THE PHYSIOLOGY OF CIRCULATION DURING SWIMMING
ACTIVITY IN RAINBOW TROUT

by

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Doctor of Philosophy

in the

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ADDENDA & ERRATA

Figure	8	Cardiac respiratory reflexes.
"	9	Approach reflexes.
"	23	Triangles 1.35(V/L).
"	29	Magnification 7.5 x
"	30	Magnification 47 x
"	33	Magnification 7.0x
"	34	Magnification 47 x
"	44	Magnification 5.0 x
"	45	Magnification 6.0 x
"	46	Magnification 118 x
"	47	Magnification 47 x
"	48	Magnification 7.0 x
"	49	Magnification 47 x
"	50	Magnification 47 x
"	51	Magnification 7.5 x
"	52	Magnification 12 x
"	53	Magnification 47 x
"	54	Magnification 47 x The main structural elements......
"	55	Magnification 25 x
"	62	X PHARYNGEAL ..... a series of ganglia are connected....

References page 119 insert;

PERKS, A.M. (1969). The Neurohypophysis.   In Fish Physiology,
(ed. W.S. Hoar & D.J. Randall) xslxIVx133   vol II   111-205.
New York: Academic Press.

Appendix III :   PRIEDE, I.G. (1974).

ADDENDUM

There are errors in this thesis which if corrected alter the conclusions which have been reached on certain points:-

Page 57

The calculation in the bottom half of the page should be:-

Therefore the pressure in the cylinder = T/r

Therefore $$BP = \frac{156960}{30}$$

$$BP = 5232 \text{ dynes/cm.}$$

This is equivalent to $\frac{5.232 \times 10^3}{1.33 \times 10^3} = 3.9$ mm Hg.

Therefore the type of breakdown being considered is very likely to occur at elevated swimming speeds.

Page 66

equation (4)

$$\frac{HR_t - HR + HR_{basal}}{HR_{basal}} = \text{Specific } O_2 \text{ pulse}$$

This equation is incorrect and the values derived directly from this for specific O_2 pulse should be ignored

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"there must be a systole and diastole
in all inquiry; a man's mind must be
continually expanding and shrinking
between the whole human horizon and
the horizon of an object-glass."

Dr. Lydgate in "Middlemarch"

by George Eliot.

ACKNOWLEDGEMENTS

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Thesis number.

Space.

Refilm p 42. Text of Fig 25

CONTENTS

INTRODUCTION

Rainbow trout (<u>Salmo gairdneri</u>) were introduced into
Europe from North America in the latter half of the last
century. They can tolerate higher water temperatures and
lower oxygen concentrations than the native brown trout
(<u>Salmo trutta</u>). Rainbows grow faster than brown trout
under similar conditions and are thus particularly attractive
for artificial rearing methods. In Denmark there is a
thriving rainbow trout farming industry producing about
9,000 metric tons annually which is largely exported for
table use. (Mills 1971).

In Britain production of rainbow trout for food is not
on such a large scale but they form the basis of a considerable
sport fishery. In Scotland and Northern England although
rainbow trout reach sexual maturity they do not generally
breed so the population is entirely dependent on restocking
with hatchery reared fish, thus although living more or less
wild in many British waters, this species is essentially an
artificially managed resource upon which man can impose
genetic selection (Donaldson and Olson 1957) as well as
normal fishery controls. A detailed understanding of the
biology and physiology of this species is hence of particular
importance.

The salmonids have long been of particular interest to
comparative anatomists and physiologists by virtue of their
primitive position in teleost evolution. In recent years
interest in their physiology has expanded partly in response
to specific management problems. For example Brett and
his co-workers have carried extensive investigations on

oxygen consumption in the Pacific salmon (<u>Onchorhyncus nerka</u>) initially in order to predict the consequences of certain proposals for the construction of dams on salmon rivers in British Columbia (Brett & Glass 1973). There has been a corresponding expansion in interest in the physiology of circulation, artificially reared trout being readily available to laboratories. Mott (1957) commented on the fragmentary nature of information available on fish circulation, much of which was from isolated comparative studies. Since then much more data has become available which has been the subject of two recent reviews by Randall (1970) and Satchell (1971).

The aim of this study was to investigate some of the changes in circulation during swimming activity and to work towards an integrated understanding of the relative importance of various regulatory mechanisms involved. Swimming activity was chosen as it probably demands some of the most rapid adjustments in circulation that the animal is likely to undergo. Telemetric studies on trout movements in lochs (Young et al 1972, Holliday et al 1974) have indicated that sustained high swimming speeds are rare in trout and this directed these laboratory studies towards concentration on the events during short periods of swimming with long periods of rest in between.

A series of papers; Stevens & Randall (1967a), Stevens & Randall (1967b) and Randall, Holeton & Stevens (1967) gave a very full account of respiratory and cardiovascular function in rainbow trout during swimming at moderate speeds. The fish were made to swim in a water tunnel during a test lasting 15 minutes at speeds of up to 51cm/sec (200g to 600g fish). During this short swimming test and the subsequent recovery period measurements were taken of: blood

pressure (in the dorsal aorta, ventral aorta and sub-intestinal vein), buccal cavity water pressure, tail beat frequency, oxygen partial pressures (in the water of the buccal and opercular cavities and in the blood of dorsal and ventral aortas), carbon dioxide partial pressures (in the blood of the dorsal and ventral aortas) and oxygen consumption. These results were aggregated from experiments on 89 fish at temperatures between $5^{\circ}C$ and $12^{\circ}C$.

Blood pressures and heart rates rose during swimming but changes in gas partial pressures in water and blood afferent and efferent to the gills were remarkably small. The cardiac output was calculated by the Fick principle and it was shown that the increase during swimming was the result of a 15% increase in heart rate and a five-fold increase in stroke volume. Stevens & Randall (1967b) conclude: "Thus the increased rate of delivery of oxygen to the tissues was largely the result of large increases in stroke volume of the heart, with adjustments in breathing to maintain saturation of the blood in the face of increased blood flow through the gills during moderate exercise." The work for this thesis hence focused on factors which might influence cardiac output in relation to changes in oxygen consumption.

Figure 1 shows a diagram of the circulatory system and some of the associated control mechanisms which are likely to control cardiac output.

Blood leaves the heart along the ventral aorta and passes through the 4 gill arches on either side of the buccal cavity. In the gills the blood can either flow through the lamellae or through the filament (Richards & Fromm 1969). These authors showed that in rainbow trout lamellar flow was induced

by catecholamines which also reduced the overall gill resistance to flow. Acetylcholine diverted the flow into the filament so that gaseous exchange with the water was reduced and the gill resistance increased. Some of the oxygenated blood from the first gill arches flows through the pseudobranch and then to the cranium. Davis (1971) has shown that a chemoreceptor site in this flow monitoring arterial PO_2 is probably important in the circulatory and ventilatory responses of rainbow trout. Laurent & Rouzeau (1972) have studied the pseudobranch in rainbow trout and have shown that there is an afferent and efferent innervation. They detected and quantified different kinds of activity in the afferent nerves in response to hydrostatic pressure, Na^+ ions, PO_2, osmotic pressure and pH. There are also end bud receptors in the buccal cavity which may monitor the PO_2 of the respiratory water flow. (De Kock 1963, Randall 1970).

From the efferent branchial arteries most of the blood flows into the anterior mesenteric artery and back along the dorsal aorta. Mott (1950) comments on the small cranial circulation in the eel. Along the anterior mesenteric artery the flows into the visceral circulation and is returned via hepatic veins to the sinus venosus (St Konair 1947).

Franz (1898) noted the presence of a ligament in the dorsal aorta of trout and salmon embryos. Burne (1909) suggested that this may be a 'blood pumping mechanism. The main detailed account of the anatomy of the circulatory system of rainbow trout was carried out by a group of Polish workers: Sikorawa (1947), Grodzinski (1947), Gorkiewicz (1947), St Konair (1947) and St Koziol (1947). These authors do not

mention the presence of a ligament in the dorsal aorta.
De Kock & Symmons (1959) reviewed the rather sparse literature
on this ligament in some teleosts and restated Burne's
hypothesis of the pumping function of this structure.
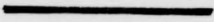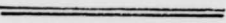
The blood in the dorsal aorta flows out through segmental
lateral branches largely to the body musculature.   Segmental
veins drain blood from the muscle into the kidney and thence
to the posterior cardinal vein (Gorkiewicz 1947).   Around the
posterior cardinal veins there is some chromaffin tissue
(Nandi 1962) which may be the source of secretion of increased
levels of blood catecholamines during activity (Nakano &
Tomlinson 1967).

In the visceral circulation, the general body circulation
and the gills plasma almost certainly filters into the lymph
system.   Wardle (1971) has shown that the volume of lymph in
plaice is much larger than that of the blood and considers
that in fish small changes in the rate of formation or drainage
of lymph could have large effects on the blood volume.
Kampmeier (1969) reviews the literature on the anatomy of the
lymph system and notes the presence of propulsive mechanisms
such as the caudal lymph heart which may help drainage of
lymph into the major veins.

Returning to consider the heart, 3 major regulatory
pathways can be recognised:-

1. Neural Mechanisms.   The inhibitory function of the vagus
innervation of the fish heart is well established (Randall
1970).   However in recent studies on the trout heart Gannon
& Burnstock (1968) have demonstrated that the vagus can have
excitatory as well as inhibitory effects.   The excitation is
mediated by adrenergic fibres in the vagus possibly originating

Figure 1. Schematic diagram of the main features
of circulation in rainbow trout and the
associated control pathways.

Blood - ——————
lymph - ==========
cholinergic innervation - ——————
adrenergic innervation - — — — — — — —
afferent innervation - ·····················

The "end bud" receptors are situated in
the water flow in the buccal cavity.

PSEUDO BRANCH

DORSAL AORTA

BRANCHIAL ARTERIES

LAMELLA

FILAMENT

END BUDS

VISCERA

PLASMA

LYMPH

PLASMA

MUSCLES

lymph 'hearts'

HEART

A.

SV

B.A.

V.

KIDNEY

CHROMAFFIN TISSUE

MAXILLARY
V

TO GILLS

VASOMOTOR ?

VAGUS
X

VASOMOTOR ?

GLOSSOPHARYNGEAL
IX

SYMPATHETIC CORD

BRAIN STEM
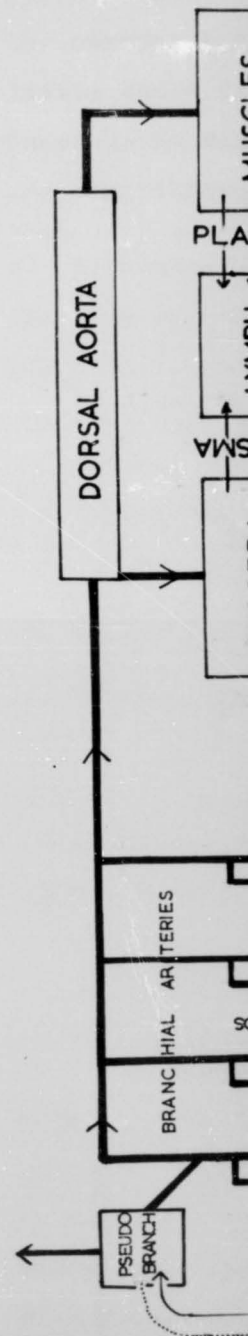
from the sympathetic ganglia.   Laurent (1962) has
demonstrated the function of afferent sensory elements in
the cardiac branch of the vagus in catfish but Randall
(1966) could find no similar function in goldfish and tench.
There is no direct evidence on the presence or absence of
afferent fibres in the cardiac nerve of trout.

2. Mechanical effects. Bennion (1968) has shown that
increase in venous pressure in an in vitro trout heart
preparation caused increases in cardiac output in accordance
with Starling's law.   Effects of peripheral vasodilation
or the action of auxiliary pumps such as the lymph propulsors
and possibly the dorsal aorta ligament may increase venous
return and cause a reflex increase in cardiac output.

3. Endocrine effects. Increases in the levels of circulating
catecholamines during activity can have a cardiac excitatory
effect as well as influences elsewhere in the vascular
system. Randall & Stevens (1967) could explain changes in
blood pressure during exercise in salmon in terms of the
interaction of adrenaline with α adrenergic receptors.

In the present study investigations were directed with
the following aims:-

1.   The extension of knowledge of changes in heart rate to
a full range of swimming speeds up to the maximum sustainable
speed at two temperatures, $6.5^{\circ}C$ and $15^{\circ}C$.   Relating this
to metabolic studies which have been carried out on trout
(Webb 1970) estimates could be made of the relative importance
of changes in heart rate in the transport of oxygen to the
tissues.

2. The investigation of the role of the vagus nerve in cardiac regulation _in vivo_ during swimming in the light of recent _in vitro_ evidence of its dual excitatory and inhibitory function in trout.

3. The investigation of the structure and function of auxiliary circulatory pumps which may increase venous return during activity which in turn would increase cardiac output.

4. The elucidation of certain aspects of the anatomy of the heart which may be of significance in relation to the large stroke volume changes it seems to undergo.

A brief outline of the actual work carried out is given below:-

Electrocardiograms were measured during and after swimming at a wide range of speeds up to the maximum which could be sustained for 30 minutes. For each test the fish was made to swim at a uniform elevated velocity against a water flow for 30 minutes and heart rates were observed during this test and the subsequent recovery period at rest. 30 minutes was chosen as the test period as this is long enough for a respiratory "steady state" to be established as in previous metabolic studies on swimming fish (Brett 1964). The experiments were carried out at two temperatures 6.5°C and 15°C which coincide closely with the temperatures of Bennions (1968) studies on the heart and are likely to be found in winter and summer respectively.

Complementary to the swimming trials measurements were made of oxygen consumption and heart rate in fish at 15°C. With these experiments further detailed information was gained on the relationship between heart rate and metabolism.

The influence of the vagus nerve was investigated by cutting it in an experimental group of fish for comparison swimming trials with the intact fish. This procedure blocked both vagal cholinergic and adrenergic innervations to the heart.

The influence of auxiliary pumps which could increase venous return automatically during swimming was considered to be of particular interest. Anatomical studies were made of the lymph system, in particular the caudal lymph heart. Also anatomical studies were made of the dorsal aorta and its ligament and a theoretical analysis was made of any possible pumping activity. Experiments on the dorsal aorta function were carried out on a machine designed to reproduce swimming movements in trout cadavers.

The anatomy of the heart was investigated using standard histological techniques and by making casts of the chambers of the heart. Measurements were made of the pressure-volume relationships of the bulbus arteriosus and a rather more detailed account of the structure of this chamber was attempted in view of its importance in maintaining blood flow in the dorsal aorta (Randall 1970).

This work began with a consideration of the function of the whole fish and more particularly the interrelationships outlined in figure 1. The bulk of this thesis represents those components of that overall conception which developed into intrinsically coherent investigations. Nevertheless the underlying theme remains one of a general consideration of the function of the circulatory system in rainbow trout.

MATERIALS AND METHODS

Introduction

In the course of these studies considerable effort was
directed to the design and development of special apparatus
and techniques. Some of the problems raised merit discussion
which would be innappropriate in the context of the final
results. These points will be discussed in this section of
the thesis which therefore needs to be longer than is
customary for an account of materials and methods.

The Fish

The fish used were rainbow trout (<u>Salmo gairdneri</u>)
obtained from Howietoun fish farm at Bannockburn, Stirling.
They were described by the farm as being of the "Shasta"
race although it is doubtful if they can be regarded as
being of a pure genetic strain (Mills 1971). Rainbow
trout have been kept at this farm since their introduction
into this country in the last century.

These fish were kept in the laboratory aquarium in
800 l circular tanks in which a circulation of water was
maintained against which the fish would tend to swim. They
were fed on standard floating fish pellets from Coopers
Nutritional Products Ltd.

All the individuals used in the experiments were thus
kept in the aquarium for at least 4 weeks at the proposed
experimental temperature $\pm 1^{\circ}$C. The experimental fish were
all 24.5 to 28.0cm long although for anatomical studies both

larger and smaller fish were used.   No criteria of sex
or sexual maturity were applied in choosing individuals
for experimentation.   It was ensured that the experimental
fish were free of disease and in as good condition as
possible.

The flume for swimming studies

DESIGN AND CONSTRUCTION.

In order to investigate changes occurring during swimming
activity a fundamental requirement was a laboratory facility
in which fish could be made to swim for periods at known
velocities.   Some sort of 'water treadmill' was envisaged
in which fish would swim against a water movement so that wires
and electrodes could be attached for physiological recording
purposes with a minimal restriction to its swimming movements.

Particular limitations governed the design of the apparatus:-

1. A fish of about 25cm long was regarded as a minimum size
on which surgical procedures could be satisfactorily carried
out.   The Home Office required that the diameter of any test
chamber should be such that a fish could turn round without
touching the sides.   This defined the minimum size of the
test chamber cross section as about 23cm.

2. Cost of the apparatus had to be kept to a minimum and no
major engineering development could be contemplated.

3. A maximum swimming velocity of at least 60cm/sec was
required to test fish over the full range of sustained swimming
speeds.

Three main types of swimming apparatus have been used
in previous swimming studies on fish:-

(a) Fish Wheel: Described by Bainbridge and Brown (1958)

in which an annular channel containing water is rotated about its vertical axis. There is no movement of water relative to the walls of the chamber which has the advantage that no problems of non-uniformity of water flow are encountered as in other forms of apparatus. The cross section of the water space in Bainbridge's apparatus is 15cm by 19cm and yet it used a very large (5 H.P.) electric motor. To build a wheel with a 23 by 23cm cross section channel would have been a major engineering project and access to the fish with wires would have been difficult.

(b) Laminar flow flume: An example of this is that described by Arnold (1969). In this straight flume, water flows by gravity from a large header tank and falls freely over an adjustable weir at the other end. By varying the slope of the flume and the height of the weir different flow rates may be obtained. Even a scaled down version of this flume which is 30cm square in cross section would be a major undertaking to construct and large pumps are required to maintain the head of water in the supply tank. Although flow is uniform over most of the cross section of the channel, close to the walls flow is inevitably slower due to the boundary layer effect.

(c) Water Tunnel: Water tunnels have been constructed mainly as respirometers such as those used by Brett (1964) and Blaska et al (1960). The design principles involved are extensively reviewed by Bell and Terhune (1970). One of the advantages of these tunnels is that designs have been produced using small volumes of water which is necessary for respirometry. By inducing turbulent flow the boundary layer effect can be reduced and a uniform flow over a large proportion of the cross section of the tunnel can be obtained. However these

and other flow correcting devices involve a considerable
head loss and relatively powerful pumps are required for
quite small test chamber diameters. A tunnel of this
type with a 23cm diameter chamber would again have been too
expensive.

With all the above designs the provision for variation
in drive motor speed over a wide range can be very expensive.
It was also apparent that any pump capable of taking the full
flow of water in a 23cm diameter cross section at 60cm/sec
would have been very large and expensive (4 to 5 horsepower).

The main feature embodied in the design eventually used
is in that the momentum of the circulating water is conserved
by minimal obstruction to the flow. For this reason no
flow correcting devices are incorporated and the grids
confining the fish to the test chamber are of the largest
mesh possible. This allows the use of a relatively small
pump (¾ H.P.) in a bypass circuit to circulate the water.
An open vaned centrifugal pump (Beresford Pumps Ltd.) was
used, the output of which was constricted by a diaphragm valve
which was used as a means of controlling the velocity of
water in the main channel. A general diagram of the flume
is shown in fig. 2 and a plan in fig. 3. Some non-uniformity
in the flow pattern was felt to be acceptable in view of the
advantages of this relatively simple design. The general
pattern of a rectangular cross section channel is very similar
to the apparatuses described by Heath & Pritchard (1962) and
Oehmichen (1958). However both these designs are entirely
enclosed and are therefore essentially water tunnels in their
operating characteristics. In the type of open channel
used in these studies with a free water surface no great

Figure 2.   The flume used for swimming studies;
diagram showing the general layout.
The arrows indicate the direction of
water flow.

PITOT TUBE

SWIVEL SUSPENSION

CABLE TO
OSCILLOGRAPH

ming studies;

eral layout.

direction of

PITOT TUBE

TEST CHAMBER

SWIVEL SUSPENSION

CABLE TO OSCILLOGRAPH

CONTROL VALVE

PUMP
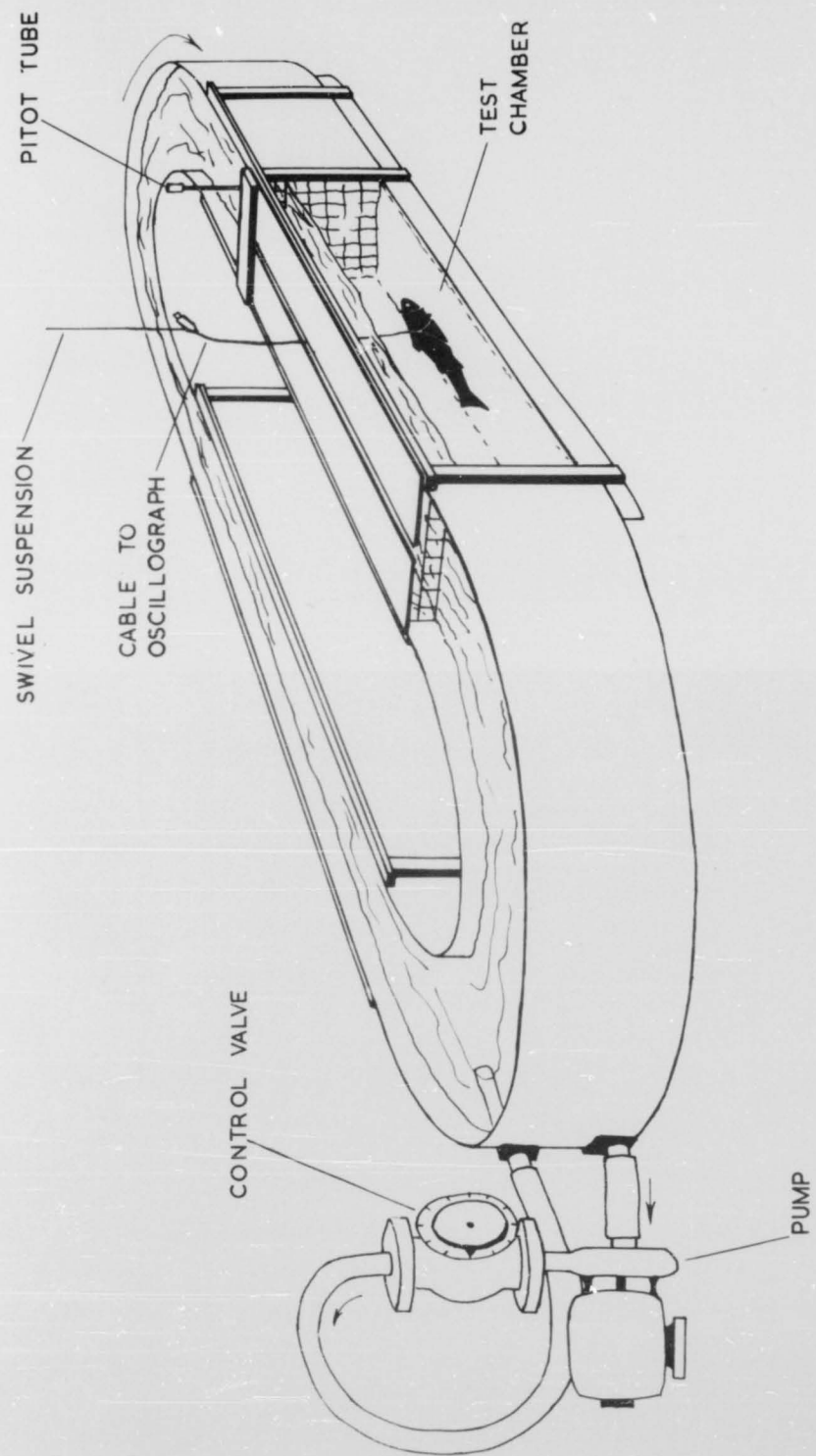
Figure 3.   Plan and elevation drawings of the flume.

PLAN

POSITION OF COOLING COIL

TO PUMP

FROM PUMP

228 mm

PLASTIC PIPES 40mm O.D.

457 mm

228 mm

THERMOSTAT SENSOR POSITION

THERMOMETER POSITION

WALLS 4 mm THICK MARINE PLY

570 mm

PITOT TUBE POSITION

914 mm

ELEVATION

FROM PUMP

TO PUMP

228 mm

BOTTOM 6 mm THICK MARINE PLY

WALLS + BOTTOM 6 mm THICK PERSPEX

SOFTWOOD FRAMEWORK
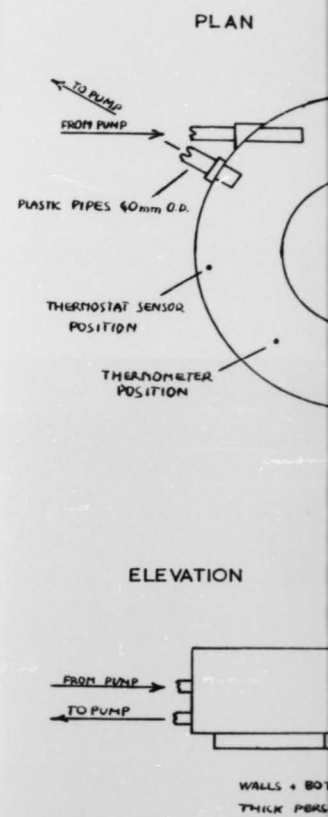
wings of the
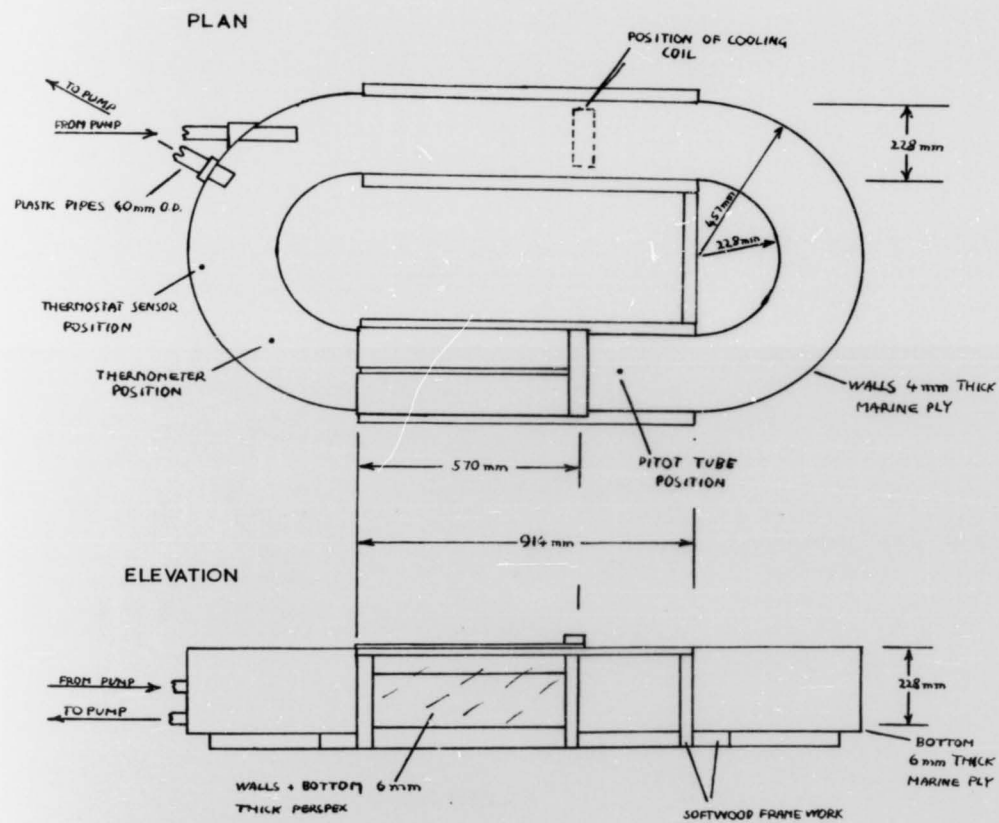
Figure 4. The sloping water manometers used
for velocity measurements front
elevation. · The assembly shown was
mounted at the correct angle on a
retort stand.

TO PITOT
TUBE

BOSS
HEAD

MANOMETER TUBES

$\text{SIN. } \alpha = 1/9$

PALMER ADJUSTABLE BOSS HEAD

BOSS HEAD

TO PITOT TUBE

INCLINED SUPPORTING ROD
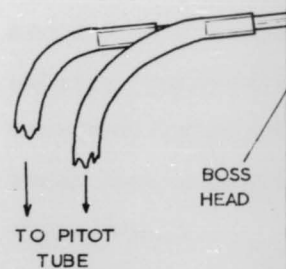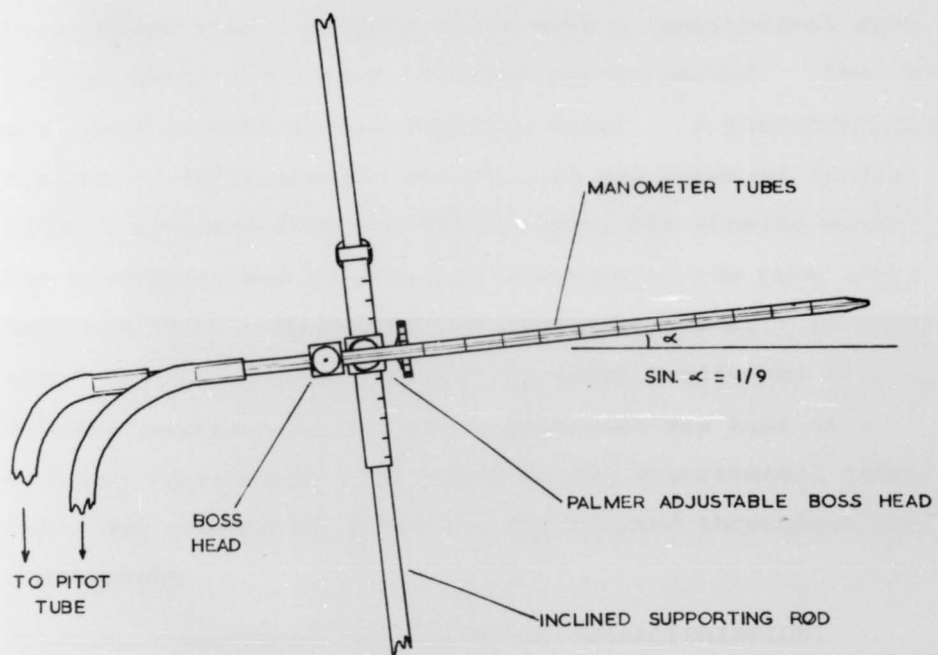
differences in the static head of water around the circuit can be allowed to occur.

The main channel is 23cm square in cross section and is constructed of timber and marine grade plywood painted to give a smooth waterproof finish. A 56cm long transparent perspex section is incorporated as a test chamber to which the fish is confined by 2.5cm mesh grids. The test chamber is provided with a plywood cover with a longitudinal slot through which wires from the fish may be passed. The flume was operated with a 21cm depth of water. A thermostatically controlled refrigerated cooling coil was immersed in the flume downstream from the pump output, the sensing element of the thermostat was placed just upstream of the pump input and kept the flume temperature constant to $\pm$ 0.5°C. In order to avoid the difficulties of cooling under conditions of high ambient temperatures the whole apparatus was kept in a constant temperature room close to the experimental temperature. Water was renewed at intervals and aerated throughout the experiments.

VELOCITY MEASUREMENT AND OPERATING CHARACTERISTICS.

A 3/16" diameter pitot-static tube (Airflow Developments Ltd.) was used for water velocity measurements in the flume. Dynamic and static pressures were measured using sloping water manometer. (fig. 4). 1ml graduated pipettes were used as manometer tubes and were chosen so that the smallest divisions on the scale (0.02ml) corresponded to 0.02cm in height when the tube was inclined at a slope of 1 in 9. The two tubes were mounted side by side on an adjustable boss head for ease of zero adjustment. The manometers were simply filled with water from the flume. Pressure could be read to an accuracy

of 0.01cm H_2O.   The water velocity was calculated using the formula:-

$$V=\sqrt{2gp} \qquad \text{(a)}$$

V= velocity cm/sec

g= acceleration of gravity 981cm/sec^2

p= dynamic pressure cm H_2O

Then the error can be analysed according to Bell & Terhune (1970)

Differentiating:-

$$\frac{dV}{dp} = \frac{\sqrt{2g}}{2\sqrt{p}} \quad \text{or} \quad \frac{dV}{V} = \frac{dp}{2p} \qquad \text{(b)}$$

As two menisci are involved in the determination of p the error in this measurement is $\pm$ 0.02cm H_2O.   However from (b) it can be seen that for a given error in velocity V the error in p can be twice as large.

The accuracy of the pitot tube velocity determination increases with velocity.   For the manometer system used it can be calculated that the minimum velocity for 10% accuracy is 14.00cm/sec and the minimum velocity for 1% accuracy is 38.50cm/sec.   Few swimming trials were carried out below 14.00cm/sec but in between trials the fish were allowed to rest at a velocity of approximately 5.0cm/sec.   The control valve setting for this was determined by timing particles in the water flow passing across the length of the test chamber. The error in this calibration was $\pm$1cm/sec due to slight variation in the behaviour of the valve diaphragm.

Figure 5. Water velocity profiles in the flume
test chamber. Measurements were taken
at the 3cm intervals indicated by the
grid. The mean and standard deviations
of these measurements at 3 speed
settings are given. (Isometric
projection.)
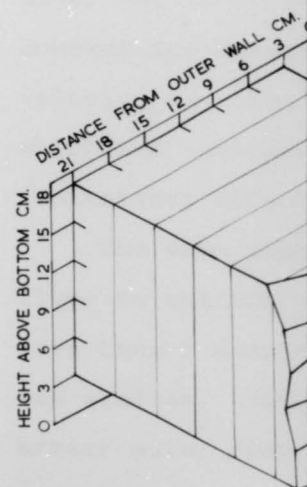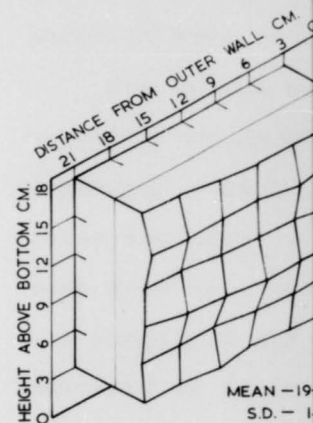
in the flume
nts were taken
icated by the
dard deviations
3 speed
metric

DISTANCE FROM OUTER WALL CM.
21 18 15 12 9 6 3
VELOCITY CM/SEC
10 20
HEIGHT ABOVE BOTTOM CM.
0 3 6 9 12 15 18
MEAN —19.47 CM/SEC
S.D. — 1.64

DISTANCE FROM OUTER WALL CM.
21 18 15 12 9 6 3
VELOCITY CM/SEC
10 20 30 40
HEIGHT ABOVE BOTTOM CM.
0 3 6 9 12 15 18
MEAN —40.61 CM/SEC
S.D. — 3.05

DISTANCE FROM OUTER WALL CM.
21 18 15 12 9 6 3
VELOCITY CM/SEC
10 20 30 40 50 60
HEIGHT ABOVE BOTTOM CM.
0 3 6 9 12 15 18
MEAN —59.20 CM/SEC
S.D. — 4.11

During a fish speed trial the pitot tube was immersed in the water flow as shown in the figures at a position where the velocities corresponded closely with those in the chamber. During such a trial pressure readings were taken at frequent intervals and the mean of these was used in calculation of the water velocity in order to minimise the influence of surge effects in the flow.

Due to the geometry of the flume some variation in velocity across the cross-section of the test chamber was expected. This was assessed by measuring the axial velocity using the pitot-static tube at points on an imaginary 3cm mesh grid in a plane tangential to the flow half way along the length of the chamber. Such velocity profiles were measured at three speed settings and are plotted isometrically in fig. 5. It was shown that the velocity of flow from the outer radius of the bends was higher than on the inside. However the standard deviation of the mean cross sectional velocity was less than 8.5% over a wide range of speeds which was considered acceptable in view of the design limitations already discussed.

The time taken for acceleration of the water in the flume to uniform maximum velocity for a speed trial was less than 3 minutes and the time for subsequent decceleration was similar. At high speeds a paddle was generally used to arrest water flow to facilitate precise determination of fish recovery times after exercise.

Surgical procedures

ANAESTHESIA.

For all surgical procedures fish were anaesthetised by immersion in 1:10000 solution of Tricaine methane sulphonate

Figure 6.   The operating table used for vagotomy
operations on trout. (isometric
projection).   The fish is held by a
folded slab of foam rubber as if between
the pages of a book.   The arrows
indicate how access can be gained under
the operculum while the gills are
perfused with anaesthetic solution.
This fish holding device was placed on
an operating table equipped with water
drainage channels etc.
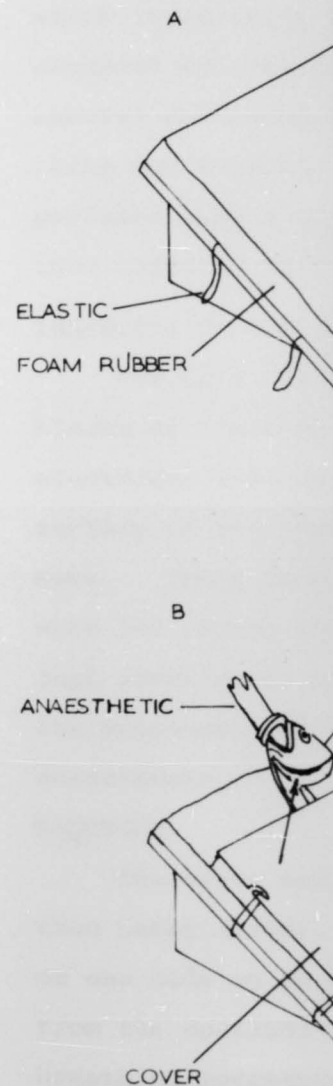
A

ELASTIC

FOAM RUBBER

B

ANAESTHETIC

COVER

A

PERSPEX
BASE

ELASTIC

FOAM RUBBER

B

ANAESTHETIC

MICROSCOPE

COVER
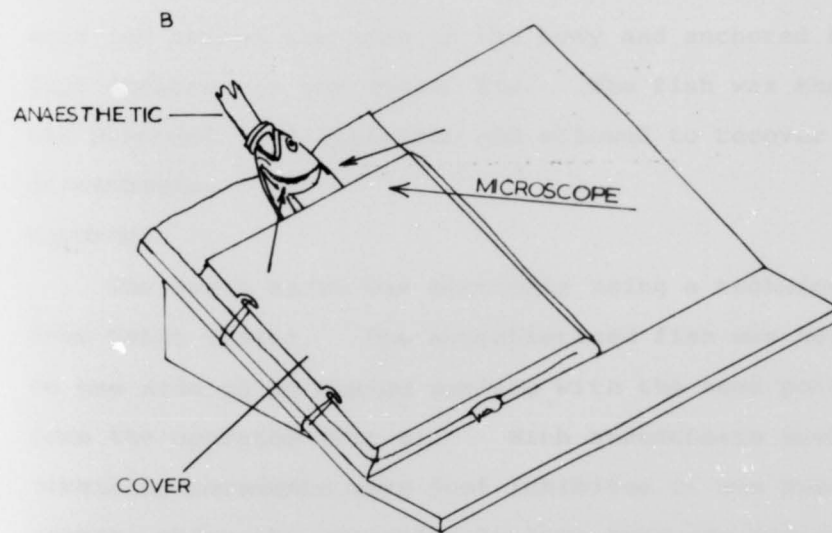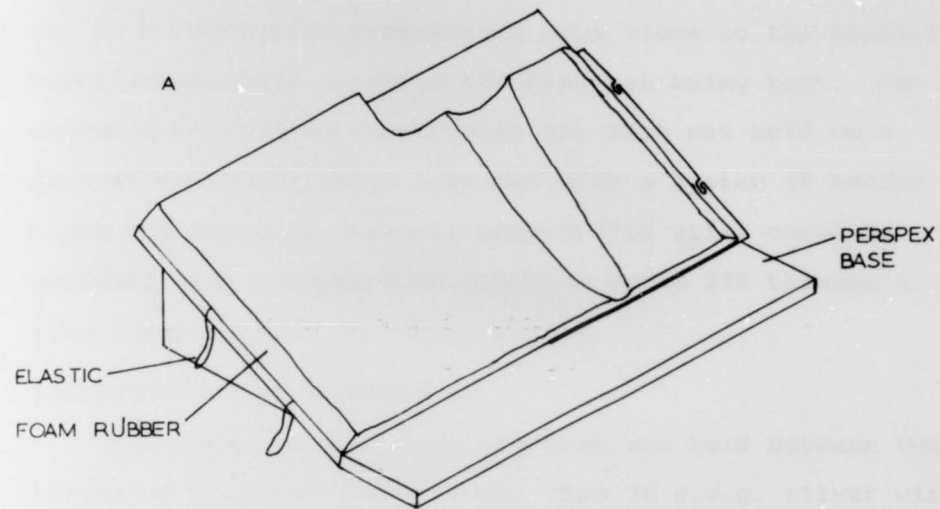
MS 222 (Sandoz). Care was taken to avoid temperature changes during anaesthesia and all operations were carried out in a controlled temperature room close to the prevailing water temperature in which the fish was being kept. For extended periods of anaesthesia the fish was held on a special operating table provided with a system of header tanks and drainage channels whereby the gills could be perfused with a controlled dilution of MS 222 through a tube inserted into the fish's mouth.

INSERTION OF ECG ELECTRODES.

For this the anaesthetised fish was held between two blocks of flexible rubber foam. Two 30 s.w.g. silver wire electrodes were implanted subcutaneously, one at the ventral surface of the pectoral girdle and the other anterior to the anus. These were sutured in place and the attached wires were led around the side of the body and anchored by suture just anterior to the dorsal fin. The fish was then put into the proposed test apparatus and allowed to recover from anaesthesia.

VAGOTOMY.

The vagus nerve was sectioned using a technique modified from Labat (1966). The anaesthetised fish was held lying on one side on an angled surface with the head pointing away from the operator (Fig 6). With anaesthesia such that the breathing movements were just inhibited it was possible to operate under the operculum looking from the posterior aspect with the aid of binocular dissection microscope.

A small incision about 5mm long was made posterior to the gills along the anterior edge of the cleithrum. A segment

Figure 7.   Diagram of a dissection of rainbow trout
            to show the vagus nerve.   The gills have
            been trimmed to give a clear view of the
            nerve.

GASTRIC NERVE

ANTERIOR CARDINAL VEIN

SEGMENT OF VAGUS REMOVED

BRANCHIAL NERVES

rainbow trout

he gills have

view of the

GASTRIC NERVE

ANTERIOR CARDINAL VEIN

SEGMENT OF VAGUS REMOVED

BRANCHIAL NERVES

PHARYNX

DUCTUS CUVIERI
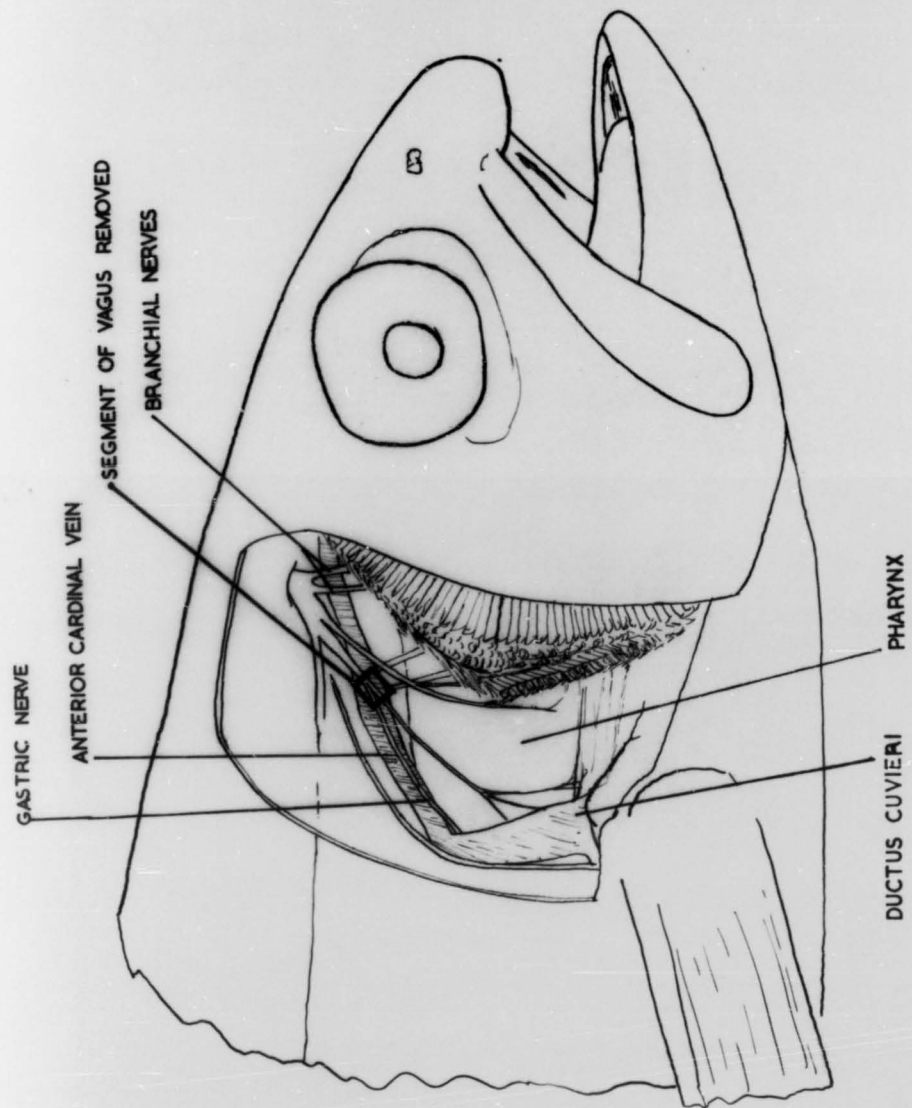
Figure 8.   ECG s of a lightly anaesthetised rainbow
trout at 15°C.   The operculi were held
shut during the times indicated by the
horizontal bars.   In the intact fish a
cardiac inhibition is observed whereas
in bivagotomised fish there is no
response.

APPRO

INTACT

BIVAGOTOMISED

APPROACH   REFLEX

INTACT

BIVAGOTOMISED

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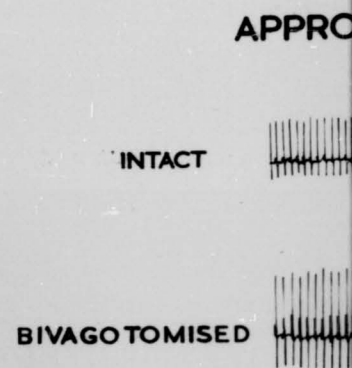
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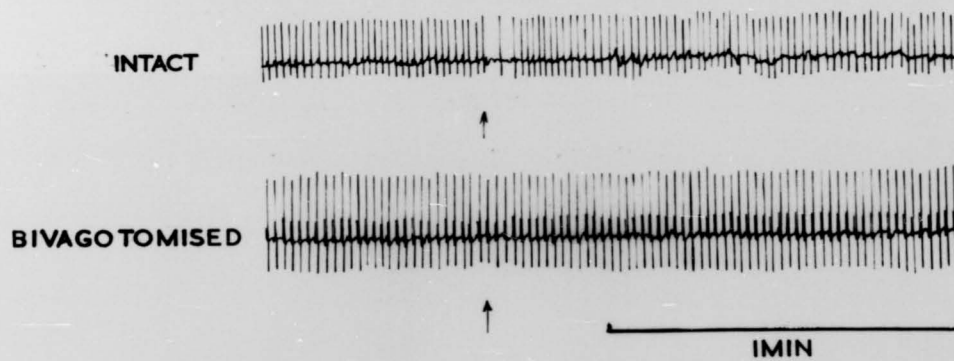
Figure 9.   ECGs of intact and vagotomised fish
             at 6.5°C.   The arrows indicate the
             presentation of a visual stimulus to
             the fish.   In the intact fish a
             cardiac inhibitory reflex is
             observed.
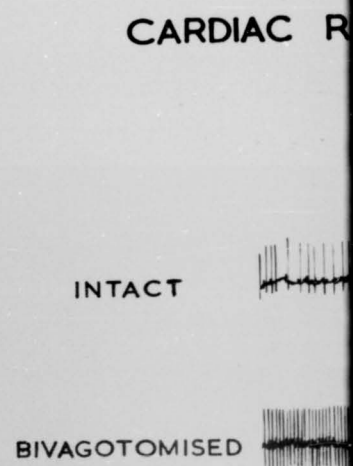
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CARDIAC RESPIRATORY REFLEXES

LIGHT MS 222 ANAESTHESIA

INTACT

BIVAGOTOMISED
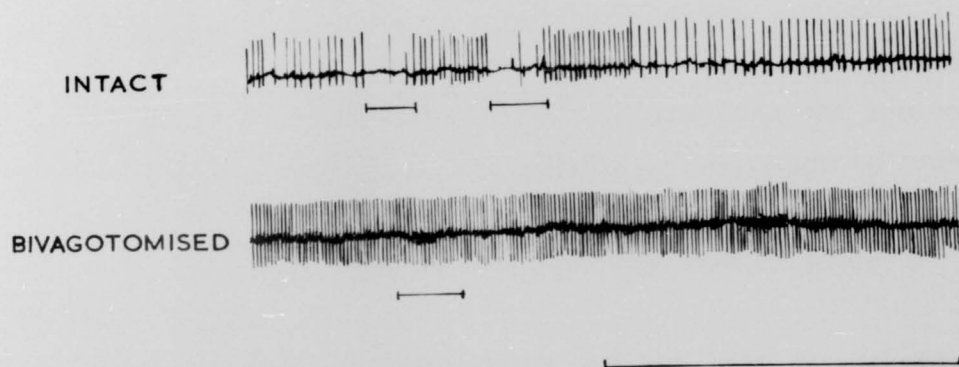
of the cardiovisceral branch of the vagus nerve was then
readily exposed and was removed taking care not to cause
haemorrhage of the major veins in this region (Fig. 7).
Two sutures were used to close the wound.   Approximately
35 minutes were required to effect a bilateral vagotomy
whereupon the fish put into a 180l tank in which it was
allowed to recover from anaesthesia.   Within one hour the
fish would regain its normal pale colour.

12 to 22 days were allowed in the recovery tank for
healing of the wound. At 15°C the sutures were often shed
within 5 days and wound healing was well advanced by the
twelth day.   At 6.5^{o}C up to 22 days were required for healing
of the wounds.

In the course of subsequent experimentation the success
of vagotomy and the absence of vagus regeneration were
tested for in the lightly anaesthetised fish by checking for
the absence of cardiac inhibitory reflexes when the operculi
were held shut (fig 8).   The absenc e of approach reflexes
in the ECGs (Labat 1966) and post mortem anatomical examination
provided additional verification of the lack of vagus
function (fig 9).

In some fish the vagus nerve was cut only on one side,
a sham operation being carried out on the other side.   None
of the fish which had been operated on could subsequently feed
but there was no evidence of post-operative infection as
reported by Labat (1966) and no mortalities occurred.

Procedure for measurement of heart rate
during swimming in intact and vagotomised fish.

Tests consisting of the measurement of heart rates during swimming activity were carried out at two temperatures 6.5°C and 15°C. At each temperature 3 groups of fish were used:- intact, bilaterally vagotomised and unilaterally vagotomised.

INTACT FISH.

Feeding was discontinued 24 hours before implantation of ECG electrodes. The fish was then put into the test chamber of the flume to recover from the anaesthesia. The screened ECG cable 'tether' was passed through the slot in the test chamber cover and attached to a swivel suspended from the ceiling. The cable was broken at this point by a plug and socket which could be disconnected to allow complete freedom of movement for the fish when not recording ECGs. Electro-cardiograms were recorded from the implanted electrodes using a Devices M2 heat pen recorder and an AC7C pre-amplifier. A screen round the test chamber prevented movements in the laboratory being seen by the fish and care was taken to avoid disturbance of the fish throughout the experiments.

The fish was kept in a water flow of about 5cm/sec in the test chamber against which it would orientate but not necessarily swim. It was thus allowed to rest overnight before the first speed trial. A speed trial consisted of exposure for 30 minutes to a uniform elevated water velocity against which the fish would swim in order to maintain station.

Two such trials were carried out daily with at least 3 hours rest (5cm/sec flow) in between.   Trials were continued for up to 6 days with each animal at a wide range of velocities up to the maximum which could be sustained for 30 minutes.   Heart rates were subsequently determined by counting the number of beats in each minute on the electrocardiogram.

In general at velocities which it could easily sustain the fish swam steadily throughout the 30 minutes without falling back or avoiding swimming by staying close to the inner wall of the flume.   At velocities close to the maximum sustainable speed the fish tended to avoid swimming or rest its tail on the rear grid.   Any trial in which the fish obviously was not swimming steadily at the test speed was excluded from the analysis of results.   Tests were often also curtailed by breakage of the wires and connections to the electrodes.

VAGOTOMISED FISH.

Bilaterally and unilaterally vagotomised fish underwent exactly the same test procedure as the intact fish after the period of recovery from the vagotomy operation (12 - 22 days).   While the fish was recovering from the anaesthesia for implantation of ECG electrodes, tests were carried out for cardiac inhibitory reflexes by holding the operculi shut. Absence of any cardiac response to this confirmed the absence of vagus function.

Measurement of heart rate and Oxygen Consumption

THE RESPIROMETER

The respirometer used in these experiments was a simple

Figure 10. The respirometer chamber used in heart
rate and oxygen consumption measurements.
(Isometric projection).

in heart

measurements.

INLET

560 MM

230 MM

190 MM

E.C.G. CABLE

ACCESS HATCH

PO$_2$ ELECTRODE
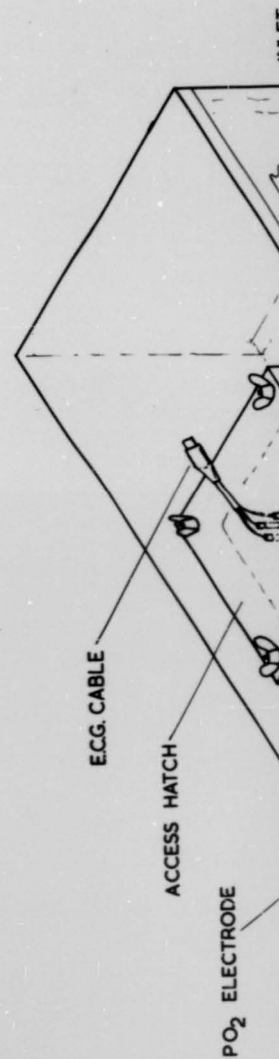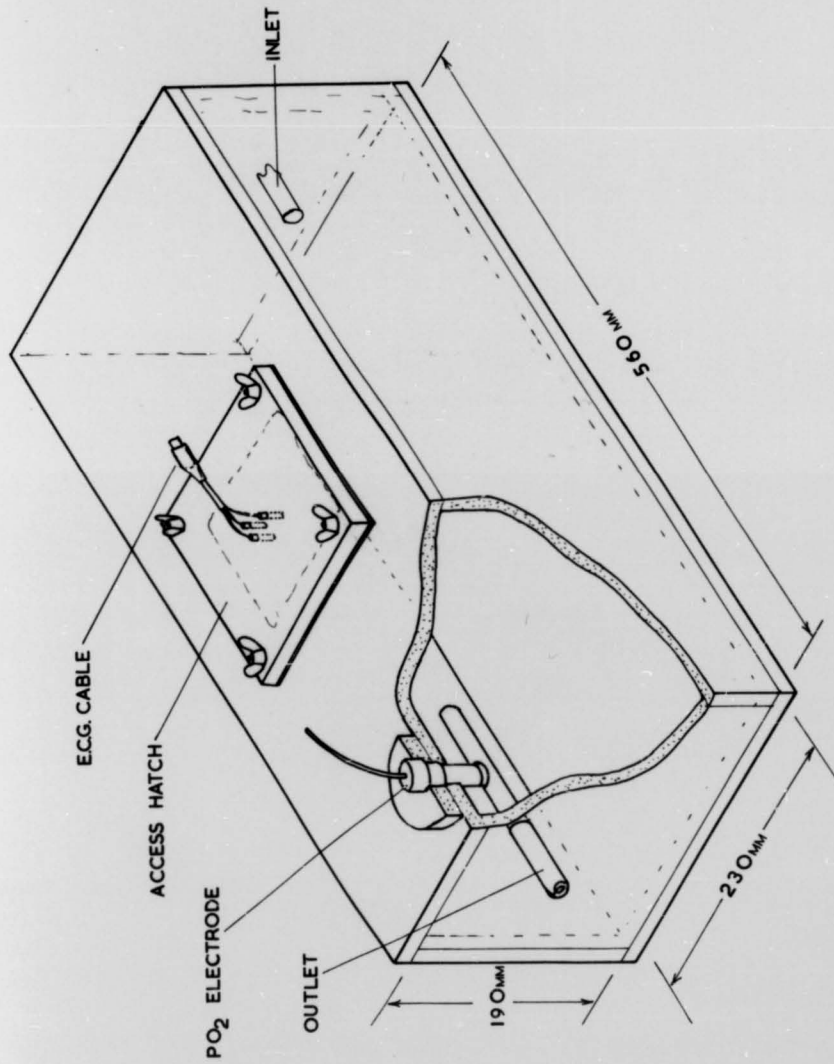
OUTLET

Figure 11. Schematic diagram of the water flow
circuit used for the respirometer.
(a) closed circuit; oxygen consumption
being measured. (b) open circuit;
system being flushed with fresh
water.

AIR BLEED
RESERVOIRS

A. CLOSED
CIRCUIT

MAIN CONTROL
VALVES

WATER
BATH

PO₂ ELECTRODE

PUMP

DRAIN

DRAIN

AIR BLEED
RESERVOIRS

B. OPEN
CIRCUIT

MAIN CONTROL
VALVES

WATER
BATH

PO₂ ELECTRODE

PUMP

DRAIN

DRAIN
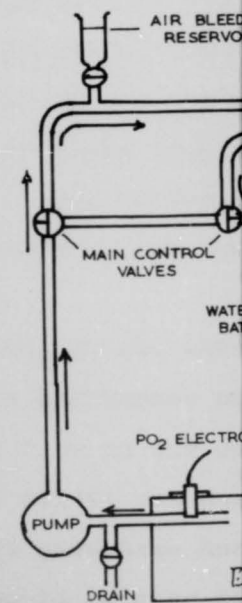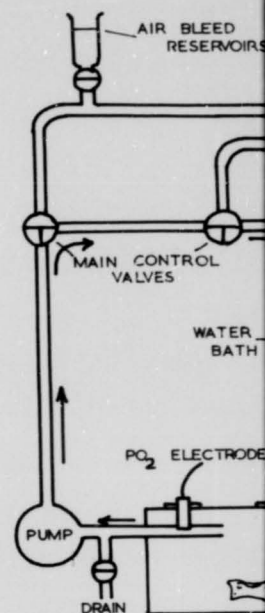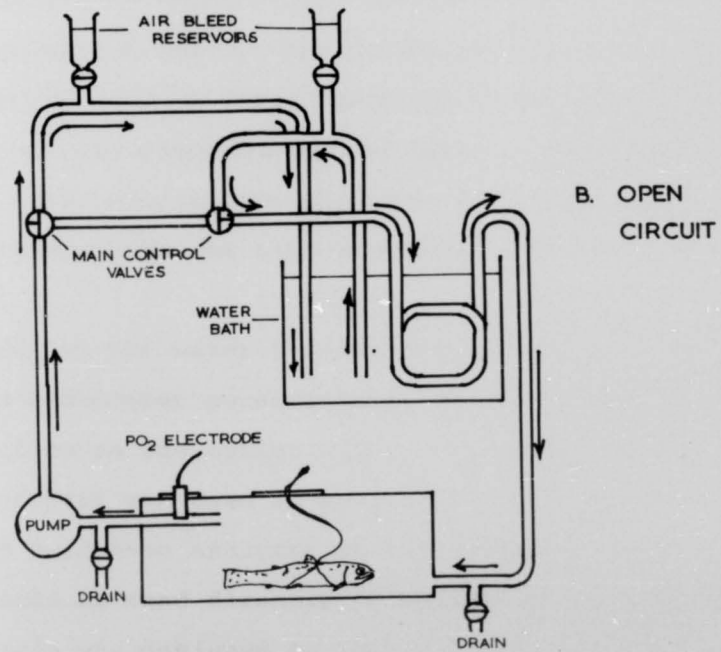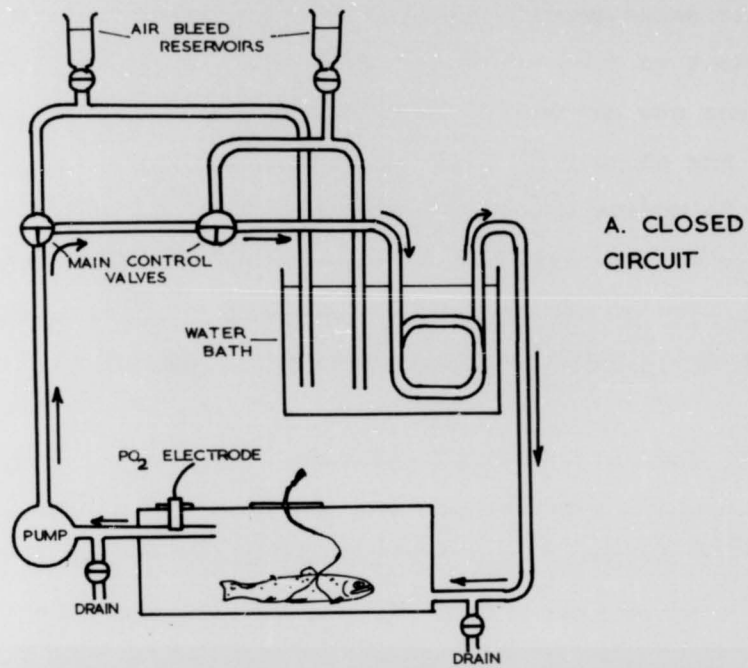
perspex box of internal dimensions corresponding closely to
the dimensions of flume fish chamber;   56cm by 23cm by
21cm (Fig 10).   An access hatch in the top was sealed by
a foam neoprene gasket and was held by 4 studs and wing
nuts.   3 brass studs passed through the centre of the hatch
to which ECG electrode wires to the fish were soldered
The electrode arrangement on the fish was exactly as used
in the flume experiments and care was taken to ensure proper
contact of the earthed screening with the water in the
chamber.   The actual electrode cables were insulated from
the water by using an aerosol lacquer spray on the soldered
terminals.   A small centrifugal pump (Charles Austen Pumps
Ltd. model C16 300) drew water from an outlet at the top
of one end of the box and circulated it through a coil
immersed in a constant temperature water bath and back to
the box via an inlet at the bottom of the other end (fig. 11).
By a system of valves this closed circuit could be opened so
that fresh aerated water was drawn from the constant temperature
bath into the respirometer and back into the water bath (fig.
11b).   For convenience the flume was used as the constant
temperature bath for this experiment and as a store of aerated
water.

PO$_2$ of the water leaving the respirometer was monitored
with a Radiometer polarographic electrode inserted into the
water flow in the outlet tube.   A Radiometer PO$_2$ electrode
(type E5046) was used in conjunction with a Radiometer type
PHM71B Acid-base Analyser on the galvanometer scale of which
PO$_2$ could be read directly in mm Hg.   The insertion of the
electrode was designed so that the body of the electrode was
immersed in and kept at the temperature of the respirometer

water.   The volume of the respirometer box and the closed

circuit plumbing was 24,620 l. and the flow through the

pump was 1.75 l/min. or one respirometer volume in 14

minutes.   This together with the mixing effect of the

flow ensured adequate sampling of the water PO_2.

EXPERIMENTAL PROCEDURE.

All experiments were carried out at $15^{\circ}C \pm 0.5^{\circ}C$ on

rainbow trout acclimated to that temperature.

The fish was anaesthetised and the ECG electrodes were

implanted as described before.   It was then transferred

into the respirometer to recover from anaesthesia.   The

access hatch was screwed down firmly ensuring that the

chamber was free from air bubbles.   The main control valves

were then set to the open circuit position and the fish was

allowed to recover from anaesthesia with the respirometer

being perfused with aerated water.

The oxygen electrode was calibrated in air saturated

water and in zero PO_2 solution (barium thiosulphate) prior

to experiments with each new fish.   The 100% saturation value

calibration was frequently rechecked but the zero PO_2 reading

was very stable (being independent of membrance properties

which are liable to variations) and was checked less

frequently.   The reading to which the 100% saturation

calibration was set was calculated from the barometer pressure:-

$\quad$ (P - SVP) x $\dfrac{21}{100}$ = PO_2 mm Hg at 100% saturation

$\quad$ P = Atmospheric pressure mm Hg

$\quad$ SVP = Saturated vapour pressure of water mm Hg

$\quad \dfrac{21}{100}$ = approximate fraction of oxygen in dry air.

Once the fish had recovered from anaesthesia the PO_2 electrode was inserted into the respirometer and the first "respiration run" was commenced with the PO_2 in the respirometer close to the saturation value.

Prior to the respirometer run the main control valves were turned to the closed circuit position and the electrocardiograph was set to start recording at an appropriate speed. After about 3 minutes the actual start of the run was marked on the ECG and the first PO_2 reading was taken. PO_2 readings were taken at 5 minute intervals for 45 minutes while the ECG was being recorded (i.e. 10 PO_2 readings per run). At the end of the run the control valves were turned to open circuit to allow replenishment of oxygen in the respirometer. If the oxygen consumption rate was low two runs were often made consecutively without replenishment of water but the PO_2 in the chamber was never allowed to fall as low as 70% saturation when the effects of hypoxia could be expected to become manifest (Randall & Smith 1967).

The number of heart beats in alternate minutes on the ECG were counted and their mean was calculated to give the mean heart rate during the particular run. The PO_2 readings were plotted graphically against time and a straight line was fitted to them by eye. Maximum deviations from the line never exceeded 2mm Hg so no difficulty was encountered in obtaining an accurate fit. The nominal starting and finishing PO_2 were then read off the line for calculation of the rate of oxygen

consumption as follows:-

$$\frac{PO_2 \text{ start}}{PO_2 \text{ sat}} \times 100 = \%C_{start}$$

$$\frac{PO_2 \text{ end}}{PO_2 \text{ sat}} \times 100 = \%C_{end}$$

PO_2 start = partial pressure O_2 mm Hg at start of run (nominal)

PO_2 end = partial pressure O_2 mm Hg at end of run (nominal)

$\%C$ start = per cent saturation at start of run.

$\%C$ end = per cent saturation at end of run.

The per cent saturation figures (%C) were then converted to
mg/litre (C) using the nomogram of Hart (1967).   Then the
oxygen consumption in mg per kilogram of fish per hour was:-

$$\frac{(C_{start} - C_{end}) 24.62 \times 1.333}{W \ 1000} = O_2 \text{consumption mg/kg/hr}$$

24.62 = respirometer volume litres.

1.333 = corrects reading to 1 hour.

W = weight of fish in grams.

Runs were repeated on each fish for up to 6 days.   Apart
from avoiding the obvious effects of anaesthesia no effort
was made to select times for runs when the fish was not
excited or disturbed.   The aim of this work was to investigate
as fully as possible the bounds of variation in the relationship
between heart rate and oxygen consumption.   Often during a
run the fish was quite active.

The Fish Bending Machine & Experiments on Dorsal aorta

function

THE FISH BENDING MACHINE

As a basis for studying the influence of fish body
movements on blood circulation a number of experiments on trout
cadavers and artificial models were carried out on a fish
bending machine which could simulate fish swimming movements.

One of the anticipated advantages of simulating fish swimming
movements was the possibility of carrying out work at high
"swimming speeds" which could not be sustained for more than
a few seconds by an actual living fish.   It was thus regarded
as very important to be able to reproduce high swimming speeds
which could not be investigated in any other way.   Thus at
the outset the specifications of the machine were laid down:-

1. A 30cm fish should be used; this is large enough to allow
   sufficiently precise dissection and cannulation of the
   blood vessels.

2. The maximum tail beat frequency attainable should be at
   least 12 hz.

3. The speed should be continuously variable over wide range
   of speeds.

The actual swimming wave to be used was derived from the
literature and is discussed in this thesis in the analysis
of dorsal aorta function (fig 38).

The design initially suggested was for a set of transverse
slides to which the fish could be attached.   These could be
moved to and fro by a system of cranks or cams.   The high

Figure 12.   The fish bending machine used for
              simulation of fish swimming movements.
              A, electric motor. B, variable speed
              drive pulleys. C, speed control lever.
              D, crankshaft. E, slide bed. The fish
              is placed between the upright pins on
              the slides.

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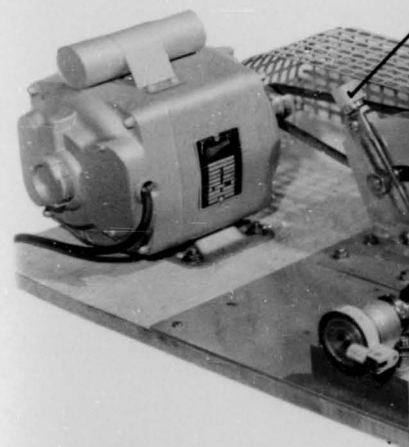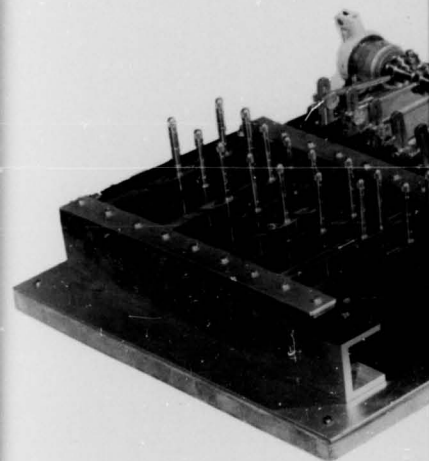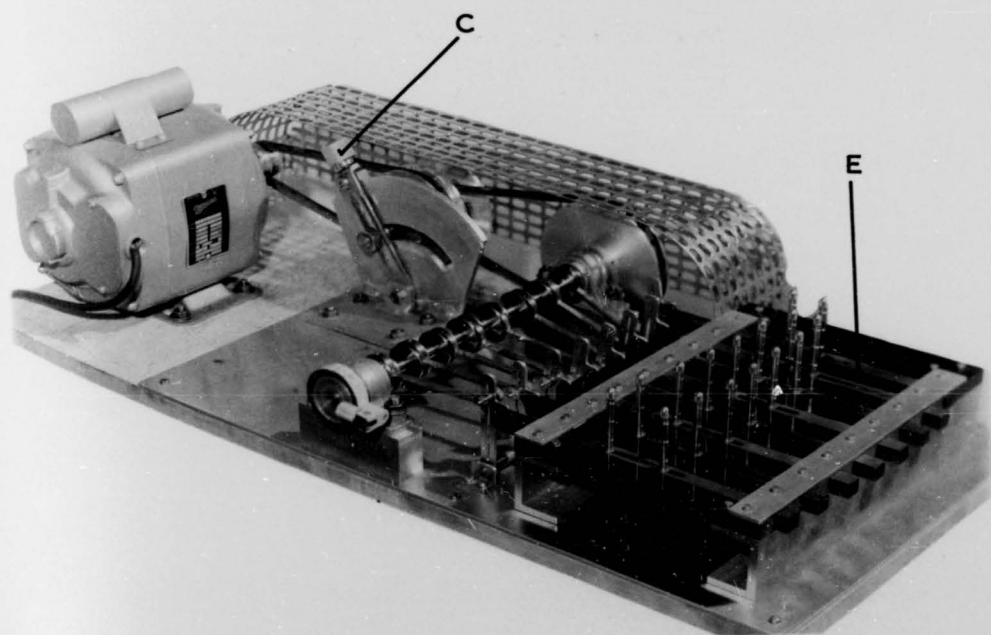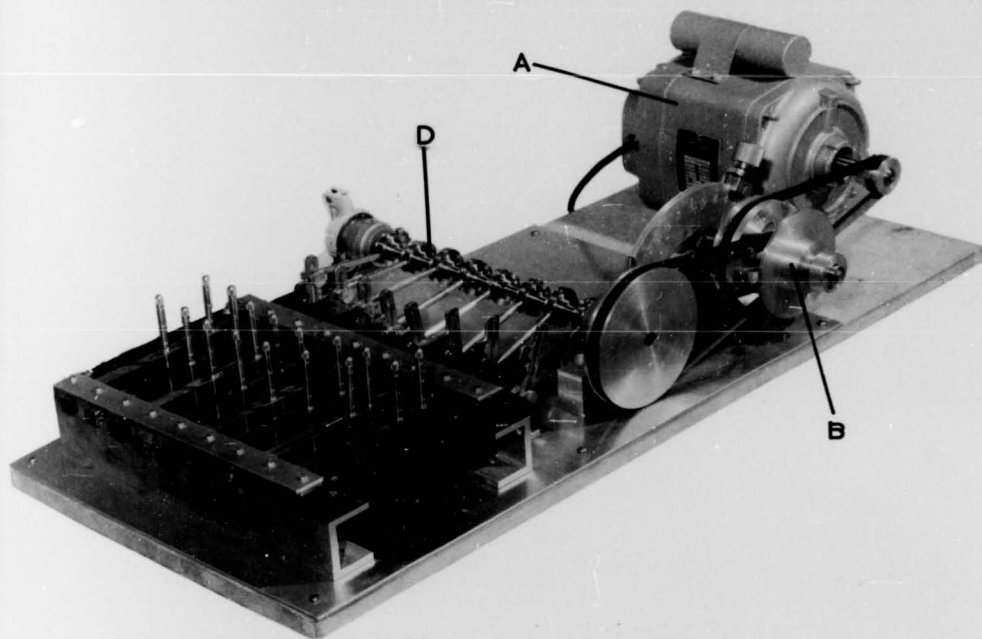
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The fish

pins on

Figure 13. Fish bending machine; Plan and transverse
section of the slide bed. Only one
connecting rod and crank assembly
is shown in the plan attached to the
anterior slide. All the linkages and
cranks for the slides are identical, the
amplitude being adjusted by moving the
gudgeon pin up or down the vertical
lever as shown.
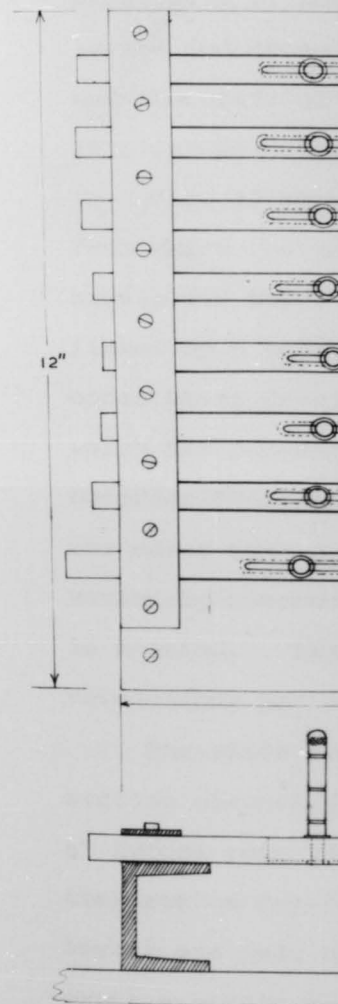
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PLAN

- spacing tube
- pivot shaft
- gudgeon locking screw
- crankshaft 1/2" dia.

12"

8"

- 1/2" square tufnol slide
- spacer (tufnol)
- aluminium alloy retaining strip
- bolts to base pieces

SECTION
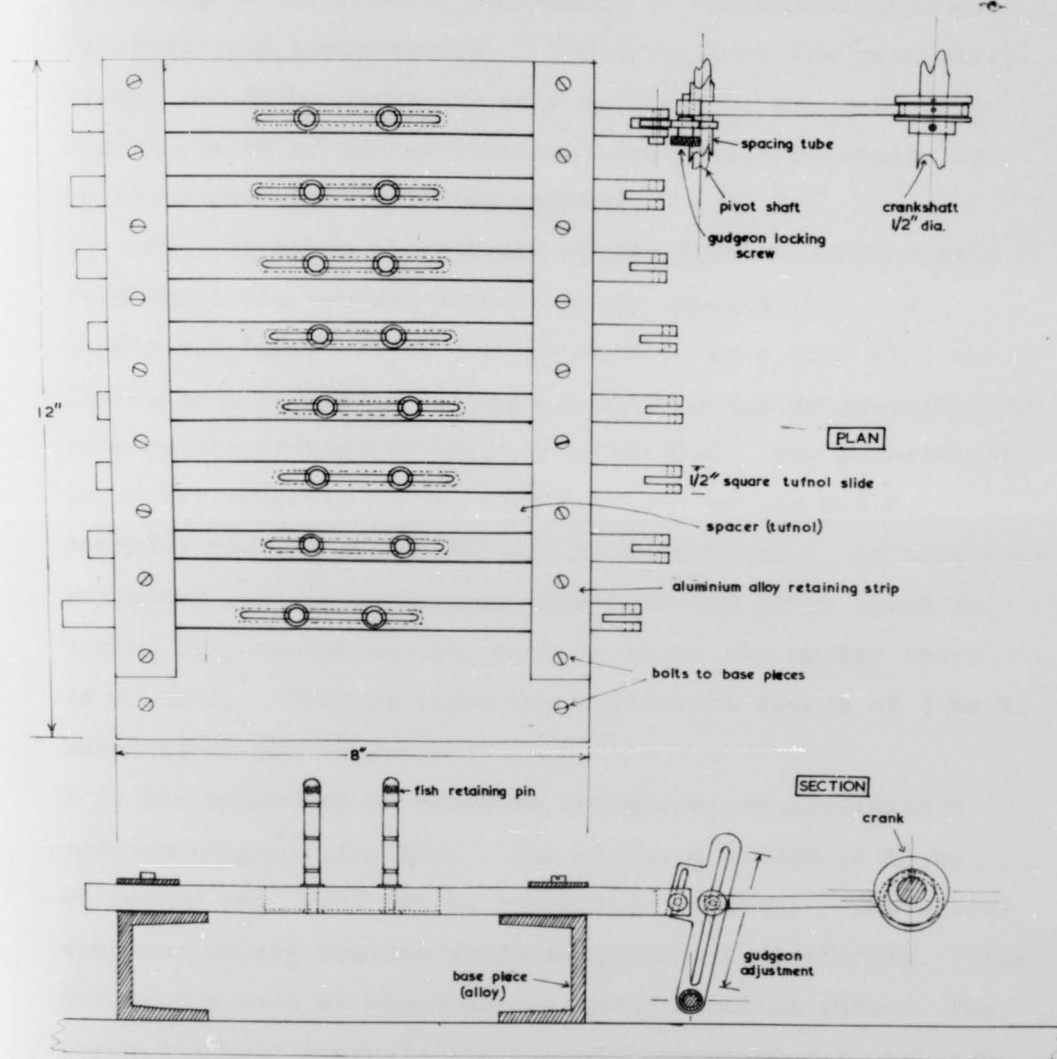
- fish retaining pin
- crank
- base piece (alloy)
- gudgeon adjustment

speeds required appeared to be major difficulty. The
final design of the main bed of the machine and transverse
slides was suggested by Mr. R. Vitols of the Textile
Machinery Research Unit, Department of Mechanical Engineering,
University of Loughborough. The details of the crankshaft,
levers and drive mechanism were designed by Mr. B.E. Povey
and the staff of Shared Technical Services, University of
Stirling who also built the machine.

Fig. 12 shows photographs of the fish bending machine
from which the general layout can be appreciated. A ¾
horsepower motor drives the crankshaft via a pair of V belts
linked by a pulley arrangement made of a set of interlocking
cones which provide a variable speed drive (the principle of
which has recently become most familiar in the D.A.F.
motorcar "variomatic" automatic transmission). In this case
the motor runs at a constant speed and the drive speed is
varied by movement on the lever on which the pulley shaft
is mounted. This provides for crankshaft speeds of 3 to 13
revolutions per second.

The slide bed is based on two pieces of aluminium U
section channel (fig 13). The slides are made of ½" by
½" tufnol rods with tufnol spacers in between. The slides
thus run on dry bearing surfaces quite satisfactorily. The
levers are made of brass as are the connecting rods. The
crank ecentric journals are turned from phospor bronze and
the crank big end bushes are steel. The phase angle of the
ecentrics relative to the shaft is readily adjustable with
set screws which lock the ecentric onto the shaft. The

amplitude of the slide movement is adjustable by altering the position of the connecting rod pivot on the lever. The position of the fish retaining pins on the slides can also be adjusted in order to centre the fish on the slide bed.

The machine is capable of reproducing a wide variety of transverse oscillatory movements (approximately sinusoid) in anything attached to the slides at frequencies of 3 to 13hz.

Two main series of experiments were carried out on the machine:-

1. Test on a cannulated dorsal aorta preparation in a dead fish.

2. Tests on an artificial model of the dorsal aorta.

Both sets of experiments were performed with the machine set up to reproduce the idealised swimming waveform of a 30 cm fish.

EXPERIMENTS ON TROUT DORSAL AORTA PREPARATIONS.

Fish of 30 cm $\pm$ 1cm were used in these experiments. A dissection was carried out from the ventral aspect to expose the dorsal aorta in the body cavity region and up to the confluence of the first two branchial arteries. The viscera and the ventral cranial region including the lower jaw were removed for this purpose. The fish was then attached ventral side uppermost to the slides on the bending machine by tying it with wires to the retaining pins. Care was taken to ensure correct alignment of the vertebral column in both horizontal and vertical planes. The third and fourth branchials, subclavian and anterior mesenteric arteries were

then ligatured. A 1.3mm O.D. flexible cannula was then inserted into the anterior part of the dorsal aorta where the first two branchial arteries join; the position used by Smith & Bell (1964) for dorsal aorta cannulation. This cannula was ligatured into place and at a distance of 5cm was connected to a 5mm bore tubing which supplied saline from a constant head device.

The caudal artery was exposed and cannulated using a 1mm diameter flexible cannula which was connected to a pressure transducer. This cannula fitted tightly in the caudal artery and its end could be pushed forward to measure pressures in the aorta in the trunk region. Occasionally a cannula was also inserted into the anterior mesenteric artery in order to monitor pressures at the anterior end of the aorta.

The aorta was thus perfused with saline with a head corresponding to the pressures measured by Stevens & Randall (1967a) and the output pressures could be measured during body movements reproduced by the machine.

Several technical problems emerged during this work:

If the inlet tubing from the reservoir was of a small diameter as soon as flow began a large head loss was noticed and the pressure actually applied to the aorta was considerably smaller than the static head apparently applied. This is to be expected from Bernoulli's theorem and in experiments with cannula tubing when flow rates are measured in terms of drop counts head losses in excess of 50% were noted. This may be the cause of unexpectedly low flow rates observed in in vitro gill perfusion experiments (Rankin 1970). This effect was

minimised in these experiments using a wide bore feed tube
and keeping the small bore section as short as possible.
Also the height of the constant head resevoir was raised to
a level which produced the appropriate pressure in the
dorsal aorta during flow.

The pressure transducer and attached cannula were
chosen to have a high enough frequency response to allow
measurement of the anticipated pressure pulses. However
cannula movement during working of the machine very readily
produced pressure pulses independent of any pumping activity
in the dorsal aorta. Careful damping of the cannulae
could minimise this effect but artefacts could never be
satisfactorily distinguished from real pumping pulses of the
aorta. As it proved impossible to reliably determine pulse
pressures, the experiments turned to the determination of
mean pressure changes, either using the pressure transducer
or simply measuring the height of the saline column in the
caudal cannula which was held vertically against a scale.
EXPERIMENTS ON DORSAL AORTA MODELS.

A 5mm i.d. transparent flexible plastic tube was used
in this work to represent a fish dorsal aorta. In some
preliminary experiments it was simply laid along the bed of
the fish bender in a groove in a block of flexible rubber
foam. The "anterior" end was put into a beaker of water
and the "posterior" end was put into a measuring cylinder.
It was ensured that the tube was filled with water and any
pressure rise due to pumping activity when the bender was
set into motion was noted as a rise in the water level in
the measuring cylinder.

Figure 14.   A. The dorsal aorta model used in basic
studies on the Burne pump mechanism.
The elastic band inside the tube is
anchored by sutures across the lumen
of the tube near each end.

B. The arrangement used for measurement
of maximum ouput head from the dorsal
aorta model.

C. The arrangement used for measurement
of flow from the dorsal aorta model
at different pressure heads.   The
outflow volume from the displacement
vessel was measured over a set time
period in order to determine flow rates.

A

5mm i.d. pvc tube
28cm long

B

C

CONSTANT
HEAD
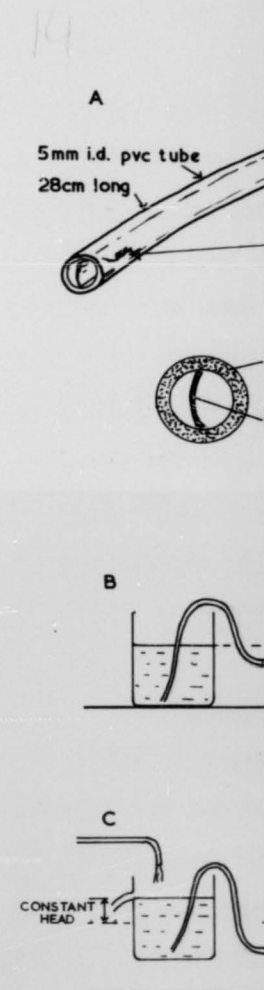
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e flow rates.

A

5mm i.d. pvc tube
28cm long

elastic band anchored by sutures

tube wall
elastic

TRANSVERSE   SECTION

B

DORSAL AORTA
MODEL

MAXIMUM OUTPUT
PRESSURE

FISH   BENDER

C

CONSTANT
HEAD

DORSAL AORTA
MODEL

FISH   BENDER

OUTPUT
FLOW
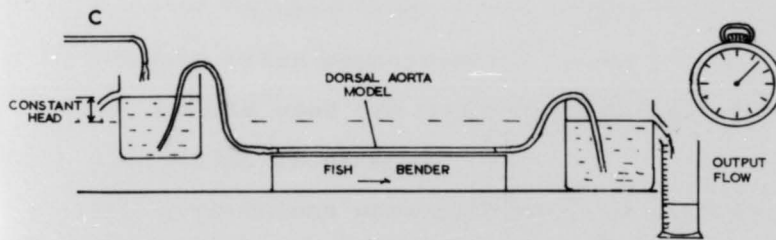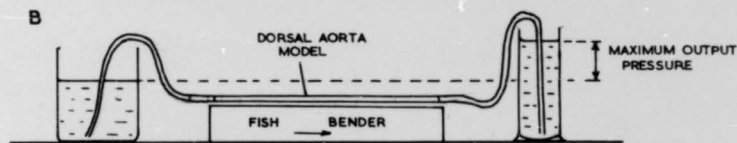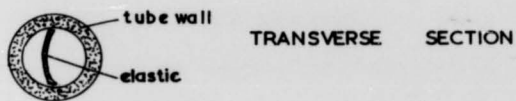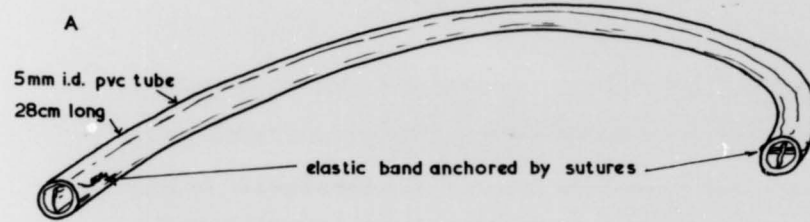
The basic work was carried out on a model consisting of the above tube with a flat rubber elastic band stretched along its bore dividing the lumen into two halves longitudinally (fig. 14).   The maximum pressure difference this was capable of producing was determined in the measuring cylinder as above.

In normal function of the dorsal aorta a pressure difference is set up across it by the heart.   This was simulated by setting up a constant head between the two ends of the tube. An Archimedian displacement vessel replaced the measuring cylinder at the "posterior" end and the overflow from this was measured during a timed period.   The flow through the model was thus measured at zero, 20 mmH_2O and 40 mm H_2O pressure drop across its length at a wide range of oscillation frequencies.   The "tail beat" frequency was monitored on the oscillograph using the tip of a water filled cannula from the pressure transducer attached to one of the slides.

Anatomical and Histological Techniques

The anatomy of the circulatory system and associated structures of rainbow trout were investigated using a variety of techniques often complementary to each other.   Some of the main methods used are described below.

BASIC ANATOMICAL TECHNIQUES

Many dissections were carried out on whole fish or parts of fish in order to elucidate particular points.   Conventional techniques were used with the aid of a binocular-zoom stereomicroscope.   Preparations were often fixed and preserved in dilute formalin solution or in alcohol which was particularly

useful for clearing in the case of nerve dissection.
Staining with osmium tetroxide was used in dissection of
the nervous system.

INJECTION AND CASTING TECHNIQUES.

To facilitate dissection of the vascular system the
blood vessels were often injected with "Silescol" silecone
rubber moulding paste.

The heavily anaesthetised fish was injected intravascularly
with Heparin usually by inserting a hypodermic needle into the
ventricle.   The structures to be studied were then partially
exposed by dissection.   The silecon rubber paste was then
mixed with the catalyst and drawn into a 1ml disposable
syringe.   The part of the vascular system being investigated
was injected using a 19 gauge needle. For the heart and
afferent branchial arteries the ventricle was a convenient
injection site.   The efferent branchial arteries and dorsal
aorta etc. could be injected by a needle inserted directly
into the dorsal aorta.   The major veins were injected via the
posterior cardinal vein in the trunk region of the kidney.
No ligatures were used but the needle was left in situ
blocking the injection hole until the rubber began to harden.
About one hour was allowed for the rubber to set and then
dissection could proceed.

Casts of particular items of interest such as the heart
and dorsal aorta were often removed for more detailed study
and measurement.   In this case the tissues were cleared from
the cast with concentrated hydrochloric acid the residue being
washed away with fine jets of water from a syringe needle.

Prolonged immersion of the rubber in acid tended to render it transparent so that it was found best to alternate short periods of immersion in acid with washing and dissection with fine instruments.

Colouring agents could also be added to the rubber. Eosin was quite successful but tends to diffuse out into surrounding tissues if the cast is left in situ; in this case pigments would be more appropriate than stains or dyes.

OSTEOLOGY

In investigating certain aspects of the caudal lymph heart anatomy and interpreting sections of the trunk region an understanding of the skeletal system was important.   The skeleton was extracted by immersing the dead fish for about 15 minutes in boiling water after which it was relatively easy to dissect away the surrounding soft tissues.   Immersion in alcohol was useful in removing fatty deposits around some structures.

THICK SECTIONS.

It was found that some fish about 25cm long which had been left in slightly acid formalin solution for more than 9 months had become entirely decalcified.   Using a razor or sharp scalpel transverse sections about 2 to 3mm thick were cut.   A series of these slices through an entire fish was particularly useful in the study of the lymph system which is difficult to see in ordinary dissection.

HISTOLOGY.

All the basic histological techniques were carried out as described in Mahoney (1969).

Structures such as the dorsal aorta and the caudal lymph heart are in very close proximity to bone so the material had to be decalcified before sections could be cut. The tissue was immersed in Gooding and Stewart's fluid for 7 to 10 days and tested for decalcification as described by Mahoney.

In some cases sections were required of the heart ventricle or bulbus in the expanded state. For this, the chamber was excised and a bluntened syringe needle was inserted through one opening and a ligature was placed round the needle. Another ligature closed off the other opening. Warm agar solution or "Vaseline" was then injected from a syringe in order to expand the chamber. The needle was withdrawn and its ligature was drawn tight. The heart chamber was then fixed and sectioned in the inflated state as for any other tissue.

All tissues in this work were fixed in formol-saline, imbedded in paraffin wax and sectioned according to standard procedures. Three staining techniques were used:-

Erlich's Haematoxylin and Eosin as general stain.

Van Gieson's stain which selectively stains collagen.

Orcein Elastica a specific stain for elastic tissue. Procedures were as described by Mahoney (1969) except that Van Geison's stain was often couterstained with Erlich's haematoxylin.

Figure 15. The apparatus used for investigation
of the bulbus arteriosus pressure-
volume relationship.

MOVABLE LIMB
MERCURY MANO

NEEDLE LUER
CONNECTOR

BULBUS IN
SALINE BATH

MANOMETER SETTING
MARK

MOVABLE LIMB OF
MERCURY MANOMETER

SALINE RESERVOIR

ΔP

NEEDLE LUER
CONNECTOR

BULBUS IN
SALINE BATH

MANOMETER SETTING
MARK

1 ML SYRINGE
0·01 ML GRADUATIONS
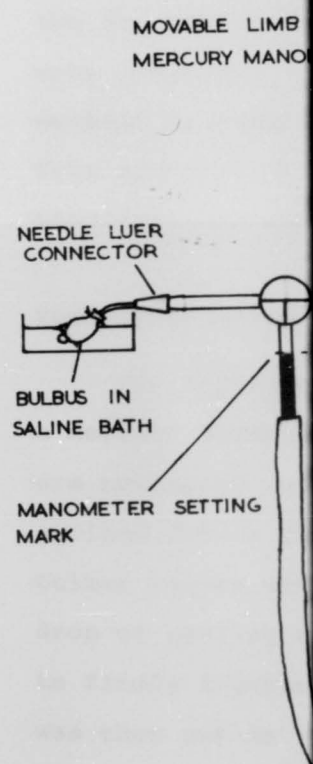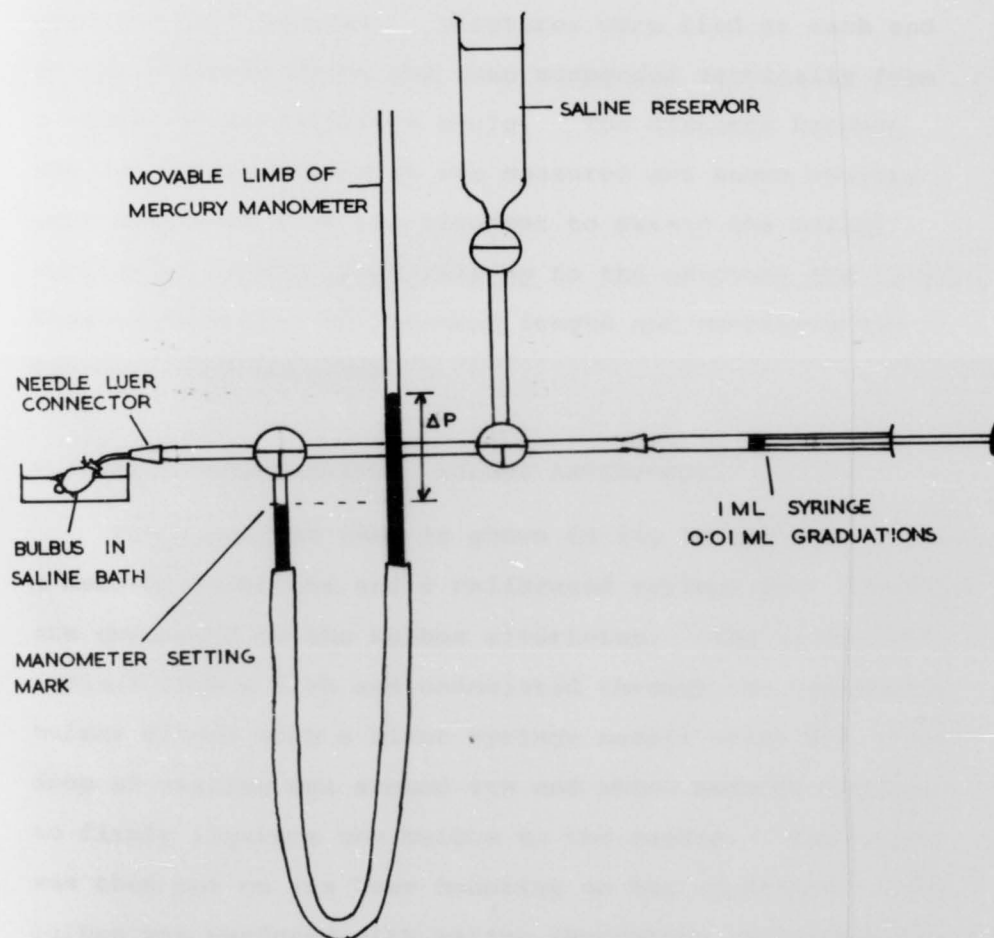
ELASTICITY MEASUREMENT - DORSAL AORTA LIGAMENT.

A fish was dissected to expose the dorsal aorta
ligament.   Two ligatures were tied around the ligament
5cm apart in the trunk region;   these acted as markers.
Then the section of ligament between the cranium and
the first hypural arch was removed taking care to keep it
moist with saline. Burnstock's (1967) trout saline was
used for this purpose.   Ligatures were tied at each end
of the ligament which was then suspended vertically from
a retort stand against a scale.   The distance between
the two marker ligatures was measured and known weights
were suspended from the ligament to extend the marked
section by regular intervals up to the original 5cm length.
Thus measurements of ligament length and corresponding
tensions were obtained. ·

ELASTICITY MEASUREMENT - BULBUS ARTERIOSUS.

The apparatus used is shown in fig 15 and consists of
a mercury manometer and a calibrated syringe both of which
are connected to the bulbus arteriosus.   The bulbus was
excised from a fish and cannulated through the ventriculo-
bulbar valves with a blunt syringe needle which had a small
drop of sealing wax around its end which made it possible
to firmly ligature the bulbus to the needle.   The needle
was then put on its Luer mounting on the apparatus.   The
bulbus was perfused with saline (Burnstock 1967) from the
reservoir and it was ensured that the apparatus was free

from air bubbles.   The ventral aorta end of the bulbus
was then ligatured off, the manometer was set to zero
pressure and the valve to the reservoir was closed off.

The experiment began with the bulbus quite flaccid.
The piston of the syringe was advanced by 0.01ml and the
free end of the manometer was moved to return the lower
end of mercury to the zero reference mark and the height of
mercury in the movable manometer limb was noted.   This
procedure was repeated for increasing volume increments
up to 120mm Hg pressure.   Then some measurements were
taken with decreasing volumes down to the original volume.
The cycle of expansion and contraction taking volume and
pressure readings took 10 to 15 minutes.

RESULTS, OBSERVATIONS & ANALYSIS

Swimming tests and Heart rate Measurements.

In order to facilitate comparison of swimming performances all speeds are expressed in terms of specific swimming speed or body lengths per second (Bainbridge, 1958).

$$\text{specific speed } (V/L) = \frac{\text{velocity (cm/sec)}}{\text{body length (cm)}}$$

The analysis may be obscured if the heart rate is different in fish of different sizes but no such effect was observed in the narrow size range used. The results of Nomura et al (1972) for much larger rainbow trout confirm that any size effect is probably small. The results of individual speed trials in each experimental fish are given in Appendix I. The final statistically condensed data in the form of mean heart rates and regression equations is given in table I.

THE RELATIONSHIP BETWEEN SWIMMING SPEED AND HEART RATE AT 6.5°C.

Intact fish

Four intact rainbow trout were tested at 6.5°C. With the fish at rest the heart rate stabilised at 25 to 35 beats/min. (Table 1). Fig. 16a shows a typical ECG at rest and it can be seen that the beat to beat interval varies considerably. During periods of excitement or if the stomach was full, the resting heart rate was elevated for long periods up to a maximum rate of 45 beats/min. Bursts of spontaneous activity gave rise to maximum heart rates of about 60 beats/min. for periods of not more than one or two minutes. During a typical burst of this kind lasting about 10 seconds, the heart

TABLE I. Comparison of heart rates in intact and vagotomised fish at 6.5°C and 15°C.

Condition of fish	Basal heart rate Beats/min.		Maximum heart rate Beats/min.		Regression line for intermediate heart rates.		
	Mean	S.D.	Mean	S.D.	Equation	Correlation coeficient	P
Intact 6.5°C	32.54	3.81	55.61	0.74	Log.H.R.= 1.3342 + 0.3038(V/L)	0.8965	<.001
Unilateral vagotomy 6.5°C	34.56	1.82	56.81	0.85	Log.H.R.= 1.3821 + 0.2658(V/L)	0.8669	<.1
Bilateral vagotomy 6.5°C	48.25	4.27	52.49*	3.70*	Log.H.R.= 1.6846 + 0.0363(V/L)*	0.3726*	<.1 *
Intact 15 C	45.52	6.18	93.76	3.78	Log.H.R.= 1.5639 + 0.1937(V/L)	0.8290	<.001
Unilateral vagotomy 15 C	37.26	3.38	96.20	2.81	Log.H.R.= 1.4894 + 0.2505(V/L)	0.9617	<.001
Bilateral vagotomy 15 C	45.13	9.46	82.67	2.28	Log.H.R.= 1.5927 + 0.1826(V/L)	0.8292	<.001

H.R. = Heart rate.   P = Level of significance of regression correlation.

* These values were calculated by incorporating all the heart rate figures above 0.2(V/L).

Figure 16.   ECGs of intact and bivagotomised rainbow
             trout at 6.5°C.   The intact fish shows
             irregularity in the beat to beat
             interval whereas the bilaterally
             vagotomised fish has a regular cardiac
             rhythm.

INTACT

BIVAGOTOMISED

INTACT

BIVAGOTOMISED

1 MIN

Fig. 17.   Intact fish at 6.5°C. Heart rates during
            the course of three speed trials. Circles -
            1.07(V/L). Triangles - 1.38(V/L). Squares -
            1.66(V/L).

rates during

rials. Circles -

(V/L). Squares -

RESTING · SWIMMING · RECOVERY
MINUTES
HEART RATE BEATS/MIN

often slowed or stopped and the peak heart rate was reached approximately 30 seconds after the peak of activity.

Speed trials at below 0.6 (V/L) had no significant effect on the heart rate apart from the cardiac inhibition often observed during the initial acceleration stages of a test.

At higher test velocities heart rate rapidly increased to a uniform level which was sustained throughout the test period after which the rate rapidly fell to the resting levels.   A typical trial of this kind at 1.07 (V/L) is shown in fig. 17; in this case there was also some spontaneous activity after the trial.   This active heart rate increased with swimming velocity up to approximately 1.5 (V/L) when the maximum heart rate of about 56 beats/min was reached.   In trials in excess of this critical speed no further increase in heart rate took place and a longer period for return to the resting rate was observed (fig 17, compare 1.38 (V/L) and 1.66 (V/L).   As the test speeds were increased above this critical speed the times for recovery increased and eventually the point was reached when the fish could not sustain the swimming speed for as long as 30 minutes.   This point where swimming could only just be sustained for 30 minutes (when a maximal recovery time was observed) was the maximum speed at which the fish were tested.

For each speed trial the mean was calculated of all the heart rate readings, excluding the first five, in order to discount the disturbances associated with the initial acceleration.

Fig. 18.   6.5°C.   The relationships between heart
rate and swimming speed.

A. Intact fish.   Open circles are data
from unilaterally vagotomised fish
inserted for comparison but not used in
the calculation of the lines shown.

B.   Bilaterally vagotomised fish.   The
line shown is the mean basal heart rate;
no significant relationship could be
derived for the heart rates during
swimming.
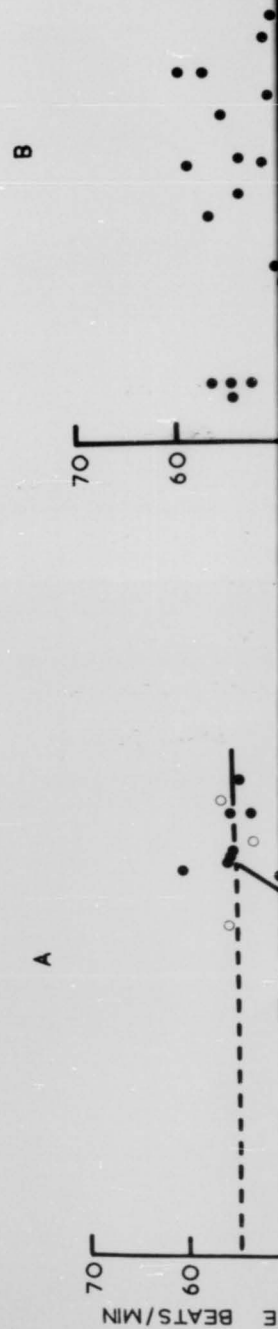
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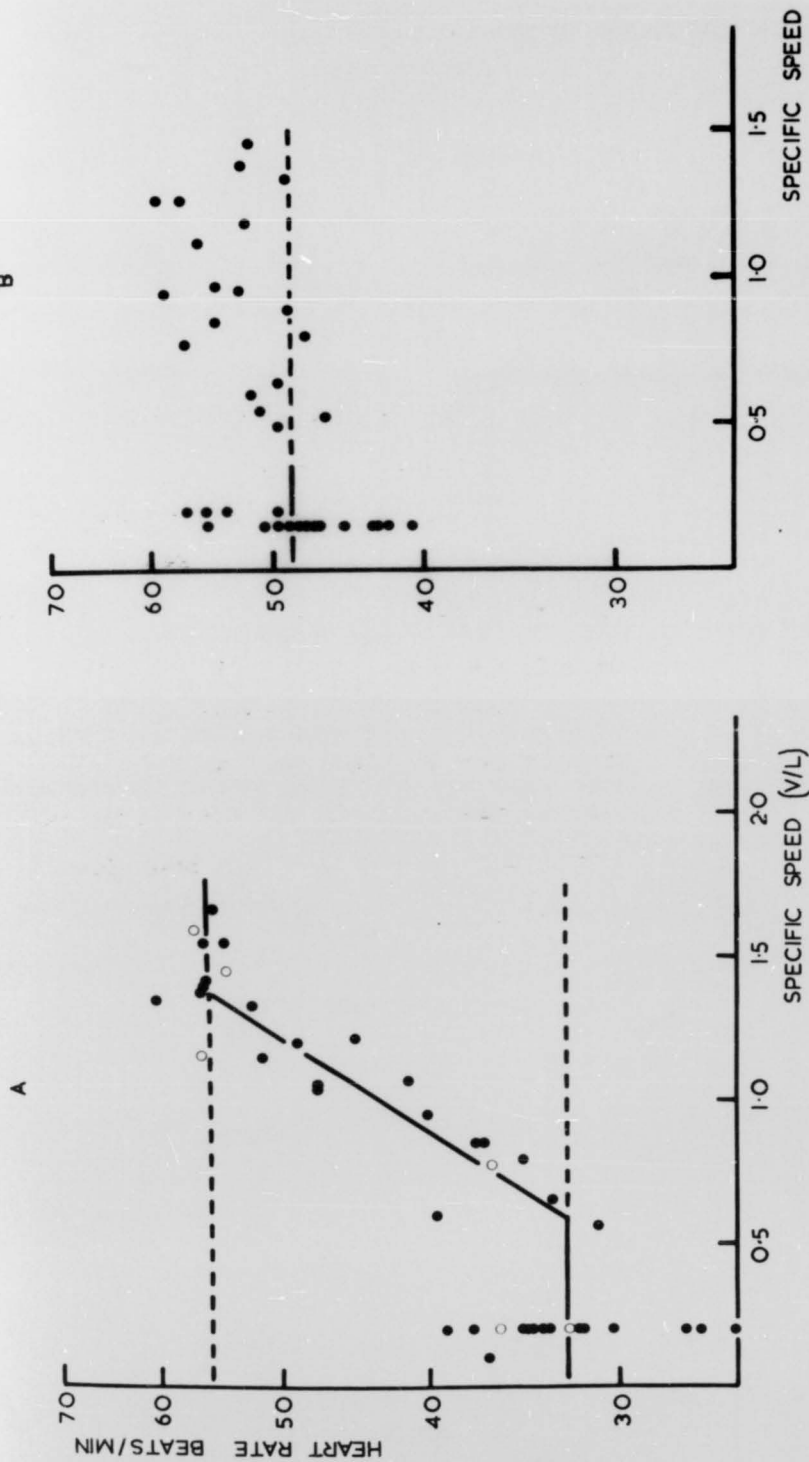
Figure 19. The relationships between swimming speed
during a trial and the time taken during
recovery for the heart rate to fall half
way back to the level prior to exercise
(50% recovery time).

swimming speed

taken during

to fall half

to exercise

A. 6.5°C INTACT

B. 6.5°C UNILATERAL VAGOTOMY

C. 15°C INTACT

D. 15°C UNILATERAL VAGOTOMY

E. 15°C BILATERAL VAGOTOMY

50% RECOVERY T. MIN.

50% RECOVERY TIME MIN.

(V/L)

PL.
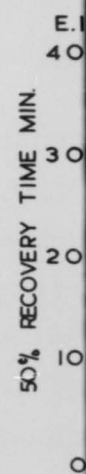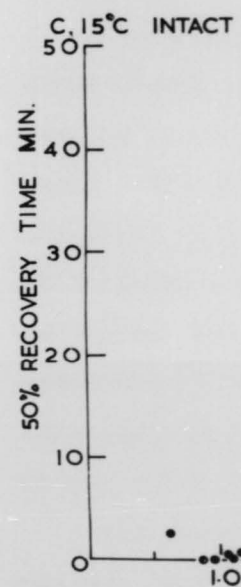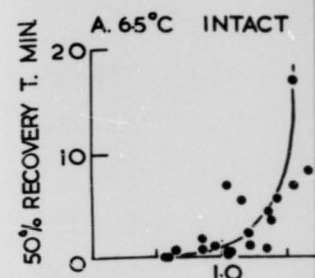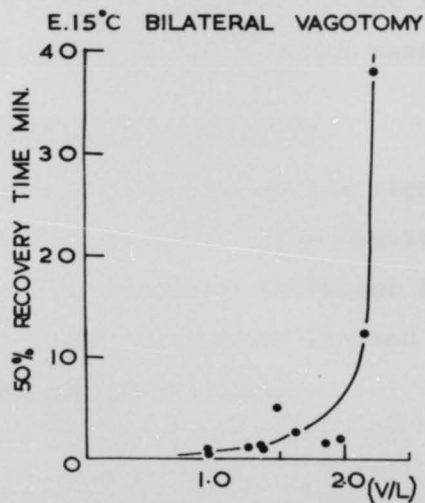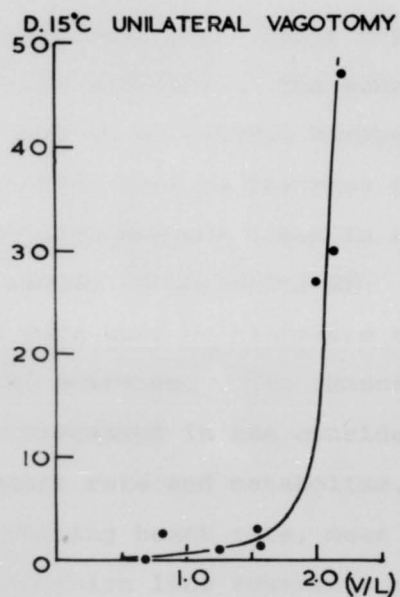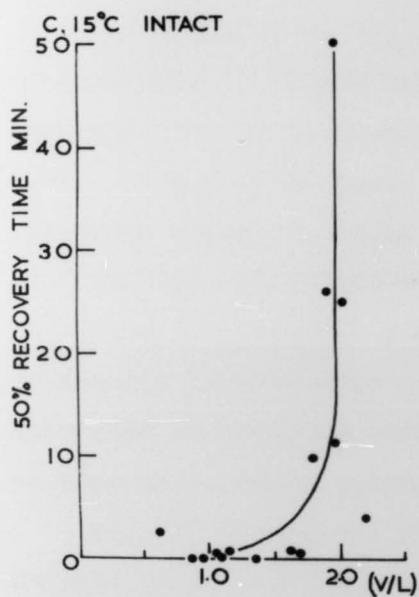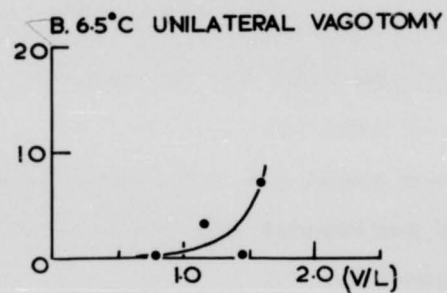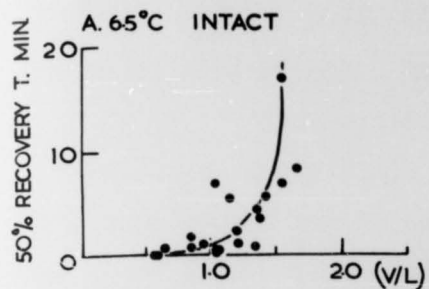
The logarithms of these mean values are plotted against swimming speed in fig. 18a. The mean of the readings at rest for 20 to 30 minutes prior to each test are also plotted

The time taken after a speed trial for the heart rate to fall half way to the prior resting level was determined and the inter-relationship between this time for 50% recovery and trial speed is shown in fig. 19a.

For analysis in fig. 18a the velocity - heart rate relationship is broken into three segments. The mean resting level (0.2 (V/L)) is shown as an initial horizontal line. The maximum heart rate was taken as the mean of all the heart rates of trials with 50% recovery times in excess of 5 minutes (i.e. speeds in excess of the critical). The remaining intermediate points were used to calculate the regression line between the two extremes. The reasons for this kind of analysis will be discussed in the consideration of the relationship between heart rate and metabolism.

All the values for mean resting heart rate, mean maximum heart rate and the regression line equations are given in table 1. In contrast to the resting levels there was little variation in the maximum heart rate.

The effect of unilateral vagotomy

One fish was vagotomised on the right side only with a sham operation on the left. The results of swimming tests were in no way significantly different from those in the intact fish as shown in figures 18a and 19b and all the features of the ECG were normal.
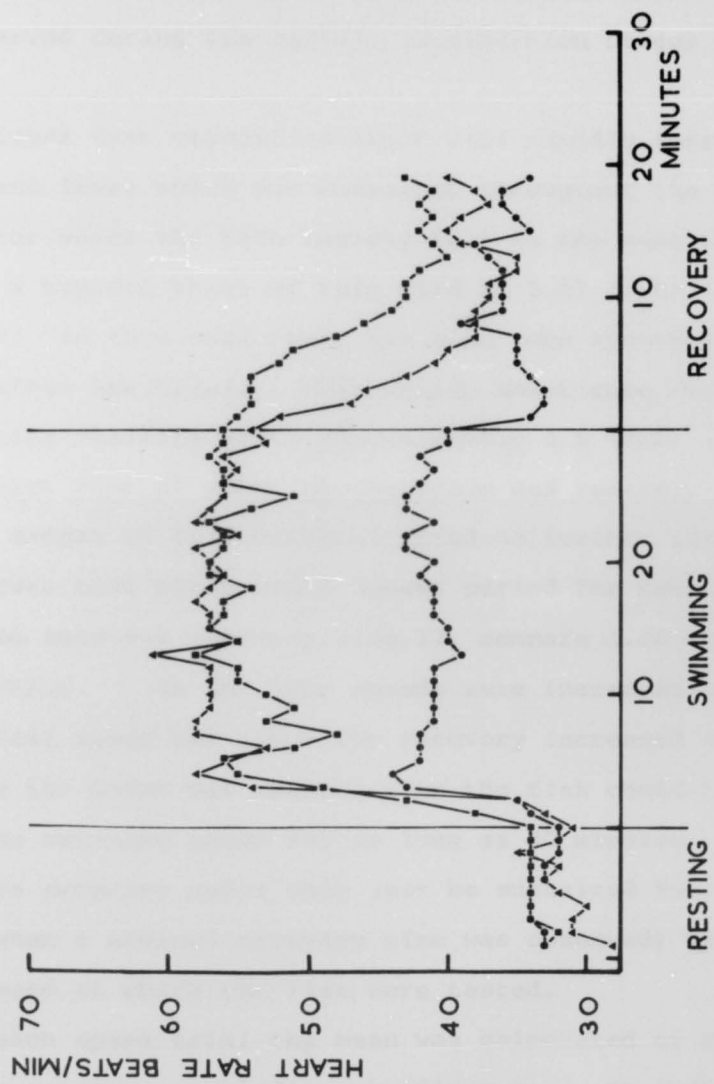
Figure 20. Bilaterally vagotomised fish at 6.5°C.

Heart rates during the course of three

speed trials. Circles - 0.97(V/L).

Triangles - 1.12(V/L). Squares -

1.97(V/L).

sh at 6.5°C.

rse of three

.97(V/L).

uares -

HEART RATE BEATS/MIN

70
60
50
40
30

RESTING   SWIMMING   RECOVERY

MINUTES
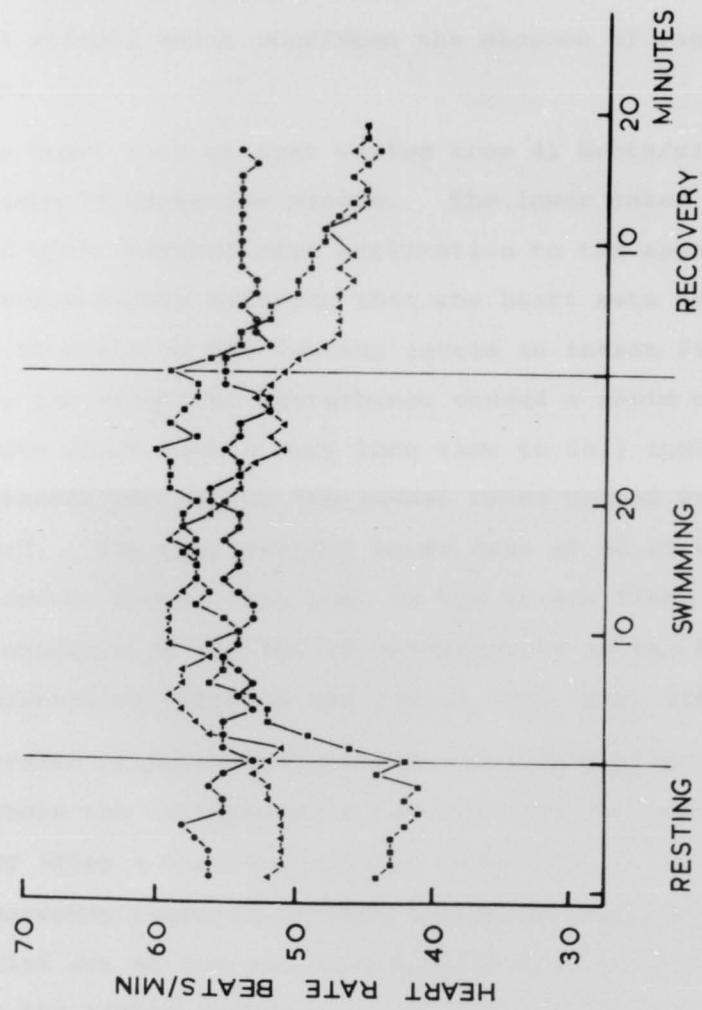
The effect of bilateral vagotomy

Bilateral vagotomies and swimming tests were carried out on two rainbow trout at 6.5°C. No cardiac inhibitory reflexes were observed in response to visual or other external stimuli which confirmed the absence of vagal function.

The heart rate at rest varied from 41 beats/minute to the maximum 56 beats per minute. The lower rates were only recorded after several days acclimation to the apparatus and then there was some evidence that the heart rate tended to decline to close to the resting levels in intact fish. However, the slightest disturbance caused a rapid rise in heart rate which took a very long time to fall again so that extended periods at the lowest rates proved impossible to record. The mean resting heart rate of 48.25 was significantly higher than that in the intact fish. There was no evidence in the ECG of irregularity in the beat to beat interval as noted in the intact fish (fig. 16b).

Cardiac response to exercise was variable and even in cases where the resting heart rate was low the pattern of recovery after a test was erratic (Fig. 20). Training was apparently required in that one or two tests had to be carried out at low swimming speeds before the fish could sustain the higher speeds without occasionally falling back in the water flow. The maximum speed attained, 1.47 (V/L), was slightly lower than that in the intact individuals. Heart rates during swimming activity were significantly

Figure 21. Intact fish at 15^oC. Heart rates during the course of three speed trials. Circles - 0.62(V/L). Triangles - 1.14(V/L). Squares 1.97(V/L).
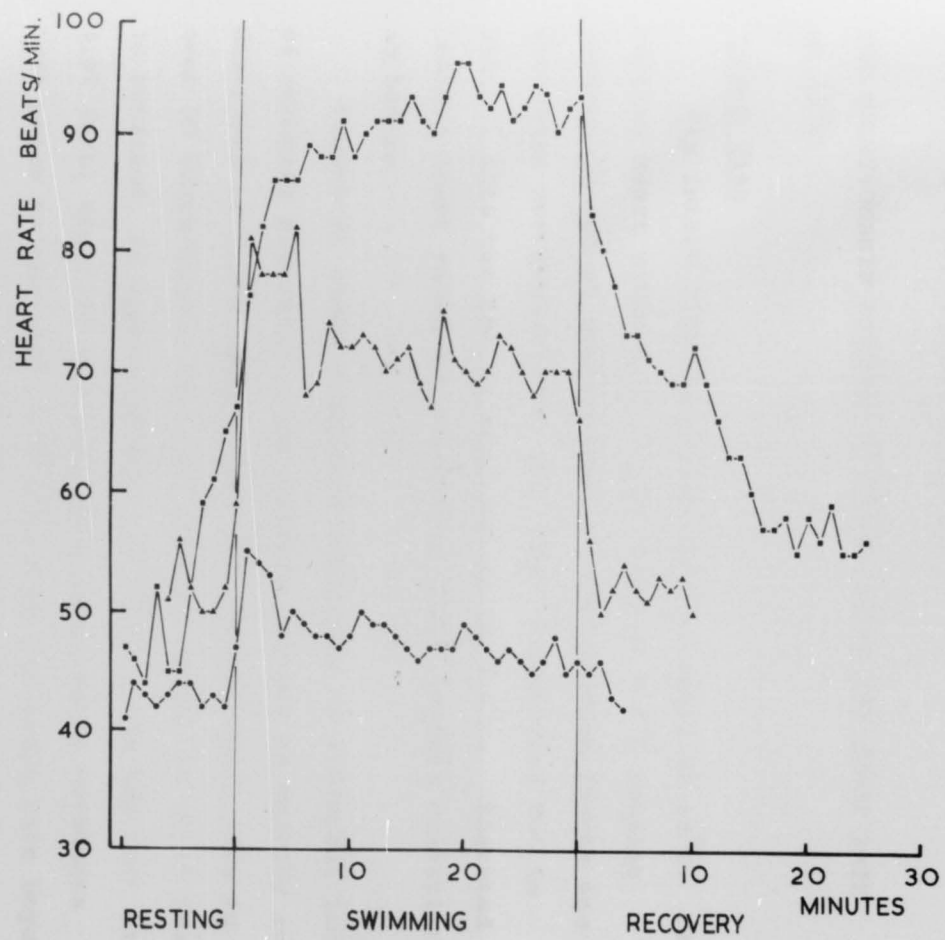
higher than at rest (P < .001) but the correlation
between heart rate and swimming speed was not significant
and no coherent analysis could be made of recovery times
or critical speeds.   Thus the only line shown in fig.
18B is the mean resting heart rate.

THE RELATIONSHIP BETWEEN SWIMMING SPEED AND HEART RATE
AT 15°C.

Intact fish

Six intact fish were subjected to tests at 15°C.   The
resting heart rates were higher than at 6.5°C ranging
between 32 and 55 Beats/minute.   The cardiac rhythm was
irregular particularly at the lower frequencies but in
general this was not as apparent as at 6.5°C.   Elevated
resting heart rates were observed under certain conditions
as before.

Figure 21 shows 3 cardiac responses to different levels
of swimming activity.   The general pattern is exactly as
described at 6.5°C except that the maximum heart rate is
over 90 beats/minute and the critical speed, at which that
is attained, is approximately 2.0 (V/L).   In the test at
1.97 (V/L) the fish executed some spontaneous movements
just prior to the test period so that the heart rate began
to rise prematurely (Fig. 21).   The steep increase in
recovery times at 2.0 (V/L) is shown in figure 19c.   The
maximum time observed for complete recovery in heart rate
from fatigue was 5½ hours.

Figure 22. 15°C.   The relationships between heart
rate and swimming speed.   A - Intact
fish.   Open circles are data derived
from unilaterally vogotomised fish
inserted for comparison but not used in
the calculation of the lines shown.
B - Bivagotomised fish.
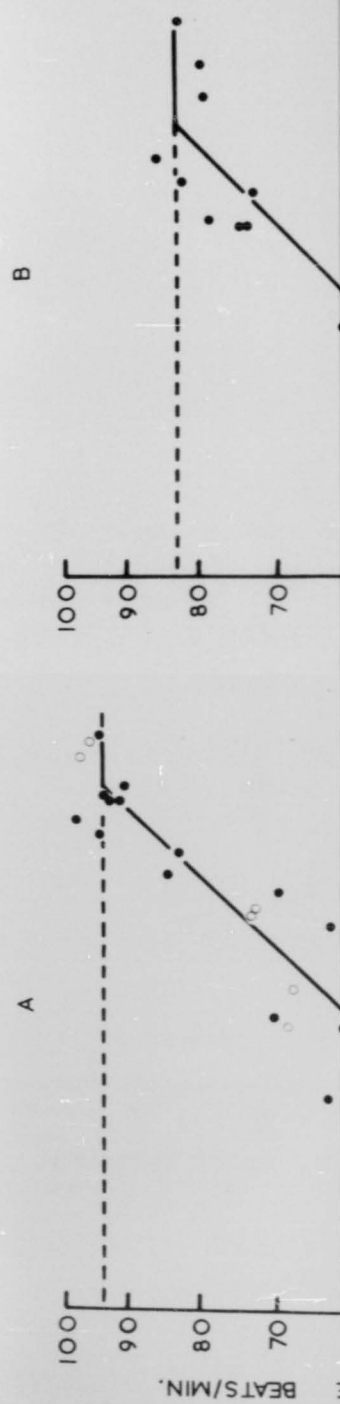
etween heart

A - Intact

ta derived

ed fish

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s shown.

A

B

SPECIFIC SPEED (V/L)
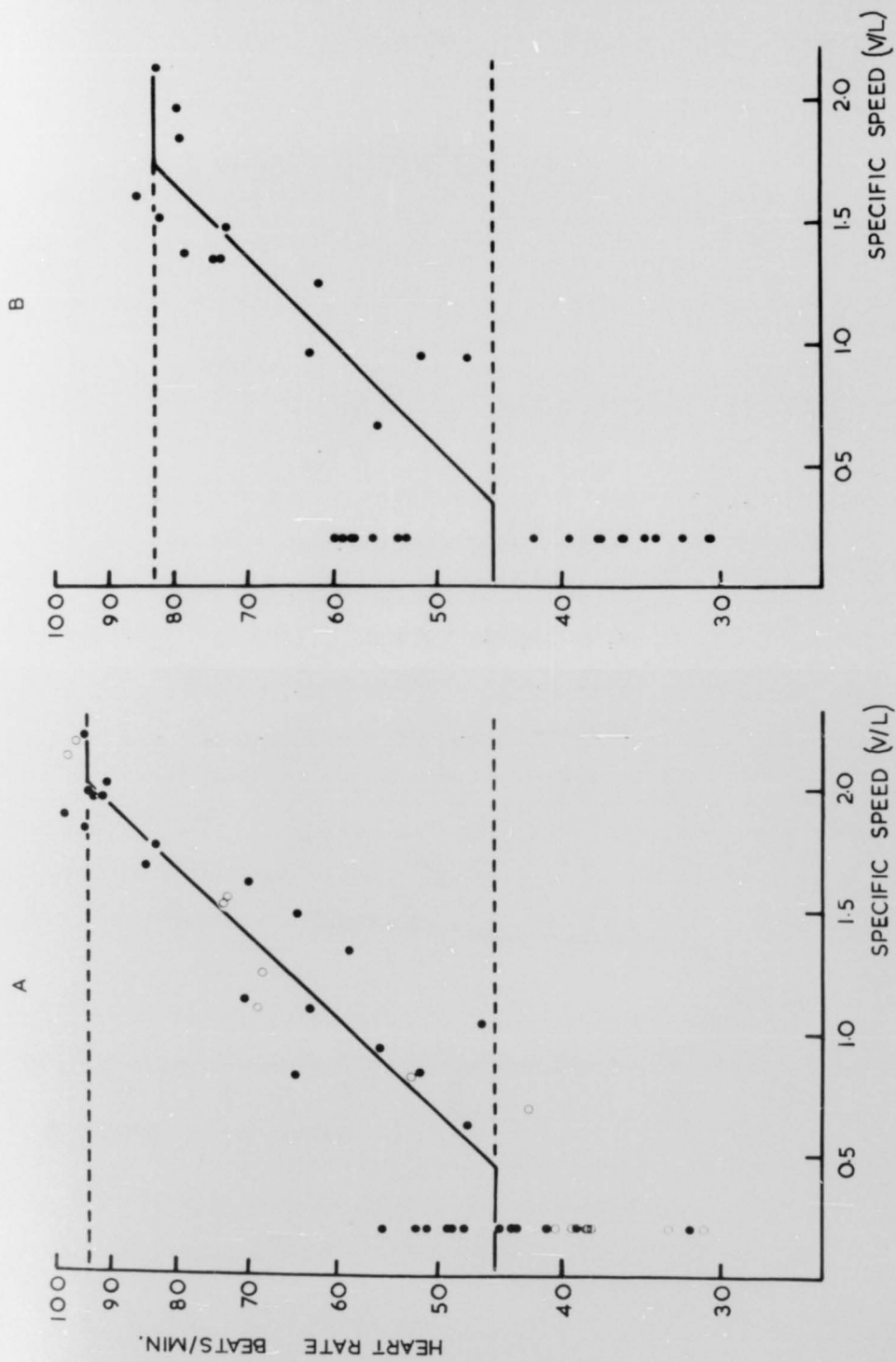
HEART RATE   BEATS/MIN.

Figure 23. Bilaterally vagotomised fish at 15°C.
Heart rates during the course of
three speed trials.   Circles -
1.25(V/L).   Triangles - (V/L).
Squares - 2.22(V/L).

BEATS/MIN RATE ART
100   90   80

RECOVERY

MINUTES

30

20

10

SWIMMING

20

10

RESTING

HEART RATE BEATS/MIN

100

90

80

70

60

50

40

30
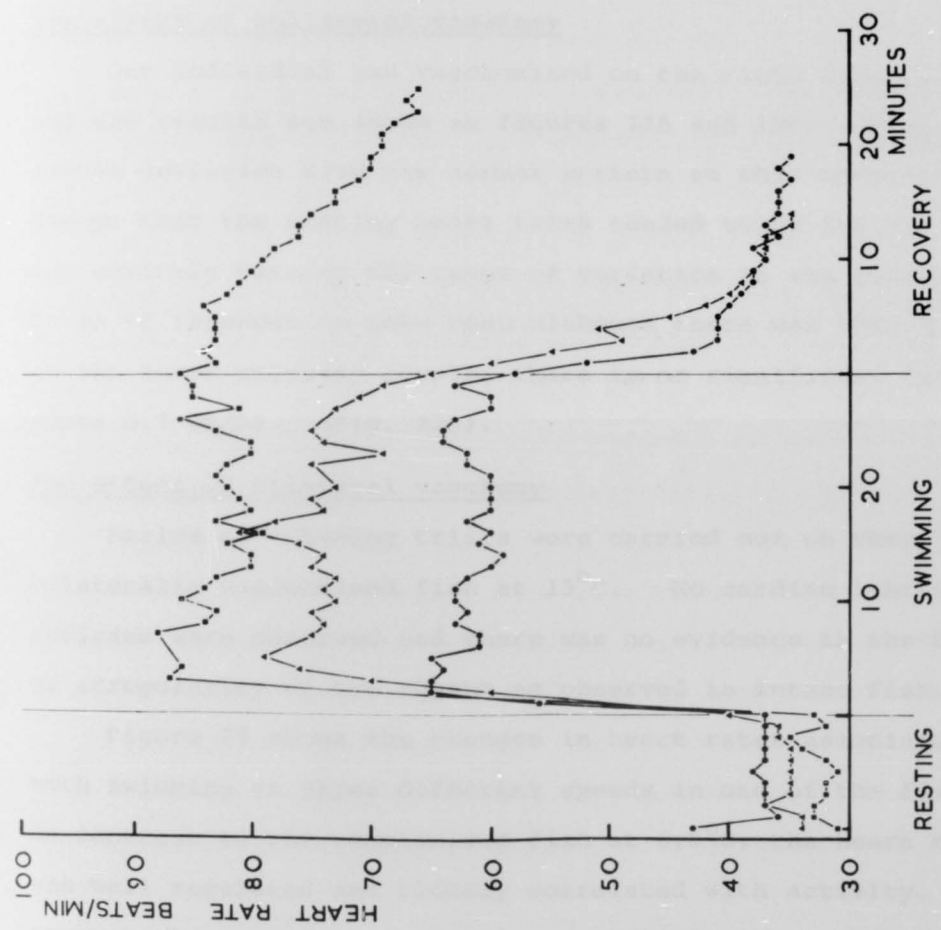
The relationship between heart rate and swimming speed was analysed as before and is shown in figure 22A. Despite large variations in resting heart rates the maximum frequencies are remarkably constant. The slope of the regression is not as steep as at 6.5°C (Table I).

The effect of unilateral vagotomy

One individual was vagotomised on the right side only and the results are shown in figures 22A and 19D. There was little deviation from the normal pattern at this temperature except that the resting heart rates tended to be low but were not entirely outside the range of variation in the intact fish. It is of interest to note that although there was this difference at the lower activity levels, there is no significant difference above 0.7 (V/L). (Fig. 22A).

The effect of bilateral vagotomy

Series of swimming trials were carried out on three bilaterally vagotomised fish at 15°C. No cardiac inhibitory reflexes were observed and there was no evidence in the ECG of irregularity of the rhythm as observed in intact fish.

Figure 23 shows the changes in heart rates associated with swimming at three different speeds in one of the fish. In contrast to the vagotomised fish at 6.5°C, the heart rate was well regulated and closely correlated with activity. However the mean maximum heart rate (83.88 beats per minute) was significantly lower than in the normals. The resting heart rate ranged from 31 to over 60 beats/minute, two fish having high resting frequencies and one a low resting rate. (Fig. 22B). The pattern of increase in recovery time at the critical speed of about 2.0 (V/L) was very close to that in normal fish. (Fig. 19e).

Thus the vagotomy did not have any significant effect at 15°C except that the maximum heart rate was depressed. This lowered maximum heart rate was further confirmed in two other vagotomised fish in which due to excessive spontaneous activity no reliable speed trials could be carried out.

CONCLUSIONS

At both 6.5°C and 15°C a general pattern of increase in heart rate with swimming speed was observed. These results will be discussed in detail in a general analysis of the relationship between heart rate and metabolism.

Unilateral vagotomy had no significant effect on heart rates during activity or on any cardiac response to stimuli. The significantly lower resting heart rates in unilaterally vagotomised fish at 15°C may be due to individual variation as heart rates of the same order were recorded in intact fish.

Bilateral vagotomy had distinctly different effects at the two temperatures. There was little effect on the general pattern of increase of heart rate with swimming speed at 15°C although cardiac inhibitory reflexes and associated periods of bradycardia were absent. At 15°C the maximum heart rates in bivagotomised fish were low. At 6.5°C the resting heart rates in bivagotomised fish were high and changes associated with swimming did not appear to be well regulated.

The effects of Feeding on Heart rate

INTRODUCTION

In the speed trials it was found that due to excitement or spontaneous activity of the fish high resting heart rates were often recorded in some fish. These were excluded from the analysis of the heart rate/swimming speed relationship.

Figure 24.   15°C.   The relationship between heart
          rate and swimming speed in recently fed
          and starved fish compared with the normal
          group of intact fish at this temperature.

● —FISH NO.26 FULL STOMACH

○ —FISH NO.26 EMPTY STOMACH

ATE   BEATS/MIN

100 90 80 70 60
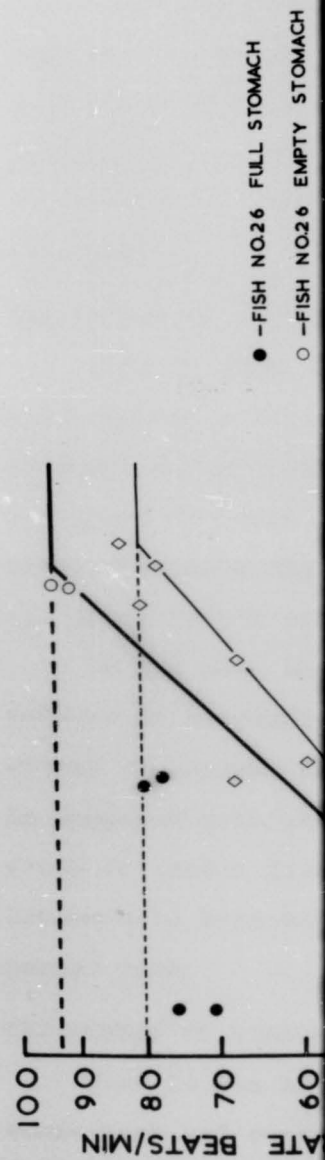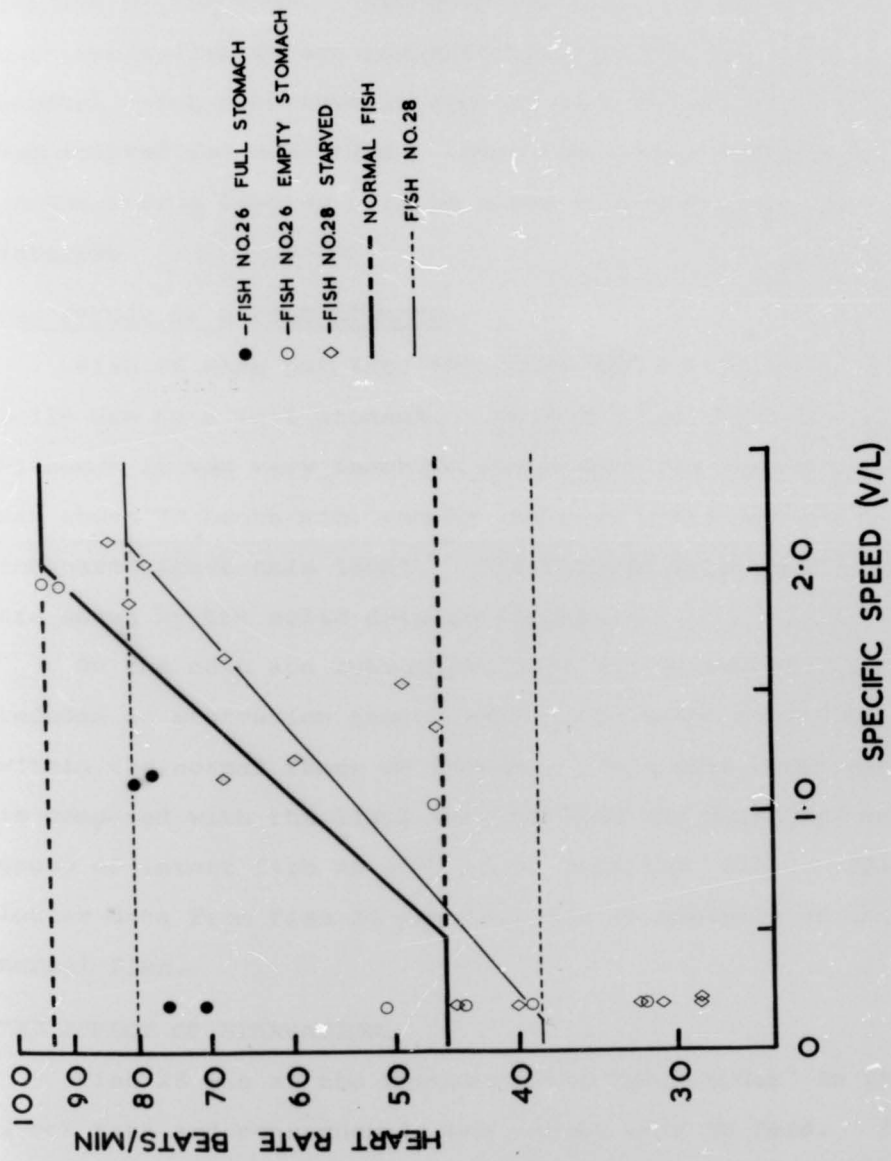
In some cases consistently abnormally high or low heart
rates could be definitely attributed to the state of
feeding of the fish.   Two such cases for which sufficient
data was collected are presented here;   one fish with a full
stomach which subsequently emptied and another fish which
had starved for some time.   Also the events occurring in
an ECG during feeding will be shown as a related point of
interest.

THE EFFECT OF A FULL STOMACH.

Fish 26 when put into the flume had an obviously distended
belly due to a full stomach.   On the first experimental day
although it was very inactive and sedate its resting heart rate
was above 70 beats/min. and in swimming tests the heart rate
increased above this level.   The results from this first day
are shown by the solid dots in figure 24.

On the next and subsequent days its distended belly
receded as starvation proceeded and the heart rate fell to
within the normal range of values.   This data (open circles)
is compared with the lines derived from the main experimental
group of intact fish at 15°C (i.e. from fig. 22A).   This
latter data from fish 26 was included in analysis on intact
normal fish.

THE EFFECT OF STARVATION.

Fish 28 was at the bottom of the "peck order" in the
stock tank and consequently was rarely able to feed.   Its
condition factor was 0.95 and analysis of lipid content of the
tissue carried out by Mr. M.G. McLeod (Dept. of Biology,
University of Stirling) indicated a condition equivalent to a

Figure 25.   ECG during feeding in the flume test
             chamber.   The fish took one
             floating food pellet at the point
             indicated.

E.C.G. DURING FEEDING (FISH 27   15°C)

E.C.G. DURING FEEDING (FISH 27   15°C)

TAKES FOOD PELLET →

COUGHING →

COUGHING →

COUGHING →

1 MIN.
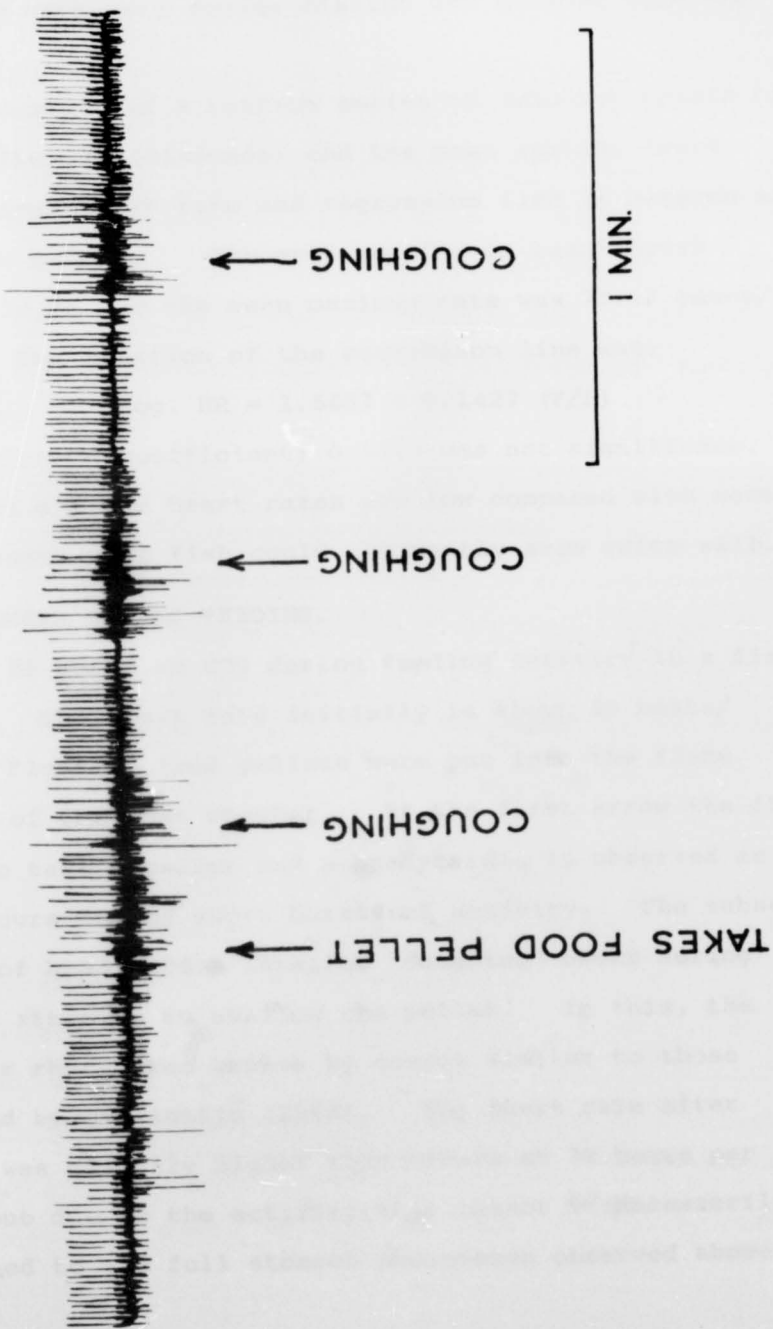
period of starvation of several weeks.   Its general
appearance with many scales missing and damaged fins was
poor.

The results of a routine series of swimming trials is
shown in fig. 24 (Diamonds) and the mean resting heart
rate, maximum heart rate and regression line in between are
plotted as before.   The mean resting or basal heart
rate was 38.28 and the mean maximum rate was 79.67 beats/
minute.   The equation of the regression line was:

$$Log. HR = 1.5657 + 0.1427 (V/L)$$

The correlation coefficient; 0.5412 was not significant.
In general all the heart rates are low compared with normal
fish although this fish could apparently swim quite well.

ECG PHENOMENA DURING FEEDING.

Fig 25 shows an ECG during feeding activity in a fish
at 15°C.   The heart rate initially is about 60 beats/
minute. Floating food pellets were put into the flume
upstream of the test chamber.   At the first arrow the fish
"rose" to take a pellet and a bradycardia is observed as
often occurs during short bursts of activity.   The subsequent
periods of bradycardia labelled "coughing" occur during
apparent attempts to swallow the pellet.   In this, the
opercular rhythm was broken by coughs similar to those
described by Ballintijn (1969).   The heart rate after
feeding was slightly higher than before at 70 beats per
minute but due to the activity this cannot be necessarily
attributed to the full stomach phenomenon observed above.

Oxygen Consumption and Heart Rate Measurements

In the course of investigation of the relationship between heart rate and swimming speed elevated heart rates were often observed when the fish was at rest.   It was assumed generally that this was attributable to the effects of a full stomach, recovery from activity, or other effects which might be likely to cause an elevated oxygen consumption.   Also much of the analysis of the heart rate-activity studies implicitly assumes a uniform relationship between heart rate and oxygen consumption. The investigation in this section was proposed in order to test these assumptions.

RESULTS.

Measurements of oxygen consumption and heart rate were carried out on 6 rainbow trout at $15^{\circ}C$.   The details of the fish, oxygen consumptions and mean heart rates are given appendix II.   The logarithms of heart rate and oxygen consumption are plotted graphically in figure 26 and a calculated regression line (solid line) is drawn through the points.   The equation of line was:-

$$LogHR = 0.4393 \, LogO_2 + 0.8171$$

The correlation coefficient was 0.7788 which with n=46 is highly significant at the .001% level.

Also in figure 26 are shown two dotted lines which were calculated using the heart rate swimming speed relationship already derived (table I) and oxygen consumption - swimming speed relationships given in the literature.   Two different sets of data were used.   Webb (1971 b) measured O_2 consumption

Figure 26. The relationship between heart rate and
oxygen consumption. The results of
individual fish are plotted and a
calculated overall regression line is
drawn through the points (solid line).
The dotted lines are "predicted" forms
for this relationship using oxygen
consumption data from the literature
in conjunction with the heart rate-
swimming speed data in this study.

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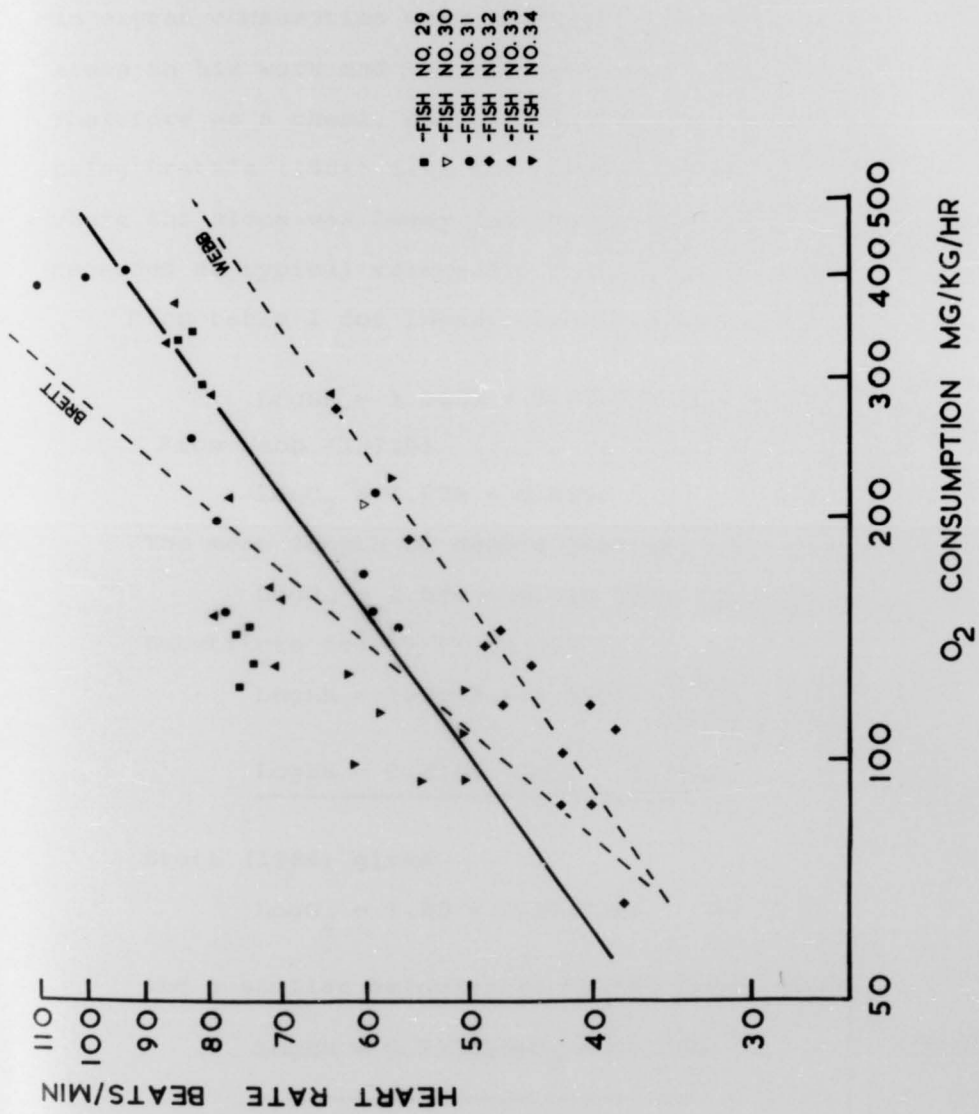
in rainbow trout from what is probably a very similar stock
to that used in this work. However the slopes of increase
in oxygen consumption with swimming speed are unusually
steep in his work and the critical swimming speeds were low.
Therefore as a check, calculations were also carried out
using Brett's (1964) data for Onchorhyncus nerka at 15^OC
where the slope was lower and closer to what has come to be
regarded as typical values for fish. (Tytler 1969).

From table I for intact rainbow trout at 15^OC :

$$LogHR = 1.5639 + 0.1937 (V/L) \qquad (1)$$

From Webb (1971b)

$$LogO_2 = 1.878 + 0.016V \qquad (2)$$

The mean length of Webb's fish was 29.2 cm. Therefore

$$LogO_2 = 1.878 + 0.016 (V/L) 29.2$$

Substitute for (V/L) in (1)

$$LogHR = 1.5639 + 0.1937 \frac{LogO_2 - 1.878}{0.016 \times 29.2}$$

$$\underline{LogHR = 0.4146 LogO_2 + 0.7852} \qquad (Webb)$$

Brett (1964) gives

$$LogO_2 = 1.85 + 0.27(V/L) \qquad at\ 15^O C$$

and a similar calculation to the above gives

$$LogHR = 0.7174 LogO_2 + 0.2365 \qquad (Brett)$$

A statistical analysis was made of the variance ratio in
order to determine whether there is any significant difference
between these two "predicted" lines and the actual O_2-HR
relationship obtained. In order to carry this out the swimming
speed values for the 15^OC HR - (V/L) relationship were transformed

Figure 27. The data from figure 26 is shown with
the limits on the oxygen consumption
and heart rate inserted for comparison.
The irregular quadrangle to the left
of the dotted line within which most
of the data points lie is termed
"The quadrangle of scope".
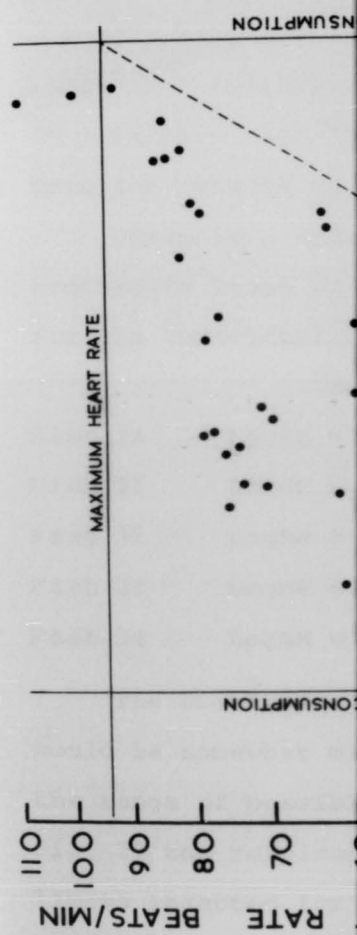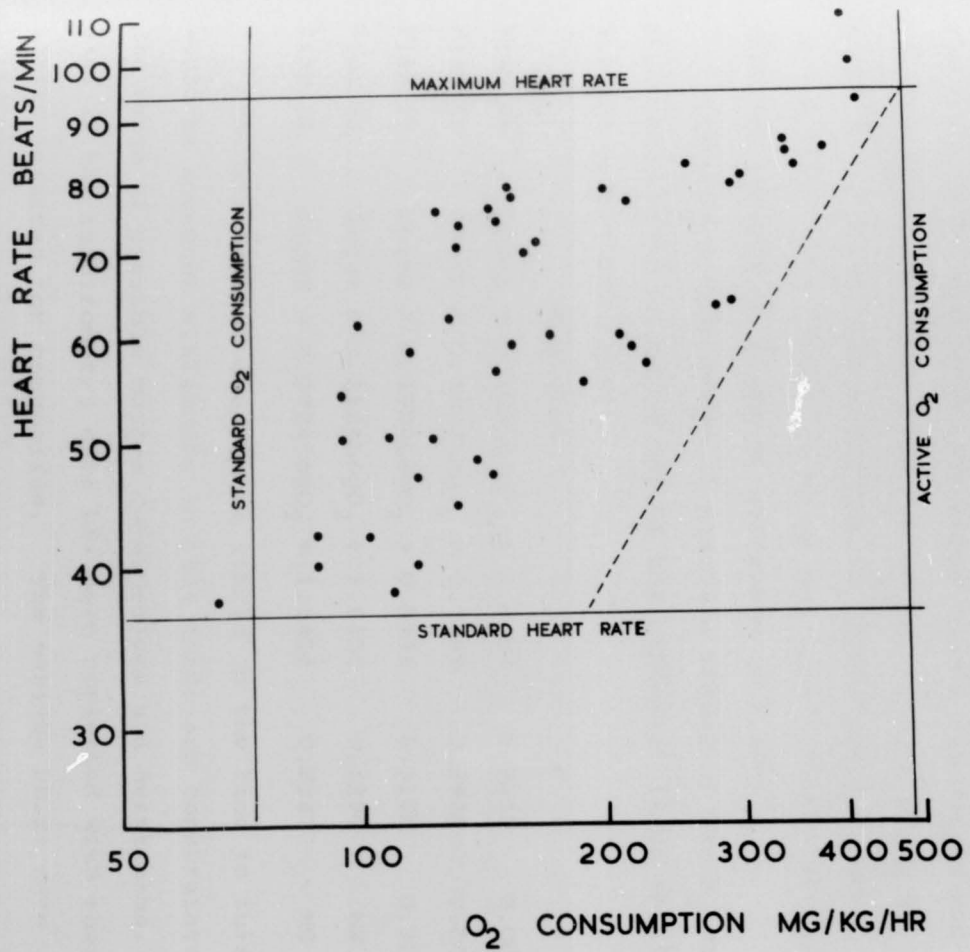
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into the corresponding oxygen consumptions using either the
above "Webb" or "Brett" equations. The resulting
transformed sums, squares etc. of X and Y values could be
used in a standard analysis of covariance of two regressions
calculation (F test) for comparison with the actual experimental
results. The predicted line using Webb's figures is not
significantly different from the actual line in terms of
slope (P > 10%) but its elevation is significantly lower
(P < 0.1%). The "Brett" line is not significantly different
from the results in slope or elevation (P > 10%).

There is a wide range of possible slopes of the O_2 HR
regression lines as borne out by the different lines obtained
for the individual fish:-

	Equation	r	P
Fish 29	$LogHR = 0.1091LogO_2 + 1.6397$	0.9233	0.001
Fish 31	$LogHR = 0.4807LogO_2 + 0.7389$	0.8539	0.001
Fish 32	$LogHR = 0.3704LogO_2 + 0.8796$	0.9158	0.001
Fish 33	$LogHR = 0.1124LogO_2 + 1.6125$	0.4530	NS
Fish 34	$LogHR = 0.0724LogO_2 + 1.5924$	0.2247	NS

The statistical confidence limits on the line in this case
would be somewhat misleading in that there are constraints on
the range of possible oxygen consumptions and heartrates. In
fig. 27 the respirometry data has been replotted with these
limits inserted for comparison. The maximum heart rate (93.76
beats/min) is the figure obtained from the intact group of fish
in the swimming experiments at $15^{\circ}C$ (Table I). The standard
heart rate (36.64 beats/min.) is the heart rate obtained by
extrapolating the active heart rate regression line to zero
swimming speed (i.e. the antilogarithm of the regression

constant in equation (1) above). By analogy with the methods used for determination of standard oxygen consumption (Fry 1957) this represents the lowest possible heart rate in a resting fish. The standard oxygen consumption (72.5 mg/kg/hr) and the active oxygen consumption (480.0mg/kg/hr) are taken from Webb (1971b).

For any given heart rate there must be a maximum possible oxygen consumption which occurs when the cardiac stroke volume and the oxygen carrying capacity of the blood are both at a maximum (i.e. maximum O_2 pulse). It was assumed that at maximum heart rate and active (maximum) oxygen consumption these optimal conditions were in existence. Thus a line could be plotted through this point which denotes the maximum oxygen consumption for any given heart rate:

$$O_2 \text{ consumption} \propto HR \quad \text{(if } O_2 \text{ pulse is constant at its maximum value)}$$

In this case $\quad O_2$ consumption = 5.1195 HR.

From these basic considerations it can be seen that all the heart rate/oxygen consumption points should lie within the irregular quadrangle to the left of the dotted line figure 27. It is also probable that at high heart rates the oxygen consumptions by the myocardium would be elevated. This would mean that as heart rate increases so the minimum possible oxygen consumption by the fish must be above the standard oxygen consumption. This effect could not be quantified precisely but is probably small (Webb 1970) and has been omitted from the diagram.

In general the points fall within the quadrangle with the notable exception of two high heart rates (fish 31). This fish subsequently died from some unknown cause possibly the

effects of stress. Despite the varied conditions under which these fish were tested (active, resting, full stomach, empty stomach etc.) the points are not scattered throughout the available area. It seems that some optimization of performance may have taken place tending to aggregate the points along the diagonal of the quadrangle. In actual fact the diagonal is probably a good approximation to the heart rate/oxygen consumption relationship.

No correlation was noted between high or low heart rates and particular aspects of the fishes behaviour or any other phenomena.

Auxiliary Circulatory Pumps - Structure and Function

Any mechanism which could act as a pump tending to increase blood flow or pressure independently of the heart could have a profound effect on the functional characteristics of the circulatory system. This section of the thesis is devoted to an investigation of the structure and possible function of such mechanisms in rainbow trout.

LYMPH PROPULSORS.

A detailed anatomical account of the lymph system was outside the scope of the present study but measurements were taken of the diameters of the major lymph vessels in the trunk and tail regions from transverse sections through a 23 cm long fish:-

> lymphaticus lateralis - 0.2 mm
>
> lymphaticus caudalis - 0.3 mm
>
> lymphaticus dorsalis   - 0.2 mm
>
> lymphaticus cardinalis - 0.15 mm

These are mean diameters as some of the vessels are eliptical in cross section.

Figure 28. Lateral view of the bones of the tail of
            rainbow trout showing the foramen
            hypurale through which the two chambers
            of the lymph heart are interconnected.
            Osteological terminology from Norden
            (1961).   CFR - caudal fin ray, E -
            Epural, EHS - expanded haemal spine,
            ENS - expanded neural spine, H -
            hypural, U - Uroneural.
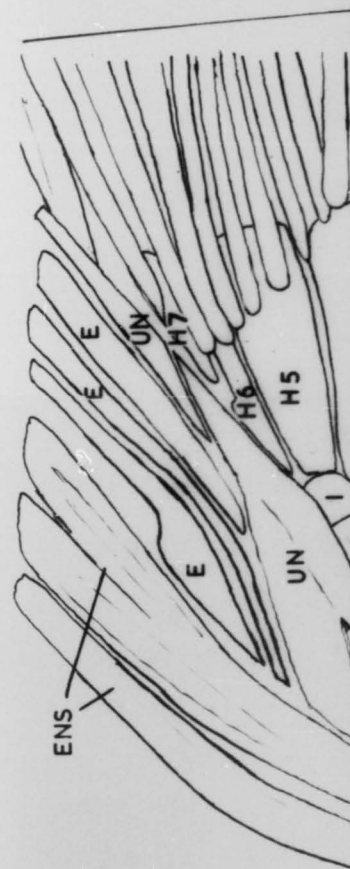
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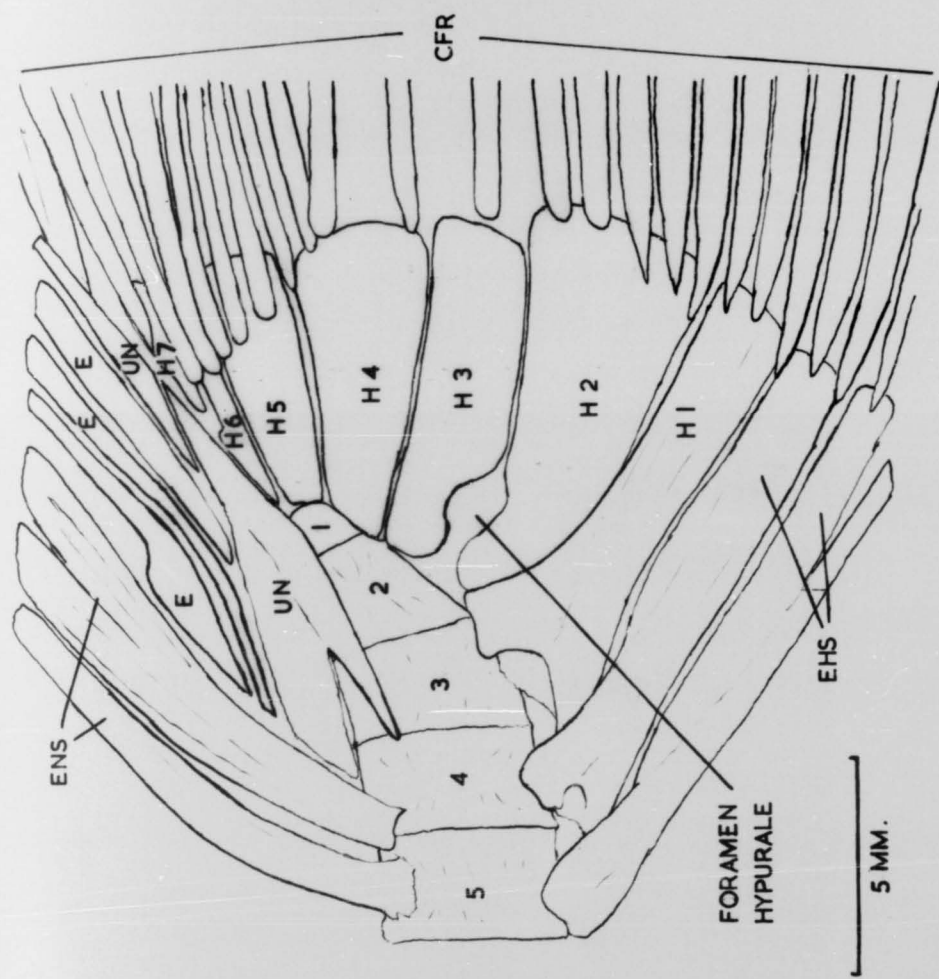
Figure 29.   Horizontal longitudinal section through
the caudal lymph heart (haematoxylin and
eosin).   CFM - caudal flexor muscle. CV -
caudal vertebra, FH - foramen hypurale,
H-hypural bone, LHC - lymph heart
chambers.

Figure 30.   Transverse section through the haemal
canal in the caudal region. (Haematoxylin
and eosin).   C - centrum of vertebra,
CA - caudal artery (injected with carbon/
agar for positive identification),
CL - caudal lymphatic, CV - caudal vein,
HA - haemal arch, X - musculature cut
away to allow penetration of the
decalcifying fluid.
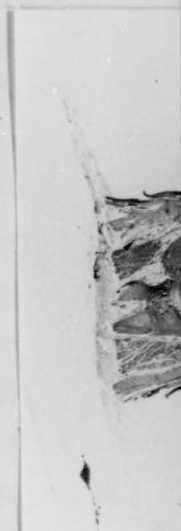
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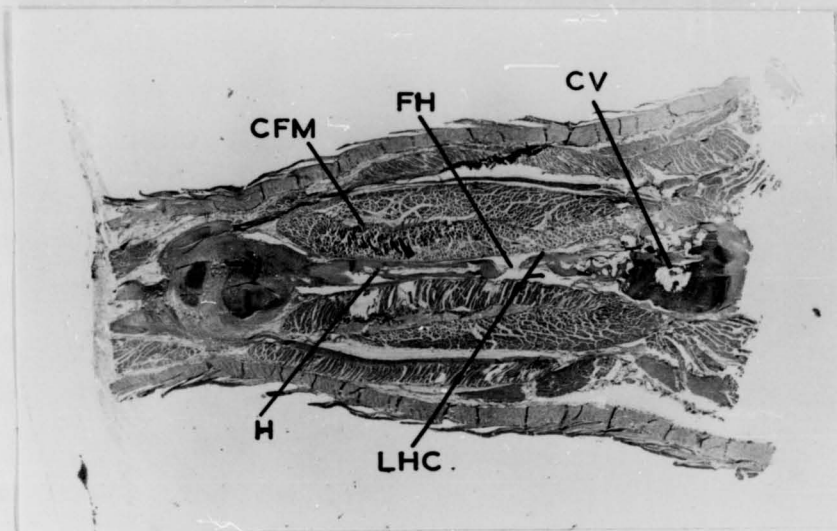
The anatomy of the caudal lymph heart was also investigated.   Figure 28 shows the bones of the tail of rainbow trout with the foramen hypurale between the hypural bones through which the two chambers of the lymph heart are interconnected.   The lymph heart consists of two small flattened sacs (about 2mm across in a 30cm fish) which lie on either side of the hypural bones below the end of the vertebral column.   Figure 29 shows a longitudinal section through in the horizontal plane this region of the fish showing the two chambers and their interconnection. There is no specialised myocardium and the only musculature associated with the heart is that of the caudal fin.   No independent pulsation of the heart was noticed.   It is probable that any pumping action of the caudal lymph heart is due to the movement of the extrinsic muscles of the caudal fin and that during swimming activity the pumping would be of the same frequency as the tail beat frequency.

The volume of each chamber of the caudal heart was estimated from silecon rubber casts from a 30 cm fish at approximately 0.002ml.

In the caudal region below the vertebral column there is a caudal lymphatic vessel running ventral to the caudal vein and artery (fig 30).   The caudal lymph heart appears to be connected to this and lymph probably flows from the lymph heart along the caudal lymphatic into the cardinal lymphatics to drain into the major veins close to the heart.   However several complex connections with the caudal lymph heart were noted and the possibility of a direct flow of lymph into the caudal vein could not be excluded.

The lymph vessels are generally an order on magnitude smaller than the corresponding blood vessels so that as high pressures are unlikely in the lymph system the flow rates are probably small in comparison with blood flow.

Kampmeier (1969) reviews the literature on lymph systems of fish and suggests that an independently pulsatile caudal lymph heart is only found in eels and similar fish. Hyrtl (1843) observed no independent pulsations of the caudal lymph heart of trout which is confirmed by the present observations. However Grodzinski (1959) who studied the development of the caudal heart in sea trout observed rhythmic contractions of the chambers. It is probable that these independent pulsations do not continue in the adult. The close proximity of the caudal skeleton and musculature would preclude any really effective pulsations independent of the tail movements. Grodzinski also clearly shows that the caudal lymph heart empties directly into the caudal vein and does not indicate the presence of a caudal lymphatic. The silecon rubber injection mass used was too viscous for entirely satisfactory study of the delicate lymph vessels but definite points of difference from the sea trout embryo emerged particularly with respect to the caudal lymphatic.

From the functional point of view it can be stated in conclusion that the caudal lymph heart pumps per tail beat approximately 0.002ml of lymph ultimately into the venous system by a pathway which is as yet uncertain. The output at different swimming speeds can be calculated by simple multiplication of the volume by tail beat frequency using the swimming data of Bainbridge (1958). This predicted output is plotted against

swimming speed in fig 41 and compared with the cardiac
output estimates of Stevens & Randall. (1967b).   It can be
seen that the caudal lymph heart output is considerably
smaller than the overall blood flow.

DORSAL AORTA LIGAMENT.

Anatomy

From the dissections of rainbow trout it was found that
the position and connections of the dorsal aorta with other
blood vessels are exactly as described by Gorkiewicz (1947)
and Sikorawa (1947).   However the uniform cylindrical form
of the aorta as represented by these authors is a simplification.

Figure 31 shows a general view of the dorsal aorta as
dissected from the ventral aspect.   It lies on the ventral
surface of the vertebral column dorsal to the kidney in the
trunk region.   It continues in the caudal region inside the
haemal arches of the vertebrae, dorsal to the caudal vein.
The aorta is flattened dorso-ventrally in the trunk region and
narrows in width towards the tail where it becomes almost
circular in cross section.   Detailed measurements of diameters
of the silecon rubber casts are shown in figure 32.   The
depth of the vessel is quite uniform throughout most of its
length.

Figure 33 shows a cross section of the aorta in the trunk
region and shows the ligament,with its attached "mesentery"
or curtain.   Staining with orcein (fig 34) shows that there
are elastic fibres in the wall of the aorta but its distensibility
is confined by the vertebrae and the kidney.   It can also be
seen that the ligament is rich in elastic fibres.   The

Figure 31. General view of a dissection of the dorsal
aorta from the ventral aspect showing the
longitudinal ligament _in situ_. Efferent
branchial arteries in the cranial region
join the aorta which runs down the length
of the body.   The segmental arteries
are not shown.

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RAINBOW TROUT
DORSAL AORTA

Figure 32. Measurements of width and depth of the
dorsal aorta of a fish 18cm long.
Measurements were taken at 5mm intervals
along the length of a silecon rubber
cast of the aorta from the point of
anchorage of the ligament (Omm) to
well into the caudal region.   The
width multiplied by depth gives an
estimate change in cross sectional
area.
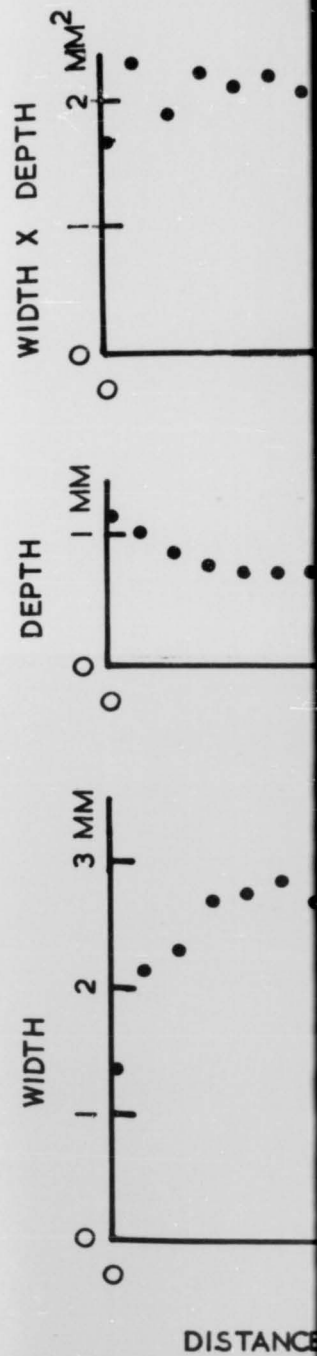
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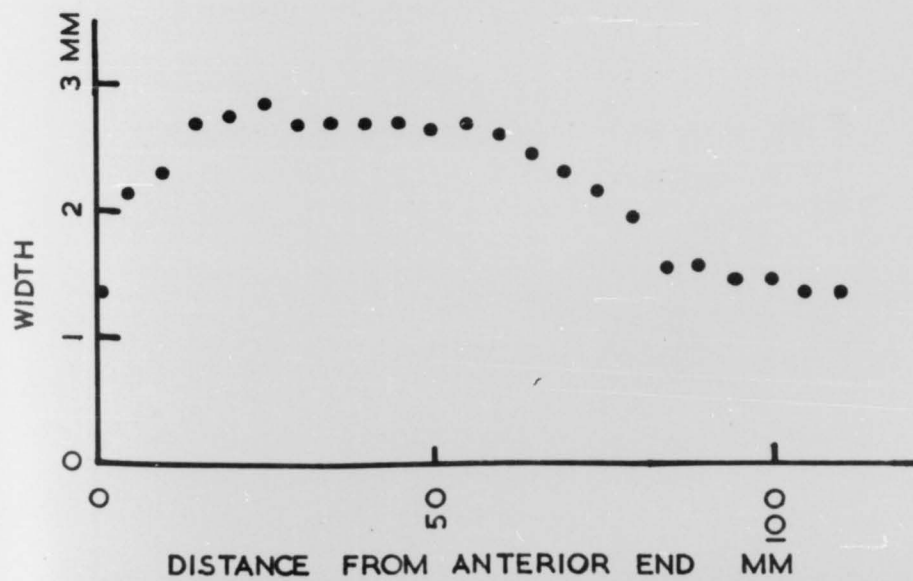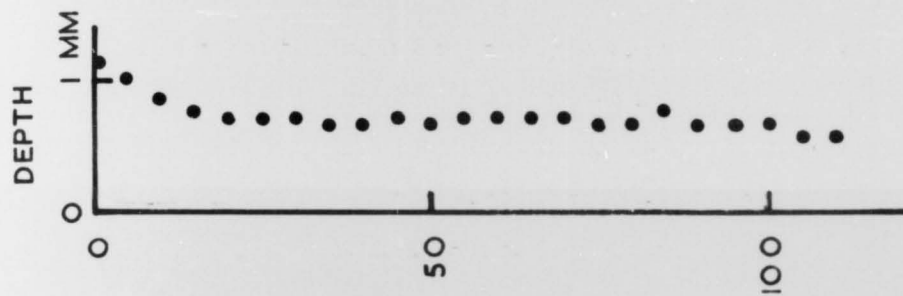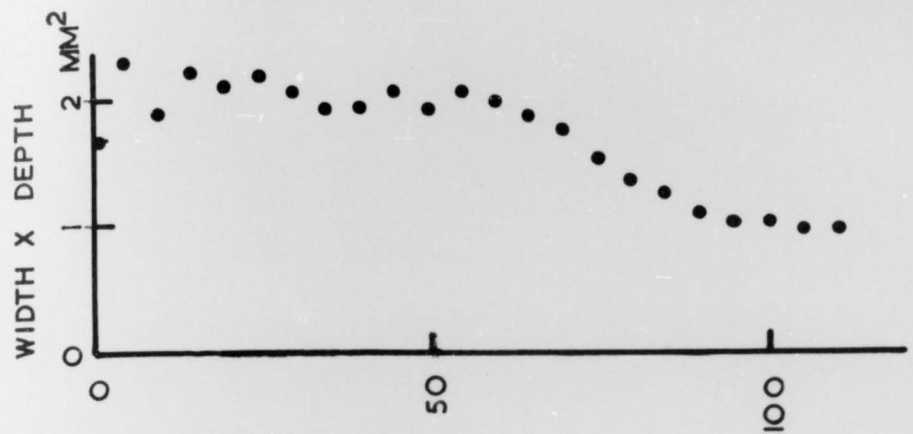
Figure 33. Transverse section through the vertebral column and dorsal aorta in the trunk region (haematoxylin and eosin). C - centrum, DA - dorsal aorta, DLL - dorsal longitudinal ligament, M - muscle, NA - neural arch, P - parapophysis, PCV - posterior cardinal vein, VLL - ventral longitudinal ligament (dorsal aorta ligament).

Figure 34. Transverse section of the dorsal aorta (orcein elastica). The orcein has selectively stained the elastic elements in the wall of the aorta and in the longitudinal ligament. Abbreviations as for fig. 33.
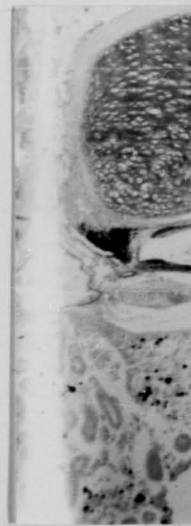
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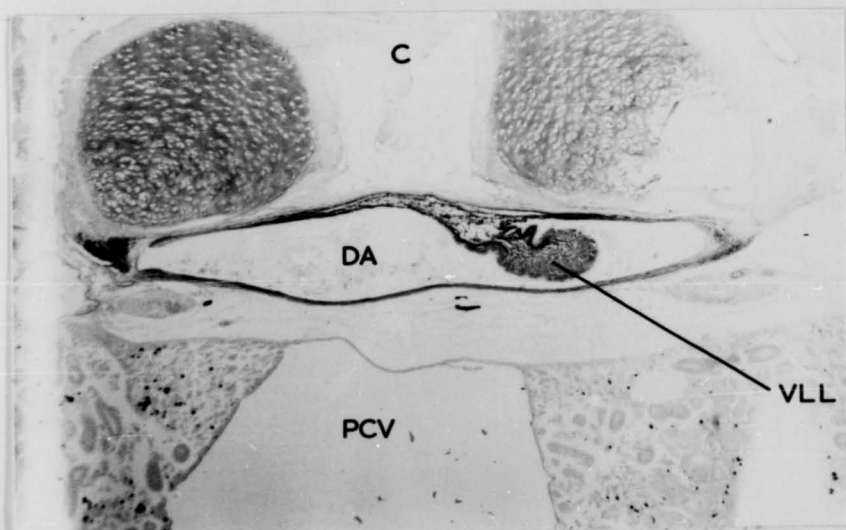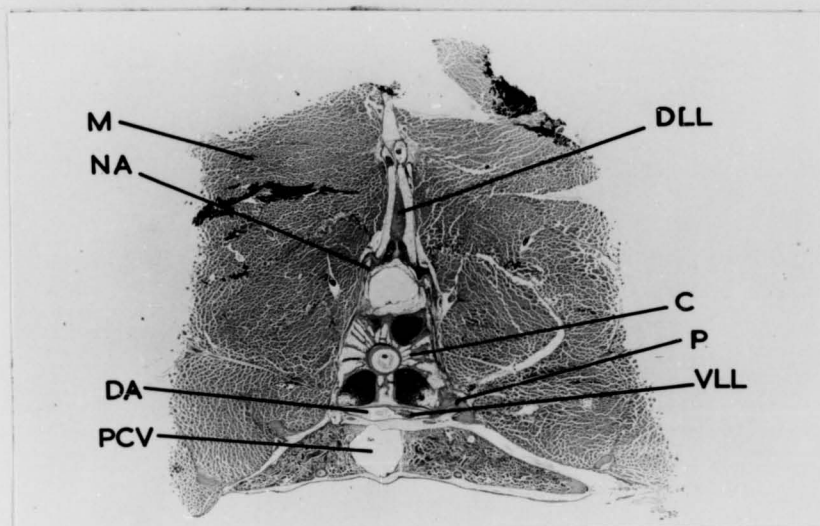
Figure 35. Anterior end of a cast of the dorsal
aorta and the efferent branchial arteries.
Side elevation of the right side. 1,2,3,4 -
efferent branchial arteries, CM - coeliaco-
mesenteric artery, DA - dorsal aorta,
DS - dorsal segmental artery, IC - internal
carotid artery, ICA - intercostal artery,
LA - lateral aorta, LS - lateral segmental
artery, S - subclavian artery, X - point
of anchorage of the aortic ligament to the
bassioccipital bone.

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ligament is attached at its anterior end to the basioccipital
bone in a blind ending anterior extension of the aorta dorsal
to the connection with the branchial arteries (fig 35). The
anterior mesenteric artery branches off ventrally close behind this
point. The posterior mesenteric artery at the posterior end
of the body cavity is very small. There is a lateral branch
(approx. 0.2mm diameter) to the body musculature at each
alternate myotome. These branch in close proximity to the
vertebrae into dorsal, lateral and ventral components as
described by Gorkiewicz (1947). In the extreme caudal region
the ligament tapers into a dorsal ridge below the vertebrae.
At the posterior end the caudal artery divides into several
branches supplying the caudal fin.

Ligament Elasticity

Figure 36 shows the length-tension relationship for a 5cm
length of ligament excised from a 27.5cm long rainbow trout.
When relaxed its length was 3cm and when extended to its
original length in the fish the tension was about 40g. Thus
the static tension in the ligament in the fish is estimated as
about 40g. The cross section of this ligament was approximately
0.75mm by 0.15 mm. Bergel (1961) gives a value of 6×10^6
dyn/cm^2 for Young's modulus of elastic tissue. From this the
force required per unit extension can be calculated for this
ligament:-

$$Young's\ Modulus\ =\ stress/strain$$
$$stress\ =\ force/area$$
$$strain\ =\ \Delta\ 1/1$$

1 = length of ligament segment relaxed = 3cm

$\Delta 1$ = extension of ligament = 1cm

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$$\text{Young's Modulus} = \text{stress/strain}$$
$$\text{stress} = \text{force/area}$$
$$\text{strain} = \Delta l/l$$

l = length of ligament segment relaxed = 3cm

Δl = extension of ligament = 1cm

Figure 36. The length-tension relationship in a
segment of the dorsal aorta ligament which
was 5cm long _in_ _situ_ in the fish. The
straight line is the theoretical
relationship assuming that Hook's law
is obeyed.

TENSION g

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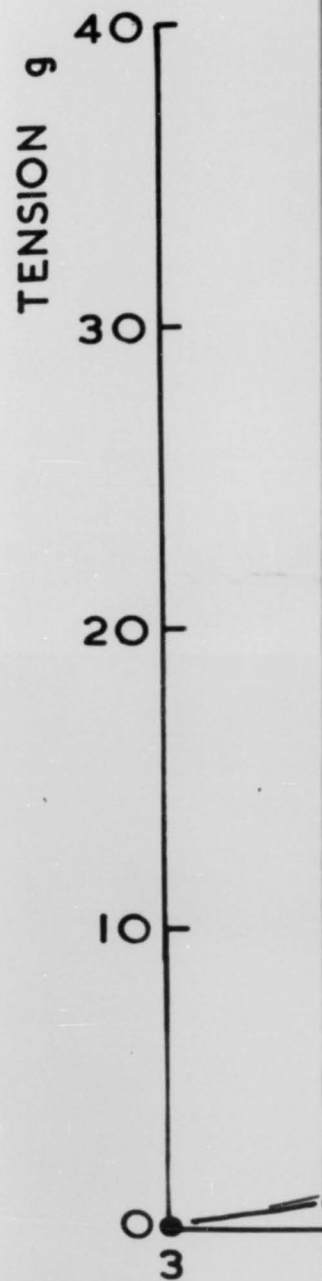
nship in a

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Y.M. = 6×10^6 DYN/CM^2

TENSION g

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LENGTH cm
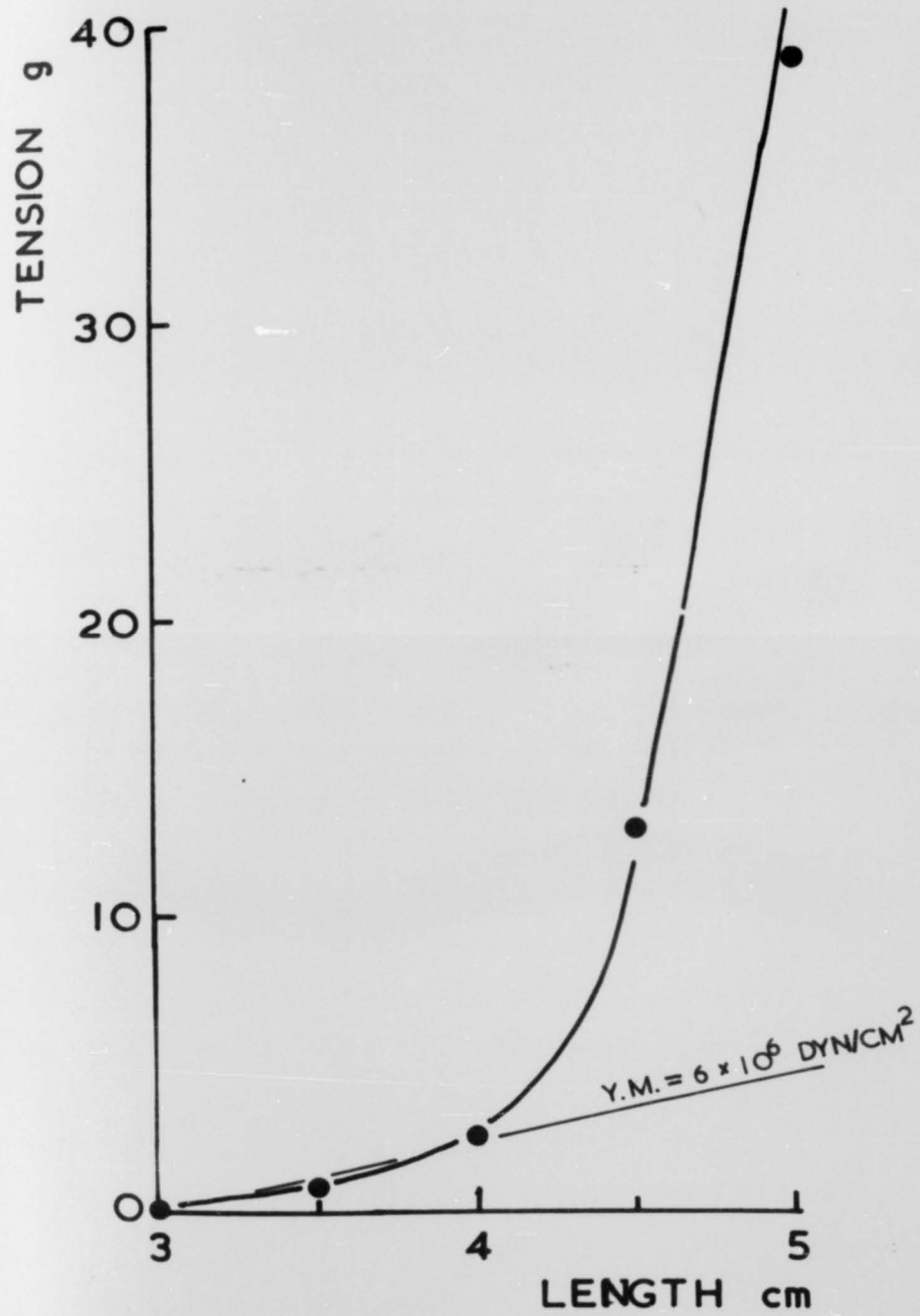
Figure 37. Function of the dorsal aorta ligament
(After Burne 1909).   In these plan
views the arrows indicate the direction
of movement of the swimming waves
along the body.   Blood is enclosed
in alternate compartments on either
side of the ligament and is carried
caudad with the wave movement.
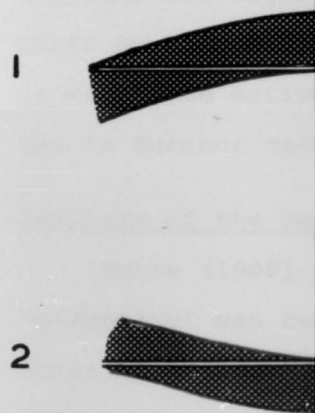
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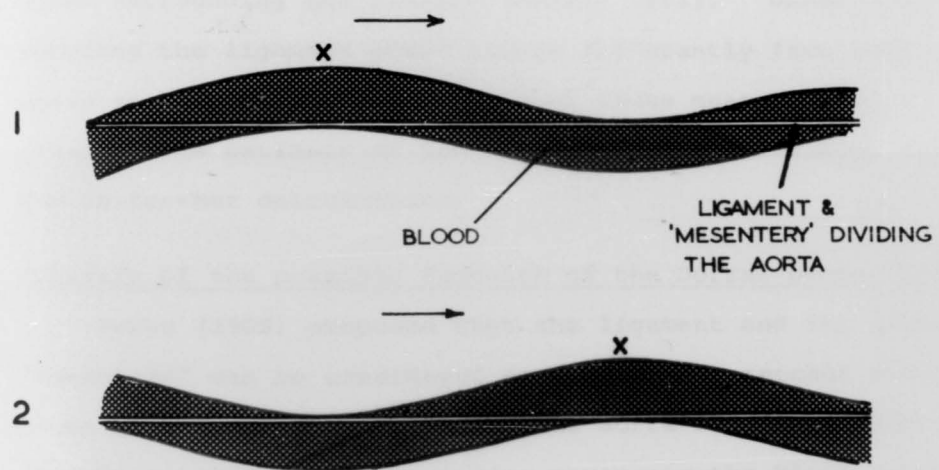
$$\frac{6 \times 10^6 \times 0.75 \times 0.15}{981 \times 3} = \text{Force /cm extension}$$

$$= 2.2 \text{ g/cm}$$

This rate of tension increase with length is plotted in figure 36 for comparison with the experimental values. It can be seen that at small extensions this ligament corresponds closely to Bergel's data but at the length in the living fish it is considerably stiffer than would be predicted.

The elasticity relations in natural fibres are complex (Alexander 1968) and can be modified by the nature of electrolytes surrounding the tissues (Bergel 1961). Under dynamic tensions the ligament would behave differently from under these sustained tensions. However these measurements do provide some estimate of the behaviour of the ligament for use in further calculations.

Analysis of the possible function of the Dorsal aorta ligament.

Burne (1909) proposed that the ligament and its attached "mesentery" can be considered as a curtain stretched along the lumen of the dorsal aorta. As the aorta bends with the vertebral column during swimming movements the blood would be enclosed in successive compartments on either side of this cutain, corresponding to the series of waves executed by the fish. (figure 37). As these waves pass posteriorly along the body blood may be carried posteriorly along the aorta augmenting the circulation during exercise.

In the light of recent knowledge of swimming movements in rainbow trout and the above anatomical studies it is proposed to investigate theoretically the feasibility of Burne's hypothesis.

Figure 38. The idealised swimming waveform used in
these studies for a 30cm long fish. The
amplitude is 1 cm trunk region and 5cm
at the tip of the tail. The wave shown
as a dotted line is 90° behind the solid
line.

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The wave shown

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HEAD

1 cm

TAIL
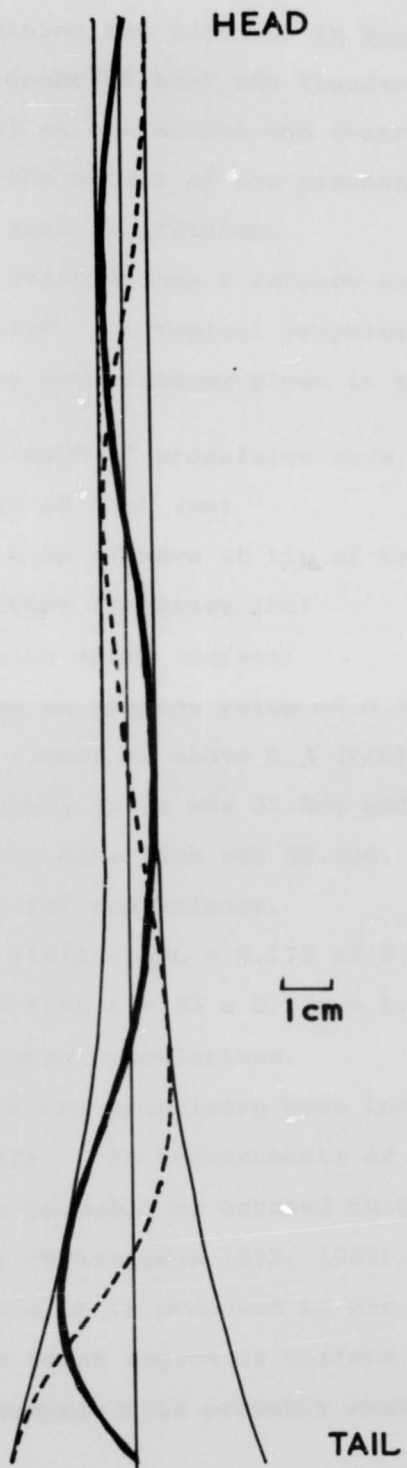
In closely examining the ligament in situ and with dye injections it seems doubtful that the ligament can act efficiently as a seal at the dorsal and ventral walls of the aorta. However at the outset of the present analysis it will be assumed that the seal is efficient.

As a basis for calculations a rainbow trout about 30cm long will be considered. A typical propulsive waveform for such a fish can be derived from figures given in the literature.

λ = wavelength of propulsive wave (cm)

L = length of fish (cm)

A = amplitude of wave at tip of tail (cm)

f = tail beat frequency (hz)

V = swimming speed (cm/sec)

Webb (1971b) obtained an average value of 0.76L for λ in trout of various sizes at speeds of above 0.3 (V/L). For a 30cm rainbow trout the actual value was 22.8cm and a theoretically derived figure for the same fish was 20.4cm. λ = 21.0cm will be assumed here for convenience.

Also from Webb (1971a) A/L = 0.175 if f is greater than 5hz. Therefore for a 30cm fish A = 30 x 0.175 = 5.25cm. A = 5.0cm will be assumed in these calculations.

The amplitude of the propulsive wave increases from head to tail (Bainbridge 1963). No measurements of this are available for trout but it can probably be assumed that trout is similar to Dace in this respect (Bainbridge 1958, 1963). Figure 38 shows the idealised waveform it is proposed to use in these studies. The amplitude in the trunk region is uniform (1cm). The error involved in this assumptio n is probably small: The dorsal

Figure 39.   Plan of the dorsal aorta during one
cycle of the ideal swimming wave. The
scale is expanded 5 times in width
relative to the logitudinal scale.
The shading traces the history of one
blood compartment assuming that Burne's
hypothesis applies.

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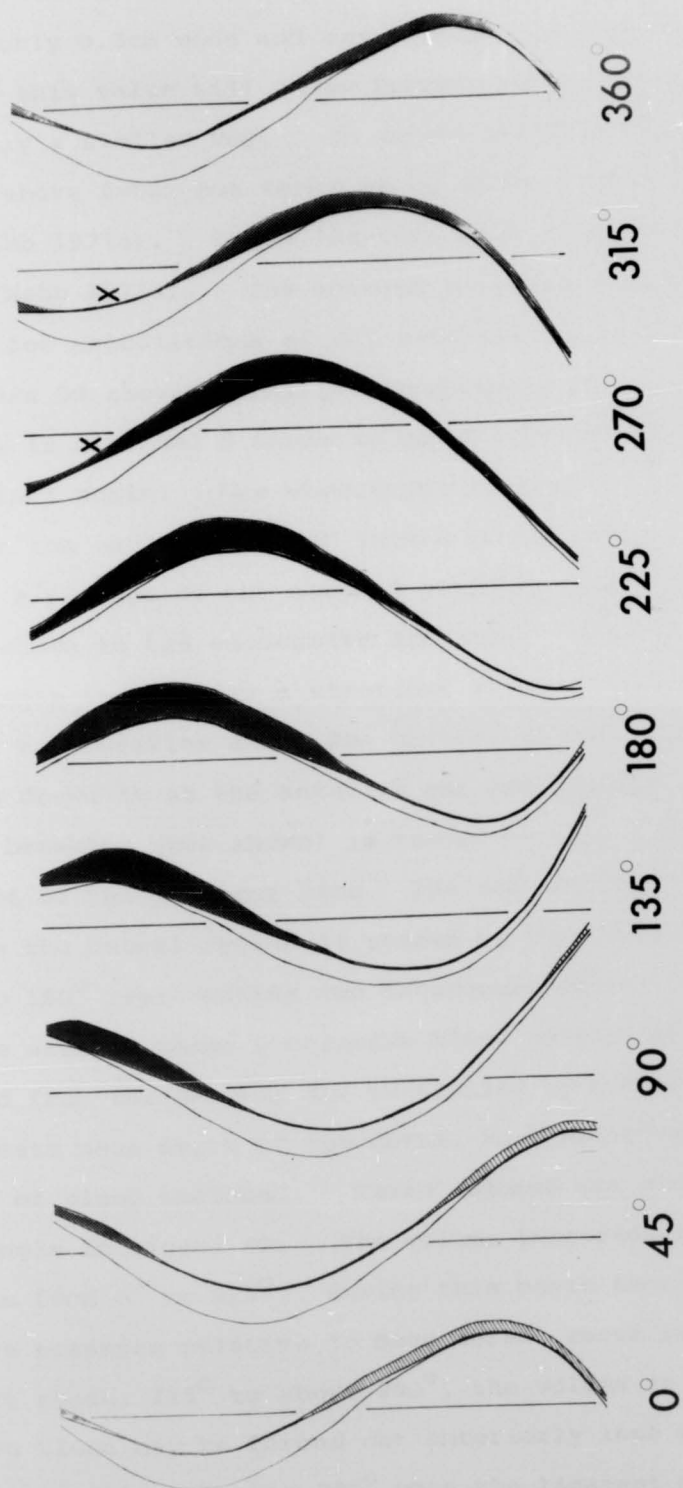
aorta is only 0.3cm wide and any swimming wave amplitude in excess of this value will allow Burne's mechanism to work in essentially a similar way. In actual swimming fish A is constant above f=5hz but tends to be smaller below this speed (Webb 1971a). Below 1hz tail beat frequency λ is not uniform (Webb 1971a). The assumed waveform is probably adequate for calculations at all swimming speeds above f=1hz.

Figure 39 shows a plan of the dorsal aorta; for clarity the scale is expanded 5 times in width relative to the longitudinal scale. One wavelength is shown which corresponds to almost the entire "active" length of the ligament. One cycle of a passage of the assumed swimming wave down the body is shown in the successive diagrams. The position of the ligament is drawn as a stretched elastic line from the anterior to posterior end. The history of one compartment of blood is drawn in at the anterior end and ejected through the lateral branches (not shown) is traced by the shaded area to the right of the ligament line. The completion of the ejection phase in the caudal region is traced by the cross hatched area in 0° to 180° representing the subsequent cycle.

The area of these successive blood compartments was measured from the drawing and multiplied by 0.05cm, the approximate mean depth of the aorta, to give an estimate of the volumes of blood enclosed. These volumes are plotted against phase angle in figure 40. .The volume increases as blood is drawn in from 0° to 225°; during this phase there would be a negative pressure relative to mean dorsal aorta pressure. In the next phase, 225° to about 290°, the volume is decreasing but some blood may be forced out anteriorly into the branchial arteries. Only at about 290° when the ligament makes a seal

Figure 40. Estimated volumes of one blood compartment
enclosed by the dorsal aorta ligament during
one cycle of the Burne type pumping action.
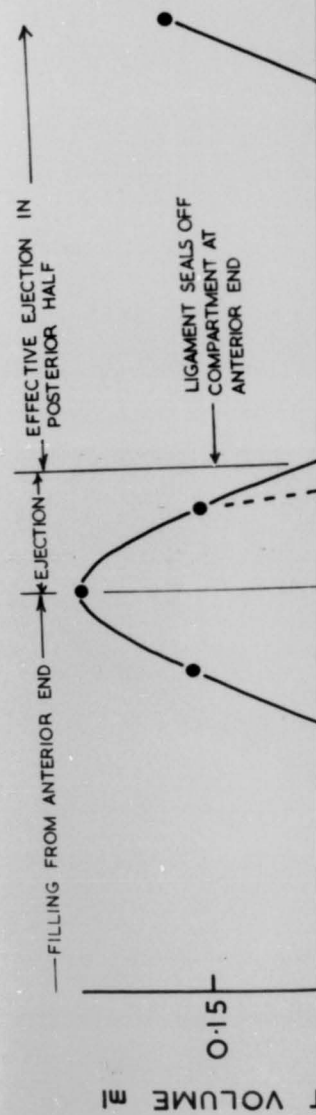
of the dorsal

he caudal lymph

at frequencies

output.

ml/minute

160
140
120
100
80
60
40
20

DORSAL AORTA 0.15 ML/TAIL BEAT

DORSAL AORTA 0.3 ML/TAIL BEAT

RANGE OF OBSERVED CARDIAC OUTPUTS
(STEVENS & RANDALL 1967b)

CAUDAL LYMPH HEART

2 4 6 8 10 12 14 16 18   tail beats/sec
1   2   3   4   5   6   speed (V/L) approx.
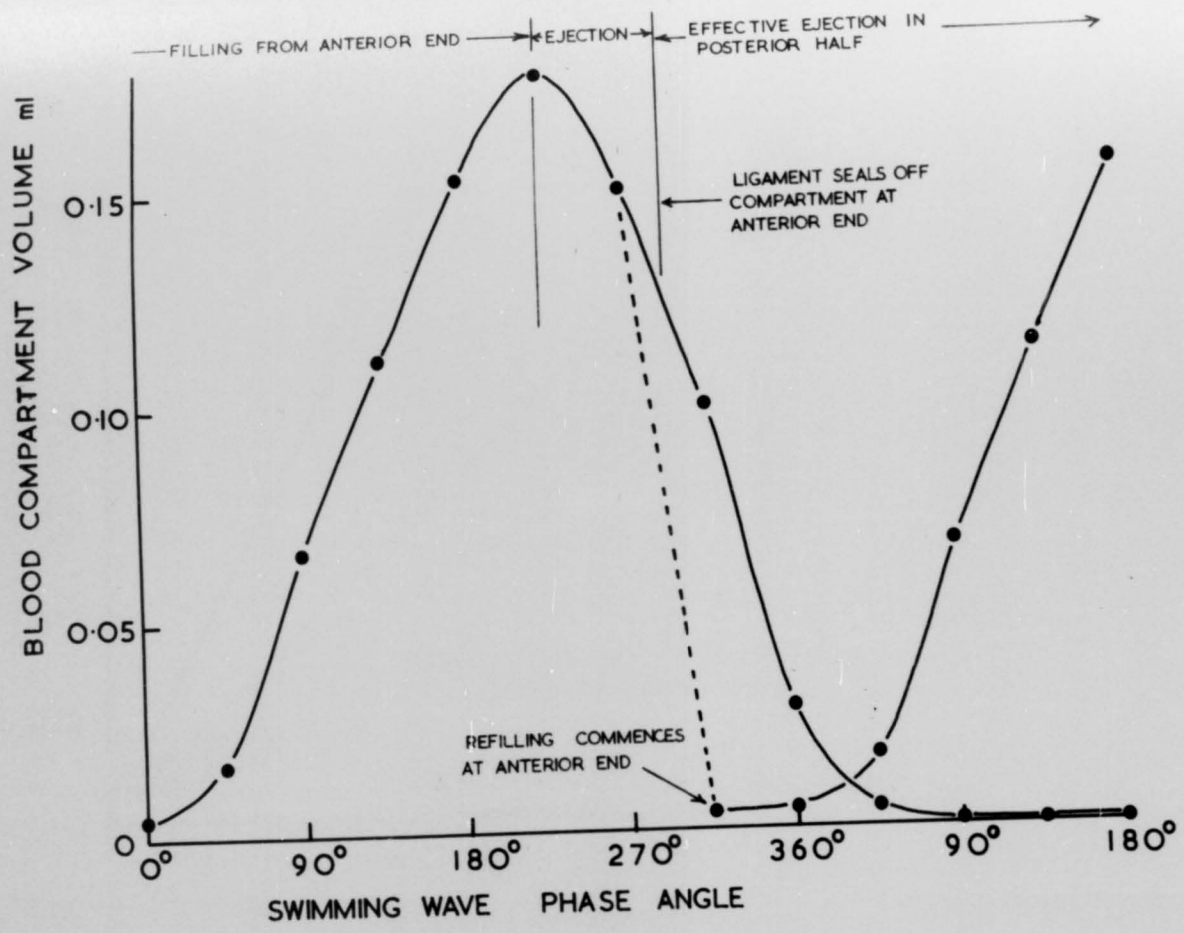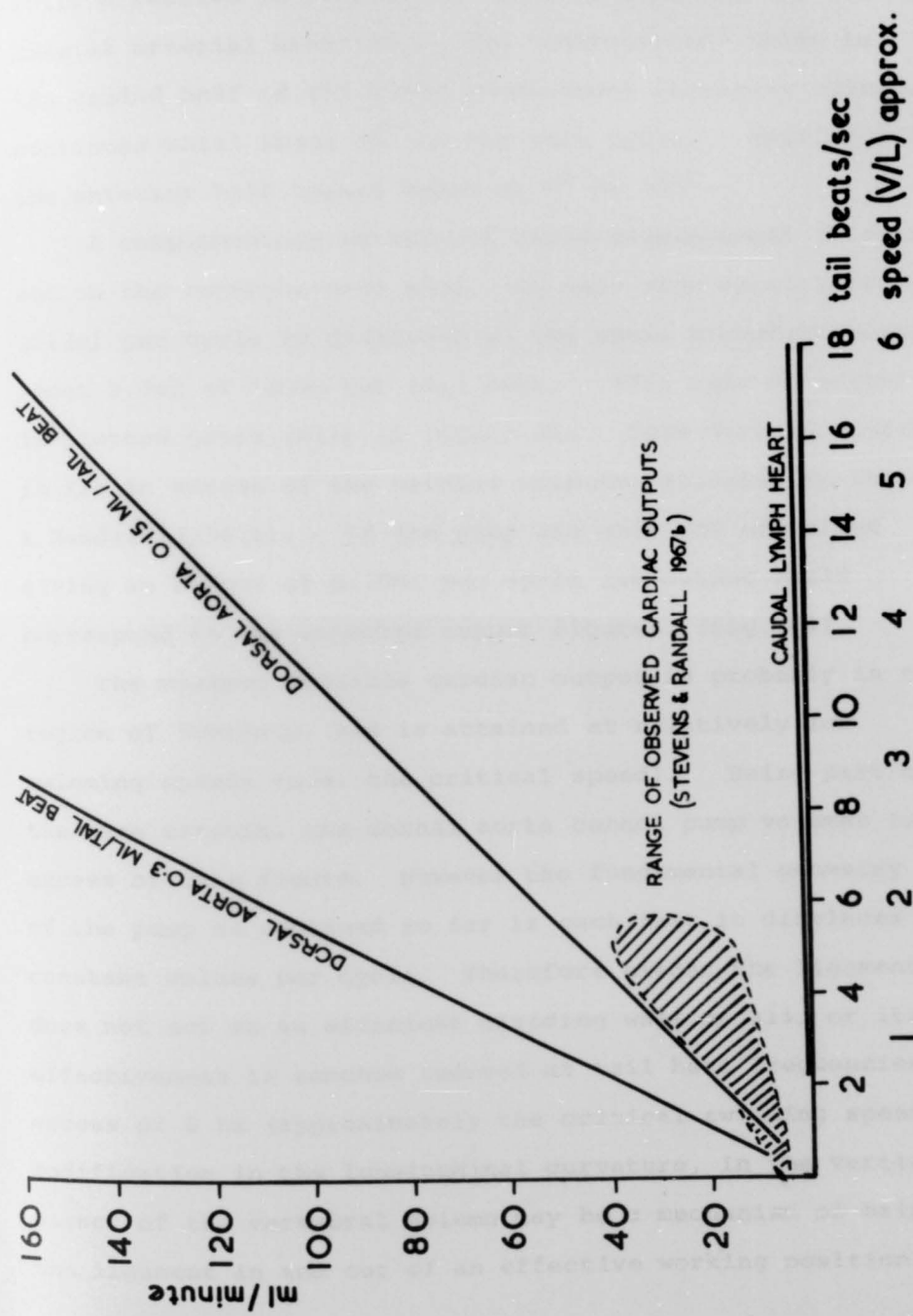
Figure 41.   Theoretical volume outputs of the dorsal
             aorta pump mechanism and the caudal lymph
             heart at different tail beat frequencies
             compared with the cardiac output.

DORSAL AORTA 0·15 ML/TAIL BEAT

DORSAL AORTA 0·3 ML/TAIL BEAT

160

140

120

100

ml/minute

of the dorsal
he caudal lymph
at frequencies
output.

ml / minute

160
140
120
100
80
60
40
20

DORSAL AORTA 0·3 ML/TAIL BEAT

DORSAL AORTA 0·15 ML/TAIL BEAT

RANGE OF OBSERVED CARDIAC OUTPUTS
(STEVENS & RANDALL 1967b)

CAUDAL LYMPH HEART

2   4   6   8   10   12   14   16   18   tail beats/sec

1   2   3   4   5   6   speed (V/L) approx.
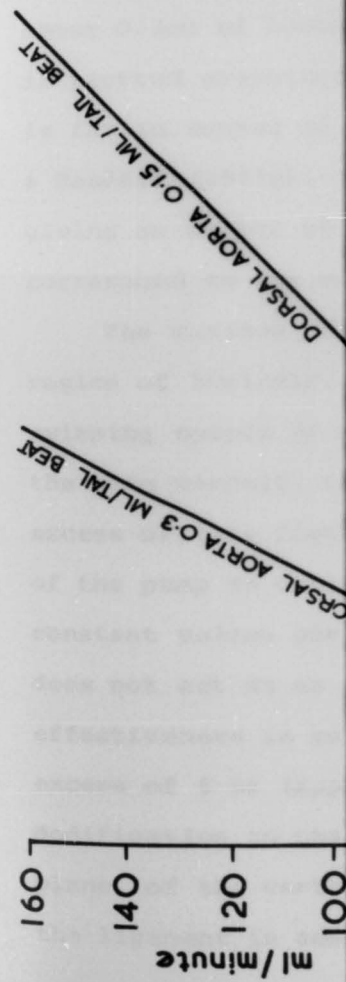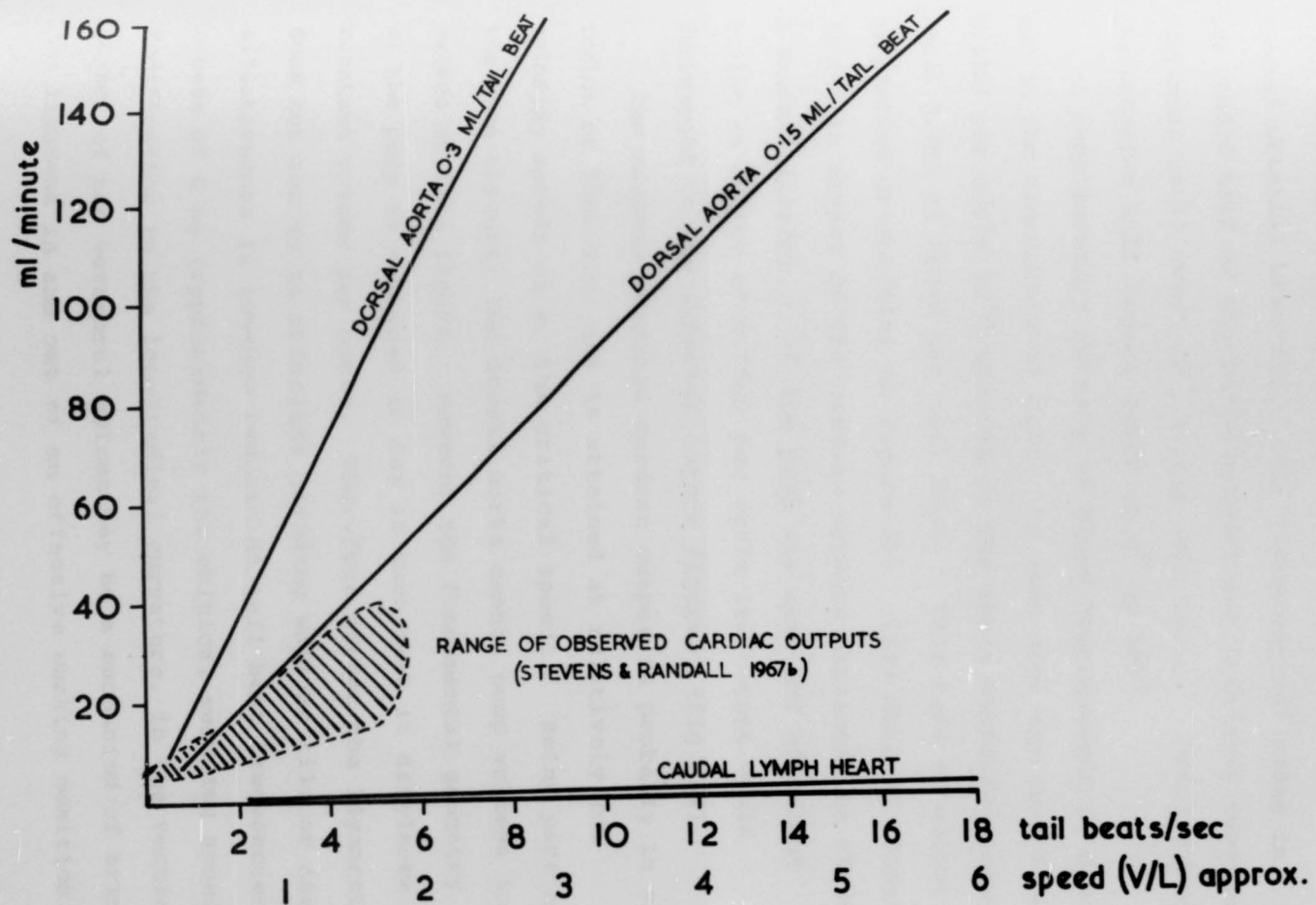
at point X on the diagram (fig. 39) could .the pump become
fully effective in propelling blood posteriorly and into the
lateral arterial branches. The "compression" phase in
the caudad half of the blood compartment initially considered
continues until about 90° in the next cycle. Refilling of
the anterior half begins again at 0° or 360°.

A complementary pattern of blood displacement is carried
out on the contralateral side. On each side approximately
0.15ml per cycle is displaced so the whole mechanism pumps
about 0.3ml of blood per tail beat. This rate of output
is plotted graphically in figure 41. This derived output
is far in excess of the cardiac outputs estimated by Stevens
& Randall (1967b). If the pump was only 50% efficient
giving an output of 0.15ml per cycle its output would
correspond to the expected output figures. (fig. 41).

The maximum possible cardiac output is probably in the
region of 50ml/min. and is attained at relatively low
swimming speeds (i.e. the critical speed). Being part of
the same circuit, the dorsal aorta cannot pump volumes in
excess of this figure. However the fundamental geometry
of the pump as outlined so far is such that it displaces a
constant volume per cycle. Therefore either the ligament
does not act as an efficient dividing wall at all, or its
effectiveness is somehow reduced at tail beat frequencies in
excess of 6 hz (approximately the critical swimming speed).
Modification in the longitudinal curvature, in the vertical
plane, of the vertebral column may be a mechanism of bringing
the ligament in and out of an effective working position.

Figure 42.   (a) one half wavelength of the swimming
              wave in the aorta showing breakdown of
              the pump mechanism (scale expanded in
              width).

              (b) The ligament and its attached
              mesentery as considered for calculations
              as a segment of a cylinder.

(b)

(a)

LIGAMENT IN
WORKING
POSITION

(a)

LIGAMENT IN
WORKING
POSITION

LIGAMENT DEFLECTED
BY EXCESS PRESSURE

10 CM

(b)

F = 375 DYN

r = 30 CM

0·05 CM
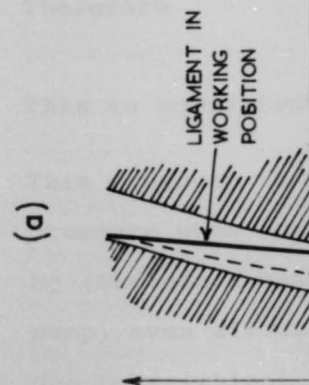
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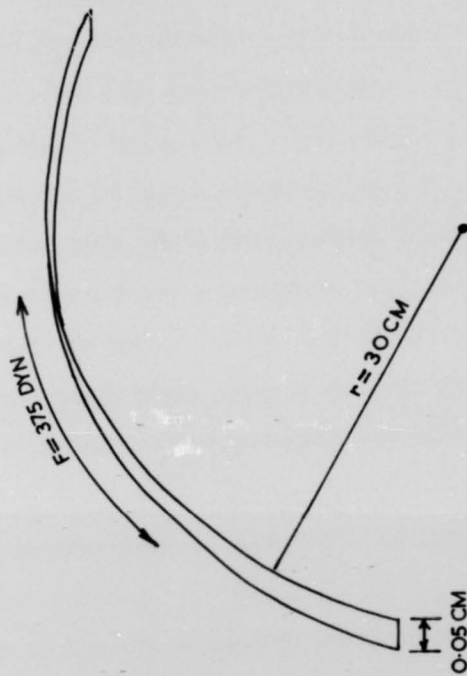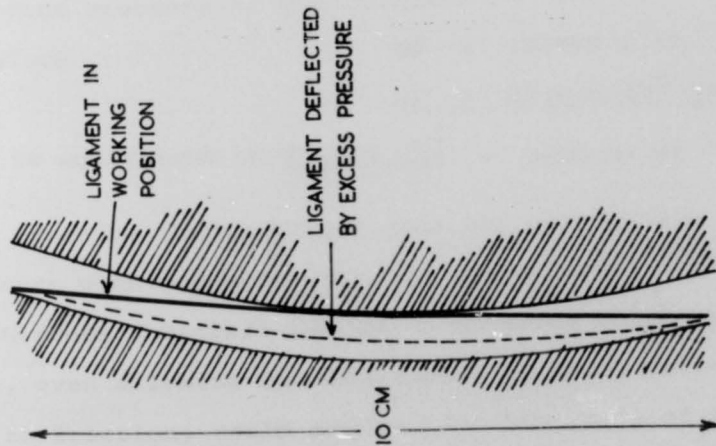
At high swimming speeds the pressure difference across
the ligament may be sufficient to deflect it so that it
becomes ineffective as a pump (fig. 42a).   This would occur
at a particular pressure produced at a particular speed which
would be determined by the elastic properties of the ligament.
This breakdown pressure can be calculated.   Taking a
segment of the ligament 10cm long ($\lambda/2$ approx.) the pressure,
BP required to stretch it by a minimal amount (say 0.1cm) will
be taken as the breakdown pressure.   From figure 36 the
tension increase required to stretch a 10cm length of ligament
in the fish by 0.1cm is 8g.   The "curtain" dividing the
dorsal aorta in one half wave length can be considered as part
of the wall of a cylinder of approximately 30cm radius (r)
(fig. 42b).

The circumferential tension in the wall of such a cylinder
1cm long = T.

$\qquad$ T $\doteqdot$ 8 x 981 x 1/o.05 = 156960 dynes/cm (Alexander 1968)

Therefore pressure in the cylinder $\quad$ = $\quad$ T r

Therefore $\qquad$ BP $\cdot$ = $\quad$ 156960 x 30

$\qquad\qquad\qquad\qquad$ BP $\;=\;$ 47.0 x 10^5 dynes/cm

This is equivalent to $\dfrac{47.0 \times 10^5}{1.33 \times 10^3}$ = $\;$ 3500 mm Hg

This pressure is far larger than any conceivable physiological
pressure when compared with the dorsal aorta pressure of 30 mm
Hg (Stevens & Randall 1967b).   It seems unlikely that the
pump, even allowing for additional ligament deflecting forces
due to friction, could suffer from breakdown of this type at
high speeds.

In conclusion on theoretical grounds it appears unlikely that the pump could be fully efficient in the sense that Burne (1909) proposed over the full range of swimming speeds. If it is effective at low speeds this effectiveness must be reduced at high speeds in order to avoid massive pressure oscillations in the dorsal aorta.

Experiments with the Fish Bending Machine.

On the trout cadaver dorsal aorta preparation.

About 20 experiments were carried out on the fish bending machine using the dorsal aorta preparation. No convincing evidence of pumping activity could be found. No increase in mean output pressure from the caudal artery could be detected. Pressure oscillations coinciding with the tail beat frequency were measured up to $\pm$ 20mm Hg about mean. These large oscillations were largely caused by movement of the cannula. With careful damping of the cannula smaller oscillations were detected but these could not be ascribed with any certainty to the dorsal aorta mechanism.

Some leakage of saline took place through the lateral segmental arteries but this was not large enough to exclude the measurement of quite high mean pressures in the aorta. Using the volume of saline in the caudal cannula held vertically the mean aorta pressure could be measured quite sensitively. Often a fall in this pressure was detected during "swimming movements". This was probably caused by increased flow through the segmental arteries. However in view of the pressure drop this increased flow was probably caused by the movement freeing more flow channels rather than a pumping action when a pressure rise would be detected.

Figure 43. Dorsal aorta model (tube and elastic)
function curves derived from experiments
with the fish bending machine. Lower
graph - maximum output pressure at
different "tail beat" frequencies.
Upper graph - volume output at different
frequencies with three different
constant pressure drops across the pump
mechanism (measured in mm of water).
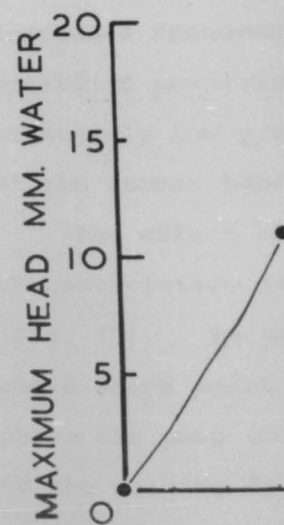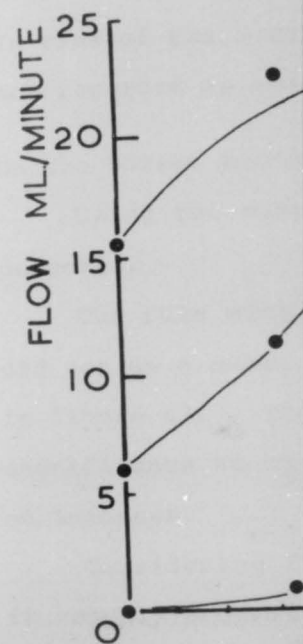
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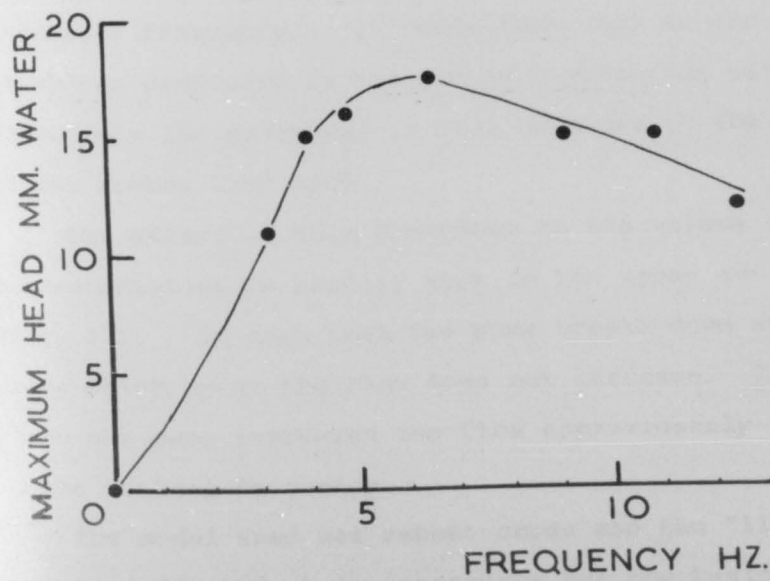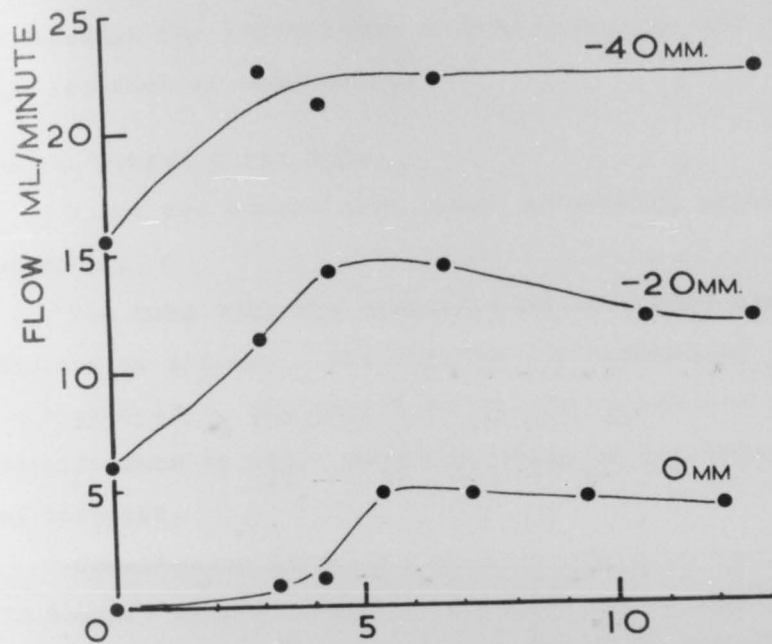
No pumping activity could be detected and the technique
was not appropriate for the study of the nature of the
oscillatory pressure pulse produced by the body movements.
In view of the limitations of the technique the results are
not regarded as conclusive.

On the Dorsal Aorta Model.

Using the simple tube model no pumping activity was
detected.

The tube with the elastic band stretched along the lumen
did act as a pump.   The results are summarised graphically
in figure 43.   The magnitude of the values has no physiological
significance at all; only the shape of the relationships is
of interest.

Considering first the maximum pressure head produced, it
is roughly proportional to frequency up to 5hz when break down
takes place and the maximum pressure produced falls with
increased frequency.   It seems that this is the type of
breakdown predicted in the theory section but which occurs at
relatively low pressures in this case due to the extensibility
of the rubber band used.

The effect of this breakdown on the volume flow
characteristics is readily seen in the upper set of curves
(fig. 43).   In each case the pump breaks down at about 5hz
above which point the flow does not increase.   In its functional
phase the pump increases the flow approximately in proportion
to the working frequency.

The model used was rather crude and the "ligament" was
readily deflected at low pressures but the basic principles of

the Burne type of mechanism were demonstrated. The geometry of the model was such that even under optimum conditions some leakage took place past the ligament.

Heart Structure

The main aim of this investigation of the heart anatomy was to elucidate the geometry and spatial relationships of the various mechanical elements which may be of importance in the interpretation of the hearts functional characteristics.

The gross morphology of the heart is probably best appreciated in figures 44 and 45 showing casts of the heart and. the immediate major blood vessels. The ducti cuvierii and hepatic veins empty into the sinus venosus which is virtually incorporated into the posterior wall of the pericardium. The other chambers of the heart; the atrium lying dorsal to the ventricle and bulbus are freely suspended in the pericardial cavity; the bulbus being anchored anteriorly by the ventral aorta.

SINUS VENOSUS.

The walls of the sinus are thin and consist of connective tissue containing large amounts of collagen as shown by its red staining with Van Geison's stain. (fig.46b). There was no evidence of muscle fibres in the sinus wall. The sinus venosus is in fact little more than the confluence of the major veins entering the heart. The volume of the cast was estimated at approximately 0.08ml.

ATRIUM.

The castings of the atrium show the internal surface to be rather irregular. The very outer wall of the atrium consists of connective tissue including collagen and some

Figure 44.   Silecone rubber cast of the heart chambers,
              lateral view.   A - atrium, ABA - afferent
              branchial artery, BA - bulbus arteriosus,
              DC - ductus cuvieri, HV - hepatic vein,
              LPCV - left posteriror cardinal vein,
              RPCV - right posterior cardinal vein, SV -
              sinus venosus, VA - ventral aorta.

Figure 45.   Silecon rubber cast of the heart chambers,
              dorsal view.   Key as for fig. 44.

eart chambers,

BA - afferent

arteriosus,

patic vein,

al vein,

al vein, SV -

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. 44.

Figure 46.   Section of part of the wall of the sinus
venosus. (Van Geison's stain). The dense
staining indicates the presence of large
amounts of collagen.

Figure 47.   Section of part of the wall of the atrium.
(Haematoxylin and eosin).   C - connective
tissue in the sub-epicardial region. M -
muscle bundles, E - erythrocytes.
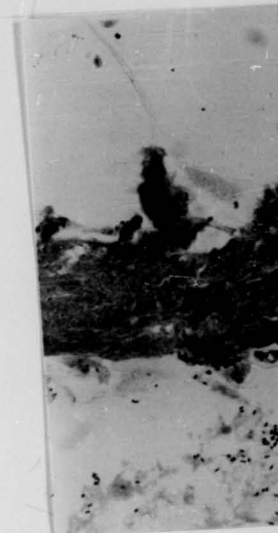
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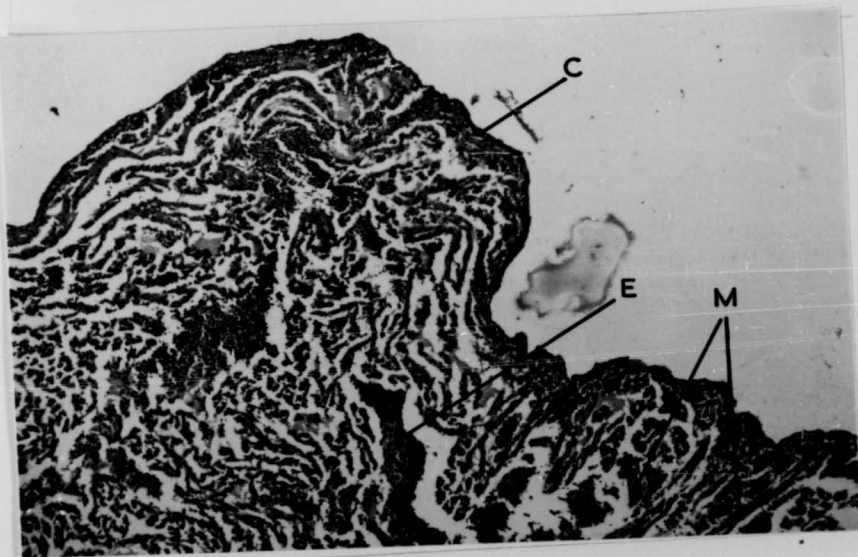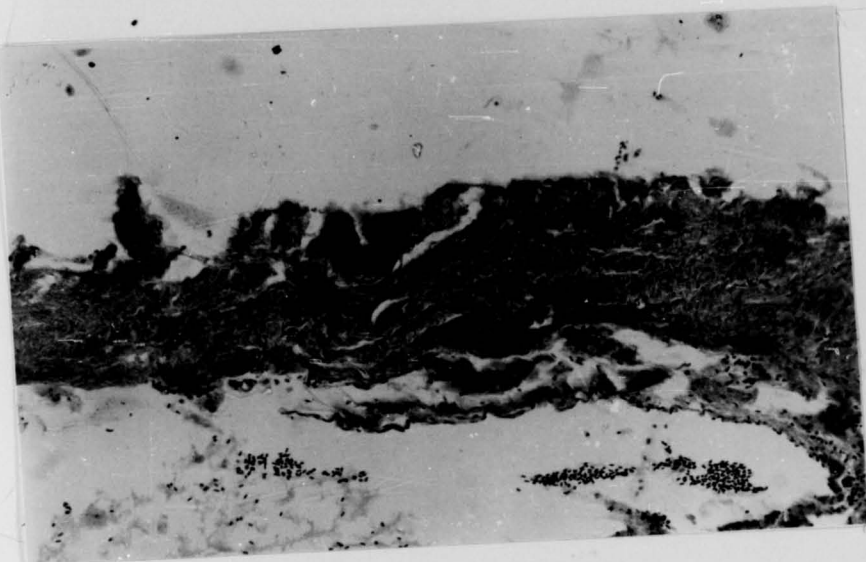
Figure 48.   Longitudinal vertical section through the
heart (Haematoxylin and eosin).   A -
atrium, BA - bulbus arteriosus, SC -
stratum compactum of the ventricular
myocardium, SM - spongy ventricular
myocardium, SV - sinus venosus.

Figure 49.   Section of the wall of the ventricle
(Orcein elastica) showing elastic fibres
in the outer part of the wall.   EF -
elastic fibres.

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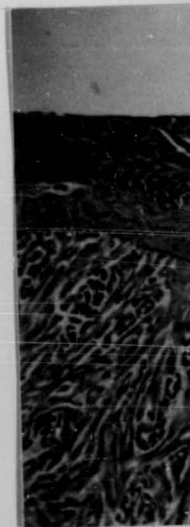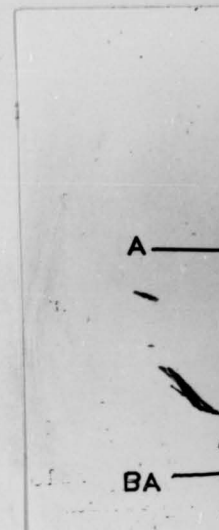
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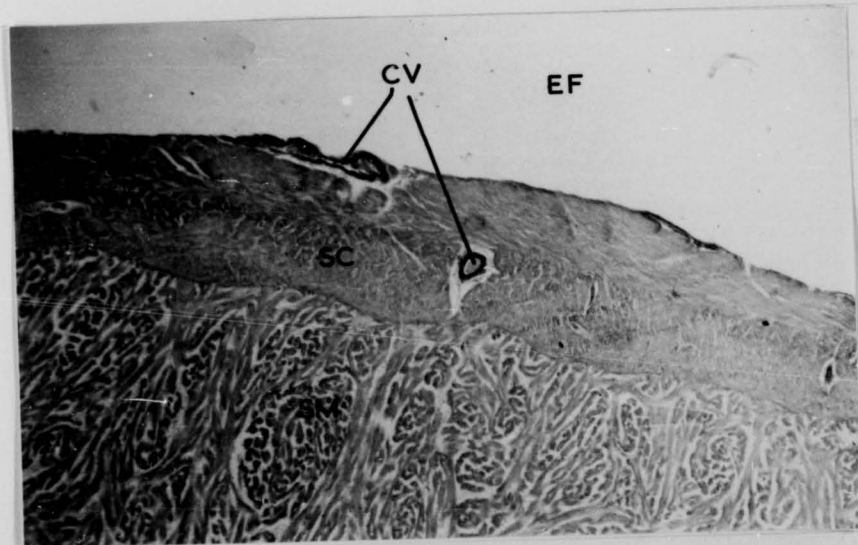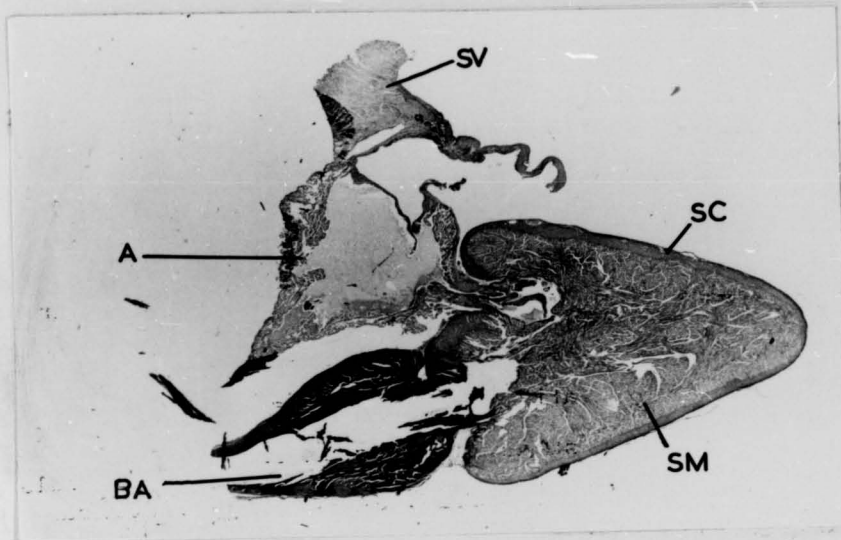
Figure 50.   Section of wall of ventricle (Van
             Geison stain) showing collagen
             fibres in the outer part of the wall
             and coronary vessels.   CF - collagen
             fibres, CV - coronary vessels.

Figure 51.   Longitudinal section of a ventricle
             expanded to a dilated diastole with
             'vaseline' (haematoxylin and eosin).
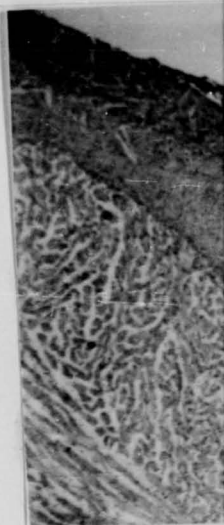
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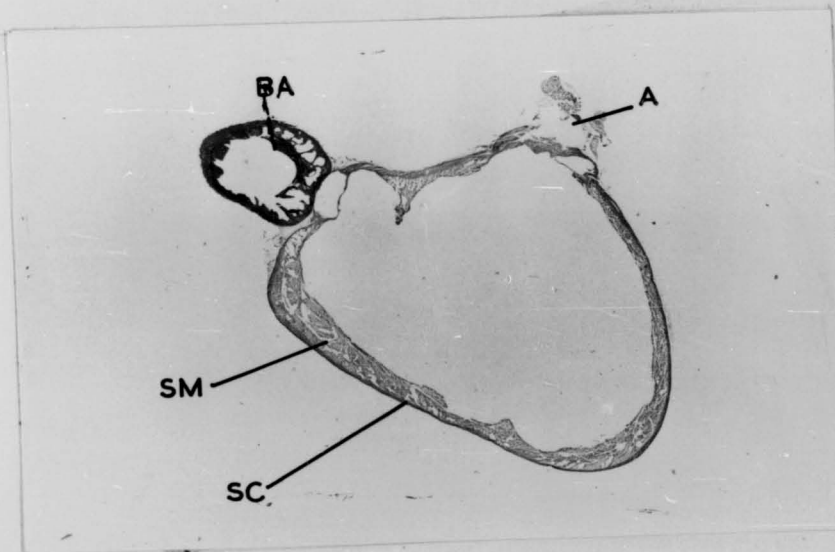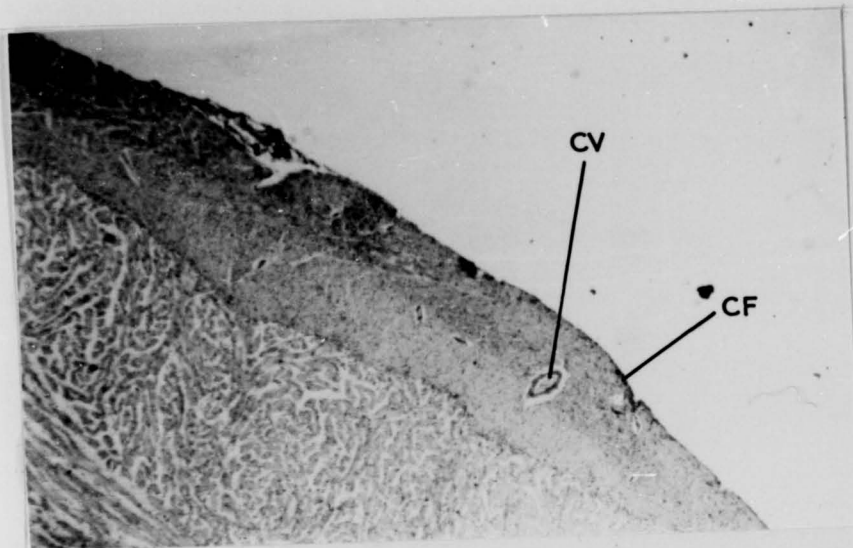
Figure 52. Silecon rubber cast of the bulbus
            arteriosus. VA - ventral aorta,
            LR - casts of longitudinal ridges.
            R - basal region with large numbers
            of radial fibres giving a spongy
            appearance.

Figure 53. Transverse section of bulbus
            arteriosus towards the anterior half
            showing elastic fibres. (Orcein
            elastica).   CF - circumferential
            fibres, LE - longitudinal elements,
            RF - radial fibres.
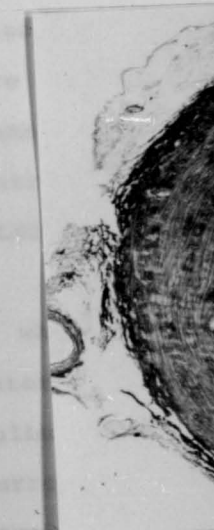
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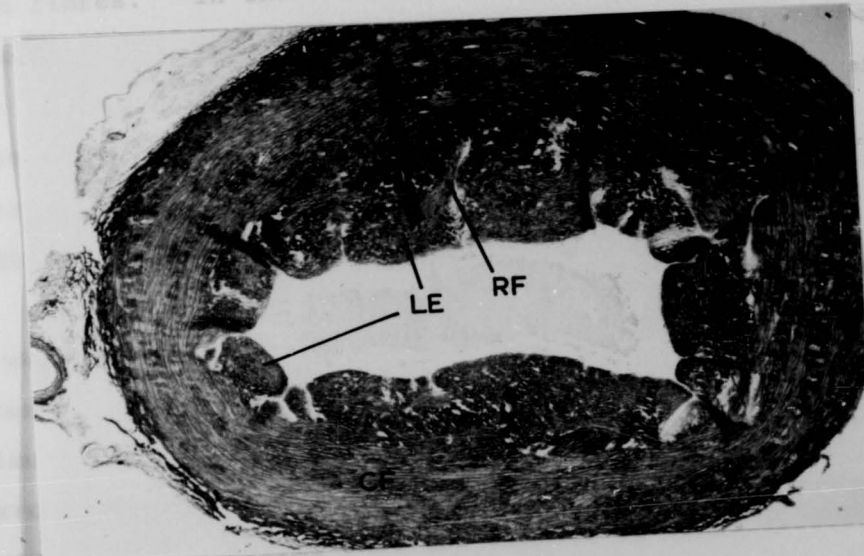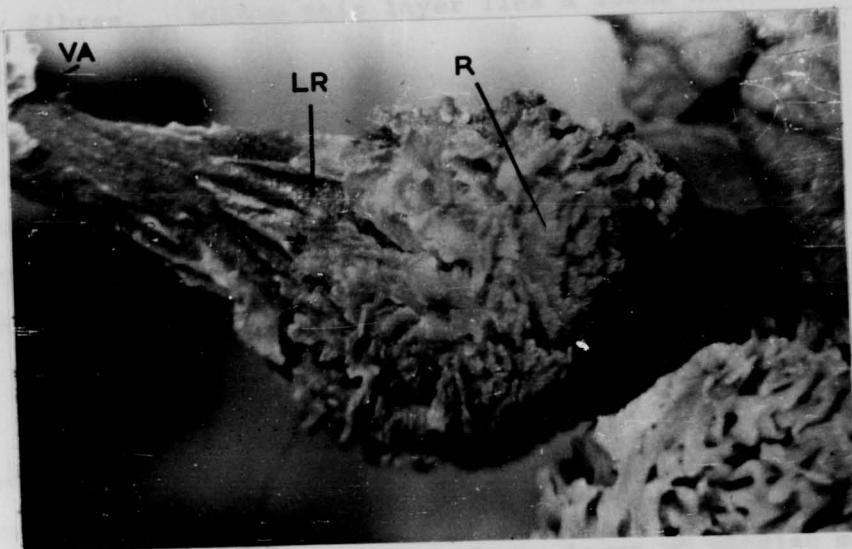
elastic fibres. Within this layer lies a loose network of cardiac muscle fibres many of which traverse the lumen of the atrium (fig. 47). A pair of simple flap valves guard the sino-atrial opening. No coronary blood vessels are present in the atrial wall.

VENTRICLE.

The ventricle is pyramid shaped and in ordinary sections there is little evidence of a lumen (fig. 48). In the very outer layers of the ventricular wall there is some connective tissue; collagen and elastic fibres (figs. 49 and 50). The coronary blood vessels also run in this outer layer. Inside the connective tissue there is a dense layer of cardiac muscle known as the stratum compactum. The rest of the ventricular myocardium is the spongy layer which extends to almost completely occlude the lumen when the heart is contracted (fig. 48). The casts in particular show that the fibres of this layer extend across the lumen and the blood runs between all these fibres. In the sections of the ventricle expanded with "Vaseline" it can be seen that the spongy layer fibres become more orientated and an open lumen appears in the centre of the chamber. (fig. 51). Valves are present at the atrioventricular and ventriculo-bulbar interconnections.

BULBUS ARTERIOSUS.

The bulbus is an onion shaped chamber the anterior end of which merges into the ventral aorta. The coronary artery entering the heart runs along the ventral surface of the bulbus. Simple dissection reveals a series of longitudinal furrows on the inner wall of the bulbus and the silecon rubber cast (fig 52) confirms that the chamber is trabeculated.

Figure 54.   Transverse section of the bulbus
              arteriosus (Haematoxylin and eosin).
              The main atructural elements are
              shown as in fig. 53 and the smooth
              muscles are shown. ' AD - adventitia.

Figure 55.   Transverse section of the bulbus
              arteriosus expanded with agar.
              (Haematoxylin and eosin).

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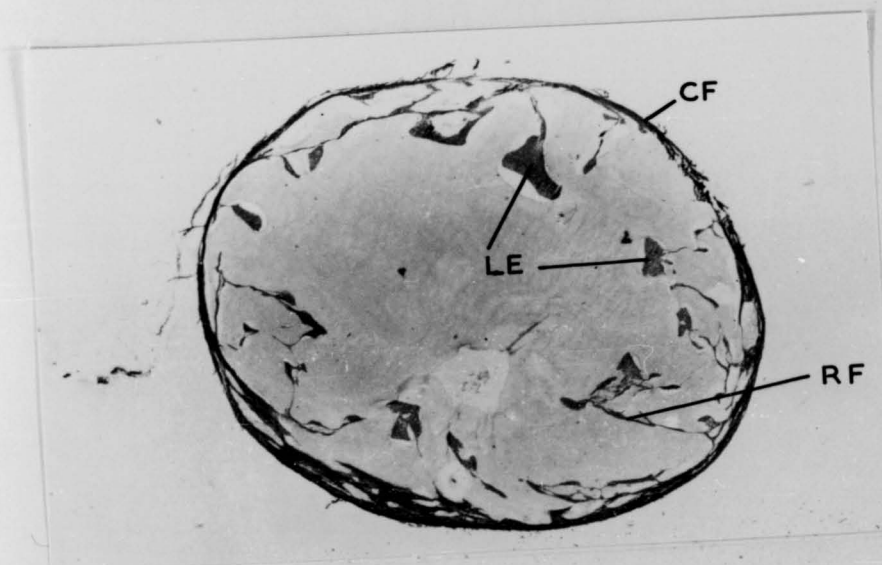
Figure 56. Isometric projection drawings of the
bulbus arteriosus as reconstructed in
the expanded and contracted states.
1 - anterior wall of pericardium,
2 - wall of ventricle, 3 - ventriculo-
bulbus valve, 4 - adventitia, 5 - main
longitudinal element, 6 - radial
element, 7 - compact layer of media with
primarily circumferential fibres.

Bulbus Arteriosus
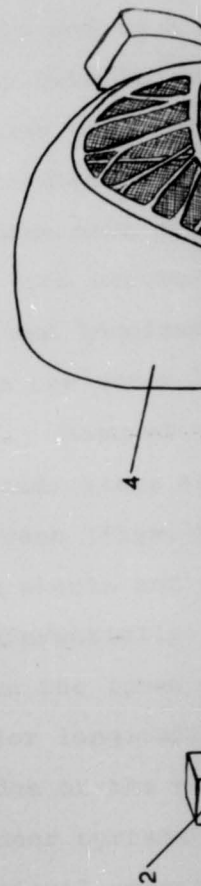
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Bulbus Arteriosus

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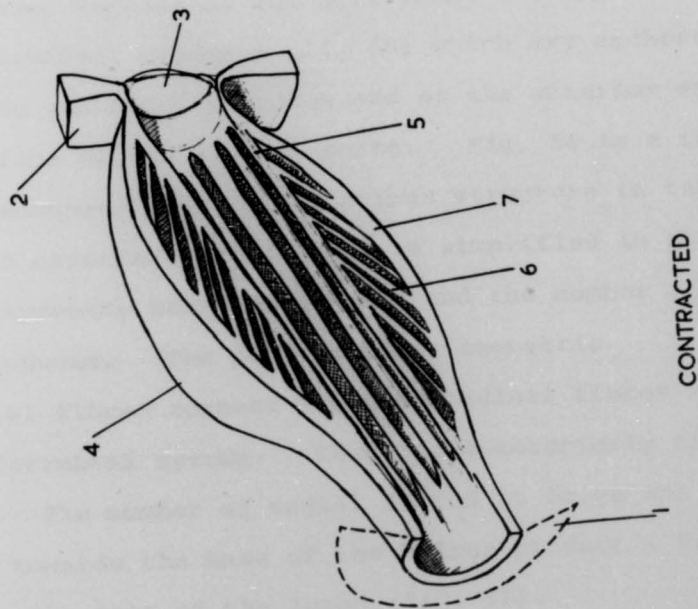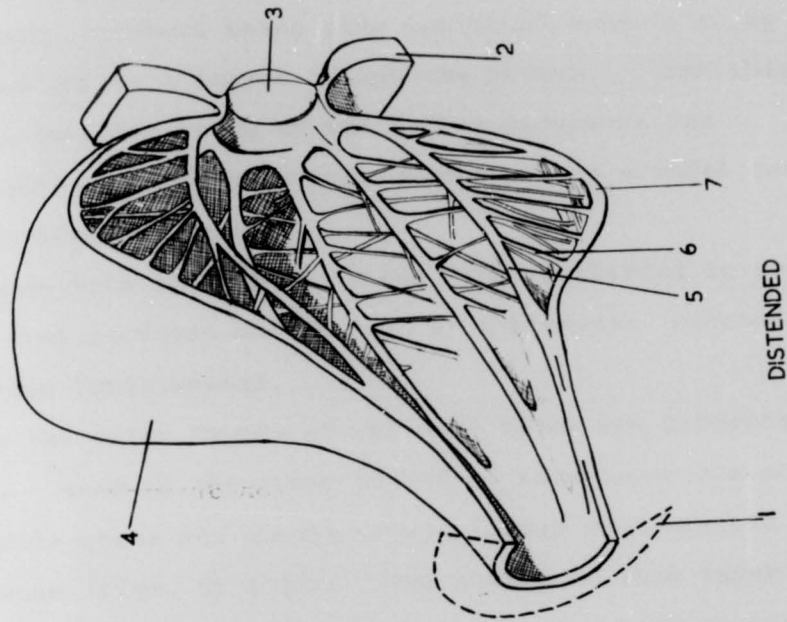
Trabeculation appears to be a significant feature of the trout heart chambers being also exhibited especially by the ventricle and to a lesser extent the atrium.   For this reason a detailed study of the bulbus structure was undertaken which could to some extent act as a model for the other cardiac chambers.

Three main systems of fibres were identified in the bulbus from sections and studies of the casts: circumferential, radial and longitudinal.

In the outer layers of the wall there are circumferential fibres.   Many of the outer fibres of this layer are collagen and inside there are smooth muscle fibres with elastic fibres in between (figs. 53 & 54). 'The fibres in this layer tend to run in whorls and spirals but the main axes tend to lie circumferentially.

On the inner surface of the wall there are approximately 10 major longitudinal elements (fig 55) which are anchored at the edge of the ventricular valves and at the anterior end on the inner surface of the ventral aorta.   Fig. 56 is a three dimensional reconstruction of the bulbus structure in the contracted and expanded states.   It is simplified in that exact radial symmetry has been assumed and the number of radial elements is reduced.   The projection is isometric.

The radial fibres connect the longitudinal fibres to the outer circumferential system.   They slope anteriorly towards the outside.   The number of radial fibres is large and particularly towards the base of the bulbus produce a fragmental appearance to the cast of the lumen (fig. 52).

Figure 57.   The relationship between pressure and
volume in the bulbus arteriosus of a
20cm rainbow trout.   Results of two
consecutive expansion and contraction
cycles are shown.

PRESSURE MM. HG.

120

100

80

60

40

20

0

0·02 0·04 0·06 0·08 0·10 0·12 0·14

VOLUME INCREASE ML.

●——● INCREASING PRESSURE
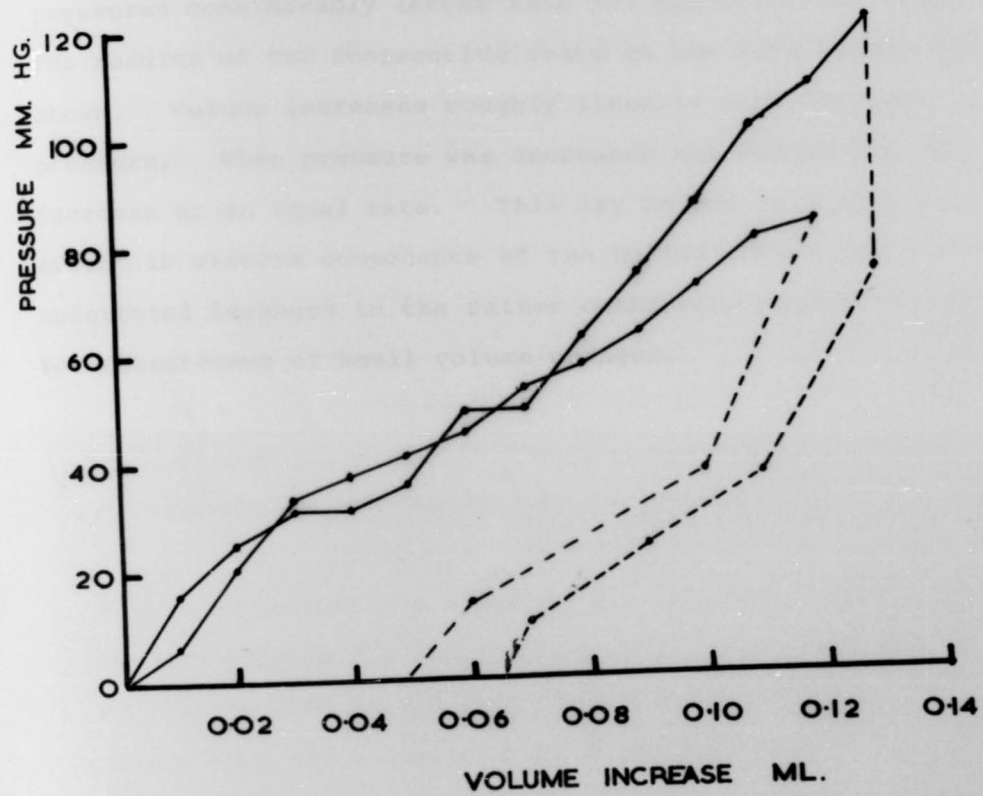
●---● DECREASING PRESSURE

BULBUS PRESSURE - VOLUME RELATIONS.

Some simple measurements of the pressure-volume
relationship were made in the bulbus of a 20cm fish.   The
results are shown graphically in figure 57.   Measurements were
taken for increasing and decreasing pressure over a range of
pressures considerably larger than the physiological range.
The results of two consecutive tests on the same bulbus are
shown.   Volume increases roughly linearly with increase in
pressure.   When pressure was decreased the volume did not
decrease at an equal rate.   This may be due to a hysterisis
effect in viscous components of the bulbus structure or due to
undetected leakages in the rather cumbersome apparatus used
for measurement of small volume changes.

DISCUSSION

Heart Rate in Relation to Metabolism

One of the main themes of this study has been the regulation of heart rate in relation to changes in respiratory transport requirements imposed upon the circulatory system. The heart rate has been measured under various conditions in which something is also known of the changes in metabolic rate. From this basis estimates can be made of the contribution of heart rate changes, to changes in oxygen transport to the tissues.

THE EFFECT OF SWIMMING ACTIVITY.

Brett (1964) carried out extensive studies on oxygen consumption in relation to swimming activity in Onchorhyncus nerka and showed that the logarithm of oxygen consumption is proportional to swimming speed:

$$LogO_2 = a + b \ (V/L) \qquad (1)$$

It was shown that the value of the constant b varied between O.34 at $5^{\circ}C$ and O.17 at $24^{\circ}C$. Tytler (1969) in similar studies on Melanogrammus aeglefinus, a marine fish, showed that the value of b could be expected to be close to O.3 in a wide variety of fish at their respective optimum temperatures. The data of Rao (1968) and Kutty (1968) for rainbow trout yield values of b of the order of O.2. However Webb (1971) gives a value of O.47 at $15^{\circ}C$ for hatchery reared rainbow trout. The critical speeds in Webb's work are low and it is possible that this is connected with the high b value. Morgan (1974) also found low critical speeds in hatchery reared brown trout but the b value was O.25. Webb (1971) suggested that the low

swimming speeds in his work were due to lack of exercise and the effect of hatchery rearing techniques. Dickson and Kramer (1971) confirm this view in their findings that maximum swimming speeds in wild rainbow trout were approximately 25% higher than in hatchery fish. The critical speeds found in the present study; 1.5(V/L) at 6.5°C and 2.0(V/L) at 15°C, correspond closely to the values obtained by Webb (1971) and Morgan (1974) and are probably typical of many hatchery reared fish.

For the purposes of discussion a convenient value of 0.3 will be assumed as the value of b for rainbow trout at all temperatures. If the amount of oxygen transported per heart beat (the O_2 pulse) remains constant then in the swimming trials the heart rate should increase logarithmically with swimming speed with a b value of 0.3 up to the critical speed where no further increase in oxygen transport to the tissues can take place.

For the intact group of fish at 6.5°C the mean basal heart rate was 32.54. Therefore if the O_2 pulse remains constant:-

$$\text{Log } HR_t = 1.5124 + 0.3(V/L) \text{ at } 6.5°C \qquad (2)$$

Where HR_t = the theoretical heart rate if the O_2 pulse remains constant at the value at rest. The critical speed (the point where the 50% recovery time curve rises steeply) was 1.5(V/L).

In the intact group of fish at 15°C the mean basal heart rate was 45.52. Therefore if the O_2 pulse remains constant:-

$$\text{LogHR}_t = 1.6582 + 0.3(V/L) \text{ at } 15°C \qquad (3)$$

and the critical speed at this temperature was 2.0(V/L).

Figure 58. Changes in heart rate with specific
speed at 6.5°C and 15°C. HR_t is the
theoretical heart rate if the O_2 pulse
remains constant at its value at
$O(V/L)$ and is compared with the actual
observed heart rates (HR).

6·5°C

HEART RATE BEATS / MIN

200
180
160
140
120
100
90
80
70
60
50
40
30

HR_t

HR

HR_{basal}

0·5   1·0   1·5   2·0
SWIMMING SPEED (V/L)

15°C

HEART RATE BEATS / MIN

200
180
160
140
120
100
90
80
70
60
50
40
30

HR_t

HR

HR_{basal}

0·5   1·0   1·5   2·0
SWIMMING SPEED (V/L)
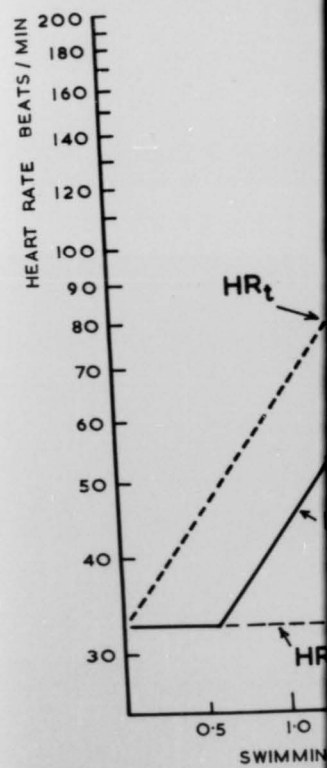
PL.
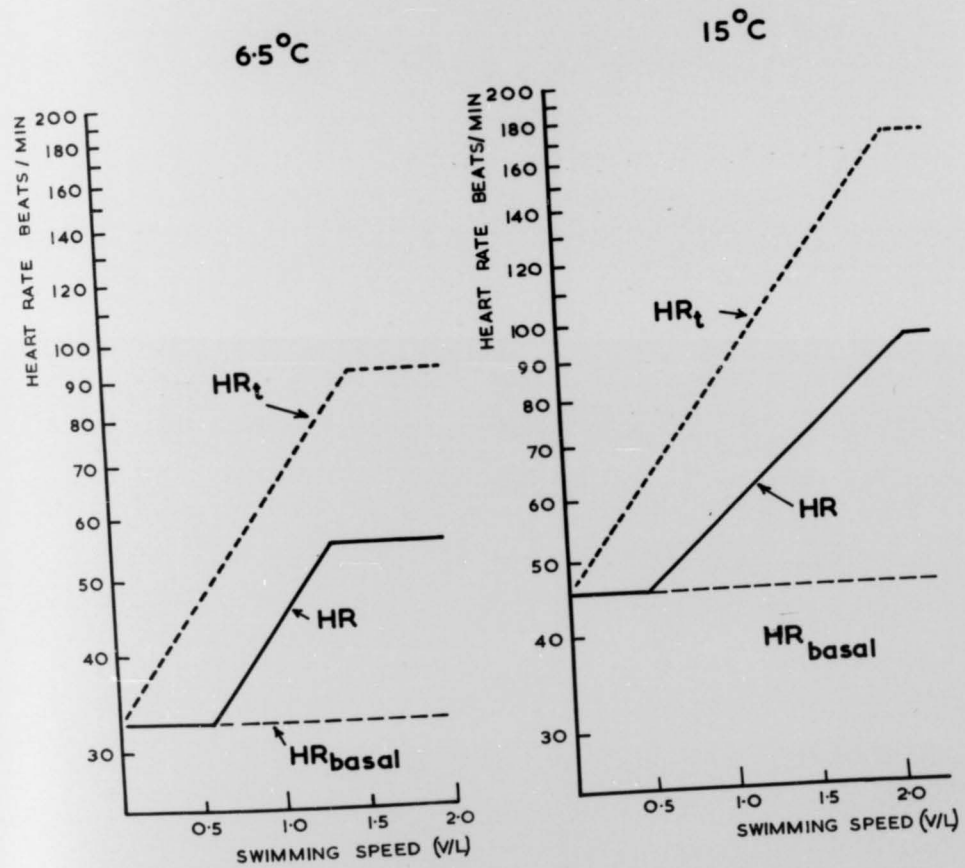
Figure 59.   The specific O_2 pulse in relation to
             swimming speed at $6.5^{\circ}C$ and $15^{\circ}C$.
             The specific O_2 pulse is a measure of
             the change in amount of oxygen
             carried to the tissues per heart beat.

relation to

nd 15°C.

a measure of

xygen

r heart beat.

a 65°C

b 15°C
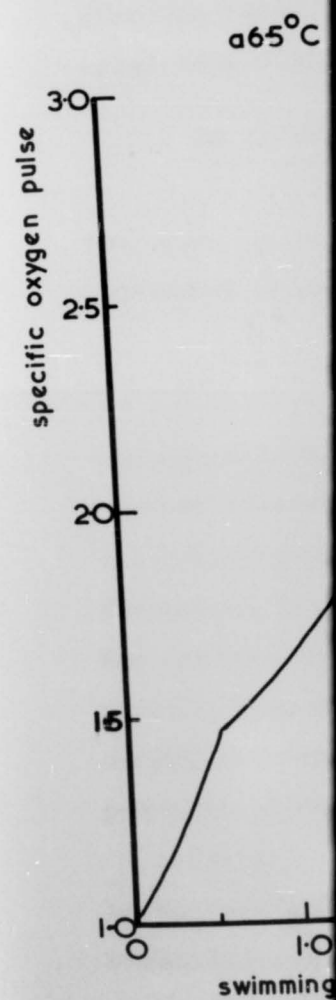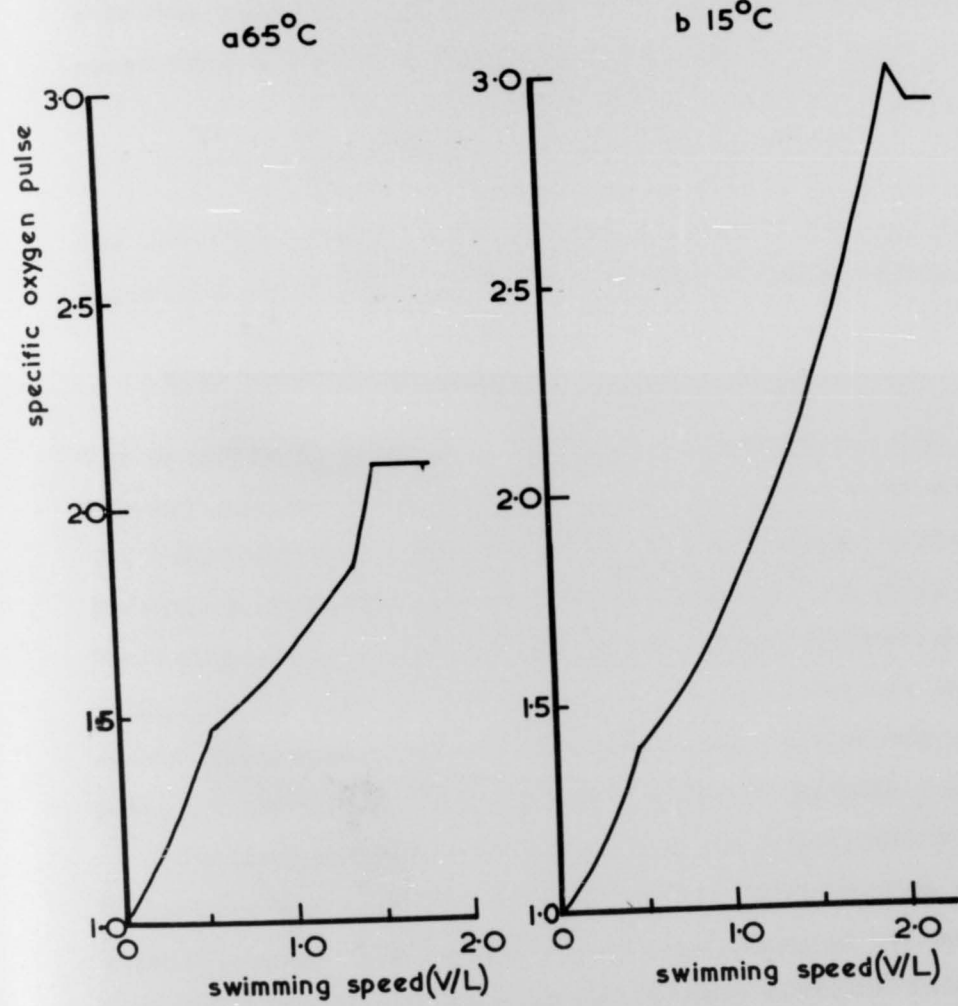
These two constant O_2 pulse heart rate relationships
are plotted in figure 58 and are compared with the actual
changes in heart rate obtained in the experiments.   The
difference between the two lines represents a deficit in
oxygen transport measured in terms of heart beats.   This
difference is a measure of how much the oxygen pulse changes
over the range of swimming speeds.   For any given swimming
speed this difference can be expressed as:-

$$\frac{HR_t - HR + HR_{basal}}{HR_{basal}} = \text{Specific } O_2 \text{ pulse.} \qquad (4)$$

The specific oxygen pulse is then defined as the O_2 pulse
expressed in units of its value at zero swimming speed.

$$\text{i.e. specific } O_2 \text{ pulse} = \frac{\text{Actual } O_2 \text{ pulse}}{O_2 \text{ pulse at zero } (V/L}$$

The specific O_2 pulse as calculated from equation (4) is
plotted against swimming speed in figure 59 at both experimen-
tal temperatures.   It can be seen that O_2 pulse increases
throughout the range of swimming speeds up to critical speed.
The inflections in the lines are due to the discontinuous
relationship used in the analysis of heart rate and the
slight discrepancy between critical speed as defined and the
point at which mean maximum heart rate is reached.

Sutterlin (1969) suggested that the relationship between
heart rate and swimming speed in brown trout assumes a
sigmoid form.   The general pattern of increase in heart
rate observed in the current experiments confirms this view,
but using graphical methods (Ricklefs 1967) none of the
commonly used growth-type curves would fit satisfactorily so
the discontinuous relationship was adopted with defined basal

and maximum heart rates.

At $6.5^{\circ}C$ there was no change in heart rate until a speed of about $0.6(V/L)$ was reached when the O_2 pulse was 1.5 times the resting value. Then both O_2 pulse and heart rate increased up to the critical speed at $1.5(V/L)$. At this point the recovery time curve (figure 19) rises steeply and it is assumed that both heart rate and O_2 pulse are maximal at speeds in excess of the critical. Actual energy expended in swimming continues to increase with increase in swimming speed and the resultant oxygen debt is reflected in the time taken for recovery after exercise at high speeds. At the critical speed heart rate increase had accounted for 39% of the estimated increase in oxygen transport and the specific oxygen pulse was 2.1.

In the experiments at $15^{\circ}C$ the heart rate began to increase at $0.45(V/L)$ at a specific O_2 pulse of 1.36. At $2.0(V/L)$, the critical speed, the change in heart rate had accounted for 36% of the overall increase in estimated cardiac output and the specific oxygen pulse was 2.9.

THE GENERAL RELATIONSHIP BETWEEN HEART RATE AND OXYGEN CONSUMPTION.

The experiments on simultaneous determination of heart rate and oxygen consumption were designed to throw further light on the inter-relationship between these parameters. It was confirmed that the relationship between heart rate and oxygen consumption is in fact quite close to that which was predicted from the heart rate/swimming speed relationship using oxygen consumption figures from the literature. This implies that any relationship between heart rate and oxygen

consumption is not greatly affected by muscular swimming movements. There was a considerable scatter in the results (figures 26 and 27) but the deviations in individual cases could not be correlated with any obvious presence or absence of activity during the period of recording.

It is evident that large changes in oxygen pulse can take place in rainbow trout so that, particularly at medium and low oxygen consumptions, a wide range of options in heart rate/O_2 pulse combinations presents itself to the fish in order to attain any particular rate of oxygen transport. The options lie within the irregular quadrangle in figure 27 which is also reproduced in figure 60. Some degree of aggregation of the points does occur towards the diagonal line suggesting some sort of optimisation of cardiac performance. From the experimental results there is a highly significant relationship between heart rate and oxygen consumption at 15°C:-

$$LogHR = 0.4393 \ LogO_2 + 0.8171 \qquad (6)$$

where $\qquad$ HR = heart rate beats/minute

$\qquad\qquad O_2$ = oxygen consumption mg/kg/hr

or $\qquad LogO_2 = \dfrac{LogHR - 0.8171}{0.4393} \qquad (7)$

taking antilogs $\qquad O_2 = \dfrac{(\ HR\ )^{\frac{1}{0.4393}}}{(6.563)} \qquad (8)$

$$O_2 = 0.0138HR^{2.2763} \qquad (9)$$

By definition $\qquad O_2 pulse = \dfrac{O_2}{60\ HR} \qquad mg/kg \qquad (10)$

Figure 60.   The relationship between heart rate and
             oxygen consumption.   The experimentally
             derived relationship compared with
             other simplified approximations
             described in the text.
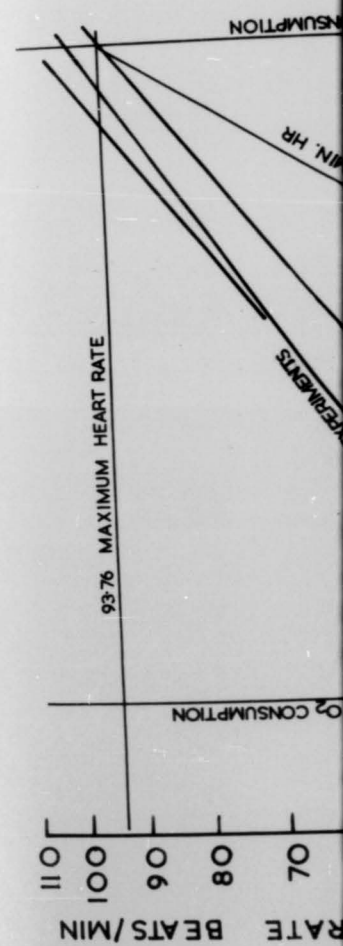
heart rate and

experimentally
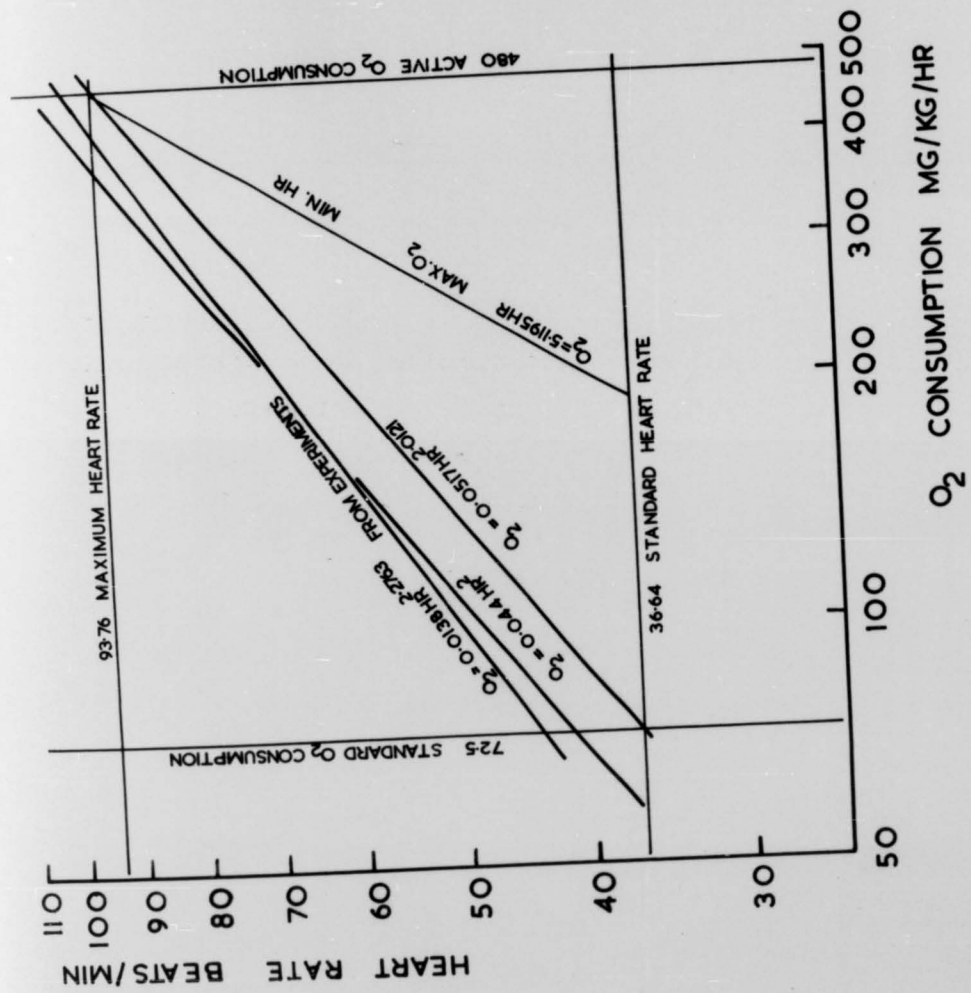
ared with

ations

Figure 61. The relationship between O_2 pulse and

heart rate. O_2 pulse $= 0.00023HR^{1.2763}$ is

the experimentally derived relationship

and is compared with a simple linear

approximation.
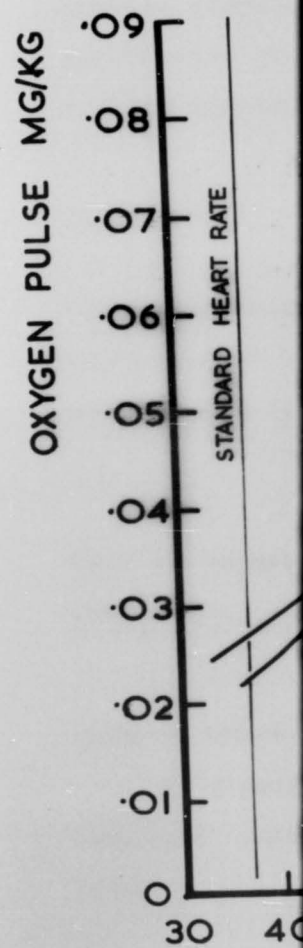
O₂ pulse and

.OOO23HR^1.2763 is

d relationship

mple linear
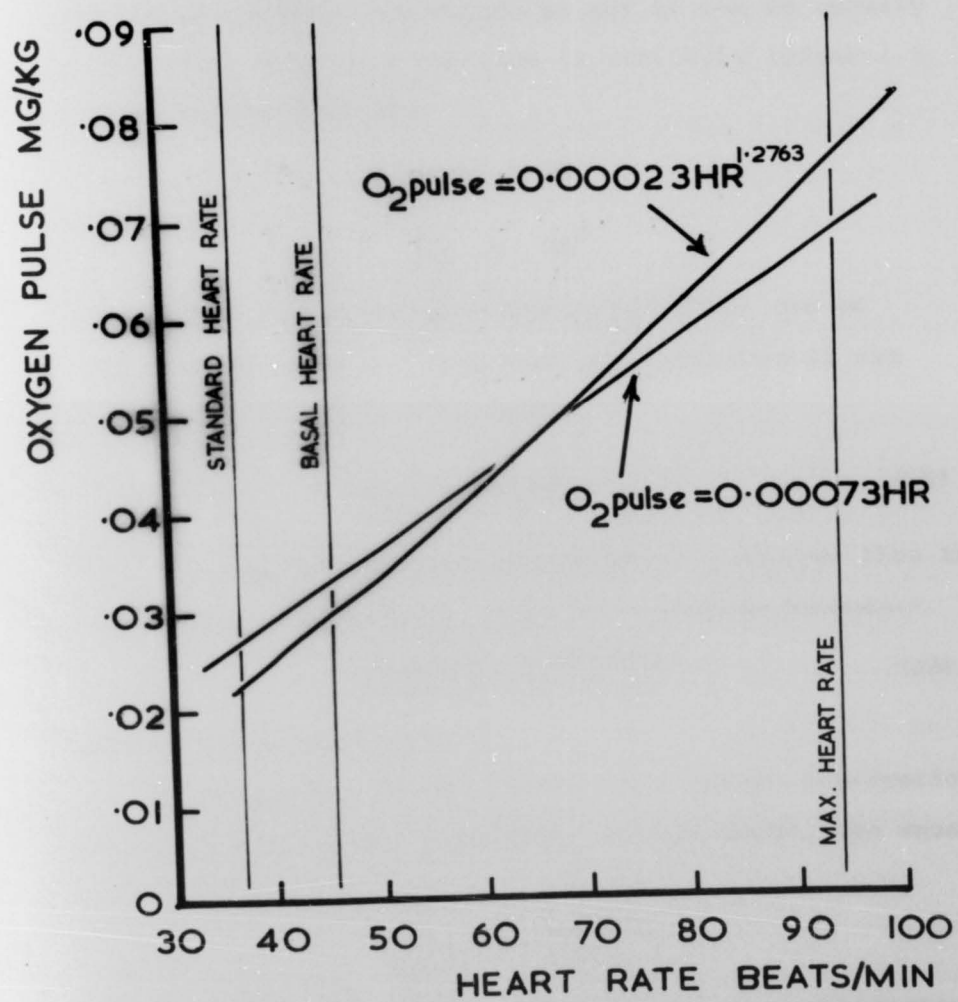
Therefore $\qquad O_2 \text{pulse} = \dfrac{0.0138HR^{2.2763}}{60HR}$

or $\qquad\qquad O_2 \text{ pulse} = 0.00023HR^{1.2763} \text{ mg/kg} \qquad (11)$

This relationship between O_2 pulse and heart rate is
plotted graphically in figure 61 and it can be readily
appreciated that this function is virtually linear i.e. to
a close approximation:-

$$O_2 \text{pulse} \propto HR$$

Then

$$O_2 \propto HR^2$$

Examining equation (9) the exponent (2.2763) can be
approximated to 2.0. The best approximation if the
exponent is fixed at 2 becomes:-

$$O_2 = 0.044HR^2 \qquad (12)$$

This is compared with the experimentally derived line in
figure 60. The oxygen pulse relationship becomes:-

$$O_2 \text{pulse} = 0.00073HR \qquad (13)$$

This is shown in figure 61.

In figure 60 the diagonal of the oxygen consumption
and heart rate scope quadrangle is also shown, the equation
being

$$O_2 = 0.0517HR^{2.0121}$$

Which also corresponds closely to a square relationship
between oxygen consumption and heart rate.

The increase in O_2 pulse from standard to maximum heart rate in the respirometry experiments is 3.3 using the statistically derived O_2/HR relationship, and 2.6 using the $O_2 \propto HR^2$ approximation. These correspond quite closely to the maximum specific O_2 pulse (2.9) determined in the 15^oC swimming experiments.

It is evident that changes in the O_2 pulse are important in varying the oxygen transport capability of the blood system. Stevens & Randall (1967b) measured dorsal and ventral aorta PO_2 in rainbow trout swimming at moderate speeds and found no significant changes during or after exercise. Thus unless there are large changes in blood oxygen carrying capacity the O_2 pulse corresponds closely to changes in stroke volume. It is probable therefore that cardiac stroke volume is proportional to heart rate. This would suggest that a relatively simple mechanism of control of cardiac output can be envisaged. However Bennion (1968) has shown that adrenaline can have different effects on heart rate and stroke volume in rainbow trout. At 15^oC stroke volume is increased and heart rate decreased by increases in concentrations of adrenaline. Also during hypoxia (Holeton & Randall 1967) decrease in heart rate is offset by increase in stroke volume. Therefore any tendancy to a constant ratio between stroke volume and heart rate appears to be readily modified. It is highly probable that much of the observed variation in the relationship between heart rate and swimming speed is due to changes in the stroke volume-heart rate relationship.

The Root and Bohr effects on the blood oxygen dissociation curve can be quite large (Randall 1970, Eddy 1971) which could radically alter the blood oxygen carrying capacity. It is difficult to estimate the magnitude of changes that are likely to occur due to metabolites such as lactic acid in the blood, but Holeton & Randall (1967) obtained an oxygen capacity value of 9.00 vol.% and Irving, Black and Safford (1941) a value of 13.8 vol.%. A change in oxygen capacity from 13.8 vol.% to 9.00 vol.% would require a 50% increase in heart rate in order to maintain a given rate of oxygen transport. These effects are particularly likely to become evident during swimming at high speeds or recovery when oxygen debt has been incurred. After exercise at high speeds anomalies in the respiratory quotient are likely to occur as carbon dioxide is expired in a series of complex interactions in the blood pH-carbonate-buffering system associated with the release of lactic acid (Morgan 1973). High heart rates are then likely although oxygen consumption may be quite low. Due to the possible variations in the mechanical function of the heart and changes in the blood it is hardly surprising that in individual fish often no significant correlation between heart rate and oxygen consumption could be found.

In general terms it can be assumed that the mean changes in O_2 pulse in the present study correspond closely to changes in cardiac stroke volume. On this basis the cardiac stroke volume approximately doubles at $6.5^{\circ}C$ and approximately trebles at $15^{\circ}C$ between minimum and maximum

This is rather smaller than the five-fold increase estimted by Stevens & Randall (1967b). In their experiments the increase in oxygen consumption was rather high for the low swimming speeds attained which could elevate the estimate of stroke volume change based on the Fick principle. Also the present estimate in the swimming trials is very sensitive to changes in the assumed b value; larger values such as that obtained by Webb (1971b) would yield larger changes in the stroke volume.

From the present respirometer experiments a general and simple relationship has been derived between oxygen consumption and heart rate, so the question arises as to the reasons for a discontinuous relationship between heart rate and swimming speed. In fish respirometry studies (Fry 1957) problems have been encountered in establishing a basal metabolic rate analogous to that used in human and mammalian physiology. This is largely, to express it simply, because fish cannot be persuaded to lie still. Therefore the convention has been adopted to determine metabolic rates at different known levels of activity and to extrapolate a series of these measurements to zero activity. This extrapolated value is defined as the standard metabolic rate. Brett & Glass (1973) point out that in tunnel respirometer experiments on Sockeye salmon, velocities below 0.5 to 1.0 (V/L) were useless because metabolism from spontaneous activity was usually in excess of that from the low imposed velocities. Thus in this and other respirometry studies the measurements at low velocities are excluded

when fitting a line for extrapolation to standard metabolic
rate.   It is this convention in effect which has been
followed in the current work in calculating the regression
line between the basal and maximum heart rate.   The
lowest swimming speed used in the regression line (about
0.5 (V/L) is the lowest water velocity in which the fish
would swim uniformly.   On this basis the regression line
only was used in the calculation of predicted oxygen
consumption/heart rate relationships (fig. 26).

The observed mean basal heart rate was above the
standard heart rate.   The standard heart rate (or metabolic·
rate) is by definition the heart rate (or metabolic rate) at
zero swimming velocity and consequently the lowest possible
mean heart rate for a fish.   In a real situation with a
fish at rest, the heart rate may be close to the standard
rate but any deviation from this must necessarily be towards
a higher heart rate.   Thus over a series of experiments
the mean basal heart rate (or metabolic rate) will always
be in excess of the standard. ·   To a certain extent
therefore the basal heart rate plateau in the heart rate/
swimming speed relationship (figures 18 and 22)reflects a
real change in the oxygen consumption characteristics
which is often ignored in the derivation of general
metabolic relationships such as that of Brett & Glass
(1973).

EFFECT OF TEMPERATURE.

The general heart rate and swimming speed relationships
at the two temperatures have already been discussed but
some comment can be made on the actual values of the

different heart rates.   The Q_{10} for the temperature effect on the heart rate between $6.5^{O}C$ and $15^{O}C$ was 1.95 for the maximum, 1.65 for the basal heart rate and 1.99 for the standard heart rate.   These values are lower than those found in experiments on relatively rapid changes in temperature in Ophiodon elongatus (Stevens et al. 1972), Cyprinus carpio and Barbus fluviatilis (Labat 1966) where Q_{10}s of up to 5 are common.   This confirms that acclimation to temperatures takes place in terms of heart rate as well as metabolic rate.

Dickson and Kramer (1971) measured active and standard metabolic rates in the rainbow trout acclimated to 5°, 10°, 15°, 20° and $25^{O}C$.   In hatchery fish the active oxygen consumptions were 384mg/kg/hr and 576mg/kg/hr at. $5^{O}C$ and $15^{O}C$ respectively ($Q_{10} = 1.5$).   The standard oxygen consumptions were 36 and 78mg/kg/hr at $5^{O}C$ and $15^{O}C$ ($Q_{10} = 2.16$).   The Q_{10} for standard heart rates in the present study corresponds quite closely to this standard oxygen consumption Q_{10} but the maximum heart rate increases rather more than the active oxygen consumption.

The Q_{10} is a crude means of comparison of rates at different temperatures but is of interest to note from the limited data available that in general changes in heart rates with temperature are of the same magnitude as corresponding changes in oxygen consumption.

EFFECT OF FEEDING AND STARVATION.

The high heart rates found in the fish with a stomach full of food are probably due to high oxygen consumptions.   That

oxygen consumptions can be elevated due to food in the
gut has been noted previously (Beamish 1964) and it has
become usual to starve fish prior to measurement of standard
oxygen consumption in order to obtain uniform results.
The mean heart rate of fish 26 (fig 24) with a full
stomach was approximately 73 beats per minute which
using equation (12) gives:-

$$O_2 = 0.044 \times 73^2$$

$$O_2 = 230mg/kg/hr$$

This is approximately 3.5 times the standard oxygen
consumption.   Beamish (1964) obtained oxygen consumptions
in fed fish of 3.0 times the standard in brook trout
(Salvelinus fontinalis) at $10^{o}C$ and values from under 2.0
times to 5 times the standard in the White Sucker
(Catastomus commersoni) according to the temperature and
season.   Muir & Niimi (1972) measured corresponding values
of 3 to 4 times the standard in aholehole (Kuhlia
sandvincensis) when fed to satiation.   Thus it seems that
the high heart rate in fish 26 can be attributed to a high
metabolic rate, when the stomach is full, due to effects
such as specific dynamic action (Muir & Niimi 1972).   By
48 hours after the last meal the heart rate in fish 26 had
fallen to normal values which corresponds to the sort of
time scale for oxygen consumption to decline during the stages of
initial starvation (Beamish 1964, Muir & Niimi 1972, Glass
1968).

Throughout the heart rate work it was found that at a given temperature the maximum heart rates were remarkably constant. The only exception to this was fish 28 which was in poor condition and had probably effectively starved for some time and had low heart rates. This may have been due to a low metabolic rate but there is little evidence that this is so. Fry (1957) reports that rainbow trout starved for 100 days at 11°C did not have significantly different active metabolic rates from a fed control group of fish. Beamish (1964) shows that standard and routine metabolic rates fall rapidly in the first few days of starvation; subsequently there was no further decrease in the standard level although routine oxygen consumption continued to decrease. Glass (1968), Muir & Niimi (1972) and Dickson & Kramer (1971) all confirm this view and it seems that no further fall in metabolic rates can be expected in fish starved for periods longer than five days. Thus in theory some of the normal group of fish which were observed under experimental conditions for up to six days almost certainly had the lowest possible metabolic rates. The metabolic rate of fish 28 is unlikely to have been lower than that of some of the normal fish and the lower heart rate must have been due to some more complex effect such as a direct effect of starvation on the myocardium. In view of the uncertain history of fish 28 comment is probably best confined to a statement that fish in very poor condition may exhibit abnormally low heart rates the precise reasons for which are obscure.

The Role of the Vagus Nerve in the Control

of Cardiac Function during Activity

INTRODUCTION.

Three kinds of nerve fibres may be found in the cardiac branch of the vagus; cholinergic inhibitory elements, adrenergic excitatory fibres and afferent sensory fibres. The evidence in the literature on the latter two is sparce but the inhibitory function of the vagus is well established.

In this thesis the role of the vagus nerve in cardiac regulation was investigated by sectioning the nerve in some fish for comparison with their intact counterparts. In order to provide controls in case the surgical intervention itself had an effect independent of removal of nerve function, one fish at each temperature was vagotomised on one side only with a sham operation on the other side. At 6.5^{o}C the unilaterally vagotomised fish was in no way significantly different from the intact fish. At 15°C all the cardiac responses were normal except that the basal heart rate was significantly lower. However this resting rate (37.26) was not lower than the standard heart rate of the intact fish (36.64) so the rate was one which might be expected in a particularly placid fish. Basal heart rates of the same order were also recorded in intact fish. Basically it seems that the operation and the subsequent recovery period had no effect on the fish which could obscure interpretation of the data from vagotomised fish.

CHOLINERGIC INHIBITORY INNERVATION.

Von Skramlick (1935) reviewed much of the early work on fish circulation and proposed the general principle that

in fish due to the absence of a sympathetic innervation
to the heart it is controlled largely by inhibitory effect
of the vagus which works in antagonism to an intrinsic
tendency for cardiac acceleration.   It is now generally
accepted that acetylcholine is the transmitter substance
in this parasympathetic inflow to the fish heart (Campbell
1970) although Jullien & Ripplinger (1957) presented
evidence showing that the actual substance is probably not
acetylcholine but something very similar.

The high heart rates in vagotomised fish at 6.5^{o}C
(fig. 18B & table I) are what might be expected from the
work of Von Skramlick when the inhibitory effect of the
vagus is removed.   The irregularity in the heart beat in
intact fish (fig. 16a) may be evidence of modulation of
a continuous inhibitory tonus as suggested by Labat (1966).
This fluctuation in tonus may be linked with the
synchronisation of heart beats with breathing movements but
Randall & Smith (1967) have shown that in trout in well
oxygenated water there is no such synchrony.

In contrast to the very regular heart beat in
vagotomised trout in this study and the vagotomised carp
of Labat (1966), Jullien & Ripplinger (1957) found that in
tench vagotomy gave rise to a slow and arrhythmic heart
beat which returned to regularity after atropine treatment.
They suggested that in vagotomised fish tonically active
vagal postganglionic cells inhibit the heart muscle and
that normally these neurons are inhibited by pre-ganglionic
neurons.   Laurent (1962) and Gannon (1971) have also
demonstrated the presence of postganglionic cells in the
cardiac nerve.

At 15°C bivagotomy had no effect on the general patterns of change in heart rate. The only significant difference from intact fish was the lower maximum heart rate (Fig 22B, Fig. 23, table I). This lowered maximum heart rate may be attributable to inhibitory post-ganglionic neurons but no cardiac arhythmas of the type noted by Jullien & Ripplinger (1957) were observed in the vagotomised fish.

The normal heart rates in the bivagotomised fish at 15°C gives rise to the paradox that the intrinsic heart rate (in the absence of vagus function) is higher at 6.5°C than 15°C. Bennion (1968) showed by in vitro studies on the trout heart that at 6°C adrenaline had a tachycardiac effect whereas at 15°C this effect was absent although stroke volume was increased. Thus the catecholamines which are likely to be released into the circulation during disturbance in the experimental situation could elevate the heart rate at 6.5°C but have no effect on heart rate at 15°C. Thus it is suggested that the high resting heart rates at 6.5°C were due to high levels of circulating catecholamines. This effect would gradually decline as the fish became used to the apparatus which would explain the observed gradual fall in heart rate. The tachycardia which can be very readily evoked by the slightest disturbance may be due to renewed secretions of catecholamines. In the intact fish these effects would presumably be masked by the bradycardiac effect of the vagus.

In the resting vagotomised fish at $6.5^{\circ}C$ the heart
rate was not maximal and some increase was observed
during exercise. These increases must have been of
aneural origin possibly again due to catecholamine
secretion during activity as observed by Nakano &
Tomlinson (1967) At $15^{\circ}C$ it is evident that a large
degree of cardiac regulation can take place in the
absence of vagus function (fig. 23). Some adaptation
to the vagotomised condition may have occurred during
the period of recovery from the operation (Labat 1966).
However this result at $15^{\circ}C$ confirms the findings of
Stevens and Randall (1967a) who found no effect on
the changes in heart rate during swimming rainbow trout
when the inhibitory function of the vagus had been
blocked by injection of atropine. They conclude that
there is no continuous vagal tonus to the heart in
rainbow trout; their experiments were carried out at
10° to $12^{\circ}C$ and they did not take the fish up to the
maximum sustainable speed.

Throughout the experiments on intact fish short
periods of cardiac inhibition were often observed, in
response to external stimuli (figure 9) or during
activity such as feeding (figure 25). These effects
were abolished by vagotomy and were taken as evidence of
the success of the operation (Labat 1966). Most
authors who have worked on fish circulation have noticed
this phenomenon and Lutz (1930) points out that in the
dogfish reflex cardiac and respiratory inhibition can be
evoked by sensory stimulation of almost every part of the
fish. Despite having been described long ago

(McWilliam 1885, Mills 1886) the functional significance of these cardiac inhibitions remains obscure. Lutz (1930) suggests that this may be an emergency function in case of injury especially in the gill region. Johansen & Martin (1965) in their review suggest that it may be of importance in providing protection for the thin-walled gill vessels during periods of increased pressure in the branchial region. Many of the stimuli which evoke cardiac inhibition such as a rise in branchial arterial blood pressure (Mott 1951) and low buccal cavity PO_2 Randall & Smith (1967) are of physiological significance and would play a role in regulation of circulation. Phenomena such as the cardiac inhibition during feeding may be a purely incidental result of the simplicity of the control system.

EXCITATORY INNERVATION.

It has been generally accepted for over a century that the fish are unique among vertebrates in lacking sympathetic innervation to the heart. Gannon & Burnstock (1969) point out that this view has persisted despite numerous observations in the literature of reflex cardioacceleration in intact fish which were usually explained in terms of modulation of the vagal inhibition.

Using fluorescent histochemical techniques Gannon & Burnstock (1969) have demonstrated an adrenergic innervation of the heart in trout from fibres running in the vagus nerve.

They also demonstrated that electrical stimulation of
the vagus could cause cardiac excitation or inhibition
according to the pulse frequency used.   Campbell (1970)
reviews this and other scattered evidence and concludes
that there probably is a cardiac excitatory function of the
vagus and that the adrenergic fibres originate from the
sympathetic ganglia.   Gannon (1971) has carried out
further detailed studies on the excitatory innervation
in rainbow trout and demonstrates a similar adrenergic
cardiac innervation in the eel (Anguilla australis).

More recently Cobb & Santer (1972) also found that
stimulation of the vagus in plaice could cause cardiac
excitation as well as inhibition.   Santer (1972) applied
the histochemical techniques to the plaice heart that were
used on the trout by Gannon & Burnstock (1969) and
Yamauchi & Burnstock (1968).   He could find no evidence
in plaice of adrenergic elements as demonstrated in trout
and concluded that the stimulatory effect of the vagus in
plaice is cholinergically mediated.   Saito (1973) found
cardiac excitatory effects during and after vagus stimulation
in the carp.   These effects together with the more typical
inhibitory effects were abolished by atropine which again
suggests a cholinergically mediated acceleratory mechanism.
There was no evidence of sympathetic innervation of the
cardiac pacemaker fibres and Saito suggested that the
excitatory phenomenon was caused by effects associated with
the deterioration of the experimental preparation.   It
seems probable that there are great interspecific differences
in the autonomic nervous system of fish and this may be at
the root of much of the controversy regarding sympathetic

Figure 62.   Dissection from the ventral aspect of
             part of the right sympathetic cord.
             The cords run on either side of the
             dorsal aorta in the trunk region and
             in the cranial region a series of
             ganlia are connected with the cranial
             nerves.   This dissection shows
             that the ramus communicans to the
             vagus nerve is very close to the
             cranium.

FLOOR OF
CRANIUM

SYMPATHETIC
GANGLIA

DORSAL
AORTA

ral aspect of

hetic cord.

side of the

k region and

series of

h the cranial

n shows

ns to the

e to the

II

V + VII

IX

FLOOR OF
CRANIUM

X PHARNGEAL
X CARDIO-VISCERAL
X LATERAL LINE

SYMPATHETIC
GANGLIA

1 2
3
4

LEFT SYMPATHETIC CORD

DORSAL
AORTA

1 CM.
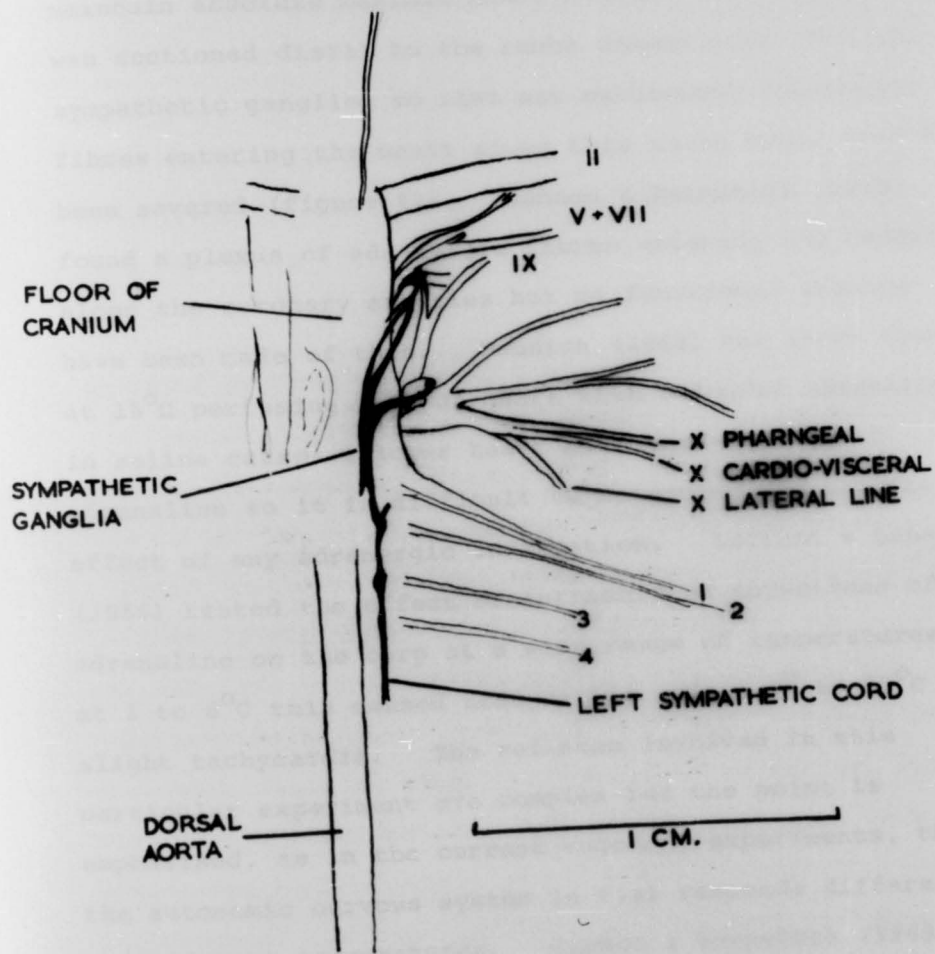
innervation of the heart.

In the present work the low maximum heart rate in vagotomised fish at 15°C may be evidence of a stimulatory function of the vagus which is required in order to maintain absolute maximum heart rates. The vagus nerve was sectioned distal to the ramus communicans from the sympathetic ganglion so that any excitatory adrenergic fibres entering the heart along this route would also have been severed (figure 62). Gannon & Burnstock (1969) found a plexus of adrenergic fibres entering the ventricle along the coronary arteries but no functional studies have been made of this. Bennion (1968) has shown that at 15°C perfusing a trout heart with 0.1ug/ml adrenaline in saline causes a lower heart rate than 0.01 ug/ml adrenaline so it is difficult to determine the precise effect of any adrenergic innervation. Laffont & Labat (1966) tested the effect of intracardiac injections of adrenaline on the carp at a wide range of temperatures; at 1 to 8°C this caused bradycardia and at 9° to 20°C a slight tachycardia. The reflexes involved in this particular experiment are complex but the point is emphasised, as in the current vagotomy experiments, that the autonomic nervous system in fish responds differently at different temperatures. Gannon & Burnstock (1969) carried out their work on a trout heart preparation at 7° to 15°C and Gannon's (1971) experiments on trout were at 8° to 11°C. As both these works demonstrated cardiac excitatory effects in the trout heart it seems probable that at both 6.5°C and 15°C some degree of cardiac excitatory function of the vagus can be expected.

The evidence that (fig. 24) starved rainbow trout
may have low heart rates suggests that the low maximum
rate in vagotomised fish is due to the fact that these
fish did not feed during the post-operative recovery
period.   The unilaterally vagotomised fish which went
through exactly the same procedure had a normal maximum
heart rate which counters this suggestion.   Despite
this, it is felt that before a continuous vagal
excitatory effect during swimming can be postulated
with certainty, detailed research is required on this
phenomenon.

AFFERENT SENSORY INNERVATION.

Lutz (1930) showed that cardio-inhibition could be
evoked by mechanical or electrical stimulation of the
ventricle;   a reflex which involved afferent as well as
efferent pathways in the cardiac branch of the vagus.
Laurent (1962) showed that the afferents in the cat-fish
are relatively large myelinated fibres and that the
efferents are fine non-myelinated fibres.   Laurent carried
out experiments on these two innervations and was able to
show distinct pulses of afferent activity which were
associated with specific events in the cardiac cycle.
Inflation of the auricle elicited inhibitory efferent
pulses to the heart and he suggested that this could form
a response to increases in venous return which would reduce
cardiac output and correct for the surge in blood flow.
This reflex seems to work in opposition to the Starling's

law reflex intrinsic to the heart whereby output increases in response to increase venous return.

Randall (1966) could find no evidence of afferent activity in the vagus nerves of tench and goldfish although he was able to detect the efferent pulses to the heart. The precise nature of any afferent cardiac innervation in trout heart is obscure and the present experiments do not show any direct evidence as to its function. It is probably appropriate merely to note the possibility of an afferent component in discussing the role of the vagus in the trout.

STROKE VOLUME CONTROL.

In the above discussion of the vagus function only effects on heart rate have been considered. This has been partly to simplify the arguments and also because there is little information on stroke volume control by the vagus nerve. Under conditions of hypoxia in trout a bradycardia develops but stroke volume increases in such a way that only minor changes in cardiac output are observed (Randall 1970). This sort of effect and observations on stroke volume relations in the isolated heart (Bennion 1968) suggest that often stroke volume and heart rate are independently variable.

Stroke volume is very much dependent on venous return characteristics but it can be assumed that an inotropic effect on the heart would ultimately cause an increase in cardiac output. Jullien & Ripplinger (1957) in experiments on the tench showed that vagal stimulation has a negative inotropic effect which is a consequence of the negative

chronotropic effect. They also showed that a distinctly separate set of fibres in the vagus had a negative tonotropic effect which made the heart elongate. Campbell (1970) suggested that this latter effect was an artefact of the kymograph recording procedure used.

Jullien & Ripplinger (1957) found that the ventricle could only respond to stimulation by complete systoles; i.e. an "all or nothing" response due to the syncytial structure of the myocardium. This would suggest that the ventricular output is largely determined by the in flow of blood from the atrium which can respond to stimulation with differing forces of contraction. However Breton et al. (1964) obtained graded responses of the ventricle to different levels of stimulation the nature of which varied considerably according to how fresh the experimental preparation was.

The work of Gannon (1971) on rainbow trout is probably of most relevance to the present study. He electrically paced isolated atria and ventricles at low voltages which were of insufficient strength to stimulate intramural nerves. These intramural nerves could then be stimulated by increasing the voltage of the driving pulses. The effect of pharmacological agents could also be observed and the inotropic responses in the preparation beating at a constant rate were readily reproducable. The atria were shown to possess both cholinergic inhibitory and adrenergic augmentor innervations. The ventricles possessed only an adrenergic augmentor innervation and were insensitive to acetylcholine. There was also evidence of post-ganglionic inhibitory neurons which were stimulated by nicotine; this confirms some of the

observations of Jullien & Ripplinger (1957).

In general there is little evidence of distinct mutually independent cardiac chronotropic and inotropic effects of the vagus in fish; these two effects are probably simultaneous. This would tend to confirm the general relationship that stroke volume is proportional to heart rate which was found in the metabolic studies. There is no actual inhibitory innervation in the ventricle so its output is controlled to some extent by the flow of blood from the atrium which has a dual innervation. It seems that any apparent independence of heart rate and stroke volume is a function of the interaction between the heart chambers and the characteristics of blood flow, especially venous return.

Auxiliary pumps and their
Influence on Circulation

The results of the experiments with vagotomised fish indicate that a considerable degree of cardiac regulation can take place in the absence of vagus function. This has directed attention to a consideration of aneural means of cardiac regulation.

Bennion (1968) carried out experiments on the in vitro heart of rainbow trout at $6^{o}C$ and $15^{o}C$. He showed that the heart obeys Starling's law in that as the input (venous) pressure is raised stroke volume, heart rate and apparent stroke work increase. Any mechanism which could increase venous return would tend to increase cardiac output. Auxiliary blood pumping mechanisms which come into operation during swimming activity could explain some of the changes in heart rate during swimming.

LYMPH PROPULSORS

Several propulsive mechanisms have been described
in the lymph system which may ultimately contribute to
the venous return flow.   Marshall-Hall (1831) described
the caudal heart in the common eel and noted that it
pulsed at a rate of 160 beats per minute as compared with
the pulmonary heart beat of 60 per minute.   This appears
to be the only quantitative information on the function of
the caudal lymph heart;   this figure being quoted by all
subsequent authors up to Kampmeier (1969) who reviews the
lymphatic system of fishes.   Surveying the function of
the caudal heart in different species Kampmeier concludes
that the caudal heart is not independently pulsatile in
trout and that it probably responds passively to the
movements of the caudal fin musculature.   Some early authors,
Marshall-Hall (1831) and Owen (1866) state that the caudal
heart of the eel pumps blood and indeed Owen uses the
term "venous heart".   Robin (1880) in a very detailed
account definitely confirms that this organ in the eel pumps
lymph as it does in salmonids.   Grodzinski (1959) shows
that the caudal lymph heart in sea trout is embryologically
of venous origin and on its initial contractions during
development empties itself of blood and becomes a lymph
heart.

The volume of each lymph heart chamber (0.002ml from
the present study) is of the same sort of size as reported
in the Conger eel (Conger vulgaris) (Robin 1880).   The
estimated output of the caudal heart, assuming passive function
with the tail beat at 2(V/L) is approximately 0.7ml/minute

as compared with a pulmonary cardiac output in the region
of 30ml/minute (Stevens & Randall 1967b) (fig 41).
Perhaps the most significant output of the caudal lymph
heart might occur during a burst of swimming with a tail
beat frequency of 15hz for example.   Then the caudal heart
output would be 0.03ml/sec.   During a burst of swimming the
pulmonary heart is often inhibited and would be working
initially at the resting stroke volume of 0.15ml with
about one beat per second.   The extra 0.03ml in the venous
return could contribute to a cardiac stimulation.   In
general however during swimming, blood pressure rises,
plasma filters into the lymph system (Wardle 1971), the
blood volume falls and haemoconcentration takes place
(Stevens 1967).   The flow of lymph from the caudal lymph
heart is probably only significant in the context of
relative lymph and blood volume regulation.   In
circumstances where large caudal heart outputs into the
veins might be expected an increased reversed flow of
plasma into the lymph system also occurs due to high blood
pressures.

Other lymph propulsor mechanisms may be active in the
cranial region (Kampmeier 1969) and the chambers of these
sinuses are much larger than the caudal lymph heart.   Any
pumping action is probably linked with the breathing
movements.   In some fish these chambers exert a suction
action on the lynph system drawing lymph forward along the
rigid walled neural lymph duct (Wardle 1971).   In
salmonids the neural lymph duct is blocked and lymph return

must be through the flaccid peripheral lymph vessels
which would restrict any suction effect.   Wardle
suggests that lymph return in salmonids is due largely
to the high arterial pressure in these fish.   It
seems probable that no significant increase in venous
pressure independent of pulmonary heart function could
be attributed to function of propulsors in the lymph
system except possibly in the initial stages of a burst
of swimming activity.

DORSAL AORTA LIGAMENT.

In teleost fishes longitudinal ligaments have been
observed immediately dorsal and ventral to the vertebral
column.   Franz (1898) in embryological studies on trout
and salmon noticed that the ligamentum longitudinale
ventrale lies within the lumen of the dorsal aorta. Burne
(1909) observed the presence of the elastic ventral
longitudinal ligament in the dorsal aorta of shad (<u>Alosa</u>
<u>alosa</u>) and suggested that it may form the basis of a blood
pumping mechanism linked with the fish's swimming movements
(fig. 37).   Antoniu-Murgoci (1940) describes the aortic
cord or ligament in Acipenserids and states that it is made
up of elastic fibres and is highly mobile.   De Kock &
Symmons (1959) described the longitudinal ligaments in
herring and discussed the possible circulatiory function
of the ventral ligament in the aorta in terms of Burne's
hypothesis.   Dornesco & Santa (1963) give a description

of the structure of the dorsal aorta in the carp.
They found a median septum attached to the dorsal wall of
the aorta which projected into the lumen but not further
than 20 to 30% of the diameter. The depth of this
ligament or septum varied being deepest where it connected
two adjacent vertebrae. The ventral longitudinal
ligament in carp thus does not divide the lumen of the
aorta and its main function seems to be as a structural
component of the vertebral column.

The ligament is not present in the dorsal aorta of pike
or perch. Observations of the presence or absence of the
ligament in the dorsal aorta of different fish are sparse
so no reliable comment can be made on its phylogeny. In
considering its function it should be noted however that
many fish do not have this structure in their dorsal aorta.

The present study has shown that a characteristic of
the Burne mechanism is that the pump output is directly
proportional to the tail beat frequency (figure 41). The
theoretical output is somewhat larger than the estimated
cardiac outputs but the discrepancy may not be significant.
Maximum cardiac output and blood flow is reached at the
critical swimming speed. During swimming above this speed
the output of the Burne type pump would tend to far exceed
the cardiac output. This would result in massive pressure
oscillations unless the pump mechanism breaks down. It
was shown that the pressure at which spontaneous breakdown
would occur in a Burne type pump is very high. It is
possible that a specific mechanism may alter the curvature
of the vertebral column to take the ligament out of its

operative position with an effective seal against the ventral wall of the aorta. The onset of white muscle activity during swimming at high speeds may act in this way.

It was concluded from the theoretical analysis that an efficiently functioning Burne type pump over the full range of swimming speeds is probably undesirable. The experiments with the fish-bending machine, although unsatisfactory in many ways, showed no positive evidence of pumping activity in a trout cadaver. This, and close investigation of the aorta perfused with dyed saline suggested that any pumping activity is probably inefficient.

The dorsal aorta is rather more complicated than a simple tube in that the outflow is largely through lateral arterial branches and a taper along the length allows for this. The residual output through the caudal artery might be quite small and possibly difficult to measure with the techniques used in the fish bending experiments.

Assuming that the Burne type of positive displacement pump mechanism does not apply to the dorsal aorta of trout the ligament almost certainly does have some influence on blood flow. An impeller type of pumping action may take place with lateral accelerations being imparted to the blood. The position of the ligament against the lateral wall of the aorta on the inside curve of a swimming wave may inhibit retrograde flow from the segmental musculature as it contracts. The carp which does not have a ligament of this type has valves at the openings of the segmental arteries (Dornesco & Santa 1959) which may fulfill this

Figure 63.   Model of the fish circulatory system

(after Satchell 1971).   CG -

capacitance of the ventral aorta and

afferent branchial arteries.   CP -

capacitance of the dorsal aorta and

peripheral vessels.

heart
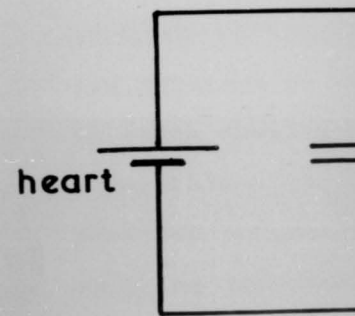
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CG -

al aorta and

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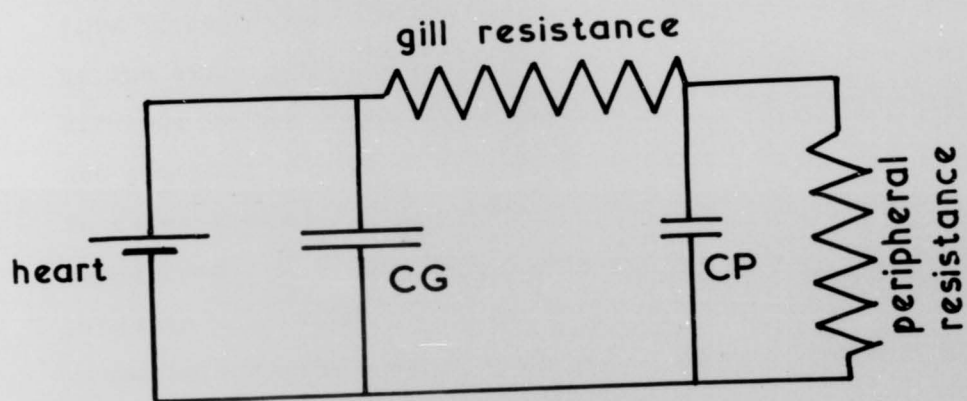
after Satchell (1971)

function.

During swimming, the movement of the ligament even
if it does not have any directed pumping action is likely
to impose pressure oscillations on the blood in the dorsal
aorta.   At anything above low swimming speeds these
oscillations would be of a higher frequency than the heart
beat.   Satchell (1971) discusses the compliance effects
in the arterial system of fishes using an electrical
analogy.   He points out that in order to maintain blood
flow through gills the capacitance (compliance) efferent
to the gills (CP) must be small relative to the capacitance
afferent to the gills (CG). (figure 63).   These capacitances
are required to smooth the blood flow and pressure pulses.
In trout despite the presence of the bulbus arteriosus
capacitance, the smoothing of flow is not complete and the
pressure pulses in the dorsal aorta increase during
swimming (Stevens & Randall 1967).   Using the electrical
analogy further:-

$$r \propto \frac{1}{f\ R\ C} \qquad \text{(Brophy 1966)}$$

where   r = ripple factor

f = pulse frequency

R = resistance

C = capacitance

The movement of the dorsal aorta ligament is likely
to effectively increase f and then r would decrease i.e.
the pressure oscillations would decrease in amplitude.

This effect would be particularly advantageous as increase in CP, the dorsal aorta and peripheral capacitance, is restricted. The smoothing of the pressure oscillations would result in an increase in efficiency of exchange with blood in the muscle capillaries.

Despite these considerations the fact remains that the tension in the dorsal aorta ligament is far larger than is required for any blood circulatory function and any such role may be secondary to that as a structural component of the axial skeleton.

CONCLUSIONS.

Satchell (1971) discusses various auxiliary mechanisms of venous return in fish but most of the information is on elasmobranchs. Sutterlin (1969) described some valves in the veins of the caudal region of trout and showed that these may promote venous return during activity.

Considering all the possible auxiliary pumping mechanisms in trout which may come into play during swimming activity it seems that no large increase in venous pressure can be expected. However the heart responds to quite small increases in venous pressure e.g. an increase in input pressure from 2mm to 6mm Hg approximately doubles the cardiac output (Bennion 1968).

Functional Morphology of the Heart

BULBUS ARTERIOSUS.

One of the characteristic features of the anatomy of the teleost heart is the bulbus arteriosus which is distinguished from the conus arteriosus of the elasmobranchs

Figure 64. Diagramatic longitudinal (A & B) and
transverse (C & D) sections of the
bulbus.

Figure 64. Diagramatic longitudinal (A & B) and
transverse (C & D) sections of the
bulbus.

A

C

2·5mm 1·0mm
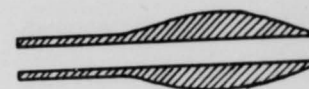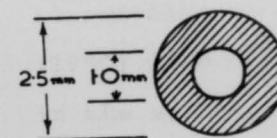
CIRCUMFERENCE
INNER 3·14
OUTER 7·85

PROBLEMS IN WINDKESSEL
DESIGN

A

B

C

D

2·5 mm 1·0 mm

7·0 mm 6·54
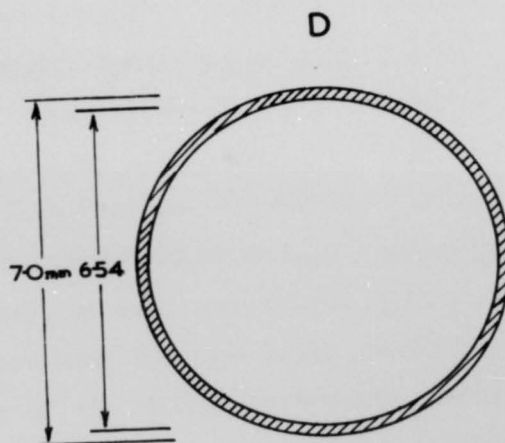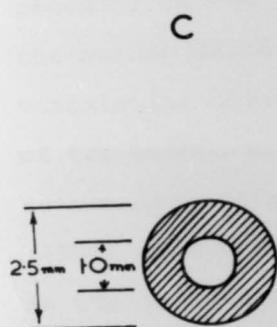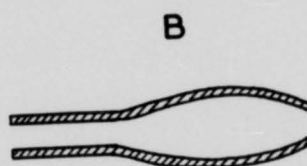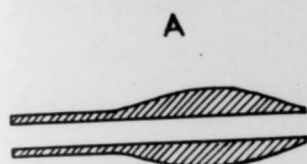
CIRCUMFERENCE

INNER 3·14 MM

OUTER 7·85 MM

CIRCUMFERENCE

INNER 20·55 MM

OUTER 22·00 MM

STRAIN

INNER 5·54

OUTER 1·80

(A & B) and

ons of the

by the nature of its walls and by the fact that it does
not contract rhythmically (Parsons 1930). The myocardium
does not extend into the bulbus which is made up of
elastic and smooth muscle fibres and acts as a passive
elastic chamber which helps to smooth the pressure pulse
from the heart.

The windkessel function of the bulbus has been
demonstrated in vivo particularly by Johansen (1962) and
Stevens et al (1969). The intravascular pressure pulse
during ventricular systole is converted to a slow rising
pressure in the bulbus arteriosus. Elastic rebound of
the bulbus maintains ventral aortic blood flow during
ventricular diastole. This effect depends on the ability
of the bulbus to expand elastically under pressure to
accommodate relatively large volumes of blood. Mott
(1950) made radiological observations on the circulation
in the eel and noted that the most remarkable feature in
connection with the heart beat was the large amplitude of
changes in the diameter of the bulbus arteriosus (from 3mm
to 5mm diameter).

In considering the design of a windkessel chamber
certain problems become immediately apparent. Figure 64a
shows a longitudinal section through a typical trout bulbus
arteriosus and it is evident that if the elastic wall was
of a uniform material the thick part in the bulbus region
would not deform at all but the ventral aorta would dilate
preferentially.. Figure 64 shows an alternative design
with a uniform thickness. In this, due to the large diameter
in the bulbus region and consequential larger tension in the

wall the bulbus would dilate as planned but as the
diameter increased so the wall would become thinner and
the possibility of a "blowout" arises.   This system
also has the disadvantage of a considerable "deadspace"
or residual volume of blood when relaxed.

Figure 64 C and D show transverse sections of the
bulbus of a 30 cm trout in the contracted and extremely
expanded states respectively, if it is assumed that the
wall is made of a uniform material.   The strain on the
fibres adjacent to the lumen is far larger than on the
outer fibres.   This is a basic problem if wall thicknesses
are large compared with the radius.   Returning to figure
56 it can be seen that in trout this problem is avoided
by the fact that the inner fibres do not follow the
outer ones as the bulbus expands.   The inner longitudinal
fibres arch outwards and help support the outer wall by
connections with the radial fibres.   If the radial fibres
were exactly radially orientated their extensions would be
very large but as they slope in the longitudinal axis
this extension is minimised.   It can be appreciated that
the complex structure of the bulbus serves to equalise the
strain in all the structural elements of the chamber wall.
Collagen, a relatively inextensible material is confined to
the outer layers where least extension takes place;   this
is typical of most blood vessels.

The amount by which the bulbus sustains flow in the
ventral aorta during ventricular diastole can be estimated
by assessing the volume change of the bulbus between systolic

and diastolic pressures. From figure 57, the bulbus
pressure-volume relationship, the volume increase of
the bulbus per mm Hg pressure increase is approximately
0.0013ml. This fish weighed 85gm so that assuming
that the volume change is proportional to the weight of
the fish the corresponding volume change in a 300 gm
fish is:-

$$\frac{300}{85} \times 0.0013 = 0.0046 = 0.005 \text{ ml/mm Hg approximately}$$

This is approximately the size of fish used by Stevens &
Randall (1967a, b) in their studies of the blood system
during swimming in rainbow trout. The effect of the
bulbus on flow can then be assessed using the blood
pressure and cardiac output figures from that study.

At rest the ventral aorta systolic pressure was
40mmHg and the diastolic 32mm Hg.

Therefore   pressure pulse = 40 - 32 = 8mmHg

Therefore the volume change in the bulbus = 8 x 0.005 =
0.04 ml

The corresponding cardiac stroke volume was 0.15 ml.
Therefore the proportion of flow in the dorsal aorta which
takes place due to elastic rebound of the bulbus is:

$$\frac{0.04}{0.15} \times 100 = 27\% \text{ approximately}$$

At the highest swimming speeds studied by Stevens &
Randall the ventral aorta systolic and diastolic pressures
were 58 and 45 mmHg respectively.

Therefore the pressure pulse = 58 - 45 = 13 mmHg

Therefore the volume change in the bulbus = 13 x 0.005 = 0.065 ml.

The cardiac stroke volume at this point was 0.70ml.

Therefore the proportion of blood flow due to bulbus rebound is:

$$\frac{0.065}{0.70} \times 100 = 9\% \text{ approximately}$$

Thus the bulbus arteriosus appears to be particularly important at low cardiac outputs in sustaining ventral aorta blood flow during ventricular diastole. Stevens et al (1969) by direct measurement of blood flow in Ophiodon elongatus showed that 29% of blood flow took place during this phase due to the action of the bulbus Arteriosus.

In the present work no account has been taken of the effects of the difference between the static and dynamic elasticity of the bulbus. In view of the few experiments carried out and assumptions used extending bulbus elasticity measurements on a small fish to much larger fish the above estimates can only be taken as an indication of the orders of magnitude involved. The smooth muscle in the bulbus could also modify the distensibility of the bulbus under different conditions. It is probable that the smooth muscle acts in a similar way to that of the ventral aorta studied by Kirby & Burnstock (1969). They showed that the ventral aorta of trout would contract in response to electrical stimulation and administration of catecholamines or acetylcholine.

The response to catecholamines was often absent and it
was suggested that the ventral aorta smooth muscle tonus
is largely cholinergically controlled in fish.   Contraction
took place over a period of about 30 seconds and relaxation
took 5 to 10 minutes.

It has been shown that the trabeculae are probably
an important functional feature of the bulbus arteriosus.
However Gegenbaur (1866) points out that in teleost evolution
there is a trend from the ridged to the smooth condition
of the bulbus wall.   Parsons (1930) states that in
trabeculate forms the conus wall is very compact but that
in the smooth types the bulbus wall is composed of a
loose spongework through which there are many interstices.
Parsons uses the term "trabeculae" to refer to prominent
longitudinal ridges apparent in simple dissection of the
bulbus.   He regarded salmon as an intermediate form in
that the anterior end of the bulbus is divided by trabeculae
but at the base there is a transition to the smooth type.
Re-examination of the cast of the trout bulbus (fig 52)
shows that at the anterior end prominent longitudinal ridges
are apparent but towards the base there is a spongy
appearance where the radial fibres predominate.   Haberich
(1965) states that the demonstration of the trabecular
structure of the heart is difficult since the meshwork
immediately collapses upon dissection.   The injection
techniques used in the present study enable some
relationships to be perceived which are otherwise difficult

to investigate.   It is apparent from these studies that
in the "smooth" regions of the bulbus wall the combination
of many radial elements with the longitudinal fibres is
an extension of the strain equalising features of the
structure.

VENTRICLE

Johansen (1965) states that the most noticeable
difference from most higher vertebrates in the ventricles of
fishes and amphibians is the almost entire lack of a
spacious central lumen.   This was noted in the present
study on trout; there being an outer compact muscle
layer and an inner spongy mass.   In amphibia it has been
shown that the trabeculation is important in maintaining
separation of venous and arterial blood.   This function
is obviously not required in fish so underlying mechanical
reasons for trabeculation must be considered.   Johansen
and Hol (1964) maintained that the myocardial arrangements
in lower vertebrates facilitates ventricular function by
reducing strain on individual muscle fibres enabling
contraction of a relatively small heart through large size
ranges.   A rigorous analysis of the ventricular function
based on the Laplace Law intended by these authors does
not appear to have been completed. · Indeed the study of the
dynamics of the heart as a muscle system in mammalian
physiology continues to use approximations of simple
geometry, isotropic wall material and uniform fibre
arrangement (Talbot & Gessner 1973).   Some progress has
been made to analysis of a natural fibre arrangement in

the canine left ventricle (Streeter et al 1969).

By simple analogy the rather qualitative analysis of the bulbus structure can be applied to the ventricle. A fragmented structure of the inner muscle is important in reducing the strain on the individual fibres of the inner layers. The trabeculation tends to equalise the amount of extension in all the muscle fibres and then they can all contract at very similar velocities which would tend to increase efficiency. It seems possible that as the ventricle contracts the outer compact muscle layer would squeeze the inner spongy mass which would not be doing any useful work towards the end of systole. At low stroke volumes if the ventricular amplitudes are small, most of the work may be done by the compact muscle layers and any increase in stroke volume would involve progressive recruitment of the fibres of the spongy muscle as contractile amplitudes increase. Only the compact muscle layer receives a coronary blood supply and the spongy muscle is supplied by diffusion from the chamber cavity (Ostadal & Schlieber 1971). This raises the possibility of an interesting parallel with the swimming musculature where only the red muscle which works continuously for long periods has a good blood supply. However Ostadal and Schlieber showed that in many fish the outer compact layer is absent, this was related to weight rather than phylogenetic relationships. Fish with a small body weight have a myocardium consisting almost entirely of the sponge like mass with no outer compact layer or coronary vessels; the plaice is an example of such a fish (Santer & Cobb 1972).

It is also of interest to note that no adrenergic nerve supply reaches the plaice ventricle (Santer 1972). This may be associated with the lack of coronary blood vessels along which adrenergic fibres apparently reach the ventricle in trout (Gannon & Burnstock 1968).

It appears in general that the thick stratum compactum with a good coronary blood supply is correlated with an ability to pump at high rates with a relatively high blood pressure. The blood pressure in trout is rather higher than in plaice (Wardle 1971).

ATRIUM

The atrium has no outer stratum compactum which is probably correlated with the relatively low pressures generated by this chamber. The myocardium is diffuse probably again to provide for large amplitudes of contraction. The atrium must be readily distensible in order to be filled by the low venous return pressures. The pressures generated during atrial systole need only be large enough to dilate the ventricle. In the section on the role of the cardiac innervation it was pointed out that the atrium has a more sophisticated control innervation than the ventricle. It is possible that the ventricular output is determined largely by simple intrinsic responses to a controlled inflow from the atrium.

SINUS VENOSUS

The sinus venosus has a volume of about 0.08ml which when compared with the likely cardiac stroke volumes indicates that at low cardiac outputs it can store 50% of the stroke volume and at high cardiac outputs this falls to

about 10%   Thus during atrial systole the sinus cannot
be just emptied into the atrium but considerable
volumes of blood must be drawn from the major veins.
The sinus venosus can hardly be regarded as a functional
chamber of the heart in the sense that the atrium and
ventricle alternately fill and empty passing on discrete
volumes of blood.   The sinus is little more than the
confluence of the major veins.   The venous pressure is
always positive in rainbow trout (Stevens & Randall 1967a)
and is sufficient to produce a regular flow of blood
through the relatively narrow veins by a _vis a tergo_
effect there being little evidence for a _vis a fronte_
as in elasmobranchs (Satchell 1971).

Conclusions

In the course of these studies information has been
accumulated on the changes in heart rate associated with
different activities and metabolic rates.   Nomura
et al (1972) have telemetered ECG's from rainbow trout
in hatcheries and were able to correlate heart rate
with patterns of activity in raceways between ponds.
Knowledge of the relationship between heart rates and
metabolism could enable assessment of metabolic rates in
free-living fish using this telemetric technique.   Caution
would have to be exercised in interpretation of results
because the range of possible oxygen consumptions for a
given heart rate is large.   Minimum estimates of metabolic
rates in free-living fish have been derived from telemetric
studies of swimming activity (Holliday et al 1974) and
laboratory studies of trout metabolism (Morgan 1974).

Using information on heart rates maximum oxygen consumption values could be accurately defined. Phenomena such as cardiac inhibitions which were observed by Nomura et al (1972) could provide a means of assessing the sensory input to the fish.   Despite the variation in basal heart rates it should be possible from ECGs to quantify the effort expended in short bursts of activity.   This could be done by observing the period over which maximum heart rate is sustained and the time for subsequent recovery when the fish is at rest.   As a measure of metabolic rate, heart rate would have a large range of error if used for overall energy budget calculations but could provide useful information on short term changes.   The effect of variation in environmental water temperatures and PO_2 etc. would have to be taken into account.

In consideration of the control of circulation this study has assessed certain aspects of the overall picture (figure 1)..   The importance of the lymph volume has been demonstrated by Wardle (1971) but the present studies indicated that the flow rates returning to the veins are small relative to blood flow.   Taylor et al (1968) developed a mathematical model to simulate the cardiovascular-respiratory dynamics of rainbow trout using largely the data of Randall & Stevens (1967 a,b.).   In these equations they used a figure for the effective volume of the body compartment blood which was 1.5 times the total blood volume.   The magnitude of this figure was

not very critical but this does suggest that fluxes
between lymph and blood are of significance in short
term (0.01 minute intervals) cardiovascular.dynamics as
well as in longer term blood volume regulation.

The fact that vagotomy had a different effect at
different temperatures is of particular importance.
Bennion (1968) noted complementary differences in the
isolated trout heart at different temperatures.   It
seems that any work on the role of the autonomic
nervous system and associated pharmacology must take
into account the temperature.   The study of fish
physiology then appears to become disproportionately
complex but some assessment of temperature as a variable
in basic physiological processes is essential.

The model of Taylor et al (1968) predicts
cardiovascular-respiratory changes during swimming
and uses three controlling system equations:-

respiratory ventilation volume $\propto$ fall in venous PO_2

cardiac output $\propto$ respiratory ventilation volume

effective gill area $\propto$ change in cardiac output

They suggest that increase in metabolic rate reduces venous
PO_2 which via receptors in the veins entrains changes in
ventilation volume, cardiac output and gill perfusion
pathways.   The model depends on monitoring venous PO_2;
no satisfactory simulation could be achieved using arterial
or venous PCO_2 or arterial PO_2 as a feedback reference.

There is little anatomical or physiological evidence for receptors in the venous circulation. The most clearly demonstrated blood PO_2 receptor site is in the pseudobranch (Laurent & Rouzeau 1972) which is in the efferent (arterial) circulation of the first branchial arch. Davis (1971) shows that the gill circulation of rainbow trout can be extensively manipulated without affecting circulatory and ventilatory responses unless the first branchial arch circulation was disturbed so as to divert blood away from the pseudobranch. The model of Taylor et al successfully predicts the behaviour of the cardiovascular system assuming continuous flow characteristics. However there is no reason to suppose that the control equations used are anything more than empirical descriptions of physiological events with no actual analogous control mechanisms being implied. The model clarifies certain aspects of respiratory exchange but as far as the control of cardiac output is concerned it is probably far too simple. For example, the pseudobranch gives rise to different efferent neural activity in response to various physiological parameters of the blood; PO_2, PCO_2, pH, hydrostatic pressure, osmotic pressure and Na^+ ions. (Laurent & Rouzeau 1972, Laurent 1967).

Vagotomy at $15^{\circ}C$ had little effect on heart rates during exercise which suggests that there is considerable redundancy in the circulatory control mechanisms.

The possible role of auxiliary pumps increasing venous
return was extensively discussed.   It may be possible
to explain the responses to exercise observed in
vagotomised fish in terms of the effect of catecholamines
circulating in the blood.   Nakano & Tomlinson (1967)
measured levels of catecholamines in blood and various
tissues of rainbow trout during and after activity.
It seems probable from this that catecholamines could
be secreted rapidly enough to explain the increase in
heart rate at the onset of exercise.   It is however
doubtful if they could be removed from the circulation
rapidly enough to explain the rapid fall in heart rate
after moderate exercise which occurs even in the absence
of vagus inhibitory function (Figures 19 & 23).
Catecholamines influence various parts of the circulatory
system in such a way that observed changes in circulation
can be explained in terms of their interaction with these
receptor sites. (Randall & Stevens 1967).   The fundamental
problem remains that little is known of the mechanism
controlling secretion or removal of catecholamines from
the circulation.   Nakano & Tomlinson (1967) point out that
the fall in catecholamine concentrations in the anterior
kidney was insufficient to account for all that appeared
in the circulation.   Either the rate of production equalled
the rate of secretion or these substances were secreted
elsewhere.   There was some evidence that noradrenaline
is secreted in the heart from the sympathetic system.
Vagotomy excludes the possibility that this could be
derived from fibres in the vagus in the present study but

the coronary adrenergic plexus (Gannon & Burnstock 1969) may be implicated. In the absence of any evidence of how catecholamine secretion is controlled, theories of circulatory homeostasis based on responses to injected drugs or the response of in vitro isolated preparations must remain essentially speculative. Anatomical and histochemical studies have been carried out on the chromaffin tissue in a shark (Gannon & Campbell 1972) and the plaice (Grove et al 1972) with a view to understanding its secretion into the circulation but no defined conclusions can be drawn which would be of direct application to rainbow trout. The role of catecholamines other than in the control of circulation is also a factor which should be considered e.g. the role of adrenaline in carbohydrate metabolism (Nakano & Tomlinson 1967, Wardle 1972).

Neurohypophysial and adrenal cortical hormones have vassopressor influences on the circulatory system and can affect blood flow patterns in the gills (Perks 1969, Rankin & Maetz 1971). However the primary importance of these hormones is probably in regulation of electrolyte exchange. In this connection the diversion of blood flow towards or away from the "chloride" cells of the gills by virtue of effects on the branchial vasculature may be of significance. Considerable fluxes of water and ions take place between the internal and external milieux in rainbow trout during swimming activity (Wood & Randall 1973 a,b,c.) and it is against this complex background (the details of which are outside the scope of the present thesis) that

endocrine influences on circulation must be considered.

Saunders and Sutterlin (1971) studied the effect of section of the IX and X cranial nerves on cardiac and respiratory responses to hypoxia in the Sea raven (Hemipterus americanus) and showed that this procedure had little effect on the observed responses.   Section of the IX nerve also denervates the pseudobranch.   In the case of hypoxia the responses may be explained by the activity of sensor "end buds" in the buccal cavity which are innervated by the V cranial nerve. (De Kock 1963). Efferent activity in the branchial elements of the V and VII nerves could account for changes in breathing.   There was evidence of peripheral vasomotor reflexes involving autonomic pathways other than the IX and X nerves.   The origin of the vasomotor supply to the ventral aorta in trout for example (Kirby & Burnstock 1969) has not been ascribed to any particular cranial nerve.   It seems probable that sectioning only the cardio-visceral branch of the vagus has left many vasomotor pathways intact and the activity of these may explain some of the circulatory changes observed in the present work.

The work carried out on the anatomy of the heart did not extend to a full analysis of mechanical relationships of the structural elements that might be hoped for.   In the context of overall cardiovascular function an estimate was made of the probable influence of the bulbus arteriosus on ventral aorta flow.   The small estimated volume of the sinus venosus emphasises that any increase in cardiac output

is very dependent on increase in venous return. The
heart cannot draw on blood stored in the sinus venosus
in order to make a significant increase in output beyond
one or two beats. A study of how the volume of each
chamber of the heart varies at different rates of cardiac
output would be of particular interest especially when
considering the function of the ventricular myocardium
with its two distinct layers. The simple static views
obtained in the present study are of limited value as
little is known of the mean amplitude about which the
heart contracts at different stroke volumes. Radiological
techniques such as those used by Mott (1950) and
Johansen & Hol (1964) may be appropriate but observation
of a perfused isolated heart would also be of value.

The amount of information available on circulatory
physiology in fish is rapidly increasing each year and it
is becoming possible to reach a general understanding of
various phenomena. However as noted at several points
in this thesis, interspecific differences between the
fishes are great and generalisations are often inapplicable
in particular instances. This is of great interest in
comparative studies but when applying physiological
knowledge in fish husbandry for example the characteristics
of each species must be independently assessed. This
thesis concentrated on rainbow trout on which much work
has been carried out and numerous problems emerged; as
attention is directed to some of the less well known
species careful consideration must be given to the most
efficient means of consolidation of information for the
purpose required.

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APPENDIX I

RESULTS OF SWIMMING TRIALS

6.5°C INTACT FISH

Fish Ref. No.	Length cm	Trial Ref. No.	Speed cm/sec	Speed (V/L)	Heart rate mean	Heart rate S.D.	50% recovery time mins.
2	27.0	I	41.8	1.55	56.08	4.26	6.8
		IVa	5.0	0.2	39.03	2.01	-
		IV	16.2	0.6	39.63	2.73	0.0
		IVb	<5.0	0.1	37.46	1.53	-
		Va	5.0	0.2	34.63	1.35	-
		V	25.65	0.95	40.13	3.83	1.0
		VIa	5.0	0.2	31.83	0.77	-
		VI	32.4	1.2	48.63	3.57	2.2
		VIIa	5.0	0.2	33.40	1.10	-
		VII	36.45	1.35	60.44	2.64	4.2
		VIIIa	5.0	0.2	31.66	1.80	-
		VIII	28.4	1.05	47.40	6.00	0.5
		IXa	5.0	0.2	30.40	1.13	-
		IX	22.95	0.85	37.00	2.86	0.6
		Xa	5.0	0.2	26.57	0.93	-
		X	17.55	0.65	33.28	2.09	0.6
		XIa	5.0	0.2	31.90	1.52	-
		XI	41.8	1.55	54.46	3.96	17.0
3	24.5	Ia	5.0	0.2	27.18	1.74	-
		I	25.2	1.15	51.37	1.30	5.4
		IIa	5.0	0.2	25.13	1.80	-
		II	20.8	0.85	37.31	1.95	1.5
6	24.5	Ia	5.0	0.2	34.37		-
		I	26.2	1.07	41.26	1.06	0.4
		IIa	5.0	0.2	37.45		-
		II	29.8	1.21	44.74	2.75	1.0
		IIIa	5.0	0.2	31.64		-
		III	33.8	1.38	56.29	1.96	3.5
		IVa	5.0	0.2	31.95		-
		IV	19.6	0.79	34.74	3.18	-
		V	14.0	0.57	31.04	3.62	0.0
		VVIa	5.0	0.2	33.70		-
		VII	40.1	1.66	55.26	1.89	8.1
		VI	34.4	1.4	56.20	2.27	-
7	24.1	IIa	5.0	0.2	34.86	0.37	-
		II	25.0	1.04	47.31	1.35	0.3
		III	32.0	1.33	53.24	2.17	0.8
		IV	34.3	1.42	56.04	1.37	5.5

6.5°C UNILATERALLY VAGOTOMISED FISH

Fish Ref. No.	Length cm	Trial Ref. No.	Speed cm/sec	Speed (V/L)	Heart rate mean	Heart rate S.D.	50% recovery time mins.
9	24.2	Ia	5.0	0.2	32.47	1.31	-
		I	28.0	1.16	56.26	1.72	3.3
		IIa	5.0	0.2	35.47	2.54	-
		II	18.6	0.77	36.41	1.57	0.2
		IIIa	5.0	0.2	35.75	1.38	-
		III	38.4	1.59	56.81	0.85	7.1
		IV	35.2	1.45	54.11	2.45	0.2

6.5°C BILATERALLY VAGOTOMISED FISH

Fish Ref. No.	Length cm	Trial Ref. No.	Speed cm/sec	(V/L)	Heart rate mean	S.D.	50% recovery time mins.
8	25.1	IIa	5.0	0.2	56.85	0.80	-
		II	16.57	0.77	56.74	0.66	-
		IIIa	5.0	0.2	53.62	0.44	-
		III	31.9	1.27	59.07	1.11	-
		IV	23.85	0.95	58.64	1.38	-
		IVa	5.0	0.2	55.07	0.26	-
		Va	<5.0	0.15	50.60	1.59	-
		V	16.2	0.64	49.54	0.64	-
		VIa	5.0	0.15	55.17	1.64	-
		VI	24.4	0.97	54.17	0.94	-
		VIIa	<5.0	0.15	43.45	0.56	-
		VII	13.05	0.52	46.08	1.14	-
		VIIIa	<5.0	0.15	48.06	3.89	-
		VIII	28.1	1.12	55.65	2.87	-
		IXa	<5.0	0.15	43.17	2.41	-
		IX	22.1	0.80	47.70	1.14	-
		Xa	<5.0	0.15	40.67	2.35	-
		X	31.9	1.27	56.89	1.91	-
10	25.5	Ia	5.0	0.2	49.56	0.96	-
		I	21.65	0.85	54.35	0.57	-
		IIa	5.0	0.2	48.00	0.57	-
		II	24.4	0.96	52.27	0.87	-
		IIIa	<5.0	0.15	47.77	0.67	-
		III	13.9	0.54	52.00	0.87	-
		IVa	<5.0	0.15	49.63	0.72	-
		IV	30.4	1.19	51.96	0.54	-
		Va	<5.0	0.15	46.53	0.57	-
		V	34.1	1.34	48.91	0.99	-
		VIa	<5.0	0.15	48.88	0.66	-
		VI	15.3	0.60	51.46	0.57	-
		VIIa	<5.0	0.15	47.00	1.01	-
		VII	35.6	1.39	52.41	0.93	-
		VIIIa	<5.0	0.15	46.66	0.66	-
		VIII	12.5	0.49	49.41	1.01	-
		IXa	<5.0	0.15	43.91	0.69	-
		IX	22.6	0.89	48.67	0.68	-
		Xa	<5.0	0.15	42.23	0.73	-
		X	36.4	1.47	51.43	0.84	-

15°C INTACT FISH

Fish Ref. No.	Length cm	Trial Ref. No.	Speed cm/sec	(V/L)	Heart rate mean	S.D.	50% recovery time mins.
11	28.2	Ia	5.0	0.2	55.17	2.28	-
		I	37.6	1.34	58.72	4.27	0.0
		IIa	5.0	0.2	47.75	1.61	-
		II	45.9	1.63	70.08	7.18	0.8
13	27.8	Ia	5.0	0.2	52.07	1.56	-
		I	35.3	1.1	63.08	3.81	0.0
		IIa	5.0	0.2	48.85	1.57	-
		II	41.3	1.49	64.11	2.93	-
		III	49.5	1.78	79.75	3.62	9.9
		IV	61.6	2.22	94.44	1.45	4.0
14	24.5	I	41.3	1.69	84.33	2.51	0.4
		II	45.2	1.84	94.67	3.33	-
		III	46.5	1.90	98.22	2.82	26.0

15°C—INTACT FISH (cont.)

Fish Ref. No.	Length cm	Trial Ref. No.	Speed cm/sec	(V/L)	Heart rate mean	S.D.	50% recovery time mins.
18	26.0	I	52.5	2.02	90.31	3.24	25.0
		IIIa	5.0	0.2	43.77	1.63	-
		III	16.0	0.62	47.41	1.42	2.5
		Va	5.0	0.2	41.14	1.39	-
		V	51.0	1.96	93.06	2.17	93.0
		IVa	5.0	0.2	49.12	2.56	-
		VI	29.7	1.14	70.71	1.97	0.5
		VIIa	5.0	0.2	43.35	2.35	-
		VII	21.8	0.84	51.64	4.32	0.0
		VIII	24.4	0.94	55.40	3.0	0.0
21	25.5	Ia	5.0	0.2	44.72	1.38	-
		I	21.0	0.82	64.67	4.08	
26	27.5	IIIa	5.0	0.2	39.00	0.93	-
		III	28.5	1.04	46.19	1.74	0.5
		IVa	5.0	0.2	43.95	1.81	-
		IV	54.3	1.97	91.19	2.59	11.5
		IVb	5.0	0.2	31.75		-
		VIa	5.0	0.2	51.06	1.39	-
		VI	54.9	1.99	93.81	4.3	-

15°C UNILATERALLY VAGOTOMISED FISH

Fish Ref. No.	Length cm	Trial Ref. No.	Speed cm/sec	(V/L)	Heart rate mean	S.D.	50% recovery time mins.
16	27.0	Ia	5.0	0.2	38.3	1.89	-
		I	22.2	0.82	52.48	3.40	2.4
		IIa	5.0	0.2	37.93	2.15	-
		II	33.9	1.25	68.58	2.99	1.0
		IIIa	5.0	0.2	33.06	1.57	-
		III	41.3	1.53	73.36	8.14	3.0
		IVa	5.0	0.2	31.00	1.45	-
		IV	53.9	2.0	95.31	3.21	27.0
		Va	5.0	0.2	39.38	2.82	-
		V	42.1	1.56	72.72	2.65	1.1
		VIa	5.0	0.2	39.67	5.08	-
		VI	57.8	2.14	97.46	2.24	30.0
		VIIa	5.0	0.2	40.50	0.80	-
		VII	18.6	0.69	42.56	2.08	0.0
		VIIIa	5.0	0.2	38.27	1.44	-
		VIII	59.3	2.2	95.72	2.51	47.0

15°C BILATERALLY VAGOTOMISED FISH

Fish Ref. No.	Length cm	Trial Ref. No.	Speed cm/sec	(V/L)	Heart rate mean	S.D.	50% recovery time mins.
22	25.7	I	38.3	1.52	82.00	2.13	0.0
		IIa	5.0	0.2	53.57	2.62	-
		IIIa	5.0	0.2	61.83	1.40	-
		IVa	5.0	0.2	52.59	1.53	-
		Va	5.0	0.2	52.53	2.62	-
		VII	17.0	0.66	55.48	2.46	0.0
		VIIIa	5.0	0.2	59.56	1.13	-
		VIII	24.6	0.96	63.00	6.16	0.0
24	25.7	Ia	5.0	0.2	34.63	0.96	-
		I	24.25	0.94	47.46	3.40	1.0
		IIa	5.0	0.2	35.85	1.23	-
		II	34.8	1.35	74.56	2.77	1.6

15°C BILATERALLY VAGOTOMISED FISH (cont.)

Fish Ref. No.	Length cm	Trial Ref. No.	Speed cm/sec	(V/L)	Heart rate mean	S.D.	50% recovery time mins.
24	25.7	IIIa	5.0	0.2	30.54	1.06	–
		III	38.0	1,48	72.70	7.60	5.0
		IVa	5.0	0.2	34.45	1.18	–
		IV	47.5	1.85	79.10	3.78	1.7
		Va	5.0	0.2	30.61	1.17	–
		V	50.7	1.97	79.45	2.40	2.1
		VIa	5.0	0.2	32.16	1.03	–
		VI	57.1	2.22	82.87	2.38	38.0
		VIIa	5.0	0.2	42.07	4.84	–
		VII	24.5	0.95	51.33	6.88	0.2
		VIIIa	5.0	0.2	35.92	1.26	–
		IXa	5.0	0.2	37.17	1,56	–
		IX	32.0	1.25	61.69	1.64	1.2
		Xa	5.0	0.2	39.46	2.15	–
		X	34.8	1.35	73.15	3.89	1.2
		XIa	5.0	0.2	37.39	1.58	–
		XI	55.0	2.14	82.48	2.21	12.3
25	27.0	I	37.2	1.38	78.57	2.66	1.0
		IIa	5.0	0.2	60.03	1.33	–
		II	43.4	1.61	85.58	1.81	2.6
		IIIa	5.0	0.2	51.59	2.74	–
		IVa	5.0	0.2	56.08	1.61	–
		Va	5.0	0.2	59.25	1.25	–

N.B. Trial reference numbers with suffixes a or b refer to periods of 'rest' in a flow of 5.0cm/sec or less. The error in these low speed estimates is ± 1cm/sec. Because of this, and due to uncertainties in the precise fish activity level (see discussion) an approximate specific speed of 0.2 or 0.15(V/L) is given. These speed values are not used in any further calculations.

RESULTS OF RESPIROMETER EXPERIMENTS - OXYGEN CONSUMPTION & HEART RATES

Fish. Ref. No.	Length cm	Weight g	Trial Ref. No.	Heart rate mean	S.D.	O_2 consumption mg/kg/hr
29	25.0	211.3	1	79.96	3.51	295.2
			3	85.10	4.51	341.8
			4	75.30	1.64	124.3
			5	73.95	2.44	147.6
			6	81.30	5.26	349.6
			7	75.73	2.05	144.5
			8	73.41	1.33	132.1
30	26.2	212.6	2	60.05	2.66	209.8
31	25.6	212.2	1	81.70	4.03	255.3
			2	78.50	1.97	201.2
			3	77.17	2.90	154.7
			4	59.00	6.22	154.7
			5	60.00	5.54	170.2
			6	56.59	1.74	147.0
			7	58.73	6.80	216.6
			8	91.96	1.64	417.7
			9	109.20	3.72	402.3
			10	99.17	2.86	410.0
32	26.3	225.1	1	55.22	8.31	189.6
			2	47.00	2.94	145.9
			3	46.86	5.31	116.7
			4	42.04	4.37	102.1
			5	39.86	1.96	87.5
			6	40.00	1.38	116.7
			7	38.06	1.91	109.4
			8	37.82	2.24	65.7
			9	42.30	3.47	87.5
			10	44.39	2.76	131.3
			11	48.39	2.67	138.6
			12	62.83	7.62	277.1
33	26.5	237.8	2	69.77	2.00	158.8
			3	71.18	1.50	165.7
			4	70.61	3.35	131.2
			5	78.71	1.63	151.9
			6	63.37	3.00	289.9
			7	76.61	2.10	214.0
			8	79.17	2.69	289.9
			9	84.00	3.92	379.7
			10	85.59	4.29	338.3
34	26.8	242.7	1	56.86	3.11	216.5
			2	61.87	3.12	128.5
			3	61.52	4.22	88.0
			4	54.26	2.26	94.0
			5	50.25	2.86	122.1
			6	50.18	2.30	94.0
			7	58.30	1.79	115.0
			8	50.29	1.40	108.3

APPENDIX III

Some of the work described in this thesis has been reported in the following article:-

PRIEDE, I.G.(1973). The effect of swimming activity and section of the vagus nerves on heart rate in Rainbow trout. J.exp.Biol. (in press)