THE RELATIONSHIP BETWEEN THE ARTERIAL BLOOD ACID - BASE STATUS AND VENTILATION IN THE RAINBOW TROUT, *Salmo gairdneri*

by

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ABSTRACT

Studies were carried out to determine the influence of the change in the acid-base status of the blood on regulation of pH in relation to control of ventilation in the rainbow trout. By placing trout in ventilation ($V_G$) boxes direct measurement of ventilation volume and rate could be made. Arterial blood was collected via chronically implanted catheters in the dorsal aorta; these catheters also allowing administration of the various acids and bases.

The first series of experiments were designed to determine ventilatory responses to high ambient $PCO_2$ levels (hypercapnia) and the effect on regulation of arterial blood pH. Both short-term (up to 8 hours) and long-term (up to 72 hours) exposures were studied. $PaO_2$ levels remained saturated, or nearly so, throughout these experiments.

The general response to high $PCO_2$ levels is an increase in the ventilatory stroke volume, this being mainly due to an increase in $V_G$ rather than $VR$. Compensation of ventilation during the sustained hypercapnia is slow, taking up to 3 days. Arterial $H^+$ levels increased during $CO_2$ exposure, increasing from a control level of $11.8 \pm 0.5$ to $41.0 \pm 3.5 \text{ nM/L}$ within 5 minutes. There is a gradual decrease in arterial $H^+$ concentration such that at 72 hours it is near normal. The time course of compensation for both $V_G$ and pHa coincide.

The hypercapnia experiments indicate that in the face of an increase in ambient $PCO_2$ trout do not adjust the $PCO_2$ difference ($\Delta PCO_2$) between arterial blood and water.
PaCO₂ changes in proportion to the change in P CO₂ such that PaCO₂ is always about 2 mm Hg above ambient, demonstrating that ΔPCO₂ is not affected by changes in ventilation.

The change in arterial blood pH is shown to be related to the transfer of CO₂ rather than by a transfer of H⁺ ions from water to blood.

Arterial blood pH is regulated via adjustment of blood HCO₃⁻ levels, adjustment being in the order of 2 - 3 days. HCO₃⁻ can be regulated, or adjusted by either the kidney or the gills. The role of the kidney was shown to be minor in the adjustment of a NaHCO₃ induced alkalosis. Uptake of HCO₃⁻ is shown to occur when fish are placed in NaHCO₃ containing water, demonstrating the role of the gills in the amelioration of arterial blood pH. These observations are discussed in relation to a HCO₃⁻/Cl⁻ exchange.

The ventilation volume is dependent on an increase in PaCO₂ and/or P CO₂ and not to pHa or pH⁺. A decrease in pH⁺, although causing a fall in pHa, has only a delayed effect on \( \dot{V}_G \).

The response in \( \dot{V}_G \) is transient. It is postulated that receptors are either adapting or are not located in the blood or water but in another compartment whose contents or properties change in proportion to ventilation.

It is hypothesized that a chemosensitive area may exist on the ventrolateral surface of the medulla as in mammals. Perfusion of the cranial cavity of trout with mock CSF, in which CO₂-HCO₃⁻ was altered, did not elicit respiratory responses. These experiments do not preclude the existence
of medullary chemoreceptors.

These results are consistent with the hypothesis that ventilation in trout is dependent on the CO$_2$ tension within the body or elsewhere and that blood pH levels are regulated via ionic exchange mechanisms at the gill surface, rather than by ion exchange at the kidney or by diffusive washout of gaseous CO$_2$ via ventilation.
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INTRODUCTION

Bodily function is dependent on a regulated arterial pH. Slight changes in arterial pH dramatically affect the dissociation state of protein moieties, thereby affecting such functions as enzyme activity and gas transport in the animal. For this reason the regulation of the blood plasma H⁺ ion concentration is one of the most important aspects of homeostasis.

This manuscript deals with the chemical reactions and the physiological responses when arterial pH and PCO₂ levels are altered by either increasing ambient CO₂ levels or by the injection of acids and bases. Since acids and bases are substances capable of delivering or accepting H⁺ ions, the concentration of H⁺ depends primarily on the amount of the various acids present in the body fluids. The most important of these is carbonic acid which is derived from CO₂, one of the major end products of metabolism. Hence, the equilibrium equations of H₂CO₃ and its reactions with other buffer substances in the blood, for example haemoglobin, form the chemical basis of CO₂ transport and acid-base balance (Albers, 1970). CO₂ and carbonic acid are related as follows:

\[ \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \]

The classical description of the acid-base status of the blood starts with the Henderson-Hasselbalch equation:

\[ \text{pH} = \text{pK}^1 + \log \frac{[\text{HCO}_3^-]}{\alpha\text{CO}_2 \cdot \text{PaCO}_2} \]

where pK¹ is the negative logarithm of the apparent first
dissociation constant of carbonic acid, $\alpha_{\text{CO}_2}$ is the solubility coefficient of $\text{CO}_2$ in plasma, and $\text{PaCO}_2$ is the arterial $\text{CO}_2$ tension. According to this equation, pH is a function of the $\text{PCO}_2$ and $\text{HCO}_3^-$ concentration. From a chemical point of view the $\text{PCO}_2$ and the $[\text{HCO}_3^-]$ are not mutually independent variables since as the $\text{PCO}_2$ rises the increase in carbonic acid is buffered by the nonbicarbonate buffers and thus the $[\text{HCO}_3^-]$ increases accordingly (Astrup et al, 1966).

In respiratory physiology the blood is considered as a buffer in equilibrium with a gas phase. The pH of the blood can thus be altered in two ways: by changing either the $\text{PCO}_2$ or the blood plasma concentration of nonvolatile acid or base. The concentration of $\text{CO}_2$ and the chemical composition of the blood are therefore the principal factors governing acid-base balance. The former can be controlled by ventilation, the latter by the action of various excretory mechanism.

It has been established that poikilotherm animals regulate a constant degree of alkalinity, or $[\text{OH}^-]/[\text{H}^+]$ ratio, in relation to the neutral point of water (Robin, 1962, Rahn, 1967, Rahn and Baumgardner, 1972, Reeves, 1969, 1972, and Howell et al, 1970). Thus as temperature increases, arterial pH decreases. This preserves a constant ratio between $\text{OH}^-$ and $\text{H}^+$ ions even though their absolute concentrations will vary greatly. It was postulated that this apparent constancy is necessary to control the dissociation state of certain protein moieties, particularly the imidazole groups of histidine, since their dissociation remains the same
when pH varies in the observed manner. In other words, arterial dpH/dT parallels dpK/dT for protein systems in poikilotherms. Homeotherms possess a blood pH which is situated on the pH-temperature continuum characteristic of the poikilotherm.

Cross et al. (1969), Randall and Cameron (1972), Truchot (1973), and Howell et al. (1973) have shown that dogfish, trout, shore crabs, and aquatic invertebrates, respectively, have a considerably lower PCO₂ than airbreathers at any temperature, as postulated earlier by Rahn and West (1963), and Rahn (1966). The PaCO₂ of waterbreathers cannot theoretically rise higher than 5.0 mm Hg above ambient. The reason for this limitation is to be found in the different solubilities of O₂ and CO₂ in water and the resultant enormous ventilation required to achieve saturated arterial O₂ tensions. According to Rahn (1966) the CO₂ tension in the gill will be 1.8 mm Hg and stands in contrast to the PCO₂ of 50 mm Hg in the alveolus with the same O₂ tension of 100 mm Hg, assuming equal exchange between O₂ and CO₂, i.e. R = \( \frac{V_{CO₂}}{V_{O₂}} \) = 1.0. Fish which are obligatory airbreathers have PCO₂ values that are approximately six times those found in waterbreathing fish (Garey and Rahn, 1970). Similar differences in CO₂ tensions are found between the strictly aquatic tadpole and the airbreathing adult amphibian (Erasmus et al., 1970).

Arterial pH can either be regulated by adjustment of the HCO₃⁻ concentration, or by manipulation of the arterial CO₂ tension in face of a rise in temperature. This is
because the $\frac{1}{dT}$ for the $\text{HCO}_3^- - \text{H}_2\text{CO}_3$ system is small compared with that for arterial blood pH (Randall and Cameron, 1972), hence $\text{PaCO}_2 \propto \text{CO}_2$ or plasma $[\text{HCO}_3^-]$ must adjust with temperature (Rahn, 1967, and Howell et al, 1970).

In various airbreathing poikilotherms, as shown by Reeves (1969) and Jackson (1973), the $\text{PaCO}_2$ varies directly with temperature, as a result of a relative hyperventilation at lower body temperatures. Hence, the observed dependence of pH on temperature in airbreathing poikilotherms is regulated physiologically by ventilatory control of $\text{PCO}_2$ (Rahn, 1967, Jackson, 1973).

Fish, however, cannot compromise $\text{O}_2$ transfer by an increase in ventilation or reduction in the $\dot{V}_G/\dot{V}_\text{CO}_2$ ratio in order to regulate $\text{PaCO}_2$, and hence arterial pH (Randall and Cameron, 1972). A reduction in the $\dot{V}_G/\dot{V}_\text{CO}_2$ is accompanied by a reduction in $\text{PaO}_2$ and an impairment of $\text{O}_2$ delivery to the tissues. Fish regulate pHa in the face of a temperature rise by adjustment of plasma $\text{HCO}_3^-$ and pH is independent of ventilation volume and $\text{O}_2$ transfer rates. Thereby the $[\text{OH}^-]/[\text{H}^+]$ ratio remains constant.

In both poikilothermic and homeothermic airbreathers the difference between ambient air and arterial $\text{PCO}_2$ levels can be adjusted by altering ventilation. Fish appear unable to regulate $\text{PaCO}_2$, and hence the $\text{PaCO}_2 - P_1\text{CO}_2$ gradient, in face of a rise in ambient $\text{PCO}_2$ by alteration of ventilation. Increases in ventilation volume above the resting level does not cause any change in arterial blood pH or total $\text{CO}_2$ content (Randall and Cameron, 1972).
The $\text{PaCO}_2 - \text{P}_1\text{CO}_2$, or $\Delta\text{PCO}_2$ difference, and CO$_2$ output in fish has been shown to be affected by changes in the ionic composition of the inspired water (Dejours et al., 1968, and Dejours, 1969). This finding is best explained by the existence of an obligatory exchange of $\text{HCO}_3^-$ for Cl$^-$ at the gill surface. When a freshwater fish (goldfish) is switched from a high to a low Cl$^-$ solution, the excretion of CO$_2$ drops off sharply (Dejours, 1969). In addition, when trout are subjected to high levels of CO$_2$, Lloyd and White (1967) observed a concomitant decrease in plasma Cl$^-$ concentration. These findings suggest then that in freshwater fish, Cl$^-$ influx is linked to $\text{HCO}_3^-$ efflux, thereby maintaining electroneutrality in the plasma. Evidence of this ion exchange system has been presented by Maetz and Garcia-Romeu (1964) in the goldfish and Kerstetter and Kirschner (1972) in trout. Regulation of pH in freshwater fish can thus be postulated to be by anion exchange rather than by adjustment of $\text{PaCO}_2$, as in mammals.

As airbreathers increase their ventilation in response to an increase in $\text{PaCO}_2$, the question arises: what occurs in a waterbreather, in which the $\text{HCO}_3^-$ concentration rather than the arterial CO$_2$ tension is adjusted to regulate arterial blood pH? Is ventilation related to changes in arterial FCO$_2$ levels or to arterial blood pH levels? What is the time course in adjustment of pH during sustained exposure to high ambient CO$_2$ levels? To test these problems rainbow trout were subjected to various acid-base disorders, namely metabolic acidosis and alkalosis and respiratory
acidosis. From results obtained it is hoped that a better understanding of acid-base regulation and its relation to ventilation is obtained in view of the animal kingdom as a whole.
ACID-BASE DISTURBANCES AND THEIR DEFINITIONS

**Metabolic acidosis**: the reduction of pH associated with accumulation of nonvolatile acid in the body, or a decrease in the bicarbonate fraction.

**Respiratory acidosis**: the reduction of pH caused by an increase in carbonic acid relative to bicarbonate.

**Metabolic alkalosis**: occurs when there is an increase in the bicarbonate fraction, with either no change or a relatively smaller change in the carbonic acid fraction. There is an increase in pH.

**Respiratory alkalosis**: an increase in pH as a result of a decrease in the carbonic acid fraction with no corresponding change in bicarbonate.

GENERAL MATERIALS AND METHODS

In order to test the relationships between the arterial acid-base status and ventilation in the rainbow trout, four series of experiments were performed. The first series was designed to determine a fish's ventilatory response to high ambient CO\textsubscript{2} levels (hypercapnia). Both short-term (up to 8 hours) and long-term (up to 72 hours) exposure were studied, measuring arterial PCO\textsubscript{2}, pH, PO\textsubscript{2} levels and ventilatory rates and volumes. The second series of experiments were executed to further illuminate the relationship between ventilation and acid-base status of blood as observed during the hypercapnic experiments. Both metabolic acidosis and alkalosis were induced through injection of acids and bases (HCl, NaOH, NaHCO\textsubscript{3}) via the dorsal aorta. Respiratory responses and arterial acid-base parameters were followed under this experimental regime. One of the compensatory mechanisms that protect an animal from acute change in extracellular pH with injection of NaHCO\textsubscript{3} is either by excretion of HCO\textsubscript{3}\textsuperscript{-} or by CO\textsubscript{2} elimination. Since fish possess both renal and extrarenal (gills) pathways, both expired water total CO\textsubscript{2} and urine total CO\textsubscript{2} were monitored following injection of labelled NaH\textsuperscript{14}CO\textsubscript{3}. Radioactivity in either expired water or urine and their relative ratios would demonstrate the route of CO\textsubscript{2} excretion in the trout. The perfusion of the cranial cavity with mock cerebrospinal fluid (CSF) comprises the fourth series of experiments. These tests were devised in light of information made available from observations made on mammals. The perfusion
of mock CSF, with altered $\text{CO}_2 / \text{HCO}_3^-$ ratios near $\text{CO}_2$, $\text{H}^+$ sensitive areas on the ventral surface of the medulla in mammals evoke responses in ventilation. The possibility of this control system existing in the trout was, therefore, tested.

All experimental work was carried out on adult rainbow trout, *Salmo gairdneri*, obtained from the Sun Valley Trout Farm in Port Coquitlam, B. C. The fish were maintained in large, circular holding tanks at the University of British Columbia, supplied with dechlorinated fresh water at seasonal temperatures. Feeding was carried out on a regular basis on a mixed diet of trout pellets (J.R. Clark Co.) and canned crab meat. Tests were conducted from early May to December, 1972, and March to July, 1973.

**OPERATING PROCEDURE**

Experiments in this study involved prolonged surgery. To accomplish such surgery an operating table similar to that described by Smith and Bell (1964) was used. Fish were netted and immersed in a solution of 1:10,000 tricaine methane sulfonate (*M.S. 222*, Sandoz). After swimming and opercular movements had ceased, the animal was then placed ventral side up on the operating table and water, containing anaesthetic, perfused continuously over the gills. At the end of surgery, the fish were transferred to ventilation boxes and within 2 to 5 minutes regular breathing movements commenced. Actual experimentation did not commence until a recovery period of 20-40 hours had passed. Such recovery is necessary as these surgical procedures lead to considerable
trauma, including acidosis (Wedemeyer, 1970).

**Dorsal Aortic Cannulation**

The dorsal aorta was cannulated in the midline at its point of intersection with the second efferent branchial arteries as described by Smith and Bell (1964). Intramedic (Clay Adams, Inc.) PE 60 tubing, tipped with a #21 Huber point needle and filled with heparinized (10 I.U./ml) Cortland saline (Wolf, 1963), was passed through a hole punched between the nares of the snout, the hole being lined with a short length of heat flared PE 190 tubing (Fig. 1). This cannulation allowed both sampling of blood for determination of arterial PO$_2$, PCO$_2$, pH, and injection of the various acids and bases. Such a cannulation proved quite secure and dependable, allowing blood sampling for up to 4 weeks.

**Urinary Cannulation**

A urinary cannula was constructed by glueing with epoxy resin, approximately 1.5 cm of PE 190 around an 80 cm length of PE 60 about 1.5 cm from the tip of the latter (Wood, 1971, Wood & Randall, 1973). Numerous small holes were punched in the proximal 0.5 cm of PE 60 with a heated #22 needle (Fig. 2). Successful cannulation was facilitated by having a variety of preconstructed catheters on hand, representing a range of flare widths, jacket lengths, and proximal tip lengths. A cannula could then be fitted to the particular urogenital morphology of a specific trout. This latter structure varies greatly between individual trout.

Cannulation was performed by opening the aperture of
the urogenital papilla with a pair of fine forceps, and inserting the proximal tip of the catheter dorsal until the proximal flange of the PE 200 jacket was just inside the papilla. Immediately following insertion, 0.5 ml of saline was infused into the urinary system and the cannula plugged with a pin. The dead space of the cannula, at 80 cm, equals 0.35 ml (4.4 μl/cm, PE 60). This operation was carried out only on those fish subjected to radioactive NaH\textsuperscript{14}CO\textsubscript{3}.

**Cranial Cavity Cannulation**

The cranial cavity, or extradural space (Davson, 1967) was perfused using a mock spinal fluid (Mitchell et al., 1963) modified for rainbow trout. #20G short bevel needles, fitted with PE 50 tubing, were passed into the foramen magnum from the dorsal surface of the head (at the margin at which scale development commences) and dorsolaterally passed through the pterotic bone structure on both sides of the cranium (Fig. 3). The former cannulation perfused the area directly dorsal to the medulla oblongata and the pterotic cannulation allowed perfusion laterally in the area between the optic lobe and the cerebellum on either side of the brain. Mock spinal fluid could be injected via any of the three cannulae; thus one cannula would serve as an inlet, the other two as outlets of perfusate.
Figure 1

Detail of the dorsal aortic cannula in rainbow trout. The dorsal aorta cannula provides sampling of arterial blood and offers a pathway for the injection of the various acids and bases used in this study.
PE 60 TUBING
PE 200 JACKET
HEAT FLARED TIP
STITCH
no.21 HUBER POINT NEEDLE IN DORSAL AORTA
Figure 2

Detail of a cannula implanted into the urinary bladder of a rainbow trout. This cannula allowed collection of urine. Placement of the ligatures was such that movement anteriorly and posteriorly was reduced to a minimum, thereby reducing chance of damage done to the delicate urogenital papilla. The diagram is after Wood, 1971.
PE 60 TUBING

PE 190 JACKET

DISTAL FLANGE

PROXIMAL FLANGE

HEAT POLISHED TIP

STICH IN VENTRAL BODY WALL

UROGENITAL PAPILLA

ANUS

ANAL FIN

LIGATURES

PELVIC FIN

ANTERIOR
Implantation of cannulae into the cranial cavity or extra-dural space of rainbow trout allowing perfusion and evacuation of fluid. Dorsal and lateral views. 2 cannulae were implanted dorsolaterally on both sides of the head. The dorsolateral cannulae pass through the pterotic bone structure, the dorsal cannula into the foramen magnum.
Detail of experimental setup used for most of this study. The first stage header tank received dechlorinated water from a tap. The two header tanks were necessary to maintain a steady waterflow to the ventilation box. A double gas regulator system was required to stabilize gas flow from the compressed CO\textsubscript{2} in air tank. The ventilation box allowed direct measurement of ventilation volume via an oral membrane stitched around the margin of the mouth to separate inspired from expired water. Movement of the fish either forward or backward is prevented by a ligature attached to the snout of the fish thereby preventing the fish from tearing loose. The holding tube is covered in black plastic.
1st STAGE HEADER TANK

2nd STAGE TANK

1° GAS REGULATOR

2° GAS REGULATOR

5% CO₂ IN AIR

OVERFLOW

ORAL MEMBRANE

HOLDING TUBE

WATER LEVEL
Figure 5

This experimental setup was used for the radiotracer study. A dechlorinator, consisting of an activated charcoal filter, was used. NaH$_{14}$CO$_3$ was injected via the dorsal aortic cannula. Urine and expired water were analyzed for radioactivity. The flow was adjusted at a rate of 200 ml/min. 50 ml of expired water was collected in a flask below a layer of hexane.
Oral Membrane Attachment

A technique which enables direct measurement of ventilation volume \( \dot{V}_G \), involving attachment of a portion of a rubber surgeon's glove to the mouth, was developed by Davis and Cameron (1970). In this technique, a 12 cm circle, which includes the thumb, was cut from a number 8½ latex glove. The tip was cut to fit the fish head so that it covered the dorsal aortic cannula at its point of emergence but left the eyes uncovered. The membrane was stitched around the margin of the fish's mouth. When properly cut and positioned the membrane exerted little tension on the head or jaws. Fish with ill-fitting membranes, or those unable to execute proper ventilatory movements were rejected. Some fish were tested for possible ventilatory impairment. No such impairment was found as the dorsal aorta blood remained oxygen saturated, or nearly so.

Fish with attached oral membranes were placed in a lucite box (Fig. 4) divided into 2 connecting chambers. Once the membrane was attached to the partition between the 2 chambers, it served as a barrier, separating inspired and expired water. The top and sides of the holding tube was covered with black plastic to prevent fish receiving visual stimuli.

Following the operation the drain at the ends of the box could be set at various levels by sliding it up or down through a rubber stopper. Positive pressure in the buccal cavity was provided by raising the drain at the head end of the box. This was used to facilitate ventilation during
recovery from the operation.

After the 20-40 hour recovery period following surgery the drains in the box were levelled and the fish were allowed to adjust for at least an hour in order to establish a normal breathing pattern. Ventilation rate (VR), ventilation volume (V̇G), inspired oxygen and carbon dioxide tension (P̄IO₂, P̄ICO₂), expired oxygen tension (P̄E₂O₂), and blood parameters (pHa, PaCO₂) were then measured at intervals. Ventilation volume was measured by collecting the water spilling over the rear drain as the fish breathed. Water was collected for 1 minute and ventilation rate was simultaneously determined by counting mouth movements.

EXPERIMENTAL SERIES

I. Hypercapnia

Fish successfully operated upon were placed in V̇G boxes. Before subjecting the fish to an increase in ambient P̄ICO₂, the animals were allowed to acclimate to the adjusted water levels in the boxes for at least 1-2 hours. Any fish which failed to demonstrate proper respiratory movements were rejected. Fish were supplied dechlorinated water directly from the tap system during non-experimental periods. To ensure stability of hypercapnic waters during the actual experiment, double systems of both gas and water were constructed. The former consisted of a primary and secondary gas regulator system, the latter of a first and second stage header tank. Accurate flows could be maintained during the entirety of the experiments, which in some cases lasted up to 72 hours (Fig. 4).
Once the level of CO\(_2\) in the secondary header tank had stabilized at 5 mm Hg PCO\(_2\), the water inlets were switched such that water containing the high CO\(_2\) level passed directly into the head end of the V\(_G\) box. In order to reduce the response time of the fish subjected to this high CO\(_2\) water, the volume of the head end of the V\(_G\) box was reduced by placing a one liter plastic bottle in it. Introduction of high CO\(_2\) water was taken as time zero.

Arterial blood, obtained by applying slight suction onto the cannula, was collected at predetermined times during the course of the experiment. Blood was analyzed almost immediately following exsanguination and analyzed for pH every sampling period and for PaO\(_2\), PaCO\(_2\) every few sampling periods. This sampling technique prevented undue hemodilution (lowering of haematocrit) during the experiment.

Water samples for P\(_T\)CO\(_2\) were taken roughly every 15 minutes. O\(_2\) levels were monitored at approximately the same time intervals. In all experiments O\(_2\) levels were kept between 70 - 100% saturation.

At the end of the experiment, cannulae and oral membranes were removed, and the fish weighed. Oral membranes were closely inspected for tears and holes and results of those with damaged membranes were rejected.

II. Metabolic acid-base disturbances

In this experimental series, metabolic acidosis and alkalosis was induced via injections of acids and bases into the dorsal aorta cannula. In order to reduce the degree of hemodilution, all compounds injected into the fish were done
so in 1 ml volumes. To produce the two acid-base disorders, the following concentrations of acids and bases were used:

- 0.025 mM HCl/ml of saline
- 0.025 mM NaOH/ml of saline
- 1.000 mM NaHCO$_3$/ml of saline

and as a control 0.025 mM NaCl/ml of saline

These concentrations were adequate to induce the two metabolic acid-base disorders.

As in the hypercapnic experiments, fish were allowed to acclimate to the adjusted water levels in the $V_G$ boxes for up to 2 hours. Fish had already recovered from the operation over a 20 - 40 hour period. Control values for $V_G$, VR, pH$a$ and PaO$_2$ were taken at the end of this time. Fish were maintained on dechlorinated tap water during the course of the experimental and control periods. To reduce unwanted stimulation of fish a perfusion rate of 1 ml/minute was used in the injection of the compounds. Commencement of administration was taken as time zero for the experiments.

At the end of each run fish were weighed and oral membranes inspected for possible leaks.

III. CSF perfusion

In this third experimental series a mock spinal fluid modified for rainbow trout, after Mitchell et al (1963), was used as a perfusate for the cranial cavity of the fish. The composition is as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>125 mM/liter</td>
</tr>
<tr>
<td>NaHCO$_3$</td>
<td>10(5,25) &quot; &quot;</td>
</tr>
<tr>
<td>KCl</td>
<td>3.5 &quot; &quot;</td>
</tr>
</tbody>
</table>
The CO₂/H⁺ responsiveness of the sensitive areas was tested by altering the PCO₂ or [HCO₃⁻] of the mock spinal fluid. This was achieved by bubbling either 1% or 5% CO₂ (a PCO₂ of 7.5 and 35.0 mm Hg, respectively) or by altering the [HCO₃⁻] of the perfusate. Injection was at a very slow rate of approximately 1 ml per 3 to 5 minutes and continued until a total of 5 to 10 ml of mock spinal fluid was perfused.

As in the previous experiments fish successfully operated upon were placed in a black plastic box, similar to the one used in Fig. 5, and allowed to recover 20 - 40 hours. All fish were maintained on dechlorinated tap water. One of the cranial cavity cannulae, filled with mock CSF, was attached to a 5 ml syringe via a 3-way stopcock. As stated, the experimental procedure consisted of slow perfusion of mock CSF with different CO₂/HCO₃⁻ ratios into the cranial cavity via either one of the 3 cannulae (Fig. 3). Recordings were made of the ventilation rate by manually counting respiratory movements. In these experiments oral membranes were not attached thus no ventilation volumes could be measured.

IV. Radiotracer studies

Fish that were successfully operated upon were placed in a watertight, lucite ventilation chamber. All sides were covered with black plastic to reduce visual stimulation,
since agitation of fish results in an increased ventilation rate and a diuresis, resulting in anomalous radioactivity readings. The ventilation box was supplied with aerated, dechlorinated fresh tap water from a system consisting of a head tank and an activated charcoal filter (Fig. 5). The temperature and $P_{O_2}$ of the system never varied more than $6.0 \pm 0.5 \degree C$ and $146 \pm 2 \text{ mm Hg}$, respectively. A steady flow rate of 200 ml/min was maintained by adjustment of a stopcock on the exit end of the ventilation box.

$\text{NaH}^{14}\text{CO}_3$ (Amersham, Searle, Inc.) was prepared such that a 1 ml sample of saline contained 1 mM $\text{NaHCO}_3$ with a specific activity of 5 $\mu C$ (see Appendix A). This amount provided adequate $\text{NaHCO}_3$ to induce metabolic alkalosis and enough activity for detection considering the water flow rate of the system and the time taken for the fish to compensate for the metabolic disorder. The total time for injection approximated 1 minute. Start of administration was taken as time zero.

Both urine and expired water samples were analyzed by a technique resulting in a $\text{BaCO}_3$ precipitate, as described in Appendix B. The principle of this technique is collection of expired $\text{CO}_2$ or bicarbonate in an alkaline trap and subsequent precipitation of the bicarbonate-carbonate with a $\text{BaCl}_2\cdot\text{NH}_4\text{Cl}$ solution. The precipitate, weighing from 1 to 3 mg was filtered using a millipore system. Millipore filters proved very suitable since accurate and reproducible readings of beta radiation ($^{14}\text{C}$) are possible due to the orientation of the precipitate on the filter surface.
Surface retention of the BaCO$_3$ prevents masking of the low energy beta. Since the refractive index of the millipore filter (cellulose ester) is 1.50 ± 0.10, it is totally transparent in a toluene based scintillation fluid, resulting in total geometry scintillation counting.

Spectrafluor PPO-POPOP, a concentrate liquid scintillator, was used as a basis of the scintillation "cocktails", at a concentration of 4 g POPOP, 50 mg PPO in toluene. Benakis (1970) found this to be a sufficiently efficient counting fluid for Ba$^{14}$CO$_3$. A blank, consisting of unlabelled BaCO$_3$ (1.5 mg) on a millipore filter was used for background count determination. The average background count varied 35 ± 2 cpm.

It was initially intended to calculate the specific activity (i.e. the $^{14}$CO$_2$/$^{12}$CO$_2$ ratio) from the weight of BaCO$_3$ precipitated. This proved to be fruitless: no correlation was obtained between the weight of BaCO$_3$ and the amount of radioactivity and therefore this analytical approach was abandoned.

Urine sampling proved fairly inconsistent, providing 2 ml of fluid one moment, none at another time during the experiment. The best sampling technique was as follows: if the fish did not contribute any urine, some saline was flushed into the bladder. The resultant saline/urine sample was then drawn into a NaOH containing (about 0.02 ml of 5 M NaOH) syringe. The urine was then injected below the hexane layer into 50 ml of water, and subsequently treated with NaOH and the BaCl$_2$-NH$_4$Cl mixture.
DATA RECORDING

Gas tensions in water and blood were measured using a Radiometer electrode system. PO$_2$ determinations were made with Radiometer type E 5046 oxygen electrode, PCO$_2$ with a Radiometer type E 5036 carbon dioxide electrode. For pH measurements, a capillary electrode, type G297/G2 was used. All electrodes were contained in thermostatted cells and maintained at the same temperature as the experimental animal. Calibration of both CO$_2$ and O$_2$ electrodes was carried out by exposure to moist gas samples of known tensions from a gas mixing pump system (Wosthoff, Germany) and a compressed CO$_2$ cylinder for CO$_2$ equilibrium and from a compressed N$_2$ cylinder and water saturated air for O$_2$ equilibration. The pH electrode was equilibrated using Radiometer precision buffers.

The oxygen and pH electrodes were hooked up to a Radiometer PHM 71 Acid-Base Analyzer. The latter, a later model of meter, features a more stable amplifier, important in measuring the low partial pressures of CO$_2$ encountered.

Blood was exsanguinated anaerobically by applying suction onto the dorsal aorta cannula and drawing the blood into a 1 ml syringe. The initial 0.05 ml of blood was rejected. Although blood pH is practically unaffected by small dilutions, the PCO$_2$ (and bicarbonate concentration) of plasma falls in direct proportion to, or slightly more than the dilution (Siggaard Anderson, 1961).

To provide consistent results, prior to introduction of a blood or water sample, the electrodes were air-dried.
The sample was then introduced into the cuvettes and readings taken after a set time. 4 minutes was required for stabilization to occur in the case of CO\textsubscript{2} measurements, 3 minutes for O\textsubscript{2}. pH values were read when the needle stabilized.

Quench curve determination and sample analysis for radioactivity were monitored in a liquid scintillation system consisting of a nuclear-Chicago Unilux IIA, as discussed in Appendices C and D.

Since it is not mathematically correct to take arithmetical means of pH values (Davonport, 1969) and that pH plotted on a linear scale distorts the physiological reality, all arterial pH values were converted to corresponding hydrogen ion concentrations. Statistics were executed on the latter, and reconverted back to pH values for comparison purposes. Standard errors shown thus apply only to hydrogen ion concentrations, not pH values.

Plasma HCO\textsubscript{3}\textsuperscript{-} concentrations were calculated from the Henderson-Hasselbalch equation, using measured values of pH\textsubscript{a} and PaCO\textsubscript{2}. Values of pK\textsuperscript{1} are those from human plasma since trout and human plasma have a similar ionic strength (Albers, 1970). αCO\textsubscript{2} is assumed the same as CO\textsubscript{2} solubility in 150 mM NaCl/L, and is expressed in mM/ ml/mm Hg (trout plasma = 130 mM NaCl/L).
SYMBOLS AND ABBREVIATIONS USED IN THIS STUDY

\[ V_G \] - volume of water passed over the gills/minute (ventilation volume)

\[ V_R \] - mouth or opercular closures/minute (ventilation rate)

\[ V_{sv} \] - volume of water passed over the gills/breathing cycle (ventilatory stroke volume)

\[ V_E \] - in airbreathers, total amount of new air into the respiratory passages/minute (respiratory minute volume)

\[ P, p \] - partial pressure of gas in mm Hg

\[ \Delta F CO_2 \] - PaCO_2 - PI CO_2 difference or gradient

\[ \alpha \] - solubility coefficient for gas in water or plasma

\[ [H^+] \] - hydrogen ion concentration, in nanomols/liter

\[ dpm \] - radioactive decay rate, disintegrations/minute

\[ dps \] - radioactive decay rate, disintegrations/second

\[ \mu C \] - microCurie, 3.700 \times 10^4 dps; based on rate of nuclear disintegration

\[ S.E. \] - standard error

Subscripts

a - arterial
pl - plasma
I - inspired
E - expired
RESULTS: EXPERIMENTAL SERIES

I. THE EFFECT OF INCREASED CO₂ TENSION ON ARTERIAL pH, PCO₂ AND ON VENTILATION.

Hypercapnia was induced and maintained by subjecting 13 fish, weighing 243.9 ± 10.5 g, to water at 5.20 ± 0.33 mm Hg PCO₂. The data presented in Figures 6 and 7 are similar, the former showing detail of short-term exposure, the latter the effect of long-term exposure. Because of the large individual variation in both ventilation rate and volume (especially in the former) and small sample size (n=5), data in Fig. 7 represent that of 1 fish only (no. 41) at both 48 and 72 hours. Values of all 5 fish are portrayed in Table I.

The general response to high CO₂ is an increase in both VR and $\dot{V}_G$ and a gradual return to normocapnic levels in 2 or 3 days. However, the increase in $\dot{V}_G$ is much more pronounced than that of VR, changing from a resting level of 47.1 ± 2.7 ml/min to a maximum of 254.9 ± 29.3 ml/min at 5 minutes. This represents a 5-fold increase in ventilation volume, compared with only a slight increase in VR. This is reflected in Fig. 20, where a linear relationship exists between ventilation volume and ventilatory stroke volume showing that the increase in $\dot{V}_G$ is chiefly due to an increase in Vsv. At 72 hours of exposure, both VR and $\dot{V}_G$ are generally back to control levels, demonstrating that compensation has occurred.

The arterial hydrogen ion concentration increased from a mean control value of 11.8 ± 0.5 to 41.0 ± 3.5 nM/L
(a pH of 7.93 to 7.39) within 5 minutes, thereby producing acute respiratory acidosis (Figs. 6, 7). With time, during continual exposure to high CO\textsubscript{2}, there is a gradual decrease in arterial \([H^+]\) such that at 72 hours it is near normal. There is suggestion of a relationship existing between \([H^+]a\) or PaCO\textsubscript{2} and ventilation because of the synchronous trends exhibited.

Arterial PCO\textsubscript{2} levels were measured relative to the PCO\textsubscript{2} of inspired water in 2 fish (no. 58, no. 59): short-term (8 hours) and long-term (72 hours), respectively. Both fish were without attached oral membranes. It is shown that fish cannot reduce their PaCO\textsubscript{2} with respect to the prevailing inspired CO\textsubscript{2} tension and that the PaCO\textsubscript{2} - P\textsubscript{i}CO\textsubscript{2} difference (ΔPCO\textsubscript{2}) is re-established in 1\(\frac{1}{2}\) to 2 hours (Figs. 8, 9). At 5 min of exposure there exists an apparent negative ΔPCO\textsubscript{2} of -0.4 and -0.1 mm Hg for fish 58 and 59, respectively. It is to be kept in mind that these PCO\textsubscript{2} values are from the dorsal aorta and is thus probably not indicative of CO\textsubscript{2} tensions actually existing at the gill.

Although the ventilation rates are lower for fish having no attached oral membranes, these 2 fish show the same general trend in response to hypercapnia. There is an initial hyperventilation which may last up to 24 hours followed by a slow return to basal ventilatory levels at 72 hours. However, the initial peak and following decrease in VR obtained in "membraned" fish is not seen, showing possible interference of the ventilatory mechanism by the oral membrane. The pattern in arterial \([H^+]\) levels are
also similar between the 2 groups of fish.

Since the capacity of trout to reduce the \( \text{PaCO}_2 - \text{PICO}_2 \) difference is small as indicated, how does the animal adjust its pH to normal levels in face of maintained hypercapnia? To answer this a pH - HCO\(^{-}\) diagram (Fig. 10) was constructed to illuminate the relation between pH, \( \text{PCO}_2 \) and HCO\(^{-}\) from the Henderson-Hasselbalch equation. Since \( \text{PaCO}_2 \) and \( \alpha \text{CO}_2 \) are held constant (both temperature and ionic strength did not change in the animal), HCO\(^{-}\) must change in order to adjust pH.

Arterial \( \text{PCO}_2 \) and pH values of 4 different fish (no. 56 to 59) were plotted following the time course during hypercapnia. The line A - C, connecting these points, is assumed to represent the blood buffer line. This line is an approximation only since it should show the characteristic upward slope to the left, where there is a slight increase in [HCO\(^{-}\)] and a large increase in the [H\(^+\)] (Refsum, 1971). The \( \text{PaCO}_2 \) values probably do not represent the actual arterial levels. This could be the result of a time lag in the HCO\(^{-}\) - CO\(_2\) system.

A fish subjected to \( \text{PICO}_2 \) levels of 5.0 mm Hg will follow this fitted line (A - C) in response to the raised CO\(_2\) levels. This is the immediate response up to approximately 1 hour (at C). After this period, as \( \text{PaCO}_2 \) has adjusted to about 7.0 mm Hg, an increase in [HCO\(^{-}\)] is seen. This increase follows the 7.0 mm Hg isopleth. Thus point C to B in Fig. 10 describes the animal's compensation to chronic, or sustained hypercapnia until pH is adjusted.
Figure 6

The effect of hypercapnia on the ventilation rate, ventilation volume and arterial hydrogen concentration. Hypercapnia was induced by subjecting 13 fish to a PCO$_2$ of 5.20 ± 0.33 mm Hg in water. The fish weighed 243.9 ± 10.5 g (8.9 ± 0.1 °C). Values are means ± 1 standard error. Time zero designates the start of the experiment. The values to the left of the dashed line designate control values.
Figure 7

The effect of hypercapnia on ventilation rate and volume, and arterial hydrogen concentration. 13 fish (243.9 ± 10.5 g, 8.9 ± 0.1 °C) were subjected to a PCO₂ of 5.22 ± 0.33 mm Hg. A single fish's values are plotted after the vertical dotted line; i.e. at 48 and 72 hours. Values are means ± 1 standard error. This figure is similar to that of Figure 6, up to 5 hours.
Table I

Ventilation rates and volumes, and arterial hydrogen ion concentration during long-term exposure to high $P_{\text{CO}_2}$. Values of fish no. 41 have been plotted in the previous diagram, Fig. 7.
<table>
<thead>
<tr>
<th>Time</th>
<th>Fish no.</th>
<th>V&lt;sub&gt;G&lt;/sub&gt; (ml/min)</th>
<th>VR (no./min)</th>
<th>V&lt;sub&gt;sv&lt;/sub&gt; (ml/breath)</th>
<th>pHa</th>
<th>[H&lt;sup&gt;+&lt;/sup&gt;]&lt;sub&gt;a&lt;/sub&gt; (nM/L)</th>
<th>P&lt;sub&gt;i&lt;/sub&gt; CO&lt;sub&gt;2&lt;/sub&gt; (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. 48 hours of exposure</td>
<td>41</td>
<td>75</td>
<td>70.6</td>
<td>1.07</td>
<td>7.85</td>
<td>14.1</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>63</td>
<td>76.4</td>
<td>0.83</td>
<td>7.82</td>
<td>15.1</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>76</td>
<td>63.5</td>
<td>1.20</td>
<td>7.89</td>
<td>12.9</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>64</td>
<td>94.5</td>
<td>0.68</td>
<td>----</td>
<td>----</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>62</td>
<td>84</td>
<td>70.6</td>
<td>1.19</td>
<td>7.81</td>
<td>15.5</td>
<td>5.1</td>
</tr>
<tr>
<td>II. 72 hours of exposure</td>
<td>41</td>
<td>59</td>
<td>71.4</td>
<td>0.83</td>
<td>7.99</td>
<td>10.2</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>55</td>
<td>73.6</td>
<td>0.75</td>
<td>7.87</td>
<td>13.5</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>48</td>
<td>64.5</td>
<td>0.74</td>
<td>7.94</td>
<td>11.5</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>54</td>
<td>87.6</td>
<td>0.62</td>
<td>----</td>
<td>----</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>62</td>
<td>102</td>
<td>70.2</td>
<td>1.45</td>
<td>7.99</td>
<td>10.2</td>
<td>4.2</td>
</tr>
</tbody>
</table>

\[ \bar{X} \pm S.E. \]

| I. 48 hours of exposure | 72.4 ± 3.5 | 75.1 ± 5.9 | 14.5 ± 0.8 | 5.00 ± 0.01 |
| II. 72 hours of exposure | 63.6 ± 10.9 | 73.5 ± 4.3 | 11.4 ± 0.9 | 4.7 ± .2 |
Figure 8

The effect of hypercapnia (5.0 mm Hg $PCO_2$) on arterial $CO_2$ tensions. Fish 58 (228 g, 9 °C) is shown. This fish did not have an attached oral membrane. Induction of hypercapnia is at time zero. The values of $PaCO_2 - PI CO_2$ ($\Delta PCO_2$) are shown immediately to the right of the vertical lines connecting arterial and inspired $CO_2$ tensions.
TIME (hours)

$P_{a\text{CO}_2}$

$P_{\text{CO}_2}$

$\text{mmHg}$

$\text{VR}$

$\text{no./min.}$

$\text{pH}_a$

FISH 58

$\text{CO}_2$
Hypercapnia and its effect on arterial CO$_2$ tensions during compensation of respiratory acidosis. Fish 59 (257 g, 9 °C) without an attached oral membrane. Values of ΔPCO$_2$ are shown adjacent to the lines connecting arterial and inspired CO$_2$ tensions.
Arterial pH - plasma $\text{HCO}_3^-$ concentration coordinates showing the isopleths for the two $\text{PaCO}_2$ values tested. The figure takes into account the changes in $\text{pK}^1$ of carbonic acid as a function of temperature and blood pH. The solubility of $\text{CO}_2$ remains constant because temperature and ionic strength did not change. $\text{PCO}_2$ values are in mm Hg. Arterial $\text{PCO}_2$, pH values of 4 fish (no. 56 to no. 59, inclusive) are included, as designated by the individual points. Letter A represents values of normocapnic fish, B those of fish which have compensated for the sustained hypercapnia.
Figure 11

The effect of low pH of water on the arterial pH and ventilation rate and volume. One fish, no. 65 (241 g, 12 ± 1 °C) was subjected to a pH of 5.0 maintained by addition of HCl to a recirculating tank system (volume 75 L). Exposure is at time zero.
Table II

Maintenance of fish in ventilation boxes for extended periods of time and the effect on ventilation rate and volume and arterial pH.
<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Fish</th>
<th>$V_G$ (ml/min)</th>
<th>VR (no./min)</th>
<th>$V_{sv}$ (ml/breath)</th>
<th>$pH_a$</th>
<th>$P_aO_2$ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>A</td>
<td>34</td>
<td>71.9</td>
<td>0.47</td>
<td>7.94</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>41</td>
<td>77.9</td>
<td>0.52</td>
<td>7.98</td>
<td>102</td>
</tr>
<tr>
<td>8</td>
<td>A</td>
<td>38</td>
<td>72.3</td>
<td>0.52</td>
<td>7.97</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>40</td>
<td>79.5</td>
<td>0.50</td>
<td>8.02</td>
<td>114</td>
</tr>
<tr>
<td>24</td>
<td>A</td>
<td>42</td>
<td>71.9</td>
<td>0.58</td>
<td>7.95</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>39</td>
<td>76.9</td>
<td>0.50</td>
<td>8.00</td>
<td>109</td>
</tr>
<tr>
<td>48</td>
<td>A</td>
<td>42</td>
<td>73.2</td>
<td>0.58</td>
<td>7.97</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>45</td>
<td>80.0</td>
<td>0.57</td>
<td>7.99</td>
<td>115</td>
</tr>
</tbody>
</table>
Table III

The effect of NaHCO$_3$ buffering of water on the responses of fish to high CO$_2$ levels. Fish no. 15 (277 g, $T = 13.0$ °C) was exposed to a 1% NaHCO$_3$ buffer system and the $P_1$CO$_2$ raised as in Figs. 6 to 9. The responses are noted in Part I. Fish no. 17 (251 g, $T = 13.0$ °C) was subjected to aerated 1% NaHCO$_3$ alone, and responses tabulated in Part II.
<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>TEMP. (°C)</th>
<th>$V_G$ (ml/min)</th>
<th>VR (breath/min)</th>
<th>Vsv (ml/min)</th>
<th>pH$a$</th>
<th>$[H^+]a$ (nM/L)</th>
<th>$P_{1}CO_2$ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. HIGH PCO$_2$ control</td>
<td>12.0</td>
<td>46</td>
<td>75.9</td>
<td>0.61</td>
<td>8.01</td>
<td>9.8</td>
<td>0.6</td>
</tr>
<tr>
<td>+ 5</td>
<td>&quot;</td>
<td>304</td>
<td>82.8</td>
<td>3.67</td>
<td>7.81</td>
<td>15.6</td>
<td>4.8</td>
</tr>
<tr>
<td>1% NaHCO$_3$ in water (fish no.15)</td>
<td>15</td>
<td>&quot;</td>
<td>212</td>
<td>90.2</td>
<td>2.35</td>
<td>7.88</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>&quot;</td>
<td>194</td>
<td>87.6</td>
<td>2.22</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>&quot;</td>
<td>206</td>
<td>92.3</td>
<td>2.23</td>
<td>7.83</td>
<td>14.7</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>12.5</td>
<td>246</td>
<td>93.0</td>
<td>2.64</td>
<td>7.86</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>&quot;</td>
<td>228</td>
<td>96.0</td>
<td>2.32</td>
<td>7.89</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>&quot;</td>
<td>224</td>
<td>96.8</td>
<td>2.31</td>
<td>7.92</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>&quot;</td>
<td>216</td>
<td>92.3</td>
<td>2.34</td>
<td>7.95</td>
<td>11.4</td>
</tr>
<tr>
<td>II. 1% NaHCO$_3$ control</td>
<td>13.0</td>
<td>48</td>
<td>92.3</td>
<td>0.52</td>
<td>8.00</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>aerated 5</td>
<td>&quot;</td>
<td>54</td>
<td>97.6</td>
<td>0.55</td>
<td>8.08</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>(fish no.17)</td>
<td>60</td>
<td>&quot;</td>
<td>70</td>
<td>103.4</td>
<td>0.68</td>
<td>8.11</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>&quot;</td>
<td>60</td>
<td>105.3</td>
<td>0.57</td>
<td>8.20</td>
<td>6.3</td>
</tr>
</tbody>
</table>
As an example, fish no. 59, under normocapnic conditions (\(\text{PaCO}_2 = 2.3 \text{ mm Hg}\)), would have a plasma \([\text{HCO}_3^-]\) of 10.0 mM/L at a pH of 7.98 (point A). Point B (on the 7.0 mm Hg PaCO\(_2\) isopleth) describes its compensation to the maintained high CO\(_2\) levels after 72 hours, gaining 21.0 mM/L of HCO\(_3^-\) to adjust its pH to 7.97 at a PaCO\(_2\) of 7.0 mm Hg. Thus regulation of pH in face of a rise in CO\(_2\) is by adjustment of the plasma \([\text{HCO}_3^-]\) during hypercapnia.

Increasing the partial pressure of CO\(_2\) in water causes a fall in the pH of water. To test the effect of low water pH, fish no. 65 (241 g, 12 ± 1 °C) was exposed and maintained at a pH\(_I\) of 5.00. Arterial pH, VR and \(\dot{V}_G\) were observed (Fig. 11). The response noted is typical of several fish tested at low pH\(_I\). Water, at a PCO\(_2\) of 5 mm Hg, never fell that low in pH, usually ranging from a pH of 6.3 to 6.6 during the hypercapnia experiments.

There was a slight decrease in arterial pH, from a pre-stress value of 7.95 to a low of 7.86 at 30 min, and a gradual increase to a pH of 7.89 at 3 hours. Upon return to normal water of pH of 7.30, the pH\(_I\) returned to control values, i.e. between 7.98 and 8.00. A pH of 5.00 represents an H\(^+\) ion gradient 100 times that normally present in water. These data indicate that during hypercapnia the arterial acidosis observed is due to CO\(_2\) and not a low pH\(_I\). It is possible that the observed, retarded increase in \(\dot{V}_G\) and VR at low pH\(_I\) could be due to mucous accumulation at the gill surface leading to an increase in \(O_2\) diffusion distance.
THE EFFECT OF NaHCO₃ BUFFERED WATER ON RESPONSE OF FISH.

Dejours et al. (1968) and Dejours (1969) observed that the effect of a change in the ionic composition of inspired water affected the ΔPCO₂ and CO₂ output in goldfish. To test if these effects existed in rainbow trout, several fish were subjected to waters containing 1% NaHCO₃ in a recirculating system of 75 liters. Short-term responses to raised levels of P₁CO₂ were observed (Table III). The fish (no. 15), whose response is portrayed, typifies the responses under these experimental conditions.

The degree of arterial blood acidosis is much less pronounced in the NaHCO₃ buffered system (Table III, part I) than in tap water system (Figs. 6,7), even though P₁CO₂ levels are similar. This implies that HCO₃⁻ must be actively taken up from the water. Fish exposed to 1% NaHCO₃ in water alone provide evidence that HCO₃⁻ is indeed actively assimilated since the arterial blood pH increases from a control of 8.00 to 8.20 in 2 hours (fish no. 17, Table III, part II).

The general trend of ventilation volume under these conditions of high PCO₂ along (Figs. 6,7), giving evidence that Vₖ is relatively independent of the arterial blood pH but dependent on arterial PCO₂ levels.

In order to determine the effect of maintaining a fish, with attached oral membrane, in a Vₖ box for extended periods of time, fish were placed in boxes for extended periods of time and observed. The results are in Table II. O₂ saturation of blood remained normal during the length of
the experiment, as did arterial pH levels. There appeared to be a slight increase in VR and $V_G$, but these were within the range of experimental error obtained in control values of fish subjected to the various acid-base disorders.

II. INDUCED METABOLIC ACIDOSIS WITH HCl INJECTIONS AND ITS EFFECT ON VENTILATION AND ARTERIAL pH.

Six fish ($264.3 \pm 19.2$ g at $11.8 \pm 0.4$°C) were used in this experiment. Injection of $0.025$ mM HCl in saline resulted in acute metabolic acidosis, affecting both $V_G$ and VR, and arterial pH. From a control value of $10.9 \pm 0.5$ mM/L, the $[H^+]$ at 90 minutes equalled $12.2 \pm 2.0$ mM/L, demonstrating the large variation existing between individual fish. Thus some fish had readjusted their pH, some had not (Fig. 12).

An acid load is dealt with in two ways; it is buffered by both the non-bicarbonate and the bicarbonate buffer system as follows:

1) nonbicarbonate component

$$H^+ + Buf^- \rightarrow HBuf$$

2) bicarbonate component

$$H^+ + HCO_3^- \rightarrow H_2CO_3 \rightarrow H_2O + CO_2$$

The former consists of plasma proteins, mainly haemoglobin. Buffering by $Buf^-$ is immediate. The added acid reacts with haemoglobin, plasma proteins and $HCO_3^-$ of blood, but some of the added $H^+$ remains in the ionized form, and thus raises the $[H^+]$a. The $H^+$ ion, in combining with $HCO_3^-$, causes an increase in carbonic acid concentration, hence there is a
rise in CO₂ in the blood. As the blood passes through the gills, some of the CO₂ is blown off.

In response to the H⁺ load, both ventilation rate and volume increased: VR from a control level of 64.7 ± 1.2 to 79.0 ± 3.8 breath/min, and V̇ from 50.5 ± 3.6 to 187.5 ± 20.3 ml/min at 2 min. Near normal levels are attained in 90 min, although there is considerable variability in the ventilation rate, i.e. 68.1 ± 4.0 breath/min. Very little variability existed at this time for V̇.

**THE EFFECT OF NaHCO₃ INJECTION ON VENTILATION AND ARTERIAL pH IN TROUT**

Intra-arterial injection of 1 mM NaHCO₃ in saline was completed on 7 fish (245.6 ± 10.8 g at 10.7 ± 0.8 °C), resulting in acute metabolic alkalosis (Fig. 13). Both VR and V̇ were affected. There was an initial hyperventilation upon completion of injection. Normal ventilation rates were attained in 2 to 3 hours. V̇ reached a peak volume of 130.4 ± 23.1 ml/min at 15 min after injection. This compares to a pre-injection control level of 45.0 ± 4.5 ml/min, showing approximately a 3-fold increase in V̇. Vsv, in accordance, rose linearly to 1.42 ± 0.23 ml/breath from 0.54 ± 0.04 ml/breath.

Injection of NaHCO₃ causes an alkalosis in the blood as follows:

1) \( \text{HCO}_3^- + \text{HBuf} \rightarrow \text{Buf}^- + \text{H}_2\text{CO}_3 \rightarrow \text{H}_2\text{O} + \text{CO}_2 \)

2) \( \text{HCO}_3^- + \text{H}^+ \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{H}_2\text{O} + \text{CO}_2 \)

causing a fall in \([\text{H}^+]_a\), hence pH increases. This was
observed, the $[H^+]$a falling from a control of $10.9 \pm 0.9$ to $4.9 \pm 0.9$ nM/L in 5 minutes after injection. This is a change of $6.0$ nM/L. A gradual return to near normal levels is observed in 3 hours, the mean pHa being $9.9 \pm 0.6$ nM/L. The NaHCO$_3$ and HCl data give further evidence that $V_G$ is not linearly related to arterial pH levels alone, since both an increase and decrease in pHa resulted in a rise in $V_G$. However, since both NaHCO$_3$ and HCl injections cause an increase in PaCO$_2$ initially, $V_G$ may be linearly related to arterial CO$_2$ tensions.

**INDUCED METABOLIC ALKALOSIS WITH NaOH AND THE EFFECT ON VENTILATION AND pHa LEVELS.**

NaOH, like NaHCO$_3$, produces a metabolic alkalosis, but unlike NaHCO$_3$ is nonvolatile. Seven fish, weighing $259.7 \pm 20.2$ g, (at $12.0 \pm 0.4$ °C) were injected with 0.025 mM NaOH into the dorsal aorta (Fig. 14). NaOH is buffered as follows:

1) $\text{OH}^- + \text{HBuf} \rightarrow \text{Buf}^- + \text{H}_2\text{O}$

2) $\text{OH}^- + \text{H}_2\text{CO}_3 \rightarrow \text{H}_2\text{O} + \text{HCO}_3^-$

by the nonbicarbonate and bicarbonate system, respectively, resulting in a rise in $[\text{HCO}_3^-]$ and hence an increase in pHa. CO$_2$ is consumed in this reaction initially, in the formation of carbonic acid.

There was a quicker return of arterial $[H^+]$ to normal values with NaOH alkalosis in comparison to that with
NaHCO$_3$ injection, even though [H$^+$]a values achieved by either injection were similar. Ventilation volume increased to a maximum of $98.0 \pm 15.4$ ml/min from a resting, control value of $53.0 \pm 4.2$ ml/min. This is slightly less than a 2-fold increase in $\dot{V}_G$, compared to a 3-fold increase obtained with NaHCO$_3$ alkalosis. That the stimulation to $\dot{V}_G$ is not an ionic effect, i.e. Na$^+$ effect, is shown in Fig. 15. NaCl, injected at equimolar amounts as NaOH, caused no significant change in $\dot{V}_G$, VR, nor pHa. These data (i.e. HCl, NaHCO$_3$ and NaOH) indicate that both a rise and fall in arterial pH result in an increase in $\dot{V}_G$, but that the system controlling ventilation is predominantly CO$_2$ sensitive.

RELATIONSHIPS BETWEEN ARTERIAL H$^+$ ION LEVELS AND VENTILATION.

In Fig. 16, mean values of VR are plotted against corresponding H$^+$ ion concentrations. The plotted values represent progressive stages of the various acid-base disorders with time during each of the experiments. Normal values represent controls for each experimental group. Due to the considerable variability, no relationship appears to exist between arterial [H$^+$] and VR. On the other hand, a relationship does exist between $\dot{V}_G$ and [H$^+$]a (Fig. 17). The 3 acid-base disorders are depicted. Values are means taken from Figs. 6,7,11,12 and 13. Metabolic alkalosis, and both respiratory and metabolic acidosis cause increases in ventilation volume. A comparison of $d\dot{V}_G/d[H^+]$ between hypercapnia and HCl acidosis is not valid since different degrees of arterial acidosis were induced. A clearer picture is obtained when all experimental values of $\dot{V}_G$ and [H$^+$]a are
plotted as a scatter diagram with a regression line drawn through these points. The equations obtained are:

\[ Y = 11.6219 + 6.1085 X \]
\[ Y = -25.6636 + 9.5517 X \]

for hypercapnia and HCl acidosis, respectively (Fig. 18, 19). These relationships should not be considered valid for statistical treatment since it contains, for one, the variable time. In addition, the functional relationship between \( \dot{V}_G \) and \([H^+]\) is far more complex than it appears. The linear regressions are thus only rough approximations.

\( \text{NaHCO}_3 \) is more of a stimulus for \( \dot{V}_G \) than \( \text{NaOH} \) is, even though both bases caused identical arterial \([H^+]\) changes. All these data suggest then, that although \( \text{CO}_2 \) appears to be the main stimulus of ventilation, the actual functional relationship is more complex than this, as demonstrated by \( \text{NaOH} \) injection. It does not appear to be by a simple ionic effect, i.e. by \( \text{Na}^+ \) ions, since \( \text{NaCl} \) injection does not stimulate ventilation.

Adjustment of ventilation itself is dominated by a change in \( \dot{V}_G \) rather than \( \dot{V}_R \), since the ventilatory stroke volume (Vsv) rose in a linear fashion between 40-240 ml/min, reflecting the rising \( \dot{V}_G \) with little or no significant change in \( \dot{V}_R \) (Fig. 20). Adjustment in mammals is similar, where the \( \dot{V}_E \) or respiratory minute volume increases rather than respiratory rate.

III. PERFUSION OF MOCK CSF INTO THE CRANIAL CAVITY AND THE EFFECT ON VENTILATION

Cerebrospinal fluid, modified after Mitchell, et al.
was perfused into the cranial cavity to test \( \text{CO}_2/\text{H}^+ \) ion sensitive areas shown to be at the ventral surface of the medulla oblongata, or pons in mammals. Slow perfusion of mock CSF with either the \( \text{NaHCO}_3 \) concentration or \( \text{CO}_2 \) tension changed caused no perceivable alteration in ventilation rate during the time course of approximately 1 hour. This suggests that either the perfusate was not circulating near the \( \text{CO}_2 \) sensitive areas or that the sensitive area is not localized superficially as in mammals. The latter is probably more correct since topical or localized application of mock CSF with high \( \text{PCO}_2 \) on the medulla did not alter ventilation (Jones, personal communication). Perfusion of the brain itself is not feasible in trout, mainly because of size.

IV. INDUCED ALKALOSIS WITH RADIOACTIVE \( \text{NaH}^{14}\text{CO}_3 \) AND PATHS OF EXCRETION OF CARBON-14.

Results from 6 fish (296.2 ± 27.4 g at 5.4 ± 0.4 °C) are presented in Fig. 21. The flux of carbon-14 is plotted versus time. The data obtained in the 6 experiments are individually shown. The curve was fitted by eye. The values of expired water equals the amount of radioactivity, as carbon-14, passed from gills to expired water in a 1 min period at time of sampling. Because of the small amounts of urine one is able to collect at any one time, each value of radioactivity in urine plotted represents the total amount of urine passed from kidney to bladder from one sampling period to another.
Figure 12

Responses of rainbow trout to induced metabolic acidosis by the injection of 0.025 mM HCl in saline. 6 fish (264.3 ± 19.2 g, 11.8 ± 0.4 °C) were tested, and the ventilation rate and volume and arterial hydrogen concentration recorded. Injection is at time zero. The values are means ± 1 standard error.
Figure 13

The effect of induced metabolic alkalosis on ventilation rate, volume and arterial pH. Alkalosis was induced by injection of 1 mM NaHCO₃ in Cortland saline in 7 fish (245.6 ± 10.8 g, 10.7 ± 0.8 °C). Values shown are means ± 1 standard error.
Figure 14

Induction of metabolic alkalosis through NaOH injection and its effect on arterial pH and ventilation rate and volume. Alkalosis was produced by injection of 0.025 mM NaOH in saline. 7 fish, weighing 259.7 ± 20.2 g, at a temperature of 12.0 ± 0.4 °C, were tested. Values are means ± 1 standard error.
Figure 15

Sham injection of NaCl in saline and the effect on arterial pH, ventilation rate and volume. 0.025 mM NaCl in Cortland saline was used as a control injection in 7 fish (278.0 ± 23.0 g, 10.1 ± 0.9 °C). Values are presented as means ± 1 standard error.
Figure 16

Relationship between arterial hydrogen concentration and ventilation rate in rainbow trout. Values represent progressive stages of the various acid-base disorders and their compensation. Thus the points plotted for elevated $PCO_2$ for example, are mean values at 5 min, 15 min, etc., up to 72 hours of exposure. Normal refers to control values.
Figure 17

Relationship between arterial hydrogen concentration and ventilation volume. Values represent progressive stages of the various acid-base disorders and their compensation.
Figure 18

Linear regression on the relationship between $\dot{V}_G$ and arterial $[H^+]$ during hypercapnia. All experimental values during the time course of the experiment have been plotted by computer. The regression line is described by the equation:

$$Y = 11.6219 + 6.1085X$$
\[ Y = 11.6343 + 6.1055X \quad \text{N} = 115 \]
**Figure 19**

Relationship between $V_G$ and arterial $[H^+]$ according to linear regression during HCl acidosis. All values during the time of the experiments have been plotted by computer. The relationship is described by the equation:

$$Y = -25.8636 + 9.5517 X$$
\[ y = -25.6626 + 9.5517x \]

\( N = 27 \)
The relationship between ventilatory stroke volume and ventilation volume in rainbow trout. Each point represents progressive stages of the various acid-base disorders and their compensation.
A plot of the percent of recovery of injected dose against time is expressed in Fig. 22, giving cumulative excretion of carbon-14. 50% excretion of the injected dose is obtained at 75 minutes after administration. The blood has circulated and passed through the gills approximately 75 times according to Davis' (1970) data on circulation time in the rainbow trout (i.e. $64.1 \pm 16.4$ sec ($n = 9$) for fish of similar weight).

Assuming that cumulative excretion of radioactive carbon in 4 hours is 90%, as Gould, et al (1948) observed in NaH$^{14}$CO$_3$ injection studies in the rat, then the percent excreted via the gill or kidneys can be calculated. The total urine activity is divided by the total activity injected. On 2 fish, from which complete sampling was made during the 4 hour run, the percent excreted via the kidney was calculated to be 0.40 and 0.03% respectively. This indicates that the kidneys play only a minor role in the elimination of a HCO$_3^-$ load. The NaHCO$_3$ load elicited a net loss of CO$_2$ and/or HCO$_3^-$ through the gills, whereas the excretion rate by the kidneys remained virtually unchanged.

There are three possible ways to restore the acid-base balance following a NaHCO$_3$ alkalosis: the passive intervention of blood buffers, or an increased HCO$_3^-$ excretion by the active intervention of either kidney or gill. Of course, the load can also be blown off as CO$_2$. That the kidney plays only a minor part in acid-base balance is supported by Smith (1939), Hodler et al (1939), and Murdaugh and Robin (1967). They found that urine pH remains constant.
despite important variations in blood pH produced by
injections of acids, alkali or acetazolamide; neither did
exposure to high CO₂ in the elasmobranch affect the urine
pH (Cross et al., 1969). Thus the gill is obviously the main
route of alkali-acid excretion thereby maintaining the
internal pH (Payan and Maetz, 1973). As Maetz (1971) states:
in freshwater fish the kidney has the task of removing
excess water and also excreting some of the organic/inorganic
acids as shown by a renal Na⁺ and Cl⁻ loss.

Unfortunately there is no analytical technique available at
present in separating the expired carbon-14 as either CO₂
or as HCO₃⁻. However, evidence suggests that the NaHCO₃
load is probably mainly excreted as gaseous CO₂ rather than
ionic HCO₃⁻ since gill tissue carbonic anhydrase levels are
relatively low (R. Milne, personal communication). Therefore,
the NaH¹⁴CO₃ experiment adds to the general theory that the
teleostean gill is a multi-purpose organ, specialized for
respiratory gas exchanges, clearance of waste products of
nitrogen metabolism and maintenance of acid-base and mineral
balances (Maetz, 1971).
Figure 21

Excretory pathways of carbon-14 as CO₂ and/or HCO₃⁻ after injection of NaH¹⁴CO₃ in 6 fish (296.2 ± 27.4 g, 5.4 ± 0.4 °C). The lines have been fitted by eye. Values of expired water equals total amount of radioactivity excreted by the fish in a 1 minute period. Each urine value represents the total amount of urine collected between 2 sampling periods; i.e. value at 90 min represents all urine excreted by the animal in the time interval from 60 to 90 min.
Figure 22

Cumulative $^{14}\text{CO}_2$ excretion pattern from fish injected with $\text{NaH}^{14}\text{CO}_3$ plotted against time. The cumulative excretion rate was calculated by the "cut and weigh" technique from the curve in Figure 21.
CUMULATIVE EXCRETION OF $^{14}\text{CO}_2$

(% OF INJECTED DOSE)
GENERAL DISCUSSION

Although there is an abundance of literature regarding pH regulation in mammals, very little is known about waterbreathers. It is only through the work of Rahn and colleagues that an interest has been focused in this latter group of animals. However, they have only concentrated on pH regulation per se, specifically in conjunction with change in temperature and the evolution of water to air-breathing. It is only in the last few years that the functional relationship between the arterial acid-base status and ventilation in waterbreathers has been studied, specifically through the work of Randall and Cameron.

I. Regulation of arterial blood pH via bicarbonate.

The hypercapnia experiments indicate that in the face of an increase in ambient PCO\textsubscript{2} trout do not adjust the PCO\textsubscript{2} difference (ΔPCO\textsubscript{2}) between arterial blood and water. PaCO\textsubscript{2}, as earlier shown by Cameron and Randall (1972), in fact increases in proportion to the change in P\textsubscript{1}CO\textsubscript{2} such that PaCO\textsubscript{2} is always about 2 mm Hg above ambient. That is, the ΔPCO\textsubscript{2} is not affected by changes in ventilation. The CO\textsubscript{2} gradient between arterial blood and water may result from either a limitation in the rate of formation of CO\textsubscript{2} in the blood (Maren, 1967, as cited from Randall, 1970) or from a diffusion resistance between blood and water.

With the increase in PaCO\textsubscript{2} there is a concomitant and significant fall in arterial blood pH at the ambient PCO\textsubscript{2} levels studied, producing respiratory acidosis. A similar response exists in airbreathing animals, a rise in
PaCO\textsubscript{2} associated with a fall in pH (Nichols, 1958). However, airbreathers are capable of reducing the difference between ambient and arterial CO\textsubscript{2} tensions during sustained hypercapnia by hyperventilation as shown by Manfredi (1962). He found that experimental subjects, during maintained high CO\textsubscript{2} levels, could reduce their PaCO\textsubscript{2} through hyperventilation to levels below 40 mm Hg. A PaCO\textsubscript{2} of 40 mm Hg is considered normal in man when ambient CO\textsubscript{2} tensions are nearly zero. Thus airbreathers, unlike waterbreathers, can adjust blood pH and PaCO\textsubscript{2} via ventilation during hypercapnia. In trout, the increase in PaCO\textsubscript{2} is in proportion to the increase in P\textsubscript{TCO\textsubscript{2}} and thus ventilation has little effect on ΔPCO\textsubscript{2}.

In trout it is shown that there is a delay in the increase of PaCO\textsubscript{2} relative to the ambient PCO\textsubscript{2}. One would expect equilibrium to be established within minutes with the commencement of high ambient CO\textsubscript{2} levels. However, if one considers movements of CO\textsubscript{2} within the fish's body during hypercapnia in relation to CO\textsubscript{2} production by the tissues, the observed time lag of PaCO\textsubscript{2} relative to P\textsubscript{TCO\textsubscript{2}} may be explained. Initially, CO\textsubscript{2} levels within body compartments will be raised due to CO\textsubscript{2} entry across the gills and by tissue CO\textsubscript{2} production itself. In addition, the reaction velocity of CO\textsubscript{2} to HCO\textsubscript{3} will affect both CO\textsubscript{2} and HCO\textsubscript{3} levels within each compartment. Eventually equilibrium will be achieved, CO\textsubscript{2} uptake across the gills will stop and CO\textsubscript{2} levels in each compartment will rise above ambient. A new steady state will thus be established. The time of this response will be dependent on the rate of exchange and magnitude of the CO\textsubscript{2}/HCO\textsubscript{3} stores within the body.
compartments. Nothing is known about many of the time constants involved.

The change in arterial blood pH is related to the change in \( \text{PaCO}_2 \) rather than the influx of \( H^+ \) ions as a result of the decrease in \( \text{pH}_1 \) associated with an increase in ambient \( \text{PCO}_2 \). Support for this comes from experiments in which the pH of water was altered. Although the decrease in \( \text{pH}_1 \) was much greater than that encountered when \( P_1 \text{CO}_2 \) was increased to 5 mm Hg, the shift in arterial blood pH was significantly smaller. Therefore, the fall in pH is the result of an increase in \( \text{PaCO}_2 \) and not a result of the transfer of \( H^+ \) from water to blood.

In order to regulate arterial blood pH in the face of a rise in ambient \( \text{PCO}_2 \), blood bicarbonate levels were adjusted. That the time course of \( \text{HCO}_3^- \) adjustment is slow during hypercapnia was also noted by Lloyd and White (1967). In trout, complete compensation to sustained hypercapnia required up to 3 days to complete. Mammals, in general, display a similar long-term mechanism during prolonged hypercapnia in the regulation of pH.

The period of hypercapnia, before compensatory mechanisms have begun, reflects the effect of blood buffering alone. In this situation, as observed in trout, reduction of \( P_1 \text{CO}_2 \) to zero leads to rapid restoration of the original, normal acid-base balance, thus reflecting a back titration along the blood buffer curve (line C-A, Fig. 10). This is as observed in mammals, the time course being similar (Refsum, 1971). Prolonged elevation of \( \text{PaCO}_2 \) leads to an increase in
pHa via $\text{HCO}_3^-$ along the actual $\text{PaCO}_2$ isopleth (line C-B, Fig.10). In this situation, rapid reduction of $\text{PCO}_2$ (as done in several fish) led to relatively marked increase in pHa above the initial value, frequently above 8.20, demonstrating the increase in the plasma $[\text{HCO}_3^-]$.

$\text{HCO}_3^-$ can either be regulated via the kidney or the gills. The kidney plays a major role in $\text{HCO}_3^-$ regulation in mammals, either by reabsorption or excretion of $\text{HCO}_3^-$ (Refsum, 1971). In fish, evidence points to the contrary, the kidney playing only a minor role in both the dogfish and trout. The renal response to NaHCO$_3$ induced alkalosis is negligible as shown by the labelled NaHCO$_3$ experiments. This is also supported by Murdaugh and Robin (1967). They found neither alkalization nor acidification of urine occurred in the dogfish following NaHCO$_3$ and HCl administration.

Evidence supports that $\text{HCO}_3^-$ is regulated via the gills. Fish subjected to NaHCO$_3$ buffered water in which $P_{i}\text{CO}_2$ was raised showed a much less severe change in arterial blood pH than fish in nonbuffered water, even though $P_{i}\text{CO}_2$ levels were similar. This implies that $\text{HCO}_3^-$ must have been taken up from the water by the gills. Fish placed in high bicarbonate water show a rise in pHa. Lloyd and White (1967) add further support to the role of the gills in $\text{HCO}_3^-$ regulation: they describe increases in blood plasma $\text{HCO}_3^-$ following acclimation to high levels of CO$_2$. They rightly conclude that the purpose of the observed increase in $\text{HCO}_3^-$ is to maintain pH. A concomitant decrease in plasma Cl$^-$ was also observed by these authors, the changes in
HCO$_3^-$ and Cl$^-$ being of approximately the same magnitude and reversed when the animals were restored to water of low PCO$_2$. Maetz and Garcia-Romeu (1964) were the first to present data supporting a HCO$_3^-$/Cl$^-$ exchange in fish. By altering the CO$_2$ level in the water they were able to vary Cl$^-$ movement across the gills. Dejours (1969) demonstrated that fish transferred to water of very low Cl$^-$ content showed a marked reduction in CO$_2$ excretion immediately following transfer.

Thus in freshwater, indications are that fish Cl$^-$ influx is linked to HCO$_3^-$ efflux. The mechanism of pH adjustment in trout may involve regulation of a HCO$_3^-$/Cl$^-$ exchange. In addition, there is evidence for a Na$^+$/H$^+$ exchange and direct H$^+$ excretion (Kerstetter et al., 1970, Maetz, 1971, 1973, Payan and Maetz, 1973). However, it has not been demonstrated that the rate of HCO$_3^-$/Cl$^-$ exchange is modified to adjust plasma HCO$_3^-$ levels in order to regulate pH$_a$. This could equally be achieved by regulation of the rate of Na$^+$/H$^+$ exchange or by H$^+$ excretion (Randall and Cameron, 1973).

CO$_2$ could be excreted by the trout either as gaseous CO$_2$ or as HCO$_3^-$. Because of some loss of ions by the kidney (i.e. Na$^+$, Cl$^-$; Maetz, 1971), HCO$_3^-$ efflux could thus be linked to a Cl$^-$ influx thereby maintaining plasma electroneutrality. However, the extent of this HCO$_3^-$/Cl$^-$ exchange may vary from species to species. In goldfish, an extensive relationship exists between HCO$_3^-$ and Cl$^-$ since CO$_2$ excretion seems very dependent on the external Cl$^-$ concentration. In salmonids perhaps this HCO$_3^-$/Cl$^-$ exchange
has a very small capacity as shown by the 2 to 3 day period before pHa was adjusted via bicarbonate. This time course is supported by R. Milne (unpublished data) in his study in transfer of trout, and their adjustment, from fresh to salt-water. In addition, he has demonstrated very low carbonic anhydrase levels in the trout gill epithelium versus high levels in the goldfish gill epithelium. Carbonic anhydrase is required in the fast conversion of CO₂ to bicarbonate (Maren, 1967). However, alteration of bicarbonate levels in the water aided pH regulation in trout in these studies. This was not confirmed by Milne, pHa not being affected by a salinity change.

II. The relationship between ventilation volume and arterial CO₂ levels.

In trout, the increase in the ventilatory stroke volume, as a result of an alteration of the acid-base status of the blood is mainly due to an increase in ventilation volume, rather than rate. In airbreathers a similar response prevails, there being a greater increase in the respiratory minute volume rather than breathing frequency (Schaefer et al., 1963).

The ventilation volume in trout is dependent on an increase in PaCO₂ and/or PRCO₂ and not to pHa or pH₁ as indicated. A decreased ambient pH level, although causing a fall in pHa, has only a delayed effect on V̇₉. Höglund and Härdig (1969) have also concluded that behavioural reactions (i.e. rise in VR) provoked by ambient pH/PCO₂ changes is essentially due to an increase in P₇CO₂ and not
to an increase in ambient pH.

The arterial acidosis may not be due to a lactate accumulation (Höglund and Börjeson, 1971) as observed when Atlantic salmon were subjected to a low pH. The observed increase in $V_G$ is probably related to conditions leading to hypoxia. It is well documented that fish exposed to low ambient pH show a decreased ability to extract $O_2$ from the water, this possibly relating to the observed increase in mucous production at the gill surface (Jones, 1964, Townsend and Cheyne, 1944; as cited from Packer and Dunson, 1970). Mucous accumulation at the gill may cause an increase in the $O_2$ diffusion distance. However, nothing is known about the rates of diffusion of gases through mucous produced by the gills (Randall, 1970). In addition a fall in arterial blood pH is associated with a decrease in the $O_2$ carrying capacity of the blood (Bohr-Root effect) (Randall, 1970).

These results are consistent with the hypothesis that ventilation is dependent on the $CO_2$ tension within the body or elsewhere and that blood pH levels are regulated via ionic exchange mechanisms at the gill surface, rather than by ion exchange at the kidney or by diffusive washout of gaseous $CO_2$ via ventilation.

III. Receptors and the regulation of ventilation.

The response in $V_G$ is rapid and transient and is shown to respond to $PCO_2$ changes at the surface or within the fish. Although the change in $V_G$ is transient, $PaCO_2$ is held constant relative to $P_{CO_2}$. Therefore, receptors are either adapting or the receptors are not located in the blood.
or water but in another compartment whose contents or properties change in proportion to $\dot{V}$. From the rapid response noted to acid-base changes in ventilation, receptors are most likely arterially located, or possibly centrally in the brain, since blood circulation time in trout is of the order of minutes rather than seconds (Davis, 1970; Jones et al., 1970). However, this does not preclude oral cavity or external gill receptors.

Since $\text{CO}_2$ still increases breathing following denervation of peripheral chemoreceptors in mammals, central chemosensitivity is of obvious importance in regulating ventilation (Hornbein, 1965). Leusen (1954) reported that ventilation could be changed by altering the $\text{PCO}_2-[\text{H}^+]$ composition of fluids perfusing the ventricles in dogs. This led to the delineation of an area on the superficial layers of the ventrolateral surface of the medulla as the chemosensitive area for ventilation (Mitchell et al., 1963). Both an increase in cerebrospinal fluid $\text{PCO}_2$ and decrease in CSF pH causes an increase in ventilation; a fall in CSF $\text{PCO}_2$ and an increase in pH causes hypoventilation in mammals. The CSF is a $\text{HCO}_3^-$ containing fluid with a very low protein content (Mitchell et al., 1963) and it has, therefore, a low buffer capacity toward $\text{CO}_2$. The barrier interposed between plasma and the CSF (blood-brain barrier) is relatively impermeable to $\text{H}^+$ ions and $\text{HCO}_3^-$ ions but highly permeable to $\text{CO}_2$ (Messeter, 1971).

The pH of the CSF is extremely well regulated in mammals (Loeschke, 1971). This regulation is exerted
by signals from the sensitive areas on the medulla (Schaefke et al., 1970), the signals depending on the local pH around these structures. These signals are responsible for the ventilatory drives by both CSF PCO$_2$ and pH in mammals. Local receptor pH is determined by blood PCO$_2$ and blood pH, the latter by way of redistribution of HCO$_3^-$ (Loeschke, 1971). The regulator acts by increasing ventilation as soon as local extracellular pH drops, the drops being counteracted by the elimination of CO$_2$ in mammals. This suggests that in trout, central receptor activity, if existing, and hence, activation of ventilation, could be the result of fast entry of CO$_2$ versus slow entry of HCO$_3^-$ or H$^+$ across the blood-brain barrier, thereby causing a fall in CSF pH. The observed transient nature of the increase in $\dot{V}_G$ could be explained by the adjustment of CSF pH to normal levels during sustained hypercapnia. Thus $\dot{V}_G$ could be dependent upon CSF pH via PaCO$_2$ in fish, the CSF pH affecting receptor activity of the respiratory mechanism. Adjustment of CSF pH is probably by HCO$_3^-$ since CSF is essentially a CO$_2$-HCO$_3^-$ buffer system.

Maren (1972) demonstrated that as the PCO$_2$ rose in the blood of dogfish an immediate rise in CSF PCO$_2$ was noted, as observed in mammals. As a result, CSF pH dropped. This drop in CSF pH is associated with an increase in ventilation in the dogfish (D.J. Randall, personal communication). CSF pH was regulated in the dogfish within 3 hours as a result of HCO$_3^-$ formation (Maren, 1972) during respiratory acidosis. This is similar to the time course of the $\dot{V}_G$ change as observed by Randall. The rate data of
bicarbonate formation and the carbonic anhydrase inhibition suggests that $\text{HCO}_3^-$ reaches the CSF by hydroxylation of gaseous $\text{CO}_2$ at the choroid plexus and probably at the glia in the dogfish.

If respiratory responses of trout to changes in the acid-base status of the blood are mediated by changes in CSF $\text{PCO}_2$-$\text{pH}$ as in mammals and as suggested in dogfish, then perfusion of the cranial cavity with mock CSF with altered $\text{PCO}_2$-$[\text{H}^+]$ should affect ventilation. Although this was attempted in trout, no response could be elicited by the perfusion technique used. D.R. Jones (personal communication) did not obtain any response in trout with topical application of mock CSF on the medulla. This suggests that the Mitchell $\text{CO}_2$-$[\text{H}^+]$ sensitive area may not exist superficially on the medulla as implied. This is supported by McCarthy and Borison (1972) who suggest that surface receptors are an addendum used to explain the rapidity of response to topically applied agents in a system where diffusion is the sole mechanism of penetration. They believe there exists a rapid access route capable of carrying substances from the surface of the medulla to more deeply lying structures. The receptors may be located anatomically more centrally in the trout brain, possibly within the ventricles themselves.

However, one should consider the anatomical differences of the brain existing between fish and mammals. The fluid surrounding the brain in mammals is CSF, the cranial cavity being the subarachnoid space. Because teleosts (and all lower vertebrates) have no true dura a true
subarachnoid space does not exist. The area surrounding the brain is rather a perimeningeal layer containing not CSF but plasma exudate or dialysate (Dayson, 1967). When labelled serum albumen was injected into the ventricles of sharks, Klatzo and Steinwall, 1965 (as cited from Davson, 1967) found no radioactivity on the brain surface or surrounding fluids. The CSF is thus confined only to the ventricles. Thus to elicit ventilatory responses in trout, one possibly needs to perfuse the ventricles directly, if central receptors are located there.

Even if central chemoreceptors were the main regulator of ventilation as in mammals, peripheral receptors, existing either within or outside the fish's body, cannot be precluded. In mammals, the activity of the medullary respiratory center is mediated by the activity of several receptors. These receptors include the aortic and carotid body chemoreceptors, mechanoreceptors in the lungs and central H\(^+\) receptors within the medulla itself (Hornbein, 1965). Analogous structures may exist in the trout, monitoring blood or water PO\(_2\), PCO\(_2\) (and to a lesser degree, pH) and maintaining respiratory rhythmicity.
SUMMARY

1) The effect of altering the acid-base status of blood on pH regulation and ventilation was studied. In addition, perfusion of the cranial cavity of trout was attempted with the hypothesis that ventilation could be affected by alteration of CSF $HCO_3^-$.

2) In the face of an increase in ambient $PCO_2$ the arterial $CO_2$ tension rose. Trout are not capable of adjusting the blood-to-water gradient of $PCO_2$ ($\Delta PCO_2$) via $V_G$, the $\Delta PCO_2$ remaining at 2 mm Hg above ambient during both normo-and-hypercapnic conditions.

3) Hypercapnia produced a severe arterial acidosis. The increase in arterial blood pH is the result of the increase in ambient $PCO_2$ rather than a result of an influx of $H^+$ ions from water to blood.

4) Arterial blood pH regulation is slow, taking up to 3 days. $pHa$ is regulated by adjustment of plasma $HCO_3^-$ levels during sustained hypercapnia along the $PaCO_2$ isopleth.

5) $HCO_3^-$ can either be regulated via the kidneys or the gills. Evidence is presented showing direct uptake of bicarbonate from $NaHCO_3$ enriched waters by the gills. The renal response is shown to be negligible in ameliorating the $NaHCO_3$ induced alkalosis.

6) $CO_2$ is shown to be the main stimulus to ventilation. However, the relationship is probably more complex. The increase in ventilation is mainly due to an increase in volume rather than rate.
7) Perfusion of the cranial cavity with mock CSF of altered CO₂-HCO₃⁻ composition did not elicit ventilatory responses. These results do not preclude the presence of centrally located chemoreceptors.

8) The results are consistent with the hypothesis that ventilation is dependent on the PCO₂ of the blood or elsewhere, and that blood pH levels are regulated via ionic exchange mechanisms at the gill surface rather than by ion exchange at the kidney or by diffusive washout of CO₂ via ventilation.
REFERENCES


APPENDIX A. ASSUMPTIONS AND CALCULATIONS FOR NaH$^{14}$CO$_3$ INJECTION AND $^{14}$CO$_2$/H$^{14}$CO$_3$ RECOVERY

In order to formulate the amount of HCO$_3^-$ to inject into the animal to induce alkalosis, the following criteria were considered. Knowing that the plasma bicarbonate concentration of rainbow trout averages 7 mM/L, a 200 g fish, containing approximately 10 ml of blood (Smith, 1966), and with a haematocrit of 30% (packed cell volume), would have about 7 ml of plasma. The calculated plasma HCO$_3^-$ concentration would be 49 μM/ml or 350 μM for a 200 g fish. Thus an injection of 1 mM NaHCO$_3^-$ was considered adequate in eliciting the desired response.

Administration of 5 μC of NaH$^{14}$CO$_3$ was calculated to be adequate for the detection of radioactivity in the expired water. This value was approximated as follows. Knowing that the flow rate of the system (Fig. 4) is 200 ml/min (less could lead to anoxia), and that the background rate is 30 cpm, the 50 ml of water sample (15 sec of sampling) should show a net count rate at least 10 times the background. Then an activity of 6 cpm/ml of water is required. If sampling is for a period of 4 hours $^1$, a rate of approximately 300,000 cpm is, therefore, required. If the overall detection efficiency for the beta particle of carbon-14 is approximately 25 to 30% (admittedly low) in the scintillation system used, the foregoing counting rate is equivalent to

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$^1$ This time period is assumed to be the time for the fish to take in order to compensate for the induced metabolic alkalosis, thus having excreted most of the labelled bicarbonate.
about 1,000,000 cpm. This, then, is the activity required in the administration of NaH$^{14}$CO$_3$, or 0.5 μC of carbon-14 to be used. Thus injection of 5 μC of NaH$^{14}$CO$_3$ should be adequate for excellent detection.
APPENDIX B. CO₂/HCO⁻₃ COLLECTION AND METHOD OF ANALYSIS

Both urine and expired water samples were analyzed by the following technique. Standard stocks of a 5M NaOH and 1 M BaCl₂ - NH₄Cl solution were prepared and kept in capped plastic containers. A high concentration of NaOH keeps the CO₂ concentration to a minimum. Expired water was collected below a layer of hexane in a 250 ml volumetric flask. NaOH was pipetted into the flask, followed by the BaCl₂ - NH₄Cl solution, resulting in a white precipitate. The NH₄Cl was added to neutralize the rather basic solution. Precipitation is via the following equation:

\[
\text{Ba}^{++} + 2\text{OH}^- + \text{CO}_2 \rightarrow \text{BaCO}_3 + \text{H}_2\text{O}
\]

The solubility product of BaCO₃ is \(1.6 \times 10^{-9}\), and is therefore very insoluble (Sinex, et al, 1955).

Filtration was completed with a Millipore filter system. The filters were allowed to dry overnight. The next day each filter was weighed and prepared for scintillation counting.
APPENDIX C. QUENCH CURVE DETERMINATION

A series of 6 quenched samples (Nuclear-Chicago, Inc.) with a disintegration rate of 238,000 cpm were used and monitored in a liquid scintillation system consisting of a Nuclear-Chicago Unilux IIA. In the procedure of channels ratio technique, one channel is used to monitor the entire carbon-14 spectrum (channel B) and the second channel (A) is used to monitor the low energy portion of the spectrum. This arrangement provides the ratio of lower energy channel A to the total energy channel B. First of all the balance point attenuator setting was determined on channel B in a 20 to 5 dynamic range window for the least quenched carbon-14 standard. This procedure consists of adjusting the amplifier attenuator on channel B so that the count rate is maximized for a given lower and upper level discriminator setting. That is, the ratio $S^2/B$ is maximized by this technique. Then a series of counts are made on channel A, adjusting the attenuator until the count rates on both channels are approximately equal.

In accordance with the quenching phenomena, the net count rate ratio for the quenched standards will increase, as the concentration of the quenching agent in the samples increases and as the counting efficiency decreases (Wang & Willis, 1965). The plot of counting efficiency versus the channels net count ratio thus provides one with a standard quench correction curve (Fig. 23) where:
% efficiency = \frac{\text{cpm (channel B)} - \text{background count}}{\text{DPM of standard}} \times 100

where background count = 30 cpm, and DPM of standard = 238,000
and the net count ratio is \frac{\text{CPM (channel A)}}{\text{CPM (channel B)}}. This ratio is used to locate the intersect on the curve and the percentage can be read on the vertical scale for the particular sample.
The actual disintegration rate is calculated as follows:

\text{DPM} = \frac{\text{count (channel B)} - \text{background count}}{\text{time}} \times 100

\times \% \text{ efficiency from curve}
Figure 23

Carbon-14 quench curve, allowing determination of the percent efficiency of a particular system once the channels ratio is calculated.
Figure 24

The effect of self-absorption on the count rate of Ba$^{14}$CO$_3$, where 100% maximum efficiency is zero sample thickness.
APPENDIX D. EFFECT OF SELF-ABSORPTION ON THE COUNT RATE OF Ba$^{14}$CO$_3$

In order to see the effect of self-absorption on the count rate of Ba$^{14}$CO$_3$, known amounts of NaH$^{14}$CO$_3$ (at a DPM of 500,000) were injected into volumetric flasks containing water of different total CO$_2$ and the maximum efficiency plotted versus the weight of Ba$^{14}$CO$_3$. The results are presented in Fig. 24, and extrapolated to 100% maximum efficiency. This plot, of course, is analogous to the quench curve, as presented in Fig. 23.

Self-absorption correction is primarily a problem in the assay of low energy beta emitters, such as carbon-14 (Wang and Willis, 1965) with the usual sample thicknesses encountered. This treatment of Ba$^{14}$CO$_3$ thus makes it possible to compare a series of counting samples of the same composition but varied thicknesses.