Nitrogen and Carbon Dioxide and counting the number of aspirator volumes transferred, gas mixtures in the desired proportions were obtained.

As this process is rather slow, a gas meter graduated in hundredths of a cubic foot was used for several experiments. The meter was kindly lent by the Glasgow Corporation Gas Department and was one of their test meters from which the coal gas had been carefully removed.

In using the meter the contained air was first swept out from the meter by a stream of Nitrogen, then some of the required Nitrogen was run into the balloon through the meter. This was followed by the desired amounts of Oxygen and Carbon Dioxide, finishing always with the balance of the Nitrogen. This ensured that the whole of the Oxygen and Carbon Dioxide measured was actually passed into the balloon.


Difficulty was experienced in the early stages in making a suitable connection to the body wall of a lamprey so that a tracing could be obtained. The lamprey's skin
is very resistant to piercing either with a needle or with a scalpel and the pressure necessary to pierce the skin is often more than enough to drive the instrument deeply into the underlying tissues.

The first tracings were obtained by inserting a small entomological pin through the branchiopore. The pin was bent so that its head pressed outward against the inner wall of the gill sac. A thread was lead from this pin to a light lever with a style writing on a smoked drum.

A cutting needle was later adopted and is used to pass a fine silk thread into the skin of a suitably narcotised lamprey to make a stitch about 1\frac{1}{2} mm. long. The ends are tied to form a loop \frac{1}{2} cm. long and this loop is left in position in the skin.

The lamprey is placed in the strait jacket which is clamped in position in the experimental chamber. Connection to the recording lever by a silk thread is made as desired, by a fine wire S-hook, 2mm. long.

The arrangement of threads and levers was adjusted until an excursion of 8mm. could be obtained.

The lever used is a straw slid over a short aluminium strip fixed to a vulcanite block mounted on a brass spindle between steel points. The straw has
Fig. 15. Diagram of Dr. Gregory's Membrane-manometer.
panels cut away to lighten it so that a thread attached 1cm. from the fulcrum can support the lever in a horizontal position by exerting a force of 440mg. A plasticine weight is added, also 1cm. from the fulcrum, to increase the load to approximately 1gm. This doubles the restoring force without very much increasing the moment of inertia of the lever.

By applying small jerks to the thread by hand it was found that the lever could produce a trace in which an excursion of 3cms. and the return together occupied 0.07 secs.

III 7. Membrane manometer.

The pressure changes occurring within the gill sac of a lamprey during the active respiration were studied by means of a small membrane manometer kindly lent by Dr. R.A. Gregory of the Physiology Dept., University of Liverpool.

The instrument (fig.15) has a thin rubber membrane, 3mm. in diameter, stretched over a depression in a brass block. The cavity below the membrane is connected to a hypodermic needle by a length of fine-bore tubing. Attached to the membrane is a small surface-silvered concave mirror. A record of the pressure changes can thus be obtained by directing a light on to the mirror and focussing
the reflected beam onto photographic paper in a constant-speed camera.

III 8. Oscillograph.

The oscillographic studies were made with Dr. O. Lowenstein's equipment in Glasgow. I am very much indebted to Dr. Lowenstein for allowing me to use this equipment and for his advice on its manipulation and control.

The equipment consists of a 3-stage resistance-capacity coupled amplifier with balanced input and high gain, together with a cathode-ray screen for visual observation and a loud-speaker monitor. A photographic record of the screen trace can be obtained on moving paper in a special camera. Flashing light-spots focussed on the paper at the sides of the trade provide a time marker and an action signal.


The reactions to the anaesthetic on the first sixteen occasions are summarised in Table 1. The advantages of Chloretone are well shown in the table and this anaesthetic was used for all the subsequent operations.
### Table I

**SUMMARY OF EXPERIMENTS WITH ANAESTHETICS.**

<table>
<thead>
<tr>
<th>Anaesthetic</th>
<th>Amount used per 100cc. water</th>
<th>Initial reaction</th>
<th>Time to produce limpness</th>
<th>Recovery</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ether</td>
<td>lcc.</td>
<td>Violent</td>
<td>3 mins</td>
<td>5 mins</td>
<td></td>
</tr>
<tr>
<td>Equal parts chloroform and alcohol of mixture</td>
<td>lcc.</td>
<td>quiet</td>
<td>5 mins. doubtful even overnight</td>
<td>Severe spasms.</td>
<td></td>
</tr>
<tr>
<td>Urethane</td>
<td>lgm.</td>
<td>Moderate</td>
<td>5 mins</td>
<td>overnight</td>
<td>lingering after-effects.</td>
</tr>
<tr>
<td>Urethane</td>
<td>lgm.</td>
<td>Vigorous</td>
<td>4 mins</td>
<td>overnight</td>
<td>lingering after-effects.</td>
</tr>
<tr>
<td>Chloral Hydrate</td>
<td>½gm.</td>
<td>slight</td>
<td>1 hour</td>
<td>overnight</td>
<td>) complete recovery</td>
</tr>
<tr>
<td>Chloral Hydrate</td>
<td>½gm.</td>
<td>restless</td>
<td>¾ hour</td>
<td>overnight</td>
<td>) complete recovery</td>
</tr>
<tr>
<td>Chlortal Hydrate</td>
<td>lgm.</td>
<td>slight</td>
<td>¾ hour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>Saturated soln. pH 5.6</td>
<td>slight; lateral line pits become prominent</td>
<td>Incomplete after ½ hour</td>
<td>—</td>
<td>Solution diluted to ½%, animal recovers in 15 minutes. conc. restored to 1%, limp after 15 minutes, did not recover. Chlortal Hydrate added slowly to effect complete narcosis.</td>
</tr>
<tr>
<td>Chloretone 6 expts.</td>
<td>0.01gm.</td>
<td>slight</td>
<td>20 mins</td>
<td>rapid and complete</td>
<td></td>
</tr>
<tr>
<td>Chloretone 2 expts.</td>
<td>0.02gm in 0.8% saline.</td>
<td>slight</td>
<td>1 hour</td>
<td>rapid</td>
<td></td>
</tr>
</tbody>
</table>
IV. EXPERIMENTAL RESULTS.

1. Kymography.

Expt. 1. The type of kymography trace obtained on a fast moving drum is indicated by figure 16. It will be seen that the expiratory movement appears to be executed in two phases and that it is followed, without a pause, by an inspiratory movement. There is a period of rest in the inspiratory position before the onset of the next expiratory movement.

The shape of the inspiratory phase of the trace is not above suspicion as the lever is not positively coupled to the body wall of the animal for movements in this direction.
If the thread from the lever is attached to a weighted spring electrically maintained in oscillation at 5 per second, it is found that the lever is unable to reproduce the movement. An earlier wave-trace obtained at 20 per second is attributed to "whip" in the lever.

Respiratory movements have been counted at rates up to 240 per minute i.e., 4 per second. Divergences of the wave-form of the movement from a sine-wave would require higher frequencies to be superposed on this and it becomes clear that the lever system is unable to reproduce the whole picture. Accelerations of the body wall which set the thread into tension can be reproduced as there is positive coupling in these conditions. Accelerations in the opposite direction can only be reproduced if they are less than that of the lever under gravity alone.

The shape of the trace for the inspiratory movement is, in some cases, very close to the shape obtained when the lever is falling freely. The method was therefore abandoned as insufficiently reliable for analysis of changes in the inspiratory movement.

IV. 2 Changes in the Respiratory Rate.

As the constant-flow experimental chamber had been
set up it was considered worthwhile to continue the investigation into the control of the respiratory rate.

2.1. Effect of Temperature alone.

Exp. 2. Estimates of the respiratory rate were taken from kymograph tracings at various temperatures and from them was derived the regression equation,

$$R = 92.5 + 7.5(T-13)$$

where $R =$ number of respiratory movements per minute and $T =$ temperature in degrees Centigrade. The fiducial limits of the regression coefficient at the 5% point were 3 to 12 so that the experiment could be regarded as confirming so diverse statements as "the $Q_{10}$ is 1.4" and "the $Q_{10}$ is 4.7".

Exp. 3. In another set of estimates, groups of 4 estimates each were made at 8 different temperatures, Table II. The rate at $5.5^\circ C$ was noticeably less than that at the higher temperatures; the groups were fairly consistent within themselves, as is shown by the analysis of variance, (Table III), but the variance between groups could not be significantly attributed to a regression on temperature.
### Table II

Estimates from kymograph traces of rate of respiration at 8 temperatures.

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Rate</th>
<th>Temp.</th>
<th>Rate.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5°C</td>
<td>67</td>
<td>10.5°C</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td></td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td></td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>73</td>
<td></td>
<td>88</td>
</tr>
<tr>
<td>7.0°C</td>
<td>106</td>
<td>11.0°C</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td></td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>103</td>
<td></td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>103</td>
<td></td>
<td>105</td>
</tr>
<tr>
<td>8.0°C</td>
<td>92</td>
<td>11.5°C</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>85</td>
<td></td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>82</td>
<td></td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td></td>
<td>110</td>
</tr>
<tr>
<td>10.0°C</td>
<td>106</td>
<td>12.0°C</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>102</td>
<td></td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>94</td>
<td></td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td></td>
<td>106</td>
</tr>
</tbody>
</table>

### Table III

Analysis of Variance.

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>dF.</th>
<th>V.</th>
<th>F.</th>
<th>5%point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>4893</td>
<td>7</td>
<td>699</td>
<td>19</td>
<td>2.4</td>
</tr>
<tr>
<td>Within Groups</td>
<td>835</td>
<td>24</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5728</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2. 2. Effect of Carbon dioxide alone.

Expt. 4 The results of preliminary experiments on the effect of bubbling CO₂ into the respiratory water have been discarded as the experimental conditions were not satisfactorily controlled.

2. 3. Effect of reduced O₂ tension alone.

Expt. 5 The aerated experimental water was drawn off and replaced by water which had been exposed in an aspirator to the low pressure produced by a Hyvac pump. The animal at once made violent escaping movements, pulling straight the wire hook connecting the ligature in the skin with the thread to the kymograph lever.

Consequently no record could be obtained of the respiratory rate in this "degassed" water. A water sample taken for oxygen determination was spoiled by an error in manipulation but from studies of similarly treated water it is estimated that the oxygen content was of the order of 2 cc. per litre.

2. 4. Three respiratory stimuli taken together.

For these and subsequent "counting experiments" the estimates of respiratory rates are obtained by measuring with a stop watch the time taken for 50 respiratory movements. The estimates are presented as the mean respiratory rate with its standard error and, in brackets
following this, the number of counts contributing to the estimate.

**Expt. 6** The results of preliminary trials, performed principally to test the reproducibility of the experimental levels of intensity of the respiratory stimuli, are given in Table IV. The gas content of the water in each case determined by the new method described in section III 5.

When the CO₂ content was raised to 39cc. per litre, the O₂ content remaining at 5cc. per litre, the respiratory rate fell off steadily, successive counts yielding rates of 127, 121, 100.... The animal was removed in distress after a short time. Much mucus was produced during the experiment.

With CO₂ content nil and O₂ at 1.8cc per litre (produced by using N₂ alone in the gas bag) the count was 91.5 ± 3.4(4) but breathing was rather irregular with occasional pauses.
Table IV

Three respiratory stimuli - preliminary trial.
CO₂ and O₂ expressed as cc. evolved from 1 litre of water, reduced to Standard Pressure.

<table>
<thead>
<tr>
<th>Temp.</th>
<th>CO₂</th>
<th>O₂</th>
<th>Respiratory Rate.</th>
</tr>
</thead>
<tbody>
<tr>
<td>15°C</td>
<td>nil</td>
<td>4.7</td>
<td>227 ± 31.2 (11)</td>
</tr>
<tr>
<td>15</td>
<td>nil</td>
<td>4.7</td>
<td>178 ± 4.9 (5)</td>
</tr>
<tr>
<td>18</td>
<td>0.06</td>
<td>4.7</td>
<td>188 ± 6.3 (8)</td>
</tr>
<tr>
<td>18</td>
<td>0.06</td>
<td>4.7</td>
<td>202 ± 5.7 (4)</td>
</tr>
<tr>
<td>12</td>
<td>5.8</td>
<td>3.7</td>
<td>102 ± 0.7 (5)</td>
</tr>
<tr>
<td>12</td>
<td>5.8</td>
<td>3.7</td>
<td>150 ± 3.6 (5)</td>
</tr>
<tr>
<td>15</td>
<td>7</td>
<td>3.7</td>
<td>127 ± 2.2 (5)</td>
</tr>
<tr>
<td>15</td>
<td>7</td>
<td>3.7</td>
<td>198 ± 3.9 (5)</td>
</tr>
<tr>
<td>12</td>
<td>0.8</td>
<td>2.2</td>
<td>203 ± 3.0 (4)</td>
</tr>
<tr>
<td>12</td>
<td>0.7</td>
<td>2.3</td>
<td>159 ± 1.3 (5)</td>
</tr>
</tbody>
</table>

It is clear from this that the three factors measured were not the only ones influencing the respiratory rate in the conditions of this experiment. The anomalies might perhaps be explained by the presence of some disturbing factor introduced by the apparatus.

2. 5. Effects of the Apparatus and Procedure.

Expt. 7. The respiratory rate of a lamprey undisturbed in its tank was estimated at 114.3 ± 0.5 (10) at 13°C. It was transferred to the experimental chamber and water from the same tank was used. Counts were taken in the intervals between sporadic bursts of activity over a period of 15 minutes.

145.7 ± 5.4(3)
180.7 ± 2.5(4)
185.2 ± 1.4(3)
187.5 ± 2.4(2)
The animal was left in the experimental chamber for 2 hours with the gas exchanger open to the atmosphere. Two more estimates of the rate were then taken.

The rate appeared to have become steady at a new rate. The temperature of the water was now 14°C and this was taken as an explanation of the difference between the steady rate in the experimental chamber and the previous rate in the tank.

A gas mixture containing 10% CO₂, 20% O₂ and 70% N₂ was made up in the balloon using the meter, and the balloon was connected to the gas exchanger.

The rate remained at

\[ 166.6 \pm 5.6(12) \]

2½ hours later it was still

\[ 164.9 \pm 1.6(7) \]

The balloon was now disconnected and the rate fell in 15 minutes to

\[ 108.7 \pm 3.1(10) \]

After half-an-hour the balloon was re-connected and the rate rose to

\[ 132.5 \pm 6.5(2) \]
Fig. 17. The respiratory rate of a lamprey in the experimental chamber.

The signal line indicates the periods during which the balloon was connected to the gas exchanger. Gas mixture: 10% CO$_2$, 20% O$_2$, and 70% N$_2$ prepared with the meter.
This change unsettled the animal and for a time it was restless. After an hour during which the gas mixture was used the rate was

$$120.1 \pm 1.4(7)$$

On disconnecting the balloon there was again a fall to

$$102.9 \pm 1.4(10)$$

These results are expressed graphically in Fig. 17.

**Expt. 8.** A control experiment was performed using a gas mixture consisting of atmospheric air passed into the balloon through the meter.

After 1 hour in the experimental chamber during which the gas exchanger had been open to the atmosphere a lamprey's respiratory rate was

$$114.3 \pm 1.2(12) \quad \text{at } 15^\circ\text{C}.$$  

45 minutes later the balloon, filled with air through the meter, was connected to the gas exchanger. There was no immediate effect, the respiratory rate being

$$113.8 \pm 1.9(10)$$

1½ hours later the animal appeared distressed; its skin had turned to a pale golden colour. The animal was periodically active and the respiratory rate had risen to

$$141.2 \pm 3.4(10) \quad \text{at } 15.5^\circ\text{C}.$$
Fig. 18. The respiratory rate of a lamprey in the experimental chamber.

Signal as fig. 17.
Gas mixture: Air passed through the meter.
The balloon was disconnected and after 20 minutes the rate fell again to

\[ 112.8 \pm 2.3(12) \]

The changes are expressed graphically in fig. 18.

**Expt. 9.** A second control experiment was performed to find out whether the increase in respiratory rate observed in the first experiment could be attributed to the effect of the meter or to that of the balloon.

An animal was placed in the experimental chamber and left for 40 minutes for the initial effects of the environmental change to pass off. The rate was then found to be

\[ 138.2 \pm 0.97(10) \]

The water was all run through the gas exchanger by the motions described on p.16 for a change of gas mixture. The animal appeared unaffected and its respiratory rate was

\[ 128 \pm 5.7(11) \]

10 minutes later the water was manipulated again and the rate was found to be

\[ 120.8 \pm 1.8(12) \]

The meter was now connected to the gas exchanger without using the balloon. Atmospheric air was now drawn into the gas exchanger through the meter.

There was no marked immediate change, the rate being

\[ 115.2 \pm 1.5(11) \]
Fig. 19. The respiratory rate of a lamprey in the experimental chamber.

The signal line indicates the period during which the air entering the gas exchanger passed through the meter.
After the meter had been in circuit for 1 hour the rate was found to have risen to

$$134.7 \pm 1.9 \ (4) \ (\text{fig.19})$$

It is inferred from these two control experiments that the use of the meter introduces some substance which affects the respiratory rate. The meter had been used previously for coal gas but it was stated to have been decontaminated. Its use was now discontinued.

The initial rise in respiratory rate which occurs after the animal has been placed in the experimental chamber as shown in fig.17, is not connected with the use of the meter as the following experiment shows.

Expt. 10. A lamprey which had remained undisturbed all day in its tank showed a respiratory rate of

$$103.6 \pm 1 \ (7) \ \text{at} \ 12.5^\circ\text{C}.$$ 

The experimental chamber was rinsed and filled with water from the same tank, the gas exchanger being open to the atmosphere. The animal was transferred to the chamber and its respiratory rate was taken

- after 5 mins. it was $170 \pm 3.8(6)$ at $13^\circ\text{C}$.
- 35 mins. " " $200 \pm 3.5(5)$
- 45 mins. " " $150 \pm 9.7(5)$

The animal was now replaced in the tank and the rate
Fig. 20. The respiratory rate of a lamprey shewing the effect of the experimental chamber alone.

The signal line indicates the period during which the animal was in the experimental chamber. The first and last counts were taken while the animal was in the stock tank.
after 5 mins. was

$$111 \pm 4.1(5)$$  (fig. 20)

**Expt. 11.** The treatment of experiment 7 was now repeated without using the meter.

A lamprey was placed in the experimental chamber at 13°C. It was still intermittently active after 1½ hours and the respiratory rate was found to be

$$142 \pm 6.4(6)$$

A further half hour was allowed for acclimatisation after which the rate was

$$125.1 \pm 1.6(10)$$

A gas mixture consisting of 1 part CO₂ to 9 parts air was prepared in the balloon without using the meter, and the balloon was connected to the gas exchanger.

During the manipulation of the water at this stage the animal appeared much disturbed. The respiratory rate fell to

$$66.9 \pm 2.4(7)$$

and the pH of the water was found to be 5.5 as against 6.9 before the balloon had been connected.

Disconnecting the balloon and manipulating the water again appeared to give the animal some relief. The respiratory rate rose to

$$115.3 \pm 1.3(11)$$
Fig. 21. The respiratory rate of a lamprey in the experimental chamber.

The signal line indicates the periods during which the balloon was connected to the gas exchanger. Gas mixtures: \( \text{CO}_2 \) in air in the indicated proportions.
### Table V

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1hr. 25 mins.</td>
<td>Air</td>
<td>142 ± 6.4(6)</td>
<td>6.9</td>
<td>13°C</td>
</tr>
<tr>
<td>2. 05</td>
<td>Air</td>
<td>125.1 ± 1.6(10)</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>2. 30</td>
<td>Change to 10% CO₂ in air.</td>
<td>66.9 ± 2.4(7)</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>2. 45</td>
<td>Change back to Air</td>
<td>115.3 ± 1.3(11)</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>3. 0</td>
<td>Change to Air</td>
<td>124 ± 0.8(10)</td>
<td>5.9</td>
<td>14°C</td>
</tr>
<tr>
<td>3. 20</td>
<td>Change to 5% CO₂</td>
<td>127.7 ± 1.2(9)</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>3. 40</td>
<td>Change to Air</td>
<td>132.9 ± 2.7(11)</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>4. 10</td>
<td>Change to 5% CO₂</td>
<td>123.5 ± 2.5(13)</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>5. 0</td>
<td>Change to 5% CO₂</td>
<td>114.6 ± 1.2(5)</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>9. 0</td>
<td>Air (4 hours)</td>
<td>71.3 ± 4.3(3)</td>
<td>7.0</td>
<td></td>
</tr>
</tbody>
</table>

*They not 6/27 as given in fig. 21.*
although the water remained acid, pH 5.7.

During the ensuing 2 hours the respiratory rate was not significantly affected by gas mixtures having smaller proportions of CO₂ as is shown by Table V and fig. 21.

The last estimate was made after the balloon had been disconnected for 4 hours. The breathing was irregular, the animal's skin showed discoloured blotches and the dorsal musculature was in spastic condition.

It is presumed that this distressed condition may have been due to poisoning, perhaps by metals dissolved from the brass body of the pump in the rather acid water.

A somewhat similar falling off of the respiratory rate with time occurred in Expt. 7.

**Expt. 12.** An attempt to study the interactions of the three respiratory stimuli, Temperature, dissolved CO₂ and O₂ lack, at three levels of intensity of each stimulus, was undertaken using a design for a factorial Experiment adapted from Yates 1937.

A complete set of observations could, however, not be obtained as the animals began to exhibit the seasonal activity described in the appendix A (page 75).
Fig. 22. Pressure changes in the gill sac.

The difference in the amplitudes is due to differences in the distances at which the camera was placed for the various records.

Time marker: steps at 5 per sec in the lower edge of the traces.

In the upper record the crests are cut off by the mask in the camera.
The results of the counting experiments can be summarised in the following terms:

1. There is an increase in respiratory rate associated with the use of the experimental chamber. This settles down in about 1 hour.

2. If the meter is used, an unidentified interfering substance causes an increase in rate.

3. Prolonged use of the apparatus may involve poisoning (possibly by dissolved metal), resulting in a fall in rate.

4. CO₂ in large doses - e.g., the use of a 10% CO₂ gas mixture - has a depressant effect on the rate.

5. The acidity produced by the CO₂ is not itself effective.

6. Smaller doses of CO₂ give no significant change in the rate of respiration in the conditions of the experiments so far.

IV 3. Pressure changes within the gill sac.

Expt. 13. Using the small membrane manometer described in section III 7, a series of records was obtained of the pressure changes at the tip of a hypodermic needle held just within the external branchiopore of a lamprey. The animal lay, lightly anaesthetised with chloretone, in a small dish.

The record is in each case of a characteristic shape (fig.22). There is a well-marked rise of pressure at expiration, with a rather variable plateau,
followed by a slightly more gradual fall of pressure. Usually the fall overshoots the resting position slightly to give a small trough. This is followed by a very gradual rise to the steady position preceding the next expiration.

The rise of pressure at the onset of the expiratory movement is quite sharp, as though there were at first a stage of isometric contraction of the gill musculature before the water starts to leave the gill sac. When a sufficient pressure difference has been set up between the inside of the gill sac and the surrounding water, a stream of water will begin to leave the sac with an acceleration proportional to the attained pressure difference. As the water leaves and the muscles continue to contract, the body-wall moves inwards in an approximately isotonic contraction.

Water is expelled from the gill sacs with considerable force, as can readily be demonstrated by placing carmine granules in the water. Jets several centimetres long can be seen. Further, an anaesthetised lamprey will often move forward steadily under the propulsive action of the jets of respiratory water.

The period of isotonic contraction appears in
the manometric record as a plateau. This is of an irregular character and is not invariably present, indicating that the principal factor in expiration is the imparting of an impulse to the respiratory water rather than a simple pumping movement.

When the expiratory muscles relax there is a fall in pressure. The water in the gill sac is at this moment moving rapidly towards the external branchiopore and it continues to move in the same direction by virtue of its momentum until the wall of the gill sac reaches the limit of its available movement. In this position the body wall is able partially to withstand the hydrostatic pressure of the surrounding water. The continued outflow of water reduces the pressure within the gill sac until a pressure difference is developed, sufficient to oppose the motion of the water. This is the transient phase of "negative pressure" appearing in the manometric records. The magnitude of this pressure difference is dependent on the momentum of the expiratory water stream and hence on the force of the expiratory movement.

Once the outflow has been checked water will flow into the gill sac to equalise the pressures again. This stage appears in the records as a gradual rise of the
pressure to the resting level.

In none of the records is there a sustained low pressure plateau such as would be expected from an active inspiratory movement. The gradual equalisation of pressures accords very well with the theory that the movement is a passive elastic recoil.

IV. 4 Extirpation Experiments.

These and subsequent operation experiments were performed on lampreys which had first been anaesthetised with chloroform until they were limp and did not react to pricking with a needle.

Expt. 14. Exposure of the branchial apparatus was performed under a binocular dissecting microscope. A saline solution was allowed to drip onto the wound just above the field of view of the microscope so that the view was not obscured by the inevitable haemorrhages. A solution containing 4gms. NaCl and 0.1gms chloroform in 500cc. of tank water was found to be satisfactory. NaCl was also added to the respiratory water in the same proportion (0.8%) in order to avoid damage to the tissues by the passage of water from the bath into the wound.

The behaviour of the muscles and cartilages
could be watched under the microscope while the breathing movements proceeded. The movements did not appear to be affected by the removal of the layer of longitudinal muscles which overlies the branchial basket. Even after practically the whole of the branchial basket of one side had been exposed the animal continued to breathe regularly for several hours.

The considerable loss of blood which occurs during an operation of this type seems to some extent to be made good by the anaesthetic saline of the bath passing into the circulation system.

At expiration the movement of the lateral commissures is most striking. The epitrematic and hypotrematic commissures move bodily towards one another without noticeable flexure. They appear to glide over the constrictor muscles to which they are only loosely attached. The extent of the movement is such as nearly to halve the distance between the two commissures at the region where they are nearest together.

The movement of the commissures is brought about by a flexure of the lateral re-entrant curves of the branchial arches.
The branchial constrictor muscles can be clearly seen and they are undoubtedly the principal factors in the expiratory movement.

4. 1. Transections of muscles.

Expt. 15. If one of the constrictor muscles be cut across, the loss of force of the expiratory movement is very noticeable. That the movements do not cease altogether is due to the action of the other constrictor muscles and to the continuity of the cartilaginous basket.

The inspiratory movement is not enhanced by cutting through the branchial constrictor; indeed it seems to be made feeble.

It was not possible to investigate the effects of cutting the diagonal muscles alone as they lie so far in that cutting them would involve complications from opening the peribranchial sinus. This would effect the action of the constrictor muscles even if they were not actually damaged during the operation.

Expt. 16. Cutting the diagonal muscles after the constrictor had also been cut did not appreciably alter the picture obtained by cutting the constrictor alone.

Expt. 17. By careful removal of the connective tissue lying in the ventral re-entrant curve of the gill arch it
Fig. 23. A left branchial bar to shew the position of the cut in Expt. 19.
is possible to expose part of the ventral diagonal muscle without opening the peribranchial sinus. The saline drip is essential for this operation as there are numerous vessels which bleed profusely.

The movements of the diagonal muscle can now be observed directly and compared with the movements of part of an adjacent constrictor muscle in the same field of view of the microscope. It is clear that both muscles are contracting at the same phase of the respiratory movement. There can be no suggestion that the diagonal muscles contract later and are responsible for the inspiratory movement. On the contrary, the onset of contraction of the diagonal muscle can sometimes be seen actually to precede the onset of contraction in the constrictor muscle by a small fraction of a second.

4. 2. Transections of Cartilages.

Expt. 18. No change in the movements occurs if the lateral commissures be cut.

Expt. 19. If the branchial arch be cut just ventral to the high hypotrematic commissure (fig. 23) the expiratory movement is unaffected but the inspiratory movement is much less forceful.
Fig. 24. A left branchial bar to shew the position of the cuts in Expt. 20.
Fig. 25. A left branchial bar to shew the region excised (AB) in Expt. 21.
Expt. 20. If that part of the branchial arch from which the second ventral-rostral process arises be isolated from the rest of the basket without damage to the ventral diagonal muscle, this chunk of cartilage can be seen to be pulled ventralwards at each expiratory movement.

For example, the 4th. branchial arch was cut at the level of the branchiopores and again just dorsal to the 1st. ventral-rostral process. (fig. 24.) The hypotrematic commissure was cut on both sides of the 4th. arch. One end of the 4th. ventral diagonal muscle was still attached to the loose piece of cartilage while the other end was attached to the branchial basket, so that the free cartilage was pulled upon by the diagonal muscle. The movements occurred at the same time as the contractions of the constrictor muscles.

Expt. 21. If a portion of the lateral re-entrant curve be excised (fig. 25 AB) some gross movement of the body wall persists through the precision of the movement is lost. The movements of the lateral commissures towards and away from one another are almost completely abolished.

Expt. 22. If the lateral corner of the ventral
Fig. 26. A left branchial bar to shew the region excised (CD) in Expt. 22.
re-entrant curves be excised by cuts above and below the 2nd. ventral-rostral process without damage to the diagonal muscle, (fig. 26, CD) the lateral region of the arch no longer moves towards the ventral region at expiration. The diagonal muscle, which is normally responsible for this movement, is intact but the two regions of the cartilage have nothing to pull them apart again after a contraction of the diagonal muscle has passed off. They remain therefore near the relative positions reached at the limit of expiration and the diagonal muscle is unable to move them further.

Shortly after these transections of cartilages the affected arches cease movement altogether though the gills on either side may continue to be ventilated.

IV. 5. Stimulation Experiments.

Faradic shocks from an induction coil having a vibrating reed in the primary circuit were applied by means of a pair of needle electrodes mounted side by side in a holder. The vibrating reed was usually set at about 7 to 10 vibrations per second, and various strengths of shock were used.

Expt. 23. The branchial constrictors of two adjacent gill pouches were cut away and the gill sacs were removed, leaving
Fig. 27. Diagram of an isolated preparation of a ventral diagonal muscle with half of a left branchial bar.

L. Re-ent. C., lateral re-entrant curve; V. Com., ventral commissure; V. Diag. M., ventral diagonal muscle; V. Re-ent. C., ventral re-entrant curve.
the branchial arch between these sacs isolated, yet with its diagonal muscles intact. The nerve supply to the diagonal muscles is presumably destroyed by this operation for the isolated arch does not continue to move rhythmically.

If one of the diagonal muscles be stimulated directly with repeated Faradic shocks, a movement of expiratory type results. The return to the resting position is fairly smart and can only be accomplished by the elasticity of the cartilage as there are no muscles present except the diagonal muscles.

**Expt. 24.** A number of isolated preparations was made of one half of one branchial arch from one side complete with its diagonal muscle undamaged yet with no other structures present at all. For instance the region of a gill arch taken was from the line of the branchiopores to the ventral commissures, (fig. 27) or from the line of the branchiopores to the parachordal sheath.

These isolated preparations gave movements of expiratory type by direct stimulation of the muscle, and there was in each case a sharp recoil.

**Expt. 25.** The isolated preparation of experiment 24 is not sufficiently robust to permit kymograph records
of its movements to be taken. The diagonal muscle can be made to lift a light lever but the recoil of the lever is probably stronger or at any rate at least as strong as the recoil of the cartilage. It has not been found possible to make a lever light enough to be lifted by the recoil of the cartilage alone.

Exprt. 26. Another type of preparation studied consisted of the branchial basket of one side, separated from the longitudinal muscles and with the gill sacs removed. This left a thin sheet of tissue containing the cartilaginous basket with the constrictor and diagonal muscles intact. Parts of the interbranchial septa and the whole of the ventral commissures were left in position, and the parachordal sheath was used as a handle for manipulating the preparation.

The stimulating electrodes could be placed on the different muscle groups from within the gill pouch with the minimum of dissection near the actual muscles. Each muscle group gave a characteristic movement to the basket when it was stimulated.

All the movements obtained on the contraction of muscle groups were of expiratory type and the
Fig. 28. Oscillograph records of potentials from the branchial musculature.

Upper record showing movement artifacts.
Lower record: bursts of muscle potentials from branchial constrictor.
Time marker: dots at 20 per sec.
basket always returned to its resting position by an elastic recoil.

Contraction of the branchial constrictors brought about flexion of the basket principally at the region of the lateral re-entrant curves.

Contraction of the diagonal muscles brought about flexion of the appropriate dorsal (or ventral) re-entrant curves only.

IV 6. Oscillographic Study.

Expt. 27. Action potentials have been recorded during respiratory movements from both constrictor and diagonal muscles. The impression is that both of these sets of action potentials appear during the expiratory movement but attempts to record both sets simultaneously, with an action signal to show the times of movement, have met with considerable technical difficulties. During these attempts a double rhythm has occasionally been heard in the loud-speaker but in each case this has been attributable to movement of the tissues over the tip of the electrodes. On one occasion a record was obtained of this phenomenon (fig. 28) and the potentials were seen to be of comparatively long duration, as
gross movements of the base line, readily distinguishable in the record from the characteristic appearance of action potentials.

**Expt. 28.** Even when one electrode was on a diagonal muscle and the other on an adjacent constrictor, there was no suggestion of a double rhythm of spikes. This establishes the fact that the diagonal muscles and constrictors contract at the same time, or at least at times certainly not sufficiently separated to justify assigning one to the expiratory and the other to the inspiratory phase.

**Expt. 29.** Spikes in rhythmic sets were obtained on one occasion only from the longitudinal muscles of the body-wall. On this occasion the electrodes were in the mid-ventral line and the skin alone had been removed in this region.

On all other occasions the only potentials obtained from the body-wall muscles (except for bursts during wriggling) consisted of large slow swings of the base line which are interpreted as movement artifacts.

**Expt. 30.** Recording of action potentials direct from the brain has given no conclusive results.
Fig. 29. Oscillograph records of potentials from central nervous system of a lamprey.

Each respiratory movement is represented by two groups of spikes.

Time marker: dots at 20 per sec.
Expt. 31. On one occasion a set of discharges was obtained from the spinal cord occurring rhythmically in phase with the respiratory movement. The region tapped was at the level of the 1st. branchiopore, near the mid-line. This is well behind the region of the choroid plexus of the medulla. The record of this discharge (fig. 29) shows a double rhythm. As this has been found on one occasion only, the significance of the result remains uncertain.

Expt. 32. On another occasion a continuous discharge was obtained from a well-defined tract running near and parallel to the mid-line of the spinal cord for 2mm. above and 2mm. below the level of the 1st. branchiopore. This animal had ceased to breathe during the initial exposure.

When the electrode was lowered into the spinal cord in the line of the tract at a level about half way between the eye and the 1st. branchiopore, a burst of spikes appeared on the screen, a "crunch" was heard in the speaker and the lamprey started to breathe regularly. The continuous massive discharge was still present and is presumed to come from a sensory pathway stimulated by some displacement produced during the initial operation and normally giving rise to reflex
inhibition of the respiratory movements via some relay centre higher up the brainstem. On interrupting this tract the inhibition ceased and normal respiration recommenced.

**Expt. 33.** On occasions subsequent to experiment 32 when breathing ceased during the initial operational procedure, the brainstem was transected behind the eyes and this was followed by a recommencement of the respiratory movements. One one occasion when transection had been tried without success, the movements restarted during a later stage of the operation.
V. DISCUSSION.

1. The Inspiratory movement.

The description of the branchial constrictor muscle given in section II 3 differs from that of Tretjakoff 1926 principally in that he speaks of an insertion into the lateral commissures where only a loose attachment by connective tissue has been found.

Dawson's (1905) description of the branchial musculature and her figure of the gill pouch (fig.2) can perhaps best be interpreted by regarding her "deep compressor muscle of the gill pouch" as the branchial constrictor and her muscular "gill pouch" as consisting only of the extremely delicate strands of muscle lying in the interbranchial and median septa.

The anatomical details which are presented in the present work as new information concern the dorsal and ventral re-entrant curves of the gill bars(fig.4) and the diagonal muscles (fig.9) which span these re-entrant curves. These new facts at first lend support to both rival theories about the inspiratory movement. The presence of the dorsal and ventral re-entrant curves distributes the bending stresses in the gill bar so that the whole arch can contribute to the restoring force when the basket is deformed by an expiratory movement. This
lends colour to the theory of elastic recoil.

On the other hand the insertions of the diagonal muscles might be just so placed in relation to the re-entrant curves as to have the effect of extending the lateral region of the arch. If this were the case, a movement of inspiratory type could be produced by muscular contraction.

In relation to the hypothesis that the inspiratory movement is produced by musculature contraction the following groups of muscles are the only ones that have to be considered.

1. The longitudinal muscles of the body-wall.
2. The Branchial Constrictor muscles.
3. The Diagonal muscles.
4. The muscles of the interbranchial and median septa.
5. The muscles in the walls of the gill sacs themselves.

The action of the branchial constrictors is obviously to produce a movement of expiratory type.

The muscles of the septa and of the gill sacs are so very much smaller than the branchial constrictors that they can play no significant part
in the respiratory movements.

The longitudinal muscles of the body-wall can be excluded on two counts. Firstly, the respiratory movements continue apparently unchanged after large sections of the body wall musculature have been removed (Expt. 14 and subsequent open operation experiments). Secondly, no action potentials having the rhythm of the respiratory movements could be picked up from the longitudinal muscles except in the mid-ventral line (Expt. 29) (The significance of these potentials from the mid-ventral line will be discussed later.)

The type of movement which the diagonal muscles are capable of producing has been shown by stimulation experiments:

(a) With the arch in position in the animal but with the constrictors removed, (Expt. 23);

(b) in isolated preparations of part of a branchial arch with the diagonal muscle alone attached, (Expt. 24);

(c) in preparations of the gill basket of one side complete with constrictors and diagonal muscles, (Expt. 26.)

These experiments conclusively show that the diagonal muscles do not produce a movement of inspiratory type.
The place of the contraction of the diagonal muscles in the time-sequence of the respiratory cycle has been studied,

(a) by direct observation of a diagonal muscle and part of a constrictor muscle in the same field of view of a microscope, (Expt. 17)

(b) by observation of the movement of a detached portion of cartilage to which a functional diagonal muscle was still attached, (Expt. 20)

(c) by oscillographic study of the action potentials from the diagonal muscles picked up simultaneously with those from the constrictor muscles (Expt. 28).

These experiments shew that the contractions of the diagonal muscles occur during the same phase of the respiratory movement as those of the constrictor muscles.

All the available muscle groups are thus accounted for by movements of expiratory type so that the inspiratory movement cannot be effected by muscular contraction.

That the cartilages play an important part in the respiratory movement is shewn by the effects of cutting out portions of a gill arch (Expts. 19, 21, & 22)

On the other hand the recoil of the cartilages, though fairly rapid in air, is not a very forceful movement (Expt. 25.) And there is no sustained low pressure phase observable in the manometric records from the gill sac (Expt. 13).
A study of these manometric records suggests that the momentum, which is imparted to the exhaled water stream at expiration, and results in a sharp fall in pressure within the gill sac as soon as the expiratory muscles relax.

The effect of this is eventually to cause water to flow in the reverse direction i.e., into the gill sac. As the pressure difference is reduced by this influx of water, the elasticity of the cartilages can come into play to move the body-wall outwards as the water enters the gill sac. Thus the final equalisation of the pressures is accomplished slowly (fig. 22).

It is suggested that the action potentials picked up from the longitudinal muscles in the mid-ventral line (Exptr. 29) indicate a "holding reaction" to compensate for the forces developed on the basket as a whole during expiration.

The respiratory water is expelled backwards with considerable force and an opposite momentum must be imparted to the branchial apparatus. It is suggested that the ventral longitudinal muscles function to secure the basket to the rest of the animal's body so that
The possibility that the last stages of the inspiratory movement may be slow provides an additional reason for discarding the results of the kymographic study. The weight of the lever may well transmit to the branchial apparatus a force in the inspiratory direction which is large compared with the forces normally developed by the cartilages.

Thus it is not to be expected that the kymograph trace (fig. 16) should show the gradual nature of the last stages of the inspiratory movement as they are indicated by the manometric record (fig. 22), any more than it could show an initial acceleration in the inspiratory direction greater than that of the lever falling freely under gravity. A system of low inertia with positive coupling in both directions would have to be devised to record such movements.

V. 2. The Respiratory Rate.

The control of the respiratory rate by various factors cannot satisfactorily be studied from experiments in which the intensity of only one of the factors is measured. This is demonstrated for instance by the preliminary trials on the effect of temperature (Expts. 2 & 3). A study of the effects and interactions of several factors is usually hampered by the difficulty of securing uniformity of experimental material. If we are prepared to restrict the study
to three factors only and consider only three different levels for each of the factors, we are already faced with 27 different combinations of treatments to try out. If only one experiment is made for each treatment-combination, it would not be possible to say with confidence that the condition of the animal at the last experiment of the set was comparable with the condition at the first experiment. Even if we thus avoid confounding the comparisons between treatments with the variation between individual animals, they are still confounded with variation in the condition of the experimental animal.

Similar problems arise from the variable soil conditions of agricultural field trials and Yates (1937) discusses experimental designs which can be used to overcome the difficulty. He describes a "3 x 3 x 3 factorial experiment" which can readily be adapted to the problems of respiratory control. The design provides 3 replications for each of the 27 treatment combinations and these are distributed over a square plot subdivided into 9 rows and 9 columns. The variation between rows and the variation between columns are both excluded from the estimate of error by which the significance of the results is...
is judged. The main effects of the factors and their interactions in pairs are all preserved from confounding. The triple interaction is recovered by a partial confounding technique.

In the proposed experiment on the control of respiratory rate, 9 animals would each be subjected to 9 sets of conditions of respiratory stimuli using an arrangement of treatments such as that suggested by Yates in his Table 50. The animals should be allotted in a random order to the columns of the table and the numbers of the experiments in order of time should be allotted at random to the rows. It is essential to the technique of recovery of the triple interaction from the partial confounding that the array of treatments should be built up in the manner Yates prescribes. It is however, not necessary to go into the principle of building up the array. His table may be used repeatedly by randomising the rows and columns amongst themselves so long as each row, or each column continues to consist of the same group of treatments.

The variation between animals and also the variation in individual animals with time can thus both be excluded from the estimate of error in assessing the main effects of the factors and their interactions.
VI. CONCLUSIONS.

The inspiratory phase of the respiratory movement of the lamprey differs from that of all higher vertebrates: it is not brought about by muscular contraction.

The elasticity of the cartilages may alone be adequate for inspiration when breathing is shallow: but in deep breathing, where the expiratory movement is forceful, there is a low-pressure region left behind by the outgoing water stream and this initiates the inspiratory phase.
SUMMARY.

The branchial apparatus of *Lampetra fluviatilis* has been studied to find out whether the inspiratory movement is brought about by muscular contraction.

Differences between existing accounts of the anatomy are pointed out, and a description is given for the first time of diagonal muscles which span re-entrant curves of the gill bars.

The changes in the inspiratory movement with changes of respiratory rate are studied in an experimental chamber designed so that the temperature, the rate of flow, and the gas content of the respiratory water can be independently varied.

The gas content of the water is determined by a new method in a special apparatus.

Experiments on the control of the respiratory rate were interrupted by the onset of periodic activity at the beginning of the breeding season.

It is found that the kymographic method is not sufficiently sensitive for recording such rapid movements as those of the lamprey's gill basket.

Exirpation and stimulation experiments provide
evidence that none of the available muscle groups can have an inspiratory function.

The pressure changes in the gill sac indicate that the momentum of the water-stream in forceful expiration leaves a region of low pressure which initiates the inspiratory flow.


* These papers have not been available for consultation.
Seasonal Behaviour.

A number of lampreys which had remained very quiet in their tanks all through the winter began quite suddenly in April to swim violently to and fro in the tanks. This violent activity occurred in spurts, a lamprey swimming strongly for about 1 minute or less and then subsiding, to lie panting at the bottom of the tank for about 5 minutes. After this there would be another burst of violent activity followed by another pause.

The sequence was repeated continually for several days and the animals then became quite quiet again. Shortly after the quiet stage had been reached the animal in every case became infected with fungus and subsequently died.

The onset of the phase of periodic activity occurred on different days for different lampreys. For example, when one of four lampreys in a tank started its sporadic activity, it was removed to another tank. The remaining three were quite quiet for two or three days until another lamprey became active. This was removed in turn and the remaining two were quiet for a few further days, and so on.

The active lampreys were sexually fully mature and eggs or sperm could easily be expressed from them. The sperm were motile, but the eggs did not cleave. There was no attempt
Fig. 30. The respiratory rate of a lamprey in the stock tank shewing the unsteadiness produced by periodic activity at the approach of the breeding season.
to pair, or to form a nest or redd in the tanks although sand and gravel were provided.

The results of a series of counts of the respiratory movements, taken over a period of about three quarters of an hour, are shown graphically in fig. 30. This figure indicates the variation in respiratory rate to be encountered at this stage in a lamprey which has not even been removed from the stock tank. It is clear that an animal exhibiting such behaviour is not a suitable subject for experiments based on the habit of regular breathing.

An explanation of the behaviour of the animals in the tanks is provided by some field observations. Specimens of *Lampetra planeri* were observed in the breeding season in a stream at Rossdhu. The stream flows fairly swiftly and the animals seemed to have difficulty holding their own against the current. As soon as they deviated to one side of the upstream direction they were rapidly carried down until they found shelter behind a stone. If they were disturbed, they turned and swam downstream, moving so rapidly that the eye could hardly follow them. When the lampreys were swimming upstream, their speed over the ground was only about 10-20 cms/sec.; whereas, when swimming downstream, they moved over the ground at a speed of the order of 2 - 3 metres/sec.
The progression upstream was slow and laborious and was interrupted by pauses during which the animals sucked on to a stone for several minutes. They swam actively for periods of about half a minute to a minute.

An upstream migration is necessary for *Lampetra planeri* before spawning, as the developing embryos and ammocoetes are continually subject to the risk of being carried downstream by the current, particularly in times of spate. *Lampetra fluviatilis* and *Petromyzon marinus* have to ascend from the sea to their spawning grounds in the rivers.

These latter species enter the mouths of the rivers in the autumn (October to November), and are taken in numbers at this time by fishermen. The lampreys do not appear at their spawning grounds until late spring (March to mid-June according to the climatic conditions). Between these two seasons, in which lampreys can be obtained quite readily, there is a period when there are none to be seen at all.

During this period the lampreys in the aquarium remained almost motionless in their tanks and it is suggested that in the wild state there is a similar period of quiescence. The animals enter the rivers in the autumn and proceed some way upstream. Then they hide away under stones and in
crannies and remain hidden all winter. During this period the gonads and genital apparatus undergo maturation changes.

When the gonads are nearly ripe the animals become active again and proceed upstream to spawn. The last upstream migration has to be performed against a rapid flow of water, so the lampreys cover the distance in a series of short sprints, resting between sprints by holding on to the stream bed with their suckers.

The gonads of the lampreys in the aquarium ripened in the normal way and accordingly the animals started to shew repeated short bouts of violent swimming activity such as would have been suitable for a last dash upstream to the spawning beds. No suitable spawning sites were found in the aquarium as there was no rapid waterflow. The intermittent swimming therefore continued until the animals became exhausted. In this condition the susceptibility to fungal attack was increased and the animals consequently died.

From figure 30 it is inferred that during the periodic activity an oxygen debt is incurred which governs the duration of the sprint. The amount of the tolerated debt, assessed by the increased rate of respiration, is
fairly constant and corresponds in this example to a respiratory rate of 170-180:\text{\textpermin}. During the rest period the debt is gradually repaid, so that the respiratory rate falls. The next period of activity starts as soon as the greater part of the debt has been repaid.
APPENDIX B.

Copulatory Behaviour.

It has been generally held by many workers that fertilisation in the lampreys is external. Loman 1912, on the other hand, suggested that there was intromission with internal fertilisation.

Spawning brook lampreys (Lampetra planeri) have been closely studied at Rossdhu and the following observations are offered as a contribution to the solution of this question.

In coupling, a male sucks on to a female which is already attached to a stone. The male attaches to the female just behind her nasohypophysial opening. Both animals now begin a violent shaking movement during which the posterior region of the male is suddenly coiled round the cloacal region of the female. The male's body is nearly always curled in the same direction, passing under the female from right to left.

Coupling occupies at most two seconds, usually much less, and throughout the coupling process the two animals are rapidly vibrating, stirring up the sand and gravel of the stream bed so that it is not easy to follow the movements precisely.
The urinogenital papilla of a mature male is a large structure when fully extended, protruding 5 to 7 mm. from the cloaca. The mature female papilla is shorter, protruding 1 to 1½ mm. only. Its walls are thickened and the lips of the cloaca are also swollen. The genital pores from the abdominal cavity open into the urinogenital sinus and this opens to the exterior by the pore at the tip of the papilla.

For internal fertilisation to take place it is, therefore, necessary for the papilla of the male to be introduced into the pore at the tip of the papilla of the female. Ejaculation into the cloaca is not effective particularly as the female papilla protrudes beyond the cloacal lips.

The male papilla can usually be clearly seen as the animals swim to and fro in the water. Only very rarely can the papilla be seen during the act of coupling. If it is visible then very often there are also repeated readjustments of the coiling.

On examining fresh ripe females under a microscope it was found that if a seeker is gently pressed against the body wall somewhere near the cloaca, or better still is actually pushed into the cloaca, the animal's body wall buckles inward before the seeker so as to guide its point
to a region immediately posterior to the papilla. Further pressure causes the papilla to bend downwards and forwards so that the point of the seeker slides along the dorsal side of the papilla into the urinogenital pore. Accurate aim is therefore not essential to ensure entry into the female urinogenital pore. The surrounding tissues assist in directing the seeker into the pore.

During the coupling, eggs can usually be seen among the sand that is stirred up. Whether these eggs were present in the sand before it was stirred or are liberated by the female during the act of coupling is not certain. Such eggs usually begin to cleave in a few hours.

Females taken from the stream in the act of coupling and segregated in an aquarium with clean sand did not produce eggs at once and those that were produced did not cleave. On the other hand, females have often been seen coiled away among the pebbles at the bottom of the nest as though for an act of oviposition; and clumps of a dozen or more eggs, all stuck together as though laid at one time, have often been found in the stream bed and also in gravel in an aquarium tank which contained both males and females.

Pending further observations it is suggested that intromission occurs, that internal fertilisation takes place
in the urinogenital sinus, and that the fertilised eggs are sometimes shaken out during the coupling, but may remain. If they do remain, they are laid later in one batch deep among the gravel. As the urinogenital sinus can accommodate only a few eggs at a time, couplings are repeated at intervals until all the eggs have been shed.


APPENDIX C.

Carbon dioxide in water.

In the control of the rate and depth of the respiratory movements in mammals the role of carbon dioxide is an important one. Many workers believe that the effective factor is the concentration of unhydrated molecules of CO₂ in the arterial blood reaching the respiratory centre. Schmidt (1945) reviews the evidence for and against this theory.

The blood is brought into equilibrium with the fluid covering the respiratory epithelium. The concentration of unhydrated CO₂ in this fluid depends on the partial pressure of CO₂ in the alveolar air and can be altered, within limits, by altering the proportion of CO₂ in the inspired air. The exchange of CO₂ concerns the unhydrated form only, as the hydrated forms cannot escape into the gas phase.

In aquatic vertebrates hydrated CO₂ can also enter into the exchange by diffusion into the respiratory water although the carbonate and bicarbonate ions presumably cannot pass the membrane separating the blood from the surrounding water. The concentration of these anions in the respiratory water should have no effect on the control of the respiratory rate. Experiments on the control of
respiration in aquatic animals are therefore likely to yield anomalous results in regard to the effect of CO₂ if this is assessed on the basis of the total concentration of CO₂ in all its forms.

The methods available for the determination of CO₂ in water were reviewed by Partridge and Schroeder (1932) and fall into five main groups:

Titration,
Evolution in a gas stream,
Equilibration with a small volume of gas,
Evacuation,
Inference from pH and mass action equations.

Examples from each group of methods will be considered in turn. **Titration Methods.**

Johnston (1916) examined the titration methods then in use and pointed out that the correct end-point varies with the constitution of the solutions titrated, so that interpretation of the results is difficult. Nevertheless titration methods are still used in routine water analyses (Amer. Publ. Health Assn., 1946). A measure of "free carbonic acid" is said to be obtained by titrating with Na₂CO₃ to phenolphthalein, and a measure of "bicarbonate" by titrating with acid to methyl orange.
Greenfield and Baker (1920) used such analyses to calculate the pH of certain natural waters using a relation derived from the mass action equation. The bicarbonate was assumed to be 85% dissociated. (This figure seems to have been arbitrarily chosen). At one point in the calculations the bicarbonate concentration at the end-point of the phenolphthalein titration is cancelled with the bicarbonate concentration in the untreated water sample. If these bicarbonate concentrations are small, any difference between them would introduce an error of unknown magnitude. In any case it appears from the results which are given that one could not undertake with confidence the reverse procedure of calculating CO₂ concentrations from the pH on the basis of their equation.

McKinney (1931) gave a neat method for calculating the total CO₂ from the amount of acid required to change the pH from 8.5 to 5.0. The method is based on theoretical ratios which the activities of each of the forms of hydrated CO₂, taken individually, bear to the activity of the total CO₂. These ratios are calculated from mass action equations using accepted values for the dissociation constants of carbonic acid. The ratios depend on the pH of the mixture considered, and the amount of hydron
required to alter the ratios from those appropriate to pH 8.5 to those at pH 5.0 can be calculated in terms of the total CO₂ present. This amount of hydrion is equated to the amount actually used in the titration and the equation is solved to give the total concentration of CO₂. From this figure the amounts of H₂CO₃, HCO₃⁻ and CO₃²⁻ can be calculated using the ratios appropriate to the pH of the original solution. McKinney and Amorosi (1944) gave a method of carrying out the two titrations on a single water sample, using a mixed indicator. Unhydrated CO₂ is not considered at any stage in the calculations.

**Evolution Method.**

The evolution method of Partridge and Schroeder (1932) was introduced to avoid the errors of the titration methods then in use. The water sample is treated with acid and a stream of air, previously freed from CO₂, is led through the heated sample into a Ba(OH)₂ solution which is later back-titrated. It is necessary to have a circulating pump so that the same air may be passed continually through the solutions until all the evolved CO₂ is absorbed. Otherwise some CO₂ may be lost after failing to be absorbed owing to the formation of a BaCO₃ layer around the gas bubbles. The method is acknowledged to give very good results for total CO₂ but the difficulty of constructing a suitable pump prevents its more general use.
No attempt was made to adapt this method to analyses for unhydrated CO₂ alone as the absorption of the gas is a slow process and some disturbance of equilibria is to be expected.

**Equilibration Method.**

Krogh (1904) obtained a measure of the tension of CO₂ with which his water samples would have been in equilibrium by shaking large samples of water (1 litre) with relatively small amounts of air (25 c.c.). The gas phase was then analysed with a modified Haldane apparatus. The nature of the method demands that the volume ratio of sample to gas phase be large. This means that either the sample is large or some micro-method must be found for the gas analyses.

**Evacuation Methods.**

Charles J. Fox (1909) introduced his water samples into an evacuated bulb, added acid, and pumped the evolved gases into a gas burette with a mercury pump. Last traces of CO₂ were swept through with H₂ and the amount of CO₂ collected was determined by a volume change on absorption with alkali. This method gives the total CO₂ only.

Swanson and Hulett (1915) exposed half-litre samples of water to a Torricellian vacuum in a vessel of 1 litre capacity and separated the evolved gas from the water without change of pressure. The gas was passed into a gas
burette and the CO₂ was determined by a volume change on absorption with alkali.

Van Slyke (1917) developed a method for applying to small samples the same principles as were used by Swanson and Hulett. A one-piece apparatus was used, consisting of a 50 c.c. evacuation chamber with a gas burette above and a trap with by-pass tube below. A 1 c.c. or 2 c.c. sample was introduced and acidified within the apparatus. By manipulating a mercury piston the sample was subjected to a Torricellian vacuum. The apparatus was now shaken and the solutions were passed into the trap. By raising the mercury through the by-pass tube the evolved gases were brought to atmospheric pressure and their volume was measured, the CO₂ being determined by difference after running in a known volume of alkali. Corrections were made by calculation for other gases present in the reagents and for the proportion of CO₂ remaining in the solution.

McClendon (1917) adapted this volumetric method of Van Slyke for 10 c.c. samples of sea water. The volumes of total CO₂ which he obtained for measurement were of the order of 0.4 to 0.6 c.c.

Shaw (1921) used another modification to take samples up to 100 c.c. The gas was measured in a separate burette. Repeated evacuations without acidification were used to
determine "free CO₂" but no account was taken of changes in the equilibria between evacuations.

Van Slyke and Neill (1924) dispensed with the trap and by-pass of the volumetric apparatus and added a manometer so that the gases could be determined at constant volume by a measurement of pressure. The solutions were not segregated after the evacuation.

Harington and Van Slyke (1924) used a modified extraction chamber permitting the solutions to be rejected, thus avoiding reabsorption.

Van Slyke (1927) modified the manometer to produce the "Van Slyke manometric gas analysis apparatus" which is now in general use. Corrections and conversion factors for use with this apparatus in analyses of the gases in blood are given by Van Slyke and Sendroy (1927). The calculations of the corrections were based on analyses for CO₂ by a constant stream evolution method without circulating pump.

This manometric method of Van Slyke is of particular application to the determination of total gases in blood and similar small samples. If one attempted to use it for "free CO₂" without acidification the reabsorption effects would be large compared with the amounts of gas to be measured.
pH Methods.

McClendon (1917), on the basis of his determinations with his modified Van Slyke volumetric apparatus, prepared curves relating the CO\(_2\) tension to the pH for various salinities.

Saunders (1923) used a theoretical relation derived by Prideaux (1915) to calculate the total CO\(_2\) in fresh water samples at various values of pH and "total base".

Bruce (1924) combined McClendon's and Saunders's figures with some fresh data and produced a family of curves relating pH to CO\(_2\) concentration for values of "excess base" ranging from N/10,000 to N x 26/10,000. A study of the curves shews that they are less useful in the pH range 6 - 7 than in more alkaline conditions. This is only to be expected as Prideaux's function, on which the curves depend, changes steeply in value in the pH range 6 - 7. The family of curves is most useful in the pH range 7.5 - 8.5.

Powers (1927), on the basis of a rather naïve argument, splits up the equation:

\[ C_{H^+} \times C_{HCO_3} = K_{H_2CO_3} \]

into the two equations:

\[ C_{H^+} = (K_{H_2CO_3})^n \]

and \[ C_{HCO_3} = (K_{H_2CO_3})^{1-n} \]
He combines the first of these equations with the equation
\[ C_{H_2CO_3} = k_{gas} \times P \]
where \( k_{gas} \) is the "solubility factor of CO\(_2\)" and \( P \) is the CO\(_2\) tension of the liquid.
He thus obtains the relation:
\[ C_{H^+} = (K_k \cdot P)^n \]
or \[ \text{pH} = -n \log(K_k \cdot P). \]
(He does not consider the fact that \( C_{H_2CO_3} \) may have different meanings in the two contexts.)

To reduce the discrepancies between observed values and the results obtained by calculation from his formula, he introduces an empirical constant \( e_1 \), and writes:
\[ \text{pH} = -n \sqrt[3]{\log(K_k \cdot P)} + e_1 \]
which reduces to \[ \text{pH} = -n \log P - ne \]
where \( e = e_1 + n \log(K_k) \).

The method which Powers proposes for the determination of CO\(_2\) tension consists then in evaluating \( n \) and \( ne \) for the particular water under consideration by taking the pH of the water after aeration with two gas mixtures having known partial pressures of CO\(_2\) and solving the simultaneous equations of the form \( \text{pH} = -n \log P - ne \). These values of \( n \) and \( ne \) are then used in conjunction with the pH of an untreated water sample to deduce \( P \).

The results of 275 analyses by this method are compared
with the known partial pressures of CO₂ used in preparing the samples and less than half of the experiments shew an error under 10%.

It will be seen from this review that there are at present no methods of analysis which will give an estimate of the CO₂ present in a small water sample in forms other than carbonate and bicarbonate. Several satisfactory methods are available for "total CO₂" but there is in each some difficulty in adapting the method to give "free CO₂" alone, i.e.; unhydrated CO₂ in solution, or CO₂ plus undissociated H₂CO₃. Powers's method for "CO₂ tension" is very attractive and simple but seems to be open to serious objections.

The new method, described in Section III, 4, is an attempt to assign a measure to the CO₂ that is available for diffusion across a gill membrane. The hydration reaction

\[
H₂O + CO₂ \rightleftharpoons H₂CO₃
\]

or \[
OH' + CO₂ \rightleftharpoons HCO₃'
\]

is known to be an appreciably slow reaction, (McBain 1912, Collingwood 1924, and Buystendyk, Brinkman and Mook 1927), and it is suggested that the amount of CO₂ liberated into the gas phase on exposing a water sample to a Torricellian
vacuum for a few seconds only can be regarded as a measure of the total amount originally present in the water sample as unhydrated CO₂. Repeated evacuations are avoided as there is bound to be an equilibrium change during the interval between evacuations.

As a measure of the efficiency of the gas extraction technique, the values obtained for oxygen concentration in the water samples were compared with values obtained by the Winkler method as modified by Whitney (1938). The values obtained by Whitney's method were consistently 1 to 2 c.c./litre higher than those obtained by using the new apparatus. On the other hand, Whitney's method always shewed at least 1.25 c.c./litre of oxygen in water samples which had been boiled for several hours in a stream of nitrogen. If this amount be accepted as a zero error of the Whitney method and is deducted from the values obtained, the agreement between the two methods may be regarded as satisfactory.

It would be possible to reduce still further the time of exposure of the water samples to the vacuum if the new apparatus (fig. 13) were altered slightly. The sampling unit could be arranged to deliver the water direct into the trap below E. A Torricellian vacuum could then be set up in the evacuation chamber, the water admitted through E and then drained back again quickly into the trap. This would
reduce the exposure time to 2 or 3 seconds and would enable the modified apparatus to be used for a fresh study of the behaviour of carbon dioxide in water.
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