FACTORS INFLUENCING THE RATE OF GAS LOSS FROM THE PHYSOCLIST SWIMBLADDER.

by

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The work presented in this thesis is the result of my own investigations. It has not been, nor will be, submitted for any other degree.

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Supervisor: Peter Lytle

Date: 28/9/77.

O scaly, slippery, wet, swift, staring wights,
What is't ye do? What life lead? eh dull goggles?
How do ye vary your vile days and nights?
How pass your Sundays? Are ye still but joggles
in ceaseless wash? Still nought but gapes and bites,
and drinks, and stares, diversified with boggles?

from "The fish, the man and the spirit."

Edward Leigh-Hunt.

CONTENTS

ABSTRACT

The aim of this study was to provide a rational account of the control of gas loss from the swimbladder of a physoclist fish Pollachius virens (L). Where possible the work has also included a study of vertically migrating mesopelagic fish and of deep-sea demersal types.

The bladder loses gas by two major routes, directed loss through the oval in buoyancy adjustment and random diffusion loss through the bladder wall. The oval is controlled by two distinct mechanisms - muscular and vascular, and the effect that each of these has on gas loss has been examined and quantified as far as possible.

The structure, histology and pharmacology of the oval were reexamined in terms of control of gas loss. The oval membrane in all anacanthines is formed from an extension of the tunica interna of the bladder and this was shown to be the layer most impermeable to oxygen. It is a good design point that the adjustable oval should be formed from this layer and this is in contrast to the arrangement found in eels.

The vascular control exerted by the oval plexus proved more difficult to assess. The effect of drugs on the plexus was confirmed, and preliminary estimates of maximum flow

rates were made from latex-injected preparations using the Poiseuille equation. The flow rates in the oval were estimated using radiolabelled microspheres and a reference organ technique in a resting and an actively resorbing fish. A significant increase in the flow rate was noted when the bladder was artificially overinflated.

The permeability to oxygen of sections of the bladder wall was estimated and low oxygen permeability was correlated with the presence of purine crystals in the tissues. It was found that substantial purine deposits occurred in the submucosa of the tunica interna and this layer had the lowest permeability to oxygen. Results by several authors indicate that the oxygen permeability of the bladder wall is inversely proportional to purine content. The purine content of the whole bladder wall was estimated in a number of species and found to be proportional to the mean depth of occurrence of the animals.

The maximum depth of neutral buoyancy was calculated in some fish using the permeability data. They were shown to have high rates of gas loss and in vertically migrating mesopelagic fish the bladder is probably inadequate to maintain neutral buoyancy at all depths. The effects of fins and ventilation thrust in reduction of sinking rate are considered and the elastic collapse of the bladder at depth may act as a safety device ensuring some static lift.

These three factors may contribute to maintenance of a stable position in the water column.

The physiological and ecological significance of these observations is discussed.

ACKNOWLEDGEMENTS

I am deeply indebted to my supervisor, Dr Peter Tytler, for the opportunity to embark on this research. His valuable advice and unfailing interest in the subject have been greatly appreciated at all stages of the work and in the preparation of the manuscript.

I wish to express my thanks to the Scottish Marine Biological Association (S.M.B.A.) and to the Marine Biological Association of the U.K. for the opportunity to take part in their 1975 joint cruise on R.R.S.Challenger to Madeira, enabling me to work on mesopelagic fish.

I am particularly grateful to Dr J.D.M.Gordon of the S.M.B.A. for the opportunity to take part in three cruises on R.R.S.Challenger to the Hebridean Terrace and the Rockall Trench. The first-hand experience gained by personal observation of many deep-sea species has been invaluable.

Thanks are also due to the many members of staff and postgraduates of the Biology department at Stirling for constructive discussions and advice during the last three years.

My wife, Barbara, has been a source of inspiration and encouragement and I cannot thank her enough for the assistance she has given me.

This research was funded by a three year studentship from the Natural Environment Research Council for which I am most grateful.

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INTRODUCTION

"The function of the swimbladder of fishes has attracted the attention of scientists for many centuries. The role that this structure plays in the life of the animal has been interpreted in almost as many ways as there have been investigators and even now there is much doubt as to the true functions of the swimbladder. Consequently any additional information concerning this organ is of immediate scientific value." (Tower, 1901).

This statement is almost as valid today, even after a further three-quarters of a century of increasingly refined research by numerous workers. A great deal more is now known of the physical properties of the organ, and the different ways in which it contributes to the lives of different groups of fish. Its roles in hearing, sound production, pressure detection, and as an accessory respiratory organ have been well documented, and there is little doubt that modern interpretations of these functions are very close to the truth. The major remaining function, that of a hydrostatic organ, has received much attention and is of paramount importance in the lives of many fish species. The means of adjustment of gas volume in the bladder have been elucidated and the fact that the bladder

does contribute to the attainment of neutral buoyancy is not in doubt, however, the means whereby dynamic changes take place in short time intervals are becoming less, rather than more, clear as work progresses.

In general, fish tissues are more dense than their environment, both in the sea and in freshwater. Many species have reduced their overall density to more closely match that of their environment by storing lipid, reducing skeletal components, or by having a high tissue-water content (Denton, 1961; Denton and Marshall, 1958). Most fish employing these methods are oceanic mesopelagic and bathypelagic species and are not really typical of the group. By far the most effective method of density reduction is the possession of a gas-filled swimbladder, and approximately half of the 20,000 known species of fish possess a bladder at some stage of their lives.

In order to attain neutral buoyancy a fish must contain in its swimbladder a volume of gas equivalent to about 5% of the body volume in seawater and about 7% of this in freshwater (Jones, 1951). This system provides the fish with sufficient lift to neutralise its own weight in water, thus avoiding the necessity to expend energy to maintain station in the water column. However, should the animal wish to alter its depth, then assuming that no compensation takes place the swimbladder will change in volume, closely

following Boyles law in most species (Alexander, 1959, 1972). This alters the hydrostatic equilibrium, and it is easy to envisage a point where the fish would sink or rise helplessly once it had moved out of its plane of operation (Jones, 1951, 1952). Apart from simple short-term movements such as this, many fish make regular vertical migrations. Seasonal changes in depth are made by perch Perca fluviatilis (L) in lakes, and the conger eel Conger conger (L) makes a vertical migration of up to 3000m in the course of its spawning activities. The herring Clupea harengus (L) makes daily vertical movements, and some species of lantern fish (Myctophidae) make quite spectacular diel vertical migrations of several hundred metres. Seasonal changes appear to be accompanied by appropriate changes in the gas content of the swimbladder. Daily changes, however, are often so rapid that it seems impossible that the fish could secrete and resorb gases quickly enough to maintain neutral buoyancy (Alexander, 1970).

In order to make adjustments of swimbladder gas content, fish are able to inflate or deflate the bladder by one of two systems. These systems have a widely different anatomical basis, and for many years this was used as a criterion in teleost classification. The review of Jones and Marshall (1953) describes how this scheme was in error and it will be sufficient to briefly describe the

two extreme cases here.

The trout Salmo trutta (L) is an example of the group known as physostomi in earlier years. Physostomes are characterised by the presence of a patent duct, the pneumatic duct, leading from the bladder to some part of the alimentary canal, usually the oesophagus. Clupeids possess two such ducts, one opening near the anus. When the external hydrostatic pressure decreases, as in a rise to the surface, physostomes are able to vent the expanding gases via this duct to the outside, so maintaining a constant swimbladder volume. The bladder is refilled at the surface by gulping air, although the problems inherent in this system are obvious. Fahlen (1959, 1967, 1968) and Sundnes et al (1958) have demonstrated the ability of some physostomes to slowly refill their bladders when kept without access to the surface, and a microanatomical basis for this has been described in Coregonids (Fahlen, 1971).

The basic physiology of hydrostatic homeostasis in physostomes, with the notable exception of the eels, is achieved by venting and muscular effort. By contrast the physoclistous fish have a completely closed bladder, and so all directed gas movement involves specialised vascular structures enclosed in an apparently impermeable bladder. This arrangement has, not surprisingly, attracted the

attention of workers for many years and warrants a more detailed description.

The secretion of gases into the physoclist swimbladder was first demonstrated by Moreau (1876) and later confirmed by many workers (Bohr, 1894; Fänge, 1953). This secretion process takes place in the anterior ventral portion of the bladder in the anatomically distinct gas gland. This structure generally comprises two more or less distinct parts; the rete mirabile- a vascular hairpin counter - current multiplier, and the secretory epithelium by way of which the gas enters the lumen of the bladder. The general blood supply to the bladder is by a branch of the coeliaco-mesenteric artery and this also supplies the rete and gas gland. Good accounts of these vascular arrangements are given by Woodland (1911), Jones and Marshall (1953), and Scholander (1954), and the generalised blood supply of a gadoid swimbladder is shown in Fig. 1 .

The swimbladder artery subdivides into many fine capillaries in the rete and gas gland and Denton (1961) describes the bipolar and unipolar rete arrangements which are shown schematically in Fig. 2 . Haldane (1922) first suggested the counter - current principle as the means of generating high partial pressures in the rete. His basic idea was developed mathematically to explain processes in the loop of Henle in the mammalian kidney by Kuhn and

Fig. 1. The blood supply to the gadoid swimbladder.

gadoid swimbladder.

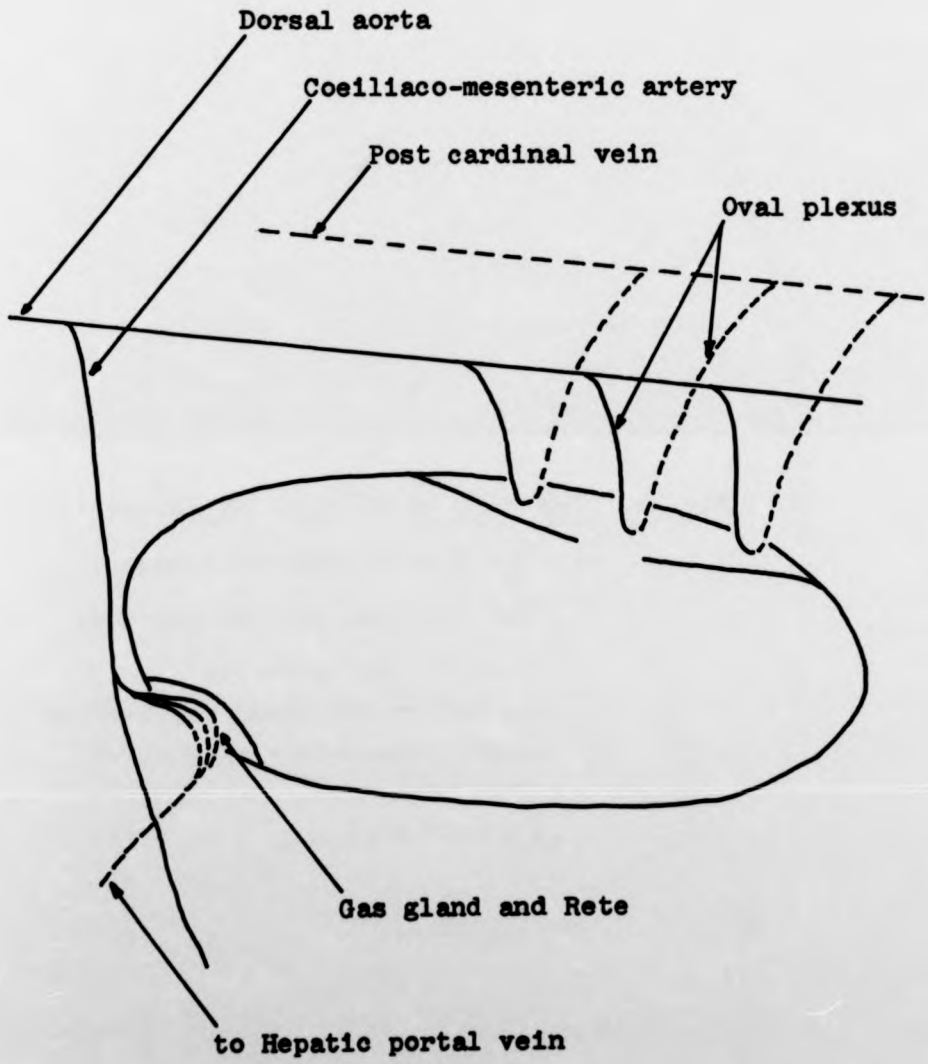

FIGURE 1

Fig. 2. The form of the Rete Mirabile.

A. The bipolar rete of apodans.

B. The unipolar rete of gadoids.

(r.m. = rete mirabile)
(g.g.c. = gas gland capillaries)
(s.e. = secretory epithelium)
(v = vein)
(a = artery)

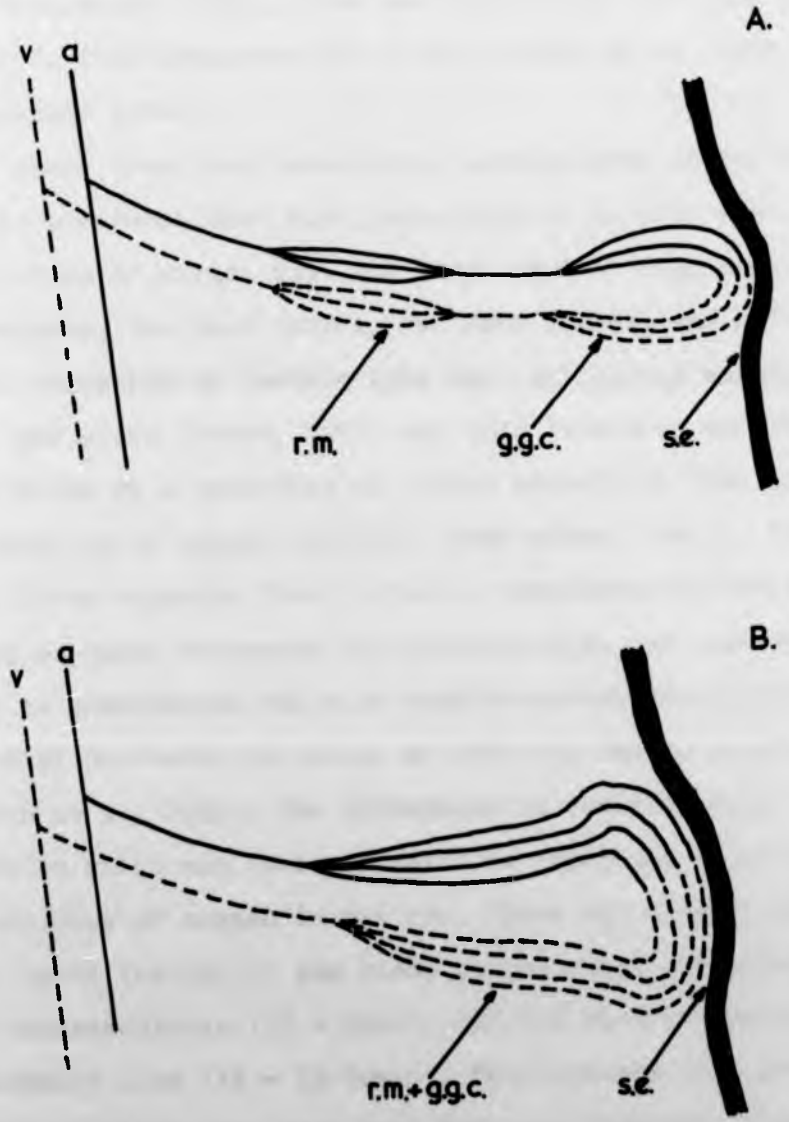

FIGURE 2

lle.

dans.

doids.

illaries)

elium)

Ruffel (1942) and Hartigay and Kuhn (1951), and was subsequently adapted for explanation of events in the rete by Scholander (1954), Kuhn and Kuhn (1961) and Kuhn et al (1963). Good summaries are given by Kuhn et al (1963) and Alexander (1966).

Apart from these anatomical arrangements in the rete three phenomena have been postulated to account for the secretion of oxygen into the lumen against high partial pressures, the Root effect, the Bohr effect, and salting out. Secretion of lactate into the capillaries occurs in the gas gland (Steen, 1963) and this liberates oxygen from the blood by a reduction of plasma solubility (salting-out) a lowering of oxygen affinity (Bohr effect) and a lowering of oxygen capacity (Root effect). Considered in isolation each of these phenomena can generate high gas pressures, but in combination and with counter-current multiplication partial pressures in excess of 2000 atm can be attained (Kuhn et al, 1963). The difference in reaction rate of the Root-on shift and Root-off shift is important in creating a build-up of oxygen in the rete (Berg and Steen, 1968). The acidification of the blood causes rapid dissociation of oxyhaemoglobin ($t\frac{1}{2}$ = 50ms), but the reverse process is extremely slow ($t\frac{1}{2}$ = 10-20sec). This ensures that the outgoing blood from rete contains less combined oxygen than the incoming blood, even though their plasma pO_2 and

pH may be almost identical. Thus the counter-current system of the rete can accumulate high partial pressures of free oxygen which eventually diffuses into the lumen of the bladder against the partial pressure gradient.

The means whereby gases leave the gas gland capillaries and move to the lumen of the swimbladder are not clear, but Fänge (1953) observed foaming of the surface of glands in actively secreting fish. The most reasonable explanation would be that gases simply diffuse in solution through the cells, however, lipids, microtubules and Golgi apparatus have all been implicated in this final part of the secretory process. Despite this, and other areas of contention it is now accepted that physoclists can secrete gases - mainly oxygen - into the bladder against very high partial pressures.

The process of resorption, by contrast, is one of passive diffusion of gas out of the bladder through the vascular area known as the oval. Not all physoclists possess an oval (Marshall, 1960, 1971), but in those that do it is a postereo-dorsally situated organ, richly supplied with blood vessels from the dorsal aorta and intercostal arteries and shown schematically in Fig. 3 . The inner layer of the bladder, the tunica interna, is modified in this area. It has an opposing set of circular and radial smooth muscle fibres forming an adjustable window over the thin epithelium covering the vascular plexus. The vascular and muscular portions of the

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Fig. 3. The gadoid oval. Ventral view.

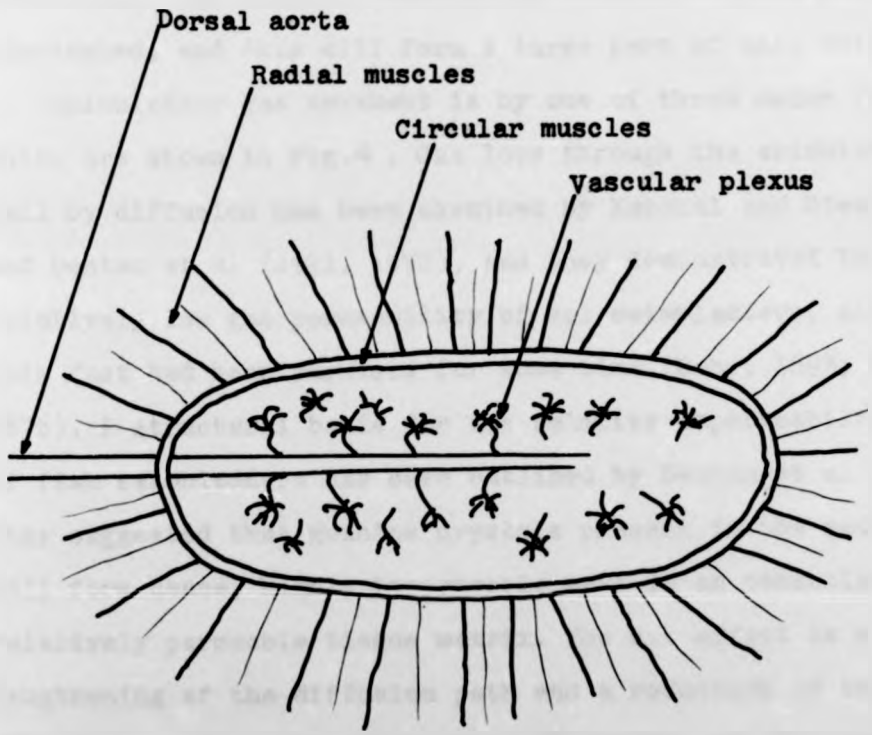

Dorsal aorta
Radial muscles
Circular muscles
Vascular plexus

FIGURE 3

oval are controlled by nerves and hormones (Fänge, 1953).

The process of resorption, being passive and easily accounted for, has not received as much scrutiny as the secretory process. Although the basic system is well understood, the means of control of resorption have not been elucidated, and this will form a large part of this thesis.

Swimbladder gas movement is by one of three major routes which are shown in Fig.4 . Gas loss through the swimbladder wall by diffusion has been examined by Kutchai and Steen (1971) and Denton et al (1971, 1972), and they demonstrated the relatively low gas permeability of eel swimbladders, although this fact had been surmised for some time (Bohr, 1894; Moreau, 1876). A structural basis for the relative impermeability of fish swimbladders has been outlined by Denton et al (1972). They suggested that guanine crystals present in the swimbladder wall form dense, highly impermeable mats in an otherwise relatively permeable tissue matrix. The net effect is a lengthening of the diffusion path and a reduction of the effective surface area over which diffusion can take place.

In considering the role of the oval, it seems a logical development to have an impermeable tissue as a basis for restriction of gas access to the vascular plexus. Denton et al (1972) described the guanine crystals of Conger conger as being in the tunica externa, and this has been confirmed in subsequent observations on Anguilla anguilla (L) and

Fig. 4. Summary of gas movement in physoclists.

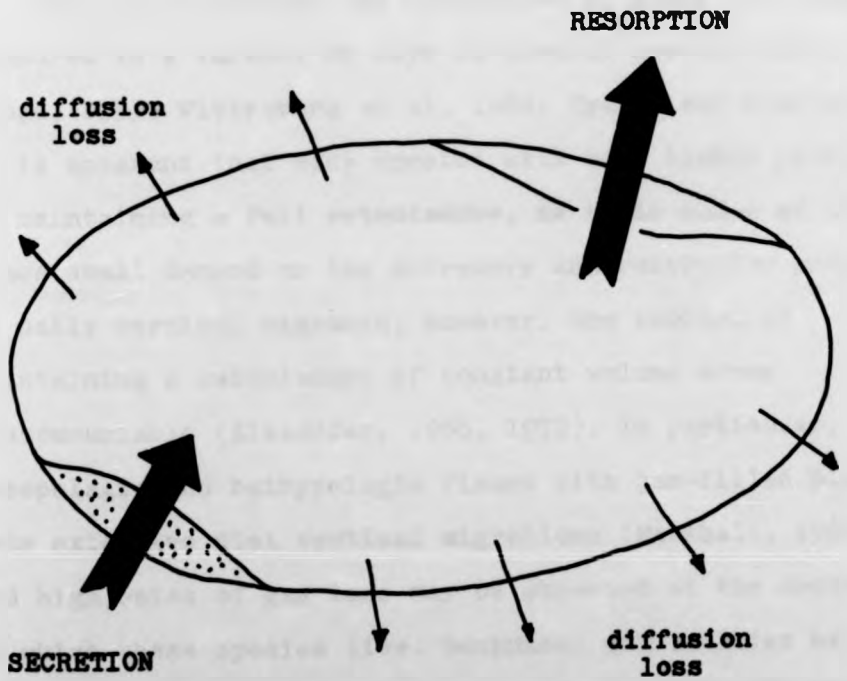


FIGURE 4

Synaphobranchus kaupi (Johnson) in this thesis. However, the dangers of generalisation in fish studies are evident here, as in a true physoclist equipped with an oval the guanine crystals would logically be located in the tunica interna, the oval being a specialised part of this layer.

Rates of secretion and resorption of gases have been measured in a variety of ways in several species (Bohr, 1894; Fänge, 1953; Wittenberg et al, 1964; Tytler and Blaxter,1973). It is apparent that many species will have little problem in maintaining a full swimbladder, as their modes of life place small demand on the secretory and resorptive processes. In daily vertical migrants, however, the problem of maintaining a swimbladder of constant volume seems insurmountable (Alexander, 1966, 1972). In particular, mesopelagic and bathypelagic fishes with gas-filled bladders make extensive diel vertical migrations (Marshall, 1960, 1972) and high rates of gas loss may be expected at the depths at which these species live. Technical difficulties have so far prevented measurement of rates of secretion and gas loss in these animals.

This thesis comprises a basic reassessment of oval function in the physoclist fish Pollachius virens (L). Morphology, histology, and pharmacology of the resorptive structures are redescribed in terms of the control of the oval. The capabilities of the vascular plexus and the oval are assessed independently in an effort to redefine

and quantify their roles in the control of gas resorption from the swimbladder. In addition a consideration of gas efflux from the swimbladder in general is presented in terms of its hydrostatic function.

The formal working link between Stirling University Biology department and the Scottish Marine Biological Association (S.M.B.A) has enabled the author to make use of research facilities aboard the N.E.R.C's research vessel R.R.S. Challenger. In this way it was possible to extend the scope of this work to consider some aspects of gas loss from representative British species of the order Anacanthini, which includes both inshore and deep-water marine fish. Similarly, it was possible to work on some aspects of swimbladder function in mesopelagic fish by participation in the 1975 joint S.M.B.A/ Marine Biological Association of the U.K. (M.B.A) cruise on R.R.S Challenger to Madeira.

Finally, a synthesis of past and present data will be made to attempt to provide a basis for a more complete understanding of the control of gas loss in the intact, true physoclist fish.

THE ANIMALS

In the study of oval function, the major experimental animal was the saithe Pollachius virens (L). This animal was chosen because it is a vertically migrating fish (Schmidt, 1955), it has a true physoclist swimbladder, and it can be obtained with relative ease in Scotland. The common eel Anguilla anguilla (L) has been widely used in the study of swimbladder physiology (Fänge, 1953; Steen, 1963a,1963b) but the morphology of its bladder makes much of its physiology sufficiently different from that of the true physoclist to preclude its further use in this thesis, apart from some minor comparative aspects.

Fairly regular saithe stocks were obtainable from the West coast of Scotland using a variety of fishing methods, including rod and line. Some animals were donated by the White Fish Authority Research Station at Ardtoe, Ardnamurchan. Others, particularly large fish, were kindly donated by Dr J.H.S.Blaxter of the S.M.B.A., Oban. The fish were transported to Stirling using the polythene bag / oxygen method and were held in recirculating, filtered sea water at $9^{\circ}C$ in large circular tanks of approximately $2m^3$ capacity. Feeding was either daily or on alternate days, on a mixed diet of chopped fish and trout pellets (Troutvit).

Freshwater eels of up to 50cm length were taken by electrofishing in tributaries of the River Forth. It was possible to keep these animals in lidded aquaria in the laboratory at room temperature for extended periods.

Two deep-frozen specimens of the deep-water gadoid Phycis blennoides (Brünnich) were provided by Dr J.D.M.Gordon of the S.M.B.A., initially for macroanatomical examination. Dissection of these prompted work on a wider range of species within the order Anacanthini. This order includes the codfishes (Gadidae), the hakes (Merluccidae), the deep-sea cod-like fishes (Moridae) and the rat-tails (Macrouridae). They are (with a single exception, the Burbot Lota lota (L)) all marine, and have a true physoclist bladder with a single chamber and completely closed from the environment. They range from coastal waters to the deep ocean in habitat and many are of great commercial importance.

Stirling University and the S.M.B.A have had a strong working association in teaching and research for some years. It was possible, because of this link, to take part in four cruises of the N.E.R.C's research vessel R.R.S. Challenger. The first of these, to Madeira in 1975, enabled work to be carried out on some aspects of swimbladder function in mesopelagic fish and some of this work has already been published (Ross, 1976). The further three cruises were to a study area including the Hebridean Terrace and the Rockall Trench (56° - 57° N and 9° - 11° W) where a seasonal study of the biology and distribution of slope-dwelling fishes is

in progress.

The deep Anacanthines examined in various sections of this thesis were;

Coryphaenoides rupestris	(Gunnerus)
Nezumia aequalis	(Günther)
Trachyrhynchus murrayi	(Günther)
Nematonurus armatus	(Hector)
Chalinura mediterranea	(Giglioli)
Coelorinchus caelorhinchus	(Risso)
Coelorinchus occa	(Goode&Bean)
Malacocephalus laevis	(Lowe)
Molva dypterygia	(Pennant)
Brosme brosme	(Ascanius)
Micromesistius poutassou	(Risso)
Merluccius merluccius	(L)
Mora moro	(Risso)
Lepidion eques	(Günther)
Halargyreus johnsonii	

In addition to these animals the deep-water eel Synaphobranchus kaupi (Johnson) and the Heteromid Notacanthus bonaparti (Risso) were examined.

Fishing methods employed in this thesis are all fairly standard procedures. Mesopelagic fish were taken by rectangular midwater trawl (RMT) of $8m^2$ or $90m^2$ mouth area (Clarke, 1969). Demersal fish were taken by long-lining and by Granton otter-

trawl.

Most of the experiments were carried out aboard ship with the exception of the guanine assays, some histology and dissections of fine nerves.

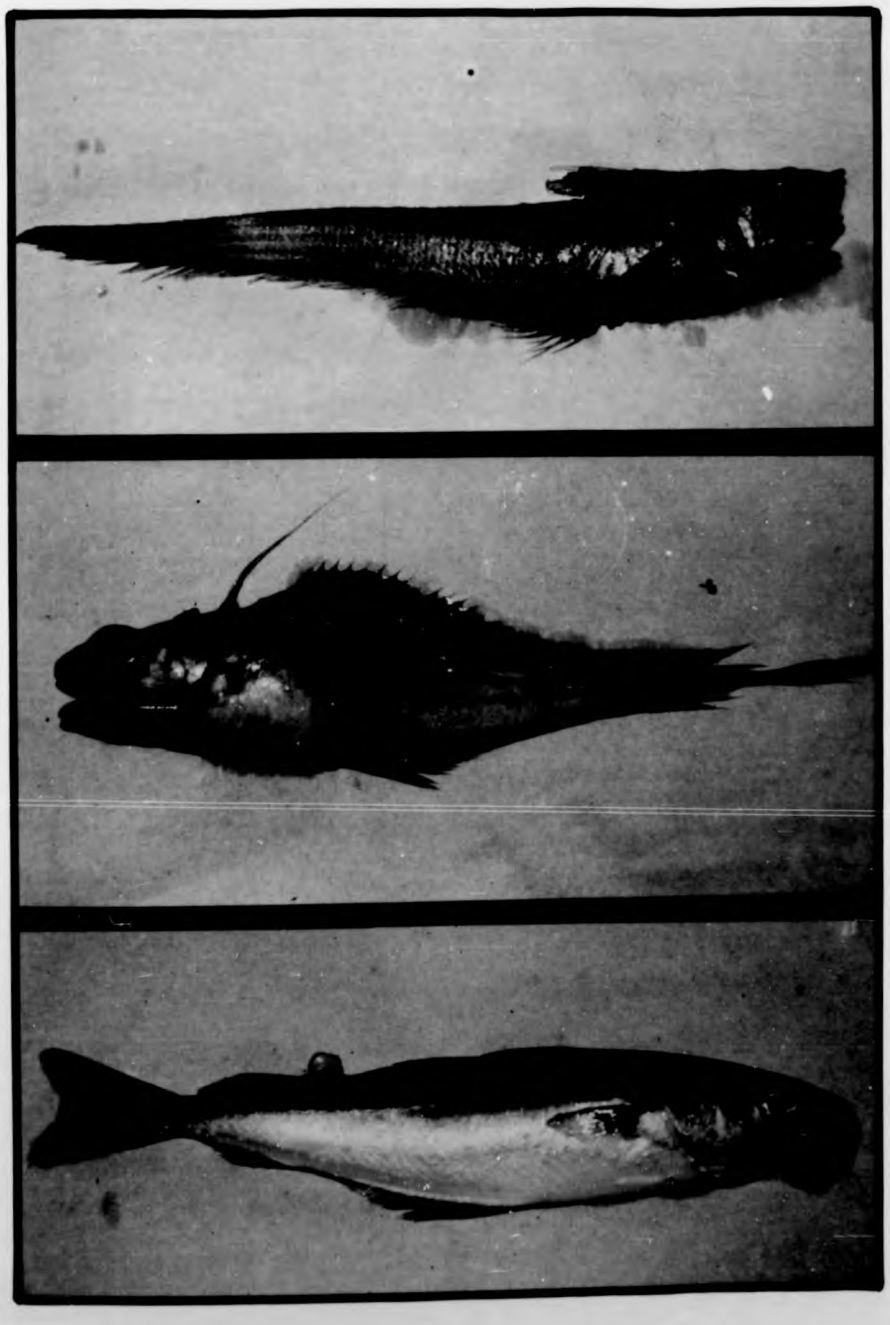
Representatives of the families Gadidae, Moridae and Macrouridae are shown in Plate 1.

PLATE 1. Representative Anacanthines.

A. Nezumia aequalis (Macrouridae)

B. Lepidion eques (Moridae)

C. Pollachius virens (Gadidae)



THE STRUCTURE OF THE SWIMBLADDER

The macro- and microanatomy of the swimbladders of many fish species have been described by many workers (Fänge, 1953, 1958; Fänge and Wittenberg, 1958; Marshall, 1960; Dorn, 1961; Fahlen, 1967; Bone, 1971, 1973). It can be said with conviction that teleosts are an extremely diverse group of animals and generalisations in terms of structure or function within the group are not advisable. With this in mind it was thought useful to survey in reasonable detail the macro- and microanatomy of Anacanthine swimbladders, with particular reference to the structures involved in regulation of gas efflux from the bladder.

The general structure of the swimbladders of all species was examined initially using standard dissection procedures. This work was largely confirmatory and served as a familiarisation procedure. Useful techniques employed involved allowing tissues to degenerate in tapwater overnight, especially where nerves were to be exposed in the anterior kidney and the general dorsal aspect of the swimbladder. In the case of large specimens a dental mirror was used to examine the interior surfaces prior to laying open. This allowed assessment of the structure of the tunica interna before all tension was removed from the wall. Methylene blue and osmic acid bulk staining were used

to trace non-myelinated and myelinated nerves respectively.

The general histology of tissues was investigated in paraffin embedded sections following buffered formalin fixation. Haematoxylin and eosin staining was employed routinely except where the nature of connective tissue layers was investigated. In this case Wiegerts and Van Giesens stain, Verhoeffs elastic stain or the specific orcein elastica stain (Mahoney,1969) was used.

Fänge (1953) gives a good description of the swimbladder of the cod Gadus morhua (L). The general outline of the saithe swimbladder is similar to that of the cod and it is an elongate organ occupying about one third to one half of the body length. Dorsally it is closely bound to the vertebral processes and the tissue of the kidney, and the posterior tip of the organ runs into the haemal arch. Ventrally it is bounded by a serosal membrane which is continuous with the peritoneum- the bladder is thus retroperitoneal. A well-defined gas gland exists in the anterior ventral aspect, and postereo-dorsally an oval is found. This oval is present in all gadoids in one form or another(Hagman, 1929;Fänge, 1953). The situation and general morphology of the saithe swimbladder is shown in Fig.5.

The vascular supply and innervation of the bladder has been described by Fänge (1953), Jones and Marshall (1953),

Fig. 5. The situation and general morphology of the swimbladder of Pollachius virens.

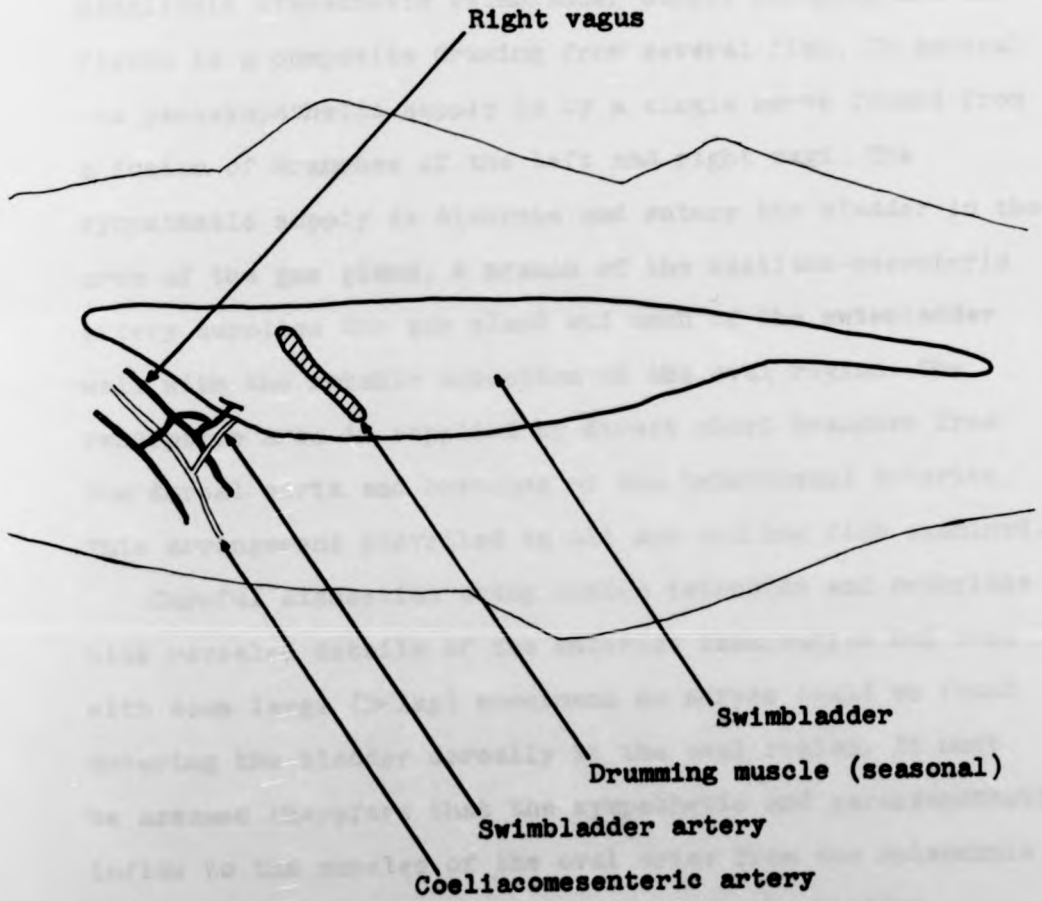

Right vagus
Swimbladder
Drumming muscle (seasonal)
Swimbladder artery
Coeliacomesenteric artery

FIGURE 5

and Steen (1970). This thesis confirms the general gadoid layout in saithe and the innervation and blood supply are shown in Fig. 6. Some variation in details of the post-ganglionic sympathetic swimbladder supply occurred and the figure is a composite drawing from several fish. In general the parasympathetic supply is by a single nerve formed from a fusion of branches of the left and right vagi. The sympathetic supply is discrete and enters the bladder in the area of the gas gland. A branch of the coeliaco-mesenteric artery supplies the gas gland and much of the swimbladder wall with the notable exception of the oval region. The resorptive area is supplied by direct short branches from the dorsal aorta and branches of the intercostal arteries. This arrangement prevailed in all Anacanthine fish examined.

Careful dissection using osmium tetroxide and methylene blue revealed details of the anterior innervation but even with some large (> 1kg) specimens no nerves could be found entering the bladder dorsally in the oval region. It must be assumed therefore that the sympathetic and parasympathetic inflow to the muscles of the oval arise from the splanchnic and vagus nerves respectively and enter the bladder anteriorly. Work by Bohr (1894), Fänge (1953) and in this thesis seems to confirm this arrangement.

The histology of the swimbladder wall is complex and varied. Fänge (1953) gave a reasonable basis for the

Fig. 6. The nerve and blood supply to the swimbladder of Pollachius virens.

(a = arterial input to bladder)
(c.g. = coeliac ganglion)
(cm.a. = coeliacomesenteric artery)
(l.v. = left vagus)
(p = parasympathetic input to bladder)
(r.v. = right vagus)
(s = sympathetic input to bladder)
(s.g. = spinal ganglia)
(s.n. = splanchnic nerve)

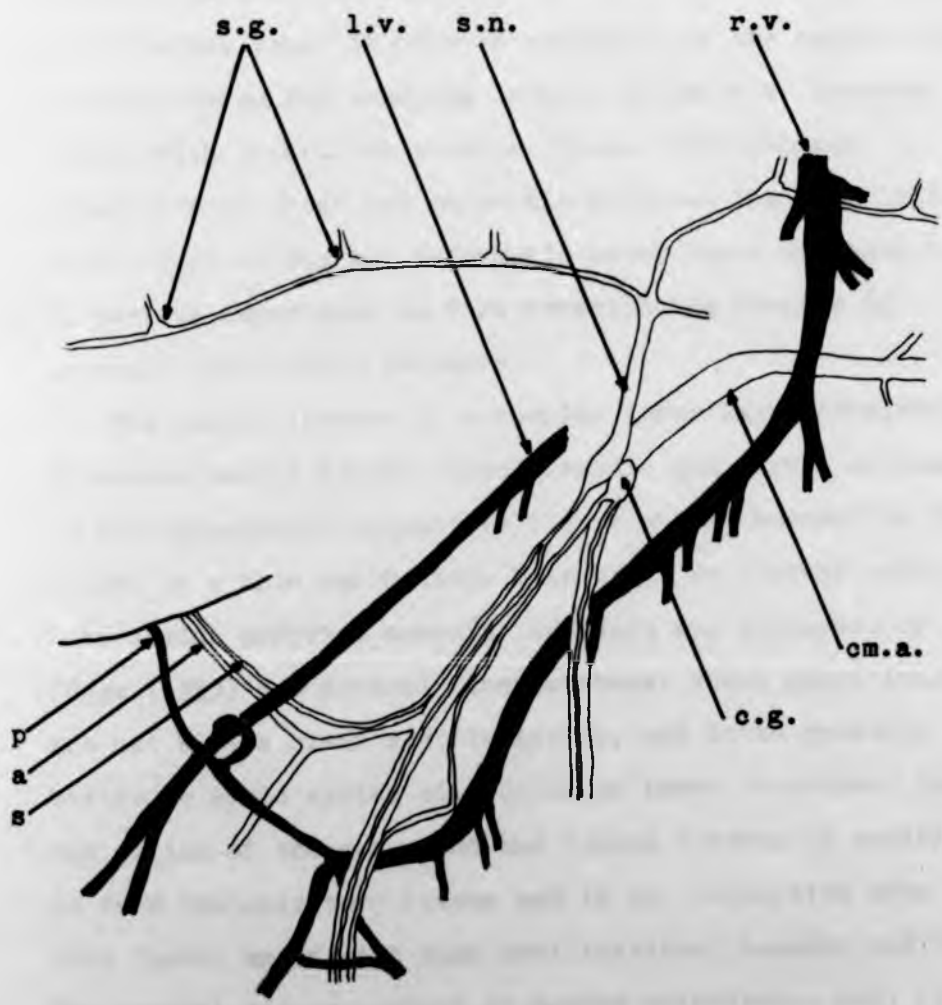


FIGURE 6

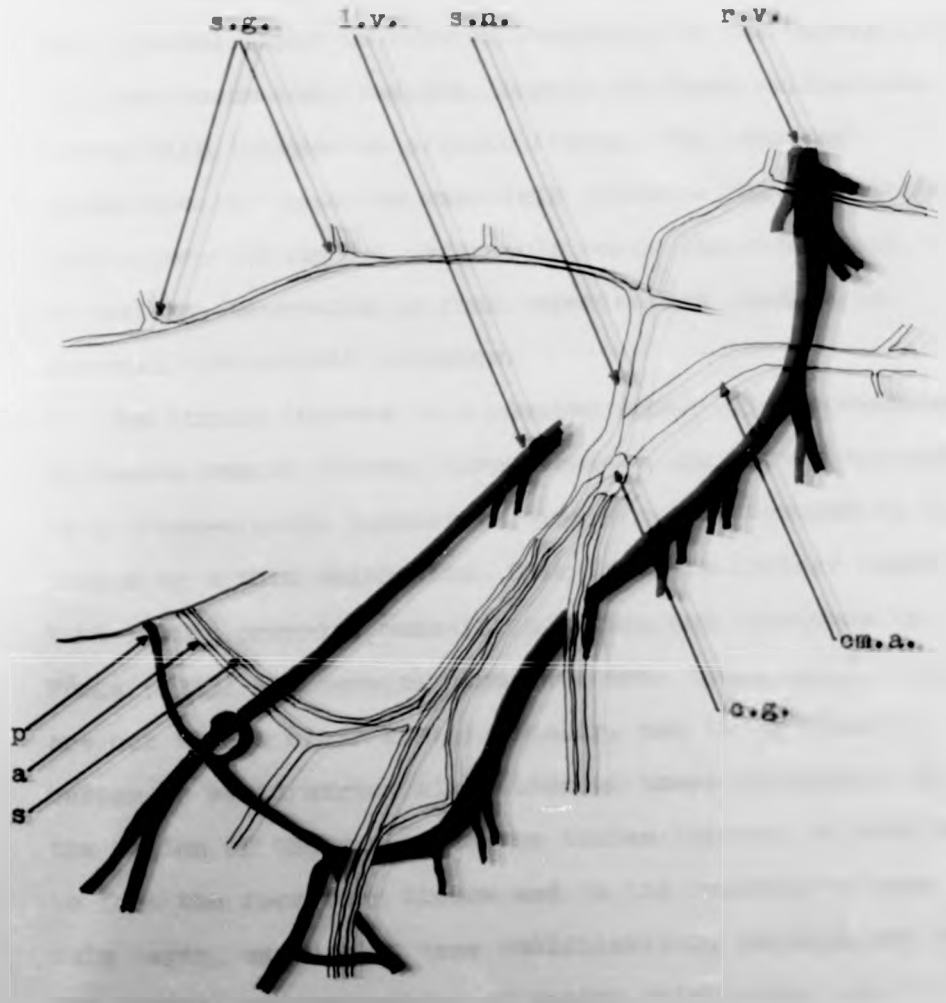


FIGURE 6

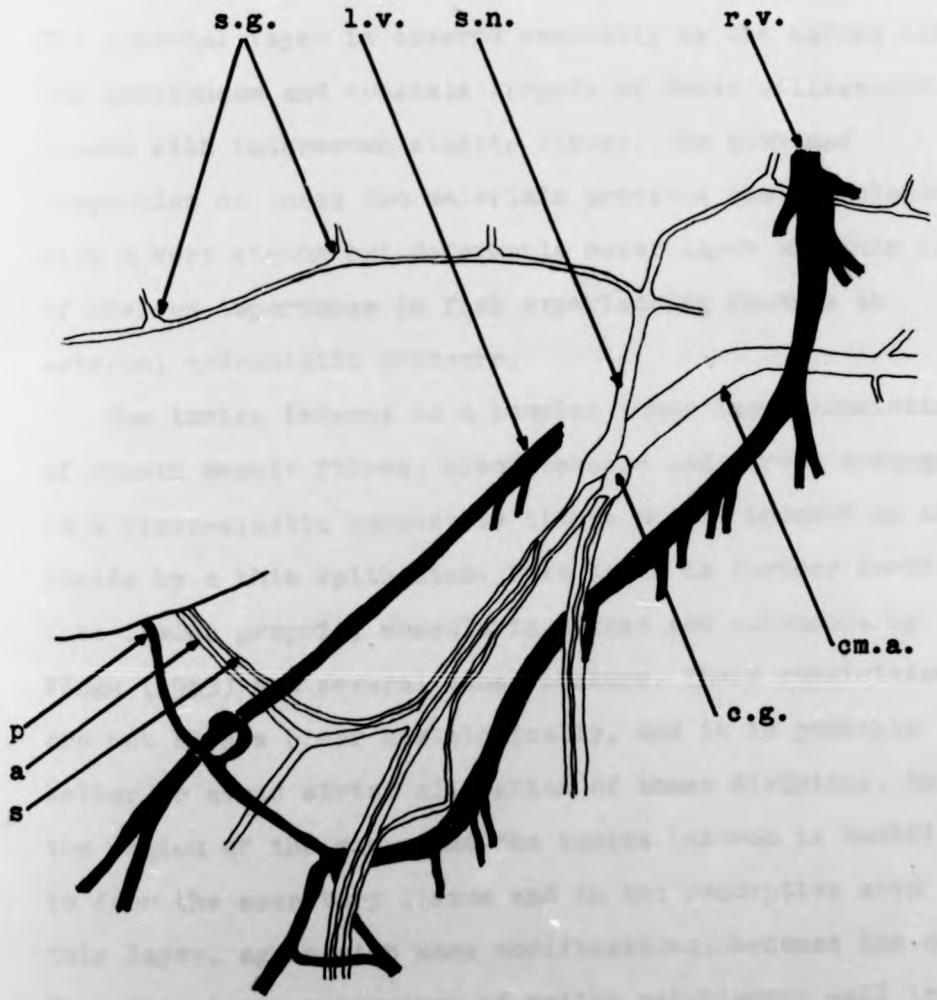


FIGURE 6

nomenclature of the layers and his system is used in this thesis. The teleost swimbladder generally has two very distinct layers, the tunica interna and the tunica externa. The external layer is covered ventrally by the serosa lining the peritoneum and consists largely of dense collagenous tissue with interwoven elastic fibres. The combined properties of these two materials provides the swimbladder with a very strong but deformable outer layer and this is of obvious importance in fish experiencing changes in external hydrostatic pressure.

The tunica interna is a complex inner layer consisting of smooth muscle fibres, blood vessels and nerves arranged in a visco-elastic connective tissue matrix bounded on the inside by a thin epithelium. This layer is further subdivided into lamina propria, muscularis mucosa and submucosa by Fänge (1953) and several other workers. These subdivisions are not always clear histologically, and it is probably better to avoid strict allocation of these divisions. In the region of the gas gland the tunica interna is modified to form the secretory tissue and in the resorptive area this layer, again with some modifications, becomes the oval. The general microstructure of saithe swimbladder wall is shown in Fig.7. and Plate 2.

In physoclists resorption is effected by opening the oval membrane to expose the vessels of the underlying plexus

Plate 2. The microanatomy of the swimbladder of *Pollachius virens*.

A. Whole swimbladder wall. (H & E)

B. Tunica externa, elastic fibres.
(Verhoeffs elastic stain)

C. Blood vessels in the oval plexus.
(Wiegert and Van Giesen, Thick section)

D. Oval membrane and adjacent tissue.
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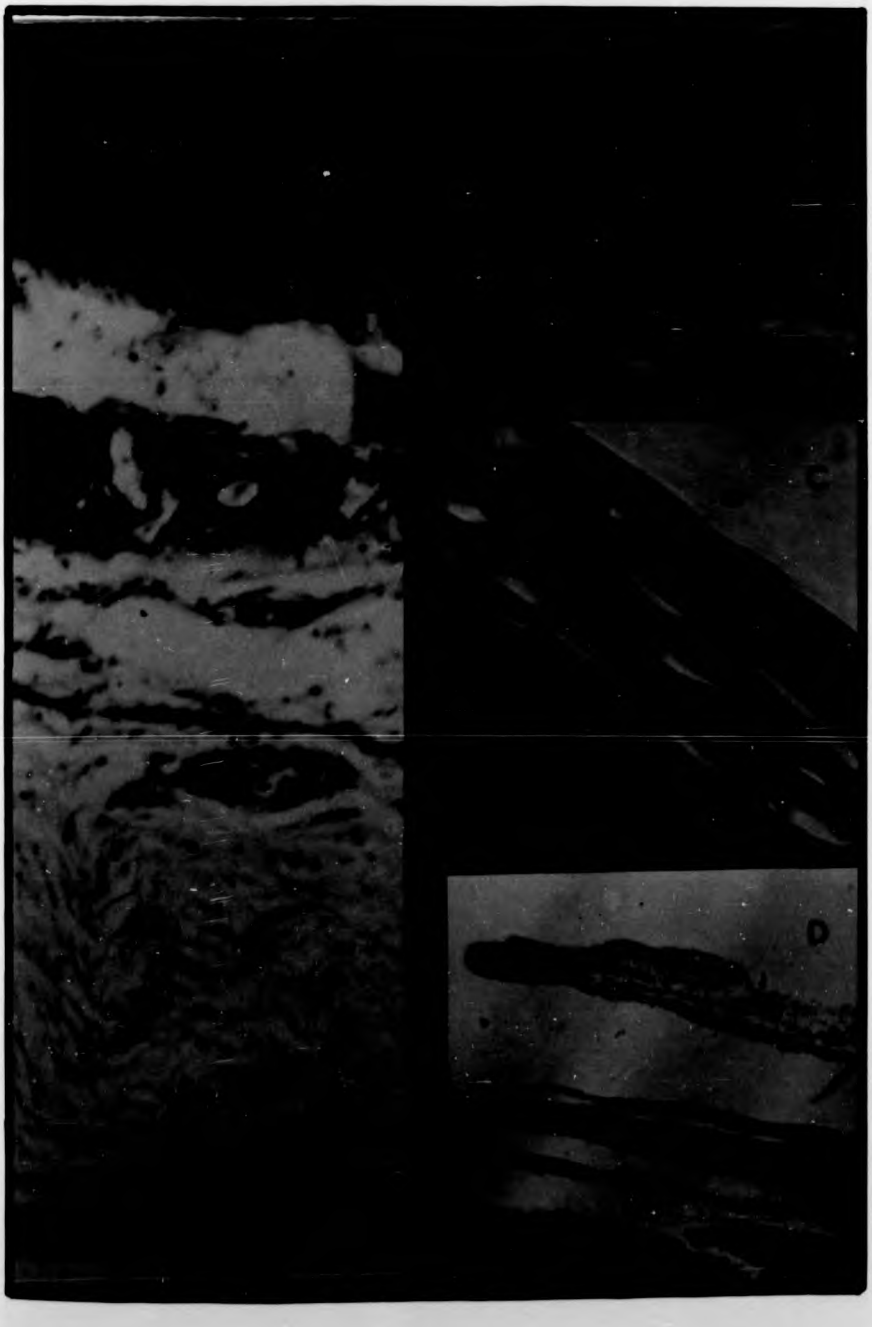
C
D

Fig. 7. The microanatomy of the swimbladder of Pollachius virens.

(Diagrammatic representation of Plate 2)

(e. = epithelium)
(e.f. = elastic fibres)
(b.v. = blood vessels in plexus)
(k. = kidney)
(o.m. = oval membrane)
(s.m. = submucosa)
(t.ext. = tunica externa)
(t.int. = tunica interna)
(L = lumen of bladder)

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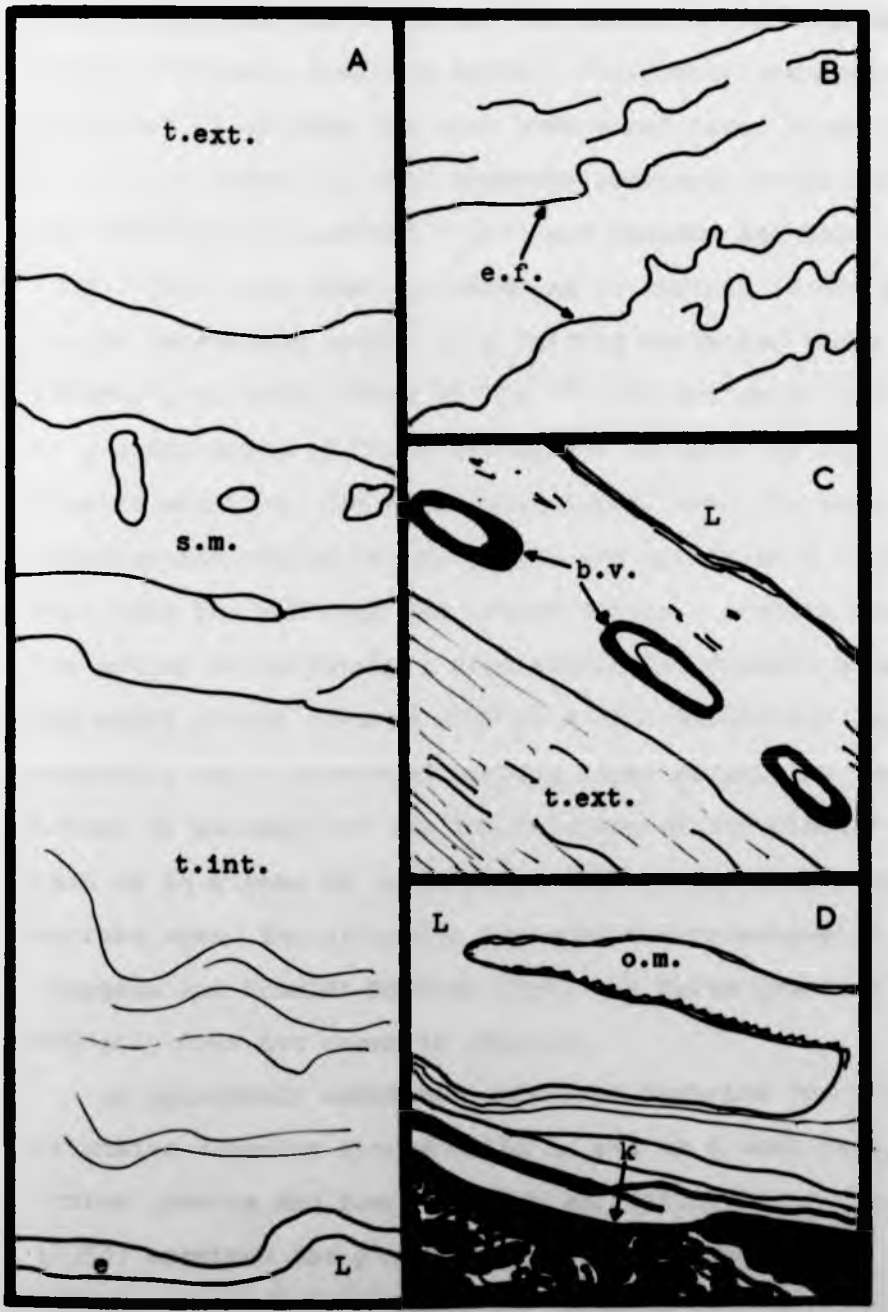

FIGURE 7

and dilating the blood vessels. In this condition gases will diffuse between the lumen and the blood for as long as a partial pressure gradient exists. Conversely resorption is prevented by closing the oval over constricted blood vessels. The way in which the oval membrane operates during resorption was disputed by Woodland (1913) and Nusbaum and Reis (1905, 1907). There was some confusion as to whether it had a simple stretching action or a folding mechanism these alternatives being shown in Fig. 8. Its action is probably by a combination of these mechanisms assisted by the visco-elastic matrix of the submucosal layer. Over the most vascularised region of the plexus the action is a folding one. Once the membrane has opened beyond a certain point the action is certainly a stretching one, finally exposing the whole plexus covered only by a thin epithelial layer centrally and a stretched mucosal layer marginally. The extent of movement of the inner layers of the bladder is such as to expose or cover about 40% of the total internal surface area. The situation depicted diagrammatically by Lappenas and Schmidt-Nielsen (1977) is quite dramatic and probably does not occur in gadoids.

As previously mentioned, the oval membrane overlies an extensive vascular plexus which is set in a much reduced tunica interna and bounded by an epithelial layer. Fänge (1953) examined the plexus in cod, Gadus morhua, and

Fig. 8. The mechanisms of oval action.

A. Stretching. (Nusbaum and Reis, 1905)

B. Folding. (Woodland, 1913)

(b.v. = blood vessels)
(c.m. = circular muscle)
(r.m. = radial muscle)
(t.ext. = tunica externa)
(t.int. = tunica interna)

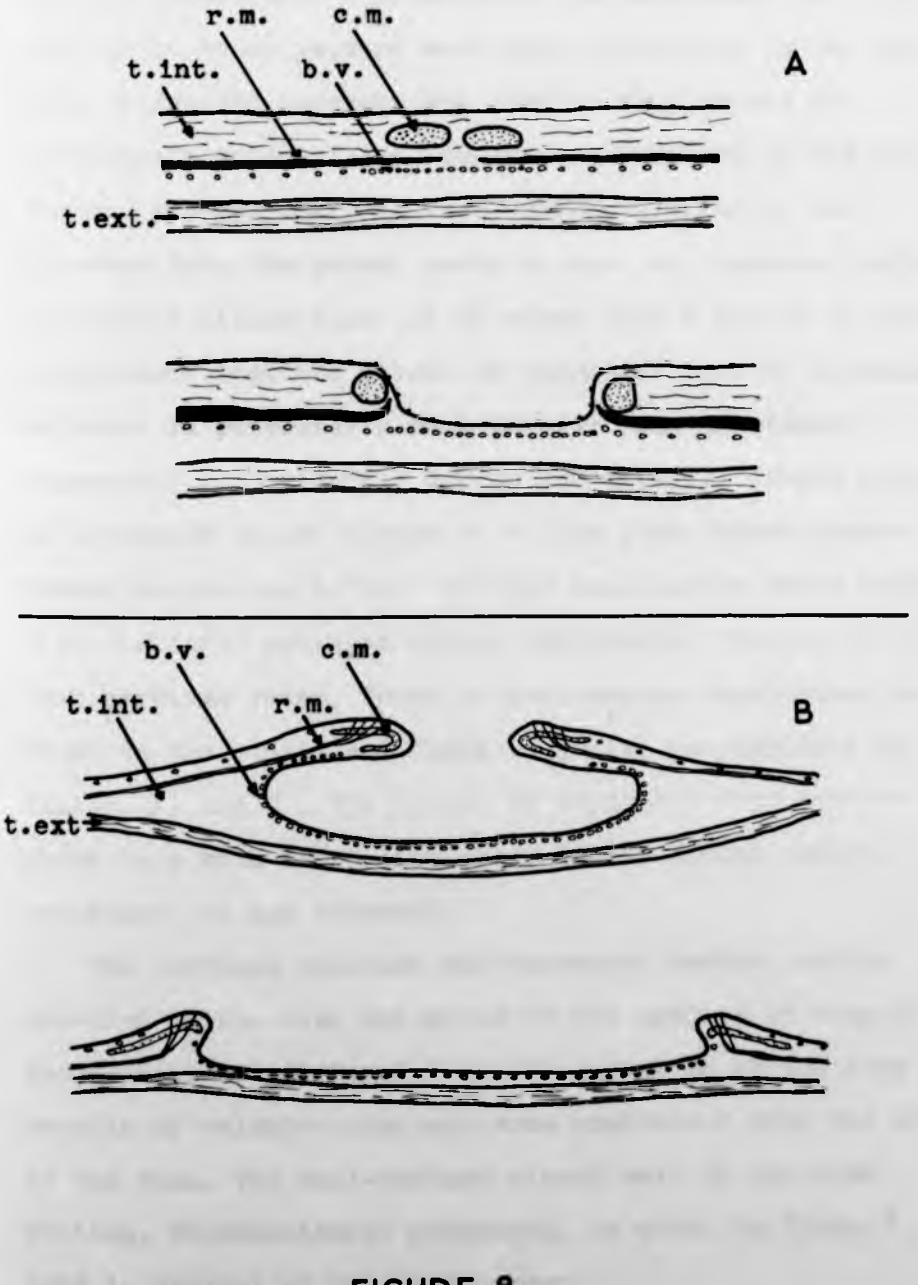

FIGURE 8

described contractile arterioles. He estimated that there were 20 to 40 or perhaps more such arterioles in the cod oval. Pigmented neoprene was used in this thesis to demonstrate arterial and venous contributions to the oval plexus. Appropriately coloured neoprene solution was injected into the dorsal aorta or the post cardinal veins in freshly killed fish. It is clear from a series of such experiments that the extent of vasomotor control in saithe at least is potentially much greater than previously suggested. Approximately 200 to 250 arterial trunks could be discerned in the plexus of a 150g fish. These trunks branch to produce a "fan" of fine capillaries which connect with similarly arranged venous capillaries leading to the post cardinal veins. These arterio-venous anastomoses are shown in the single and double injected preparations in Plates. 3 , and 4 . The plexus is separated from the gas phase by a thin epithelium which itself offers little resistance to gas movement.

The combined muscular and vasomotor control system embodied in the oval was noted in six species of deep-sea Anacanthine and differed from the layout in saithe only in details of relative size and area consistent with the size of the fish. The well-defined closed oval of the Blue Whiting, Micromesistius poutassou, is shown in Plate. 5 , and this is typical of the whole group.

Plate 3. The vascular plexus of the oval.

Demonstrated by injection of red latex into the dorsal aorta.

PLATE 3

PLATE 3

Plate 4. The vascular plexus of the oval.

Arterio-venous anastomoses shown by injection of red latex into the dorsal aorta and blue latex into the post cardinal veins.

PLATE 4

PLATE 4

Plate 5. The oval of the Blue Whiting.

Micromesistius poutassou.

Plate 5. The oval of the Blue Whiting.

Micromesistius poutassou.

PLATE 5

PLATE 5

The ultrastructure of swimbladders has been described by several authors (Dorn, 1961; Morris and Albright, 1975), and was not reexamined in this work. An interesting discrepancy appears in the interpretation of electronmicrographs by certain authors. Phleger and Holz (1973) show photographs of lipid foams from Coryphaenoides fernandeziensis swimbladder, and they suggest that it is a bilayered reformed membrane structure. Morris and Albright (1975) describe cell processes occurring in the submucosa of Opsanus tau (L) and Lappenas and Schmidt-Nielsen (1977) decided that similar structures in Leiostomus xanthurus (Lacepede) were probably folded guanine crystals. Phleger and Holz (1973) are certainly correct in their statements but Lappenas and Schmidt- Nielsen (1977) and Morris and Albright (1975) appear initially to be at variance.

Denton et al (1972) noted that guanine crystals visible by plane-polarised light in fresh material could not be seen in tissue prepared for histology. They suggested that the crystals were dislodged on sectioning and similar attempts in this thesis produced no better results, even with thick sections of up to 30 μm. There is no reason to suppose that fixation and sectioning for electronmicroscopy is any better in this respect and it could be concluded that Lappenas and Schmidt-Nielsen were looking at cell processes - effectively bilayered membranes.

Polarised light microscopy of thick frozen sections was used to partly resolve this problem. When viewed in this way the guanine crystals could be clearly seen in the submucosal region of the tunica interna. The crystal layer is quite thick in saithe, constituting about half the thickness of the tunica interna, the position of the birefringent crystals in fresh sectioned material is shown in Fig. 9 . The location of this crystal layer by electron-microscopy involves several processing steps and although Lappenas and Schmidt-Nielsen have interpreted their results correctly, the use of fresh material as in this study is far more reliable. The importance of the extent and location of these purine crystals and their role in the control of gas loss is discussed in a later section. The typical appearance of purine crystals in the tunica interna of saithe swimbladder is shown in Plate. 6 .

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Fig. 9. The location of purine crystals in frozen-sectioned material by polarised light microscopy.

(c.f. = collagen fibres)*
(s.m. = submucosa)
(t.ext. = tunica externa)
(t.int. = tunica interna)

*The orientation of the collagen fibres confirms the direction of sectioning.

L.S.

T.S.

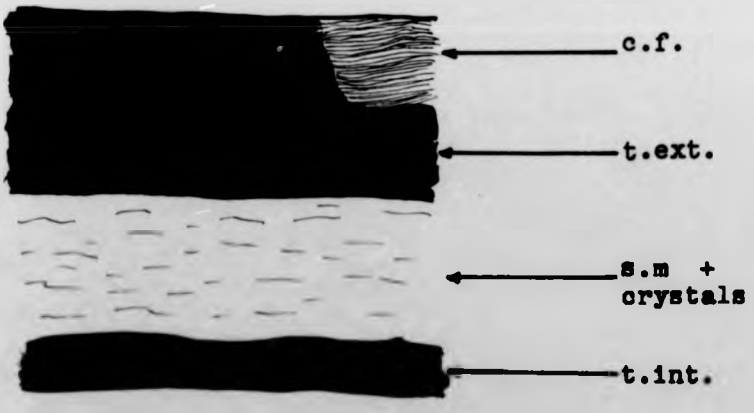
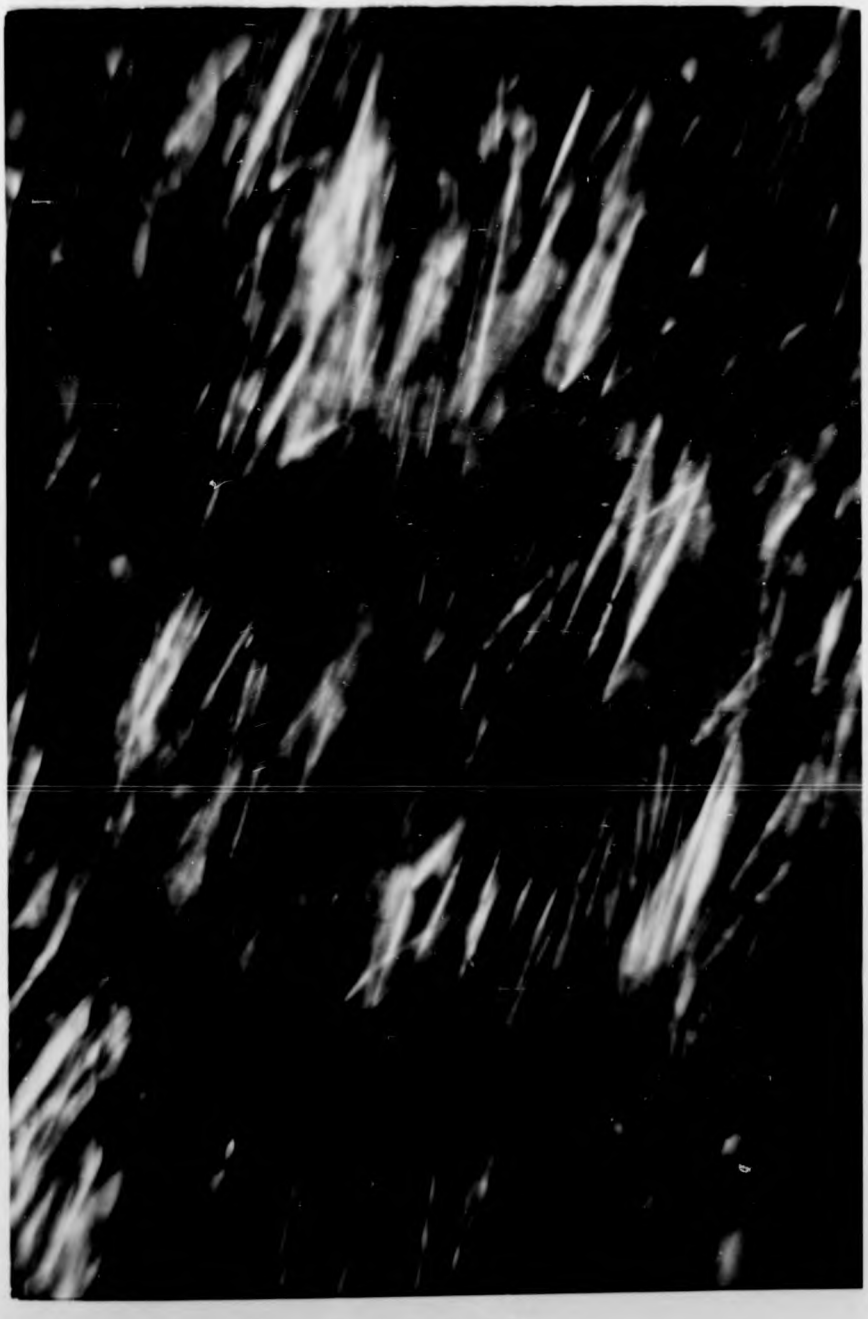


FIGURE 9

Plate 6. Purine crystals in the tunica interna of Pollachius virens.



THE INNERVATION OF THE RESORPTIVE STRUCTURES

In the investigation of the innervation and pharmacology of saithe swimbladder use was made of a saline specially developed for the purpose. This was based on a blood ion analysis of six fish which had been kept in the Stirling aquarium. The blood chemistry results and derived saline formula are given in Appendix. 1. This saline was able to maintain cleaned saithe intestine in a contractable state for seven days at 4°C. The formulation of Young (1933) is used widely by those working on marine teleosts, but in view of its specificity the saithe formula was thought to be more useful.

Throughout the pharmacological investigations, only the influence of drugs and blocking agents of major significance was assessed. This work was considered to be confirmatory in nature as the pharmacology of these tissues has been examined in other species by other workers (Fänge, 1953; Nilsson and Fänge, 1967; Nilsson, 1971).

In fish of a reasonable size, the vagal and splanchnic innervation of the bladder can be exposed satisfactorily enabling electrical stimulation of these nerves. The nerves were stimulated using a Palmer stimulator and silver or stainless steel hook electrodes. Vagal stimulation produced

a marked opening of the oval and this is consistent with results obtained by Nilsson (1971). Stimulation of the splanchnic nerve caused slight opening of the oval, and not the expected closure. This may have been due to electrical leakage within the preparation stimulating the radial muscle fibres. It is interesting to note, however, that Nilsson (1971) was unable to produce contraction in the circular muscle electrically, and he reported relaxation of his preparations. Closure of the oval appears to be related to cessation of vagal output (Bohr, 1893; Nilsson, 1971). No nerve-mediated contraction of the circular muscle was produced in this work, but it could be induced to contract by direct electrical stimulation, as could the radial muscle. Thus the proposal by Nilsson (1971) that the oval closes by cessation of vagal action rather than by a direct nervous effect seems to be largely substantiated.

Simple muscle pharmacology was investigated initially using isolated whole preparations of the oval pinned to a cork board, Fig.10a. The preparation was drip-fed with saline in which drugs were included as required by injection into the supply pipe. This scheme allowed observation of drug effects on the complete muscle system.

Fänge (1953) showed the responses of the whole isolated oval. His findings are largely upheld in this work in that adrenaline causes rapid opening of the oval and acetylcholine

Fig.10. Systems used to investigate the pharmacology of the oval muscles.

A. Isolated oval preparation.

B. Balanced frontal-writing lever.

C. R.C.A. valve-transducer.

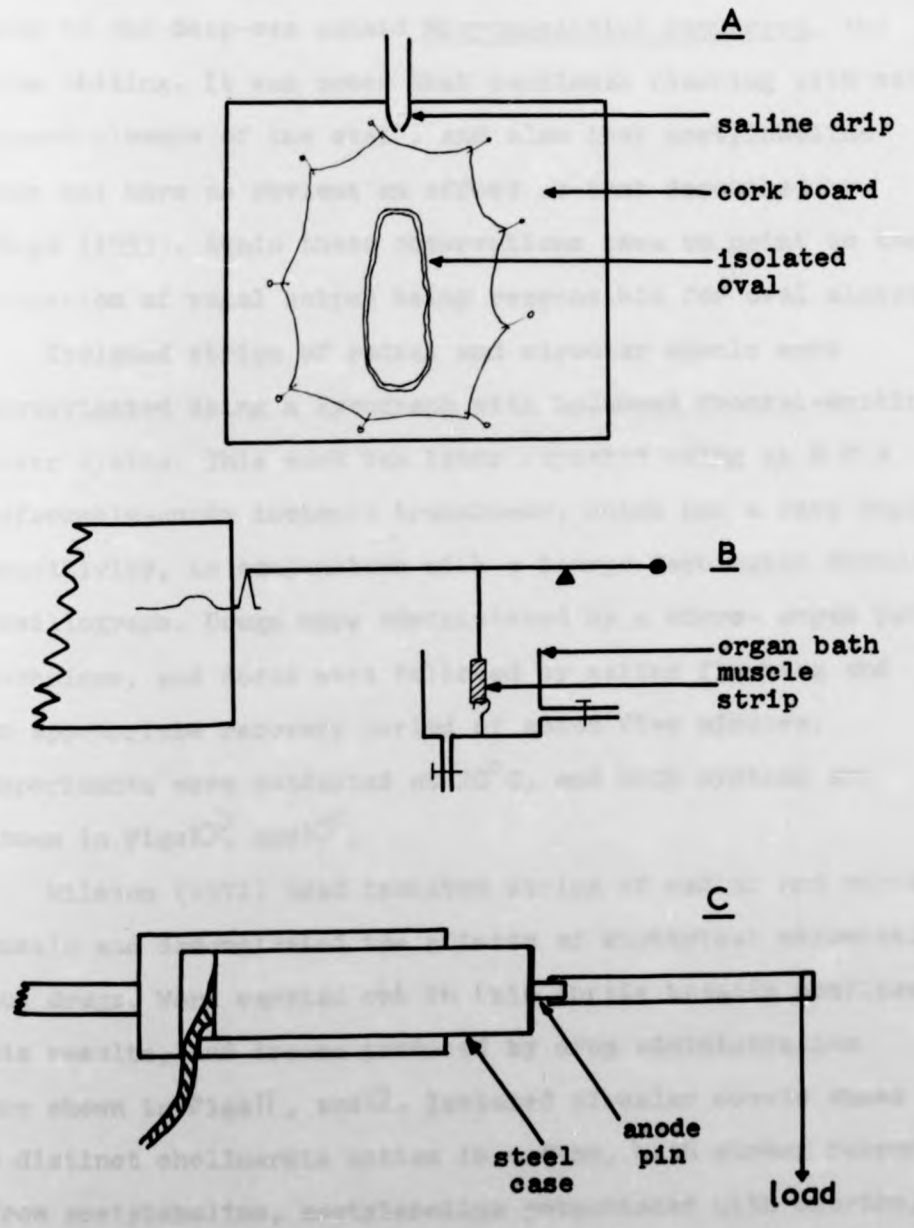

A
saline drip
cork board
isolated
oval
B
organ bath
muscle
strip
C
steel
case
anode
pin
load

FIGURE 10

has a closing effect in saithe. A similar observation was made in the deep-sea gadoid Micromesistius poutassou, the blue whiting. It was noted that continual flushing with saline caused closure of the oval , and also that acetylcholine does not have as obvious an effect as that described by Fänge (1953). Again these observations seem to point to the cessation of vagal output being responsible for oval closure.

Isolated strips of radial and circular muscle were investigated using a kymograph with balanced frontal-writing lever system. This work was later repeated using an R.C.A deformable-anode isotonic transducer, which has a very high sensitivity, in conjunction with a George Washington MD400/2 oscillograph. Drugs were administered by a micro- organ bath technique, and doses were followed by saline flushing and an appropriate recovery period of about five minutes. Experiments were conducted at 20°C, and both systems are shown in Figs10^{b}, and10^{c}.

Nilsson (1971) used isolated strips of radial and circular muscle and demonstrated the effects of electrical stimulation and drugs. Work carried out in this thesis broadly confirms his results, and traces produced by drug administration are shown in Figs11 , and12. Isolated circular muscle shows a distinct cholinergic action in saithe, with strong responses from acetylcholine, acetylcholine potentiated with eserine, and carbamylcholine potentiated with eserine. The response

Fig.11. The pharmacology of saithe oval circular muscle.

1. Acetylcholine (2×10^{-5})
2. Adrenaline (2×10^{-4})
3. Hexamethonium bromide (10^{-4})
   Acetylcholine (10^{-4})
4. Atropine (10^{-4})
5. Eserine (2×10^{-5})
   Acetylcholine (2×10^{-4})
6. Eserine (2×10^{-4})
   Carbamylcholine (10^{-4})

Drug concentrations in parentheses in g/cm^3.

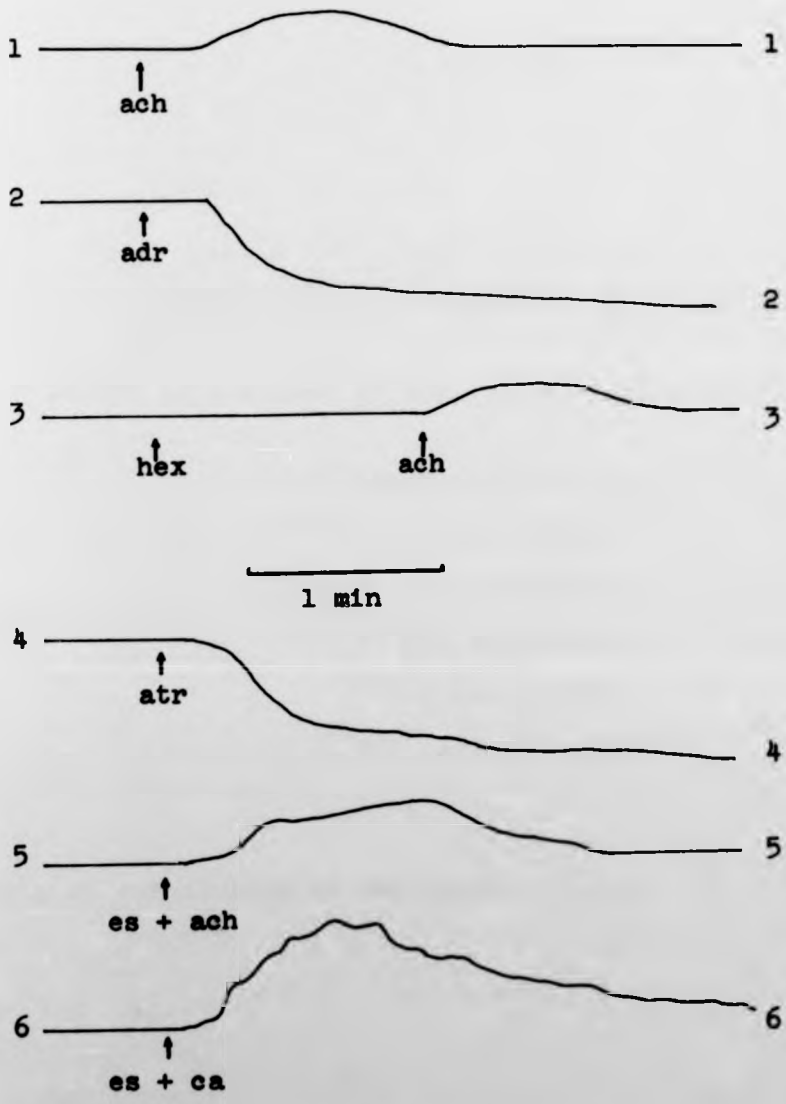


FIGURE 11

Fig.12. The pharmacology of saithe oval radial muscle.

1. Adrenaline ($2x10^{-4}$)
2. Acetylcholine ($2x10^{-4}$)
3. Noradrenaline ($2x10^{-4}$)
4. Yohimbine (10^{-4})
   Adrenaline (10^{-4})
5. Isoprenaline (10^{-4})

Drug concentrations in parentheses in g/cm^3.

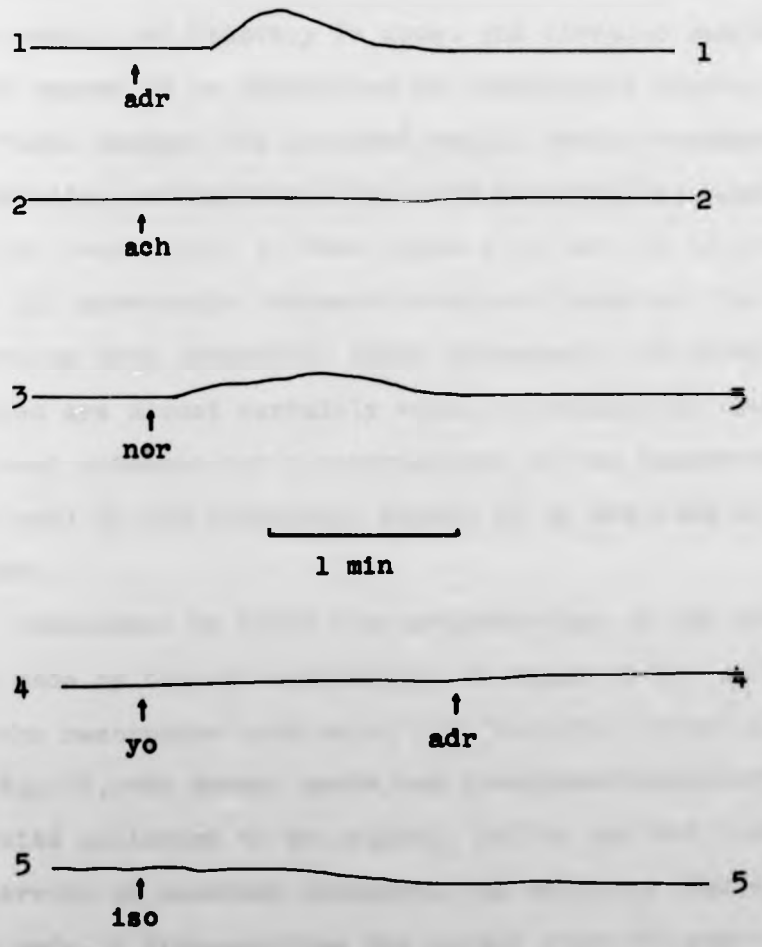


FIGURE 12

is not blocked by hexamethonium, a largely ganglionic-blocking drug, but strong relaxation of the muscle is produced by atropine, suggesting a steady discharge of acetylcholine from active post-ganglionic terminals within the preparation. Adrenaline produces a strong relaxation of the muscle and recovery is slow. The circular muscle would thus appear to be innervated by cholinergic fibres, possibly of vagal origin. The isolated radial muscle responds to adrenaline and noradrenaline with isoprenaline causing slight relaxation. It thus appears to have an alpha- action and all adrenergic responses were abolished by the alpha-blocking drug yohimbine. These adrenergic post-ganglionic fibres are almost certainly vagal in origin and there is no real evidence for a contribution to the innervation of the oval by the splanchnic nerve, as in the case of the gas gland.

Assessment of blood-flow pharmacology in the plexus was made by topical application of drugs to the epithelium of the resorptive area using the "isolated trunk" preparation of Fig.13. The dorsal aorta was cannulated anteriorly and ligated posterior to the plexus. Saline was fed from a reservoir at constant pressure. The relative flow estimation was made by drop-counting the output from the post-cardinal veins, the results being recorded on a drop-counter and coupled ratemeter (George Washington oscillograph). The technique was hampered by output from the intercostal

Fig.13. The "isolated trunk" preparation used to assess the pharmacology of the oval plexus.

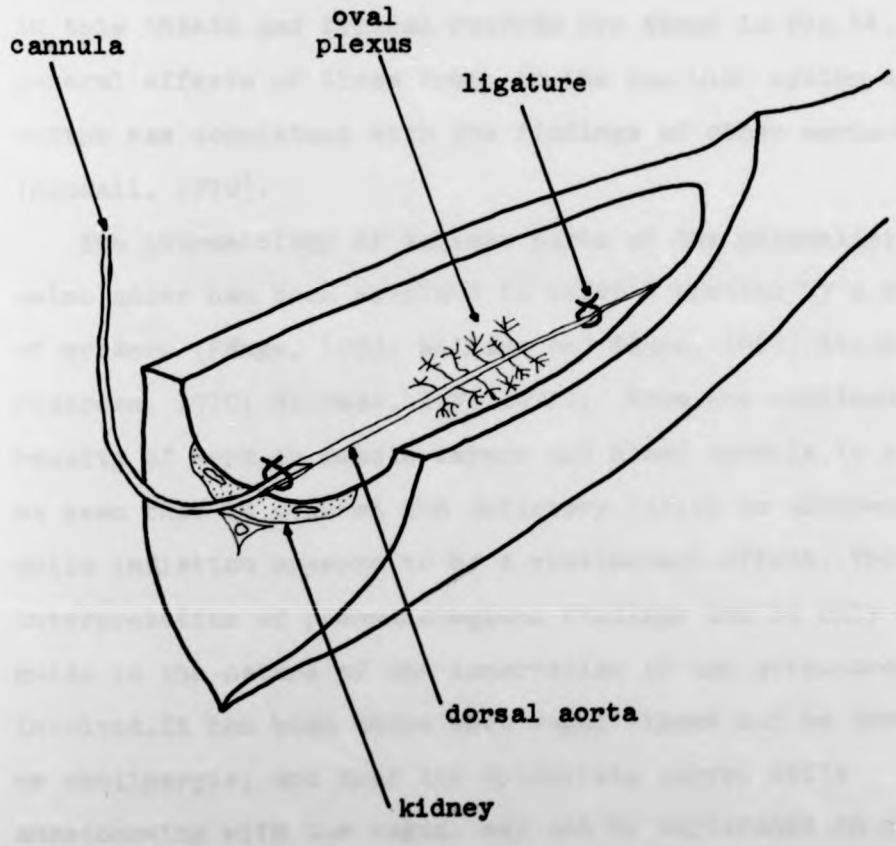


FIGURE 13

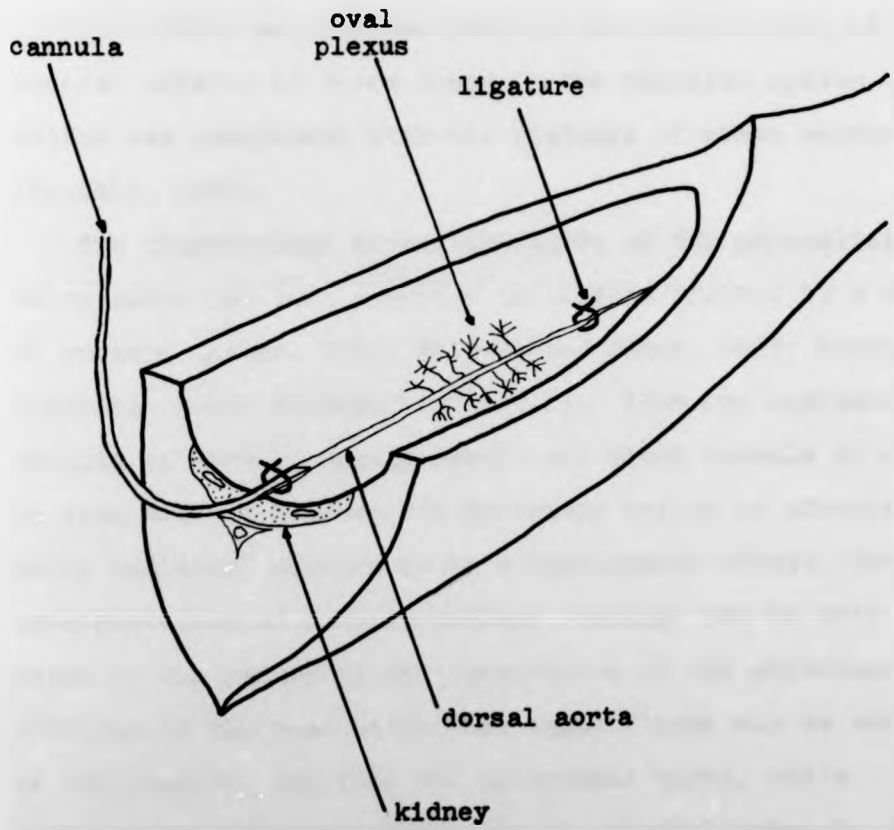


FIGURE 13

vessels, but nevertheless gave an approximate indication of drug effects. In cod and in the common eel, Fänge (1953) found that vasoconstriction was cholinergic and that vasodilation was adrenergic. These results were confirmed in this thesis and typical records are shown in Fig.14. The general effects of these drugs on the vascular system of saithe was consistent with the findings of other workers (Randall, 1970).

The pharmacology of various parts of the physoclist swimbladder has been examined in several species by a number of workers (Fänge, 1953; Nilsson and Fänge, 1967; Stray-Pedersen, 1970; Nilsson, 1971,1972). From the combined results of work on muscle layers and blood vessels it can be seen that in general the deflatory reflex is adrenergic, while inflation appears to be a cholinergic effect. The interpretation of pharmacological findings can be only a guide to the nature of the innervation of the structures involved.It has been shown that vagal fibres may be adrenergic or cholinergic, and that the splanchnic nerve, while anastomosing with the vagus, may not be implicated in control of the oval. In addition, although the major ganglia occur outside the bladder, it is evident that some ganglionic processing may take place at, or near, the oval edge (Saupe, 1940). Cholinergic varicosities observed by Fahlen et al (1965) indicate the existence of a variety of ganglionic

Fig.14. The pharmacology of the oval plexus.

(upper record = drop-counting,
lower record = ratemeter.)

A. Vasodilation.

B. Vasoconstriction.

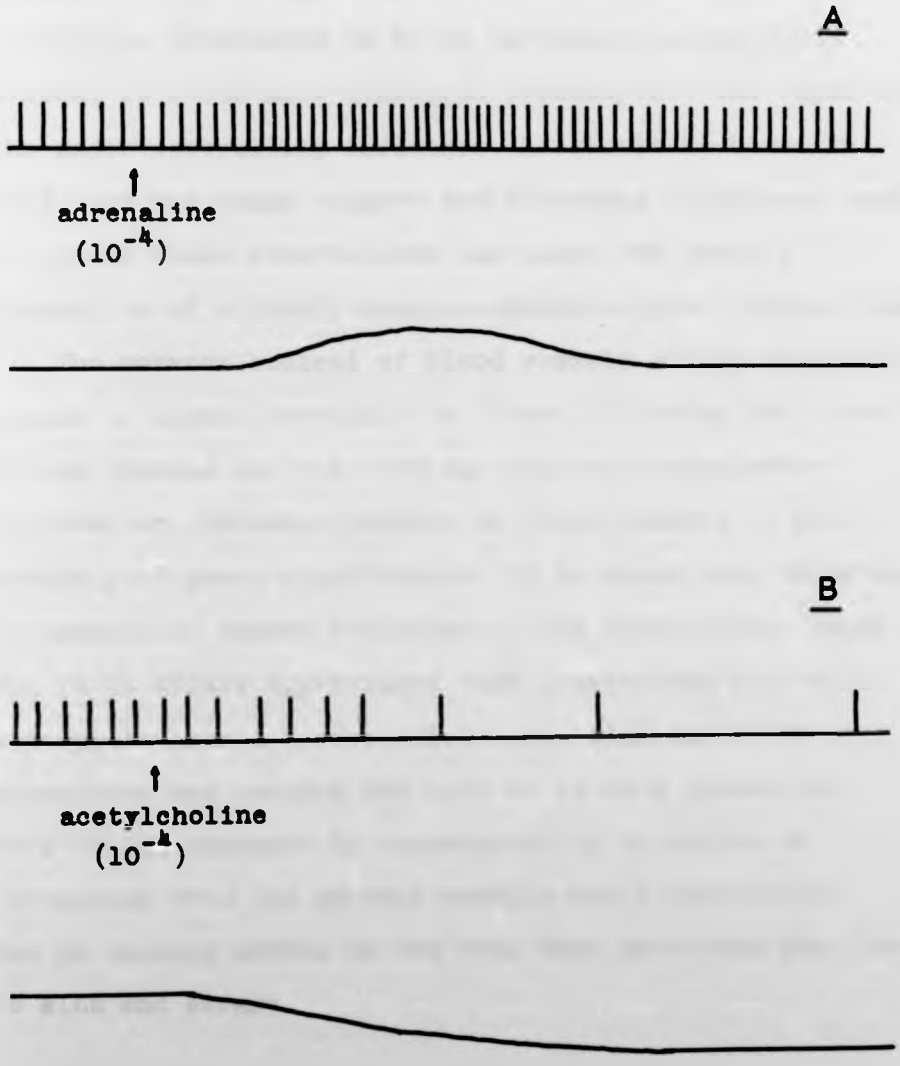


FIGURE 14

structures of uncertain function in different areas of the bladder. It is possible that the nervous control of the resorptive structures is by an autonomic antagonistic system, it seems more likely, at present, that the vagus is the major controlling influence. It should be borne in mind that the rough surgery and recording techniques used in making these observations may cause too great a disruption of a fairly complex system to give precise results.

The nervous control of blood vessels of the resorptive plexus is almost certainly by fibres following the course of the vessels and not from any discrete swimbladder innervation. Hormonal control of these vessels is also probably of great significance. It is known that injection of adrenaline causes deflation of the swimbladder (Fänge, 1953) and it is widely appreciated that a disturbed fish will attempt to escape by "sounding", Spitting gas if it is a physostome and opening the oval if it is a physoclist. This flight response is accompanied by an output of adrenaline from the adrenal medulla which undoubtedly has an opening effect on the oval thus assisting the fish to sink and escape.

THE HAEMODYNAMICS OF THE OVAL PLEXUS

The structural basis of an oxygen absorption system has been demonstrated in this thesis by dissection and latex injection of the oval plexus. Control of these blood vessels by drugs applied topically was demonstrated both in this work and by Fänge (1953), and a logical further step in this investigation was to estimate blood flow rates through the plexus. This vascular bed is a system which cannot be evaluated by the more usual methods of dye-dilution or ultrasonic Doppler shift because it is not sufficiently discrete and it is very inaccessable. Two alternative systems were used, application of the Poiseuille equation and radiolabelled microsphere injection.

Our understanding of mammalian, and particularly human, haemodynamic principles is based on the findings of several pioneer workers of the early nineteenth century. One of these was Poiseuille (1842) who was able to demonstrate the relationship between rate of blood flow, blood viscosity, and physical dimensions of the blood vessels. In engineering and precision viscometry the basic assumptions of the Poiseuille equation are rarely met (McDonald, 1974), but in microcirculation studies the simpler form of the equation appears to describe the flow rates adequately (Keele and Neil, 1971). The equation used is ; $\dot{Q} = \frac{P_1 - P_2 \cdot r^4}{8L\eta}$

where Q = flow rate, $P_1 - P_2$ = the pressure drop experienced along the length of the tube, r = radius of the tube, L = length of the tube and η = the viscosity of the fluid.

For the purpose of investigation of the oval plexus these terms had to be reinterpreted to suit the construction of the capillary bed. Latex-injected specimens were used to estimate the dimensions of the vessels. The flow rate of the bed will be a composite of the flow rates in the narrowest capillaries. The number of arterial trunks in the preparation was obtained by counting all the trunks and dividing by two, on the assumption that half of these were venous returns. The number of capillaries per trunk was then estimated from a latex-injected section of the plexus which was cleared in xylene and then observed microscopically by transmitted light. Using this same preparation the radius of the capillaries at their narrowest points could be estimated. The length of the capillaries was taken as the length of the narrowest section and in this estimate half the distance between arterioles and venules was used.

The apparent viscosity of blood varies according to haematocrit and diameter of capillaries. Very narrow vessels ($> 150\mu m$) show great anomalies, and Fåhreus and Lindqvist (1931) showed that in tubes of $40\mu m$ diameter, the relative viscosity was only 70% of normal. Haynes (1961) has shown that human blood of various haematocrit values has a viscosity of about 0.018 Poise in a capillary of $6\mu m$

diameter, this being close to the diameter of the oval plexus capillaries. Jones et al (1974) showed the maximum systolic pressure in the dorsal aorta of Gadus morhua to be about 32mm Hg, and Randall (1970) suggests a maximum venous pressure in most fish of about 10 mm Hg. These figures indicate a maximum potential pressure drop across the oval plexus of 22 mm Hg (= 29330 dyn/cm^2), and this figure is used in the calculation. Table 1 gives a summary of values used in this determination.

Because more than one vessel is involved, the equation is revised to; $Q = \frac{P_1 - P_2 \cdot r^4}{8L} \cdot N_t \cdot N_c \cdot 60$

where N_t is the number of arterial trunks and N_c is the number of capillaries arising from each arteriole. The result is multiplied by 60 to give a flow rate in cm^3/min. The flow rate calculated in this experiment for a fish of approximately 150g was 0.07613 cm^3/min.

One of the standard methods of assessment of regional blood flow is the use of dye-dilution techniques. This method depends on rapid and efficient detection of change in concentration of an injected dye at a point downstream of the organ in question. The technique has been used in the estimation of cardiac output and local blood flow. Rudolph and Heyman (1967) first introduced the use of injected radiolabelled microspheres for assessment of

Table 1. Values used in the determination of oval blood-flow using the Poiseuille equation.

TERM	MEAN VALUE	NUMBER OF DETERMINATIONS
N_t. Number of arterioles	206	5
N_c. Number of capillaries	230	10
L. Length of capillaries	0.0635 cm	30
r. Radius of capillaries	2.2705 cm $\times 10^{-4}$	30
P_1-P_2. Pressure drop	29330 dyn/cm^2	from literature
η. Blood viscosity	0.018 Poise	from literature

regional perfusion rates, after numerous workers had used a similar technique to demonstrate arterio-venous shunting (Prinzmetal et al, 1948). The technique has been reviewed recently by Wagner et al (1969) and they upheld the general validity of the method.

Blood flow distribution in the arctic grayling Thymallus arcticus has been examined recently by Cameron (1975) using radiolabelled microspheres introduced directly into the dorsal aorta. The general usefulness of this technique in fish studies was demonstrated, but this approach may suffer from failure of one of the basic assumptions of the technique, that mixing of the microspheres with the blood is complete and non-laminar (Wagner et al, 1969). In this thesis the flow rate was assessed by injection of microspheres into the heart, which gave complete mixing. This system suffers, however, from the disadvantage that much of the dose is trapped in the gills and in order to overcome this the total injected dose was increased, thus allowing a countable fraction of the radiolabelled microspheres to pass into the general circulation.

The flow rate through the saithe oval was assessed using ^{58}Co - labelled Tracer Sephadex ^{R}, suspended in Ficoll 70 injection medium. These microspheres are dextran-based beads marketed by Pharmacia Ltd. The particle size used was 15μm diameter and the size distribution of the experimental

batch is shown in Fig.15. (from Pharmacia data). The radionuclide is used as the carrier-free metallic chloride and it is taken up by the microspheres which are pre-hydrated in Tris / acetic buffer. The metal chelate complex thus formed is fixed by heating to 95°C for one hour. The beads are washed in buffer and finally resuspended in a suitable injection medium ready for use.

To determine regional perfusion rates using microspheres a reference flow-rate is needed, and Bartrum et al (1974) have pointed out the advantages of using a peripherally placed withdrawal pump as a reference organ. Following the microsphere injection, blood is withdrawn at a constant rate from a peripheral vessel, and the total radioactivity in the collected sample can be related to the activity in the organ under investigation and the reference flow- rate. The flow-rate in the organ can be determined using the relationship; $\frac{np}{fp} = \frac{n}{f}$, where np = counts in the reference organ, fp = reference flow-rate, n = counts in the organ under investigation and f = flow-rate to be determined. Thus $f = \frac{n \cdot fp}{np}$. In order to use this technique, a simple but effective withdrawal pump was constructed, and this is shown schematically in Fig.16.

Fish were anaesthetised with benzocaine (Laird and

Fig.15. Particle size distribution of Tracer Sephadex.

(Pharmacia data)

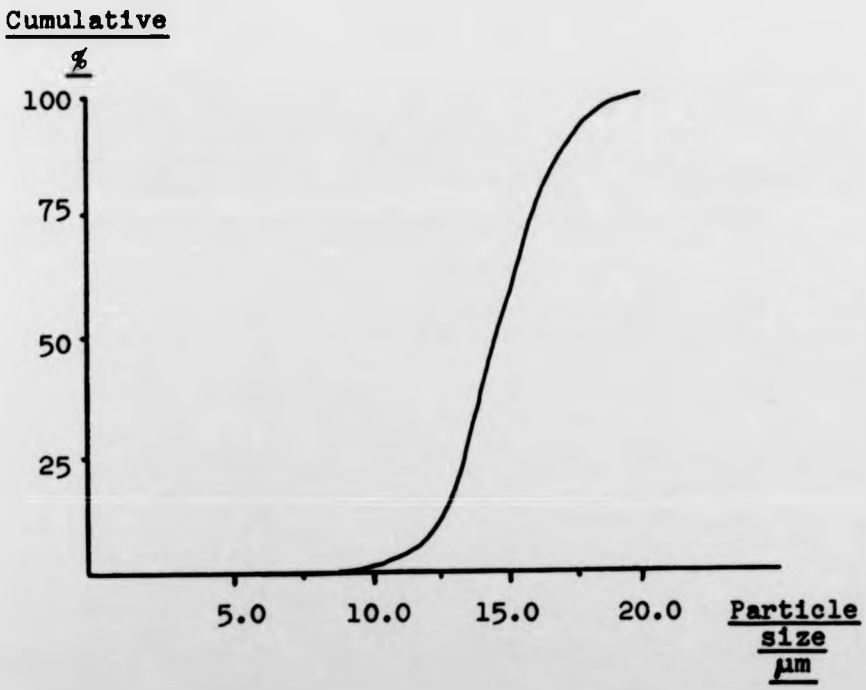


FIGURE 15

Fig.16. Schematic diagram of the withdrawal pump.

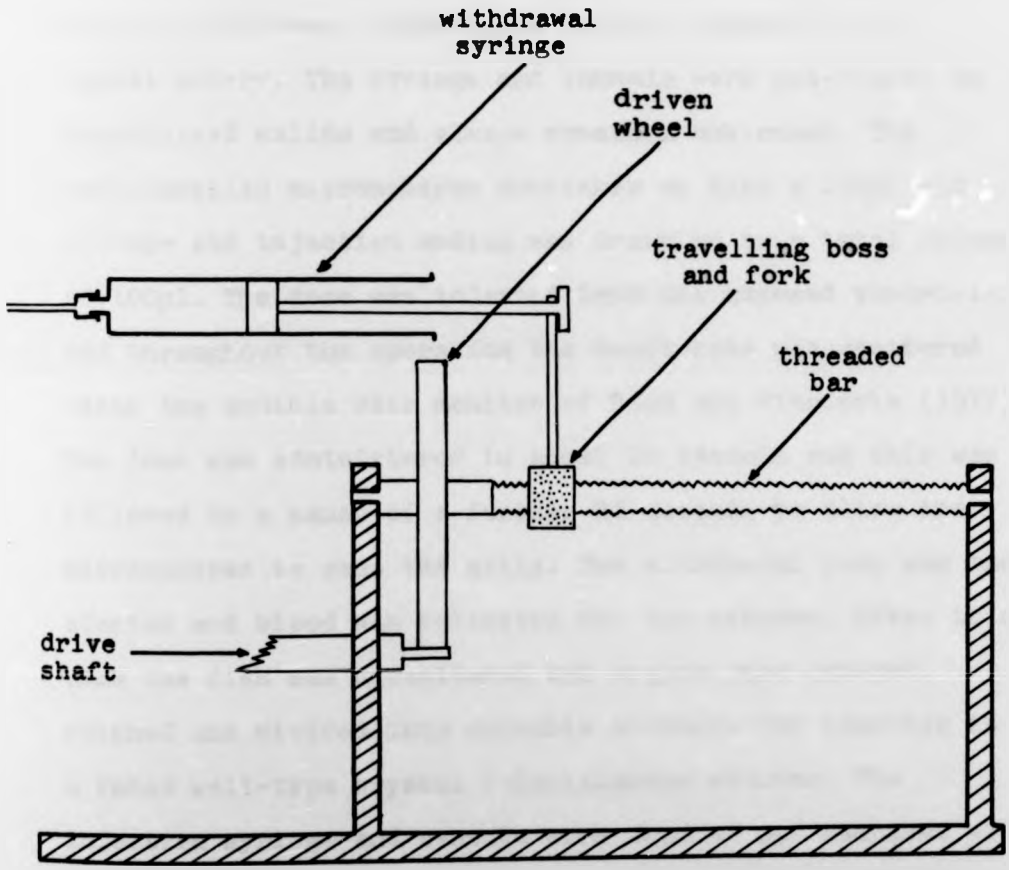

withdrawal
syringe
driven
wheel
travelling boss
and fork
threaded
bar
drive
shaft

FIGURE 16

Oswald, 1975) at a concentration of 1:30000, and were then placed on an operating table with sea water, containing anaesthetic, passing over the gills. The tail was severed and the withdrawal cannula was rapidly located in the caudal artery. The syringe and cannula were pre-rinsed in heparinised saline and always remained unblocked. The radiolabelled microspheres were taken up into a 100µl GLC syringe and injection medium was drawn up to a total volume of 100µl. The dose was injected into the exposed ventricle, and throughout the operation the heart-rate was monitored using the audible rate monitor of Ross and Wiewiorka (1977). The dose was administered in about 20 seconds and this was followed by a pause of a further 20 seconds to allow the microspheres to pass the gills. The withdrawal pump was then started and blood was collected for two minutes. After this time the fish was decapitated and organs were removed, weighed and divided into suitable aliquots for counting in a Panax well-type crystal scintillation counter. The injection syringe and cannula were counted and compared with a standard dose to calculate the total activity administered. The reference syringe and cannula was also counted, and knowing the withdrawal rate (0.49 cm^3/min) the flow-rate through various organs could be calculated. A schematic diagram of the experimental system is shown in Fig.17.

After establishing the "normal" flow-rate under the

Fig.17. Schematic diagram of the experimental system used in estimation of regional blood flow.

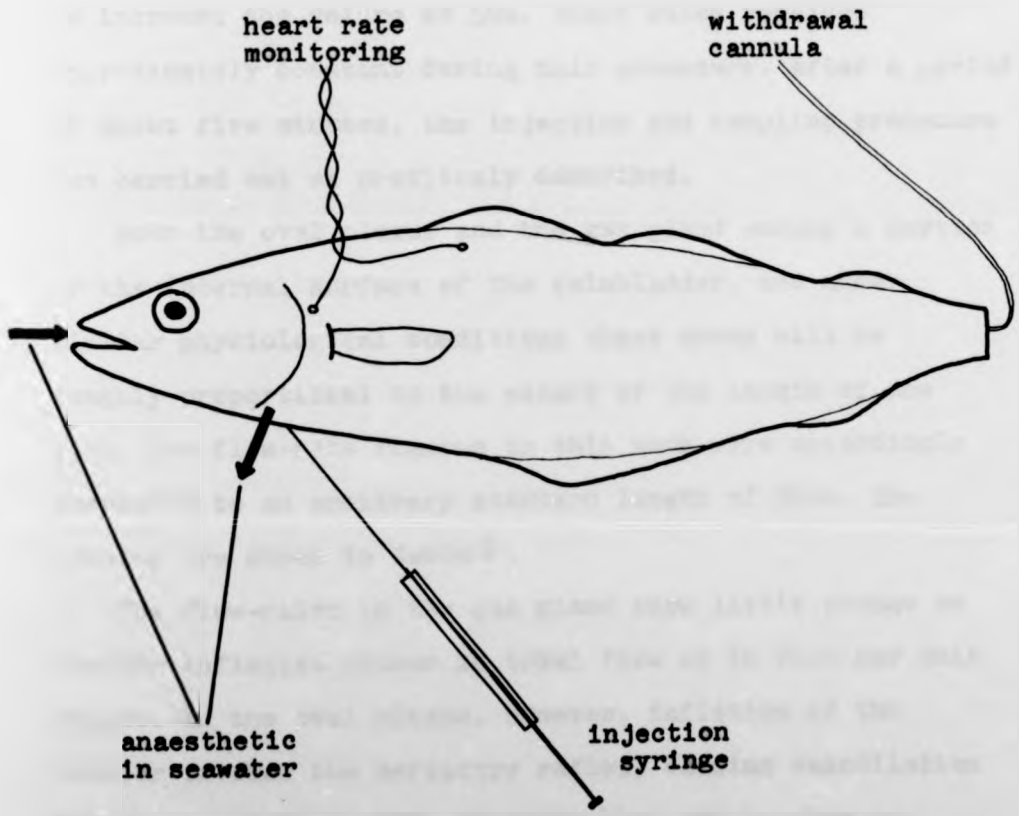

heart rate
monitoring
withdrawal
cannula
anaesthetic
in seawater
injection
syringe

FIGURE 17

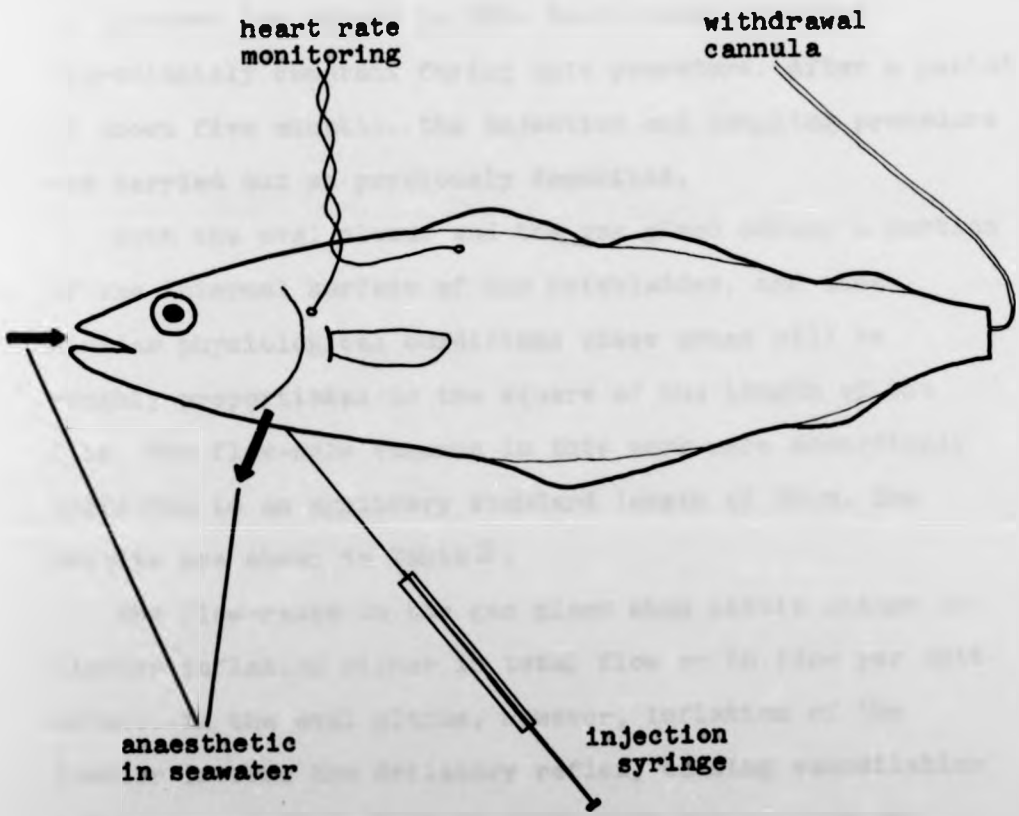

heart rate
monitoring
withdrawal
cannula
anaesthetic
in seawater
injection
syringe

FIGURE 17

conditions of the experiment, an attempt was made to alter the flow-rate in the resorbent area by inflation of the swimbladder. A cannula was introduced into the bladder lumen via the epaxial musculature, and following exposure of the ventricle and preparation of the fish for injection a quantity of air was introduced into the bladder sufficient to increase the volume by 50%. Heart rates remained approximately constant during this procedure. After a period of about five minutes, the injection and sampling procedure was carried out as previously described.

Both the oval plexus and the gas gland occupy a portion of the internal surface of the swimbladder, and under similar physiological conditions these areas will be roughly proportional to the square of the length of the fish. The flow-rate figures in this work were accordingly corrected to an arbitrary standard length of 30cm. The results are shown in Table 2.

The flow-rates in the gas gland show little change on bladder inflation either in total flow or in flow per unit weight. In the oval plexus, however, inflation of the bladder invokes the deflatory reflex, causing vasodilation and increased flow. Both in total flow and in flow per unit weight of tissue there is an apparent increase of about 50%. It was possible to repeat these experiments on only four fish in each case, and although the results seem to

Table 2. Blood flow rates estimated by the Poiseuille equation and radiolabelled microspheres.

(figures marked * obtained by calculation)

TISSUE		TOTAL FLOW µl/min	FLOW PER UNIT WEIGHT OF TISSUE µl/min/g
Oval by Poiseuille equation	*100g	57.8	-
	150g	74.7	-
	*200g	90.4	-
Oval by tracer microspheres	C	18.3 (0.99)	64.3 (6.61)
	I	29.2 (3.78)	94.7 (12.59)
Gas gland by tracer microspheres	C	16.1 (0.89)	68.0 (0.69)
	I	15.6 (0.24)	74.0 (11.0)

confirm the operation of the oval plexus, further replicates would be a great improvement. Statistical tests are not generally reliable with such small sample sizes and unfortunately cost limitations precluded further work during this study.

Fänge (1953) lists numerous authors who have induced inflatory or deflatory reflexes by altering the specific gravity of the fish. He appears to contradict himself in an attempt to refute the possibility that changes in swimbladder wall tone induce reflex deflation or inflation. It is quite apparent that most of the methods quoted which alter the specific gravity of fish will, of necessity, alter the wall tone, and this of course includes the introduction of excess gas into the bladder.

If the injected dose is known, it is possible, using this method, to calculate the cardiac output. Cardiac outputs calculated from these experiments were rather high (16 - 400 cm^3/min) and a major problem exists in being able to account for the fate of the injected dose. The method of calculation of cardiac output is fairly simple and is described by Wagner et al (1969). It is based on the relationship; $C.O. = \frac{N}{np} \cdot fp$ where C.O. = cardiac output, fp = reference organ flow-rate and np = counts in the reference organ. A major assumption is that all the

injected dose enters the systemic circulation, but because of minor clots around the wound in the heart and the massive influence of the gills, this is definitely not so in fish.

Cameron and Davis (1970) quote the cardiac output of rainbow trout as 6.56 cm^3/min in a 200 g fish at $10^{\circ}C$. As the blood flow figures in this thesis are corrected for a 30 cm fish of approximate weight 235 g, it is reasonable to assume a cardiac output of say 5 cm^3/min. In this case the amount of blood diverted to the oval under resting conditions is about 0.36% of the total flow and increases to about 0.6% when the deflatory reflex is invoked. The flow-rate suggested by the Poiseuille equation work must be considered as a maximum figure as latex injection undoubtedly causes some ballooning of the capillaries. Assuming that the maximum flow, in vivo, of about 100ul is attained, then the flow-rate in the oval would be about 2.34% of the total.

Kanwisher and Ebeling (1957), in calculations on gas secretion in deep-sea fish, assumed that one-third of the work done by the animal was available for filling the bladder. This would mean that one third of the total blood-flow was available for diversion to the gas gland. It is clear from this work, even though the experimental conditions were not typically those of a free-ranging fish, that their guess was perhaps an order of magnitude too large.

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THE PERMEABILITY TO GASES OF THE OVAL AND SWIMBLADDER WALL.

Moreau (1876), Bohr (1894), and Fänge (1953) are among those authors who have demonstrated that the swimbladder walls of certain fish are highly impermeable to gases. This property assists in the maintenance of high partial pressures of gases inthe bladder and enables fish to use the bladder as a buoyancy mechanism, often at great depth. It has been shown experimentally that the bladder contains a high percentage of oxygen (Fänge, 1953; Kanwisher and Ebeling, 1957) and that this percentage increases with depth in most fish (Scholander and Van Dam, 1953; Scholander, 1954). Since diffusion of gas through a tissue increases with increasing partial pressure difference across the tissue (Krogh, 1919), and this may be as high as 50 to 60 atm in mesopelagic fish (Kanwisher and Ebeling, 1957), a low value for oxygen diffusion is required if the bladder is to be used as a buoyancy mechanism at any depth.

Fänge (1953) showed that the secretory mucosa of Ctenolabrus rupestris (L) was impermeable to oxygen, and implied that the resorbent mucosa was also impermeable. Denton et al (1970, 1972) and Kutchai and Steen (1971) showed that the swimbladders of Conger conger and Anguilla anguilla are relatively impermeable to oxygen, nitrogen and carbon dioxide, and Denton et al (1972) showed that a

single layer of the bladder was responsible for the low overall permeability. This work was repeated recently by Lappenas and Schmidt-Nielsen (1977) on seven North American species of fish, with much the same result. Even when the resorbent plexus is constricted, some blood flow probably takes place, and the tissues forming the oval membrane must be relatively impermeable to gas to avoid losses into the resting plexus and the overlying kidney. It was thought useful to test this hypothesis, and to determine which layer of the bladder was responsible for restriction of gas movement in saithe.

The permeability to oxygen of pieces of swimbladder wall excised from fresh specimens was measured using a cuvette modified from a basic design of Kutchai and Steen, (1971) and shown in Fig.18. Pieces of tissue were held lightly between two concentric perspex rings which were then placed in the cuvette holding the temperature-stabilised oxygen electrode. The spaces above and below the tissue were flushed with oxygen-free nitrogen, the lower chamber was sealed, and the upper chamber was flushed with oxygen at a rate of about 25 cm^3/min. This established a pO_2 difference of 1atm across the experimental tissue. Gases were humidified by bubbling through water and cooled to the same temperature as the cuvette before use. The experiments were carried out at a constant temperature

Fig.18. Cuvette used for oxygen permeability measurements.

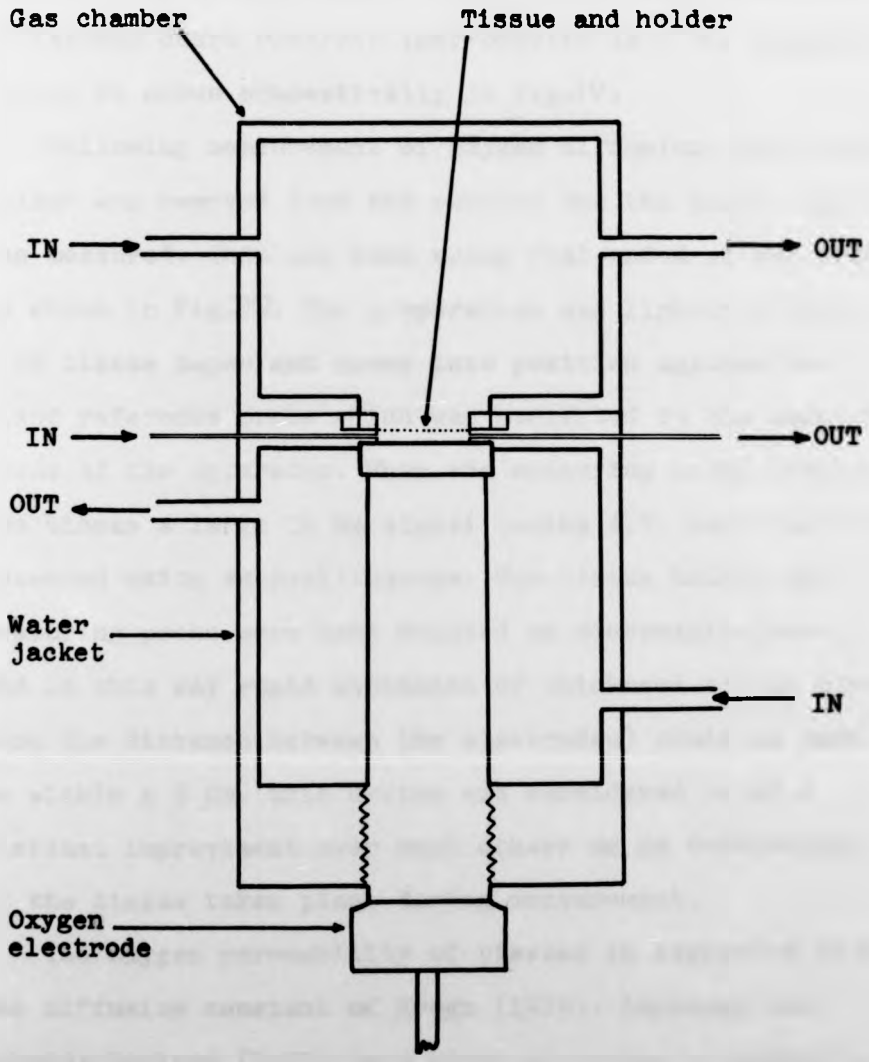

Gas chamber
Tissue and holder
IN
OUT
IN
OUT
OUT
Water jacket
IN
Oxygen electrode

FIGURE 18

in the range 19°C to 26°C. The output from the oxygen analyser (Radiometer PHM 72) was continuously monitored on a flat-bed chart recorder (Servoscribe 1s). The complete system is shown schematically in Fig.19.

Following measurement of oxygen diffusion, the tissue holder was removed from the cuvette and the tissue thickness was measured. This was done using flat-ended silver probes as shown in Fig.20. The preparation was lightly blotted with tissue paper and moved into position against the fixed reference probe which was connected to the mounting frame of the apparatus. When the measuring probe touched the tissue a large 50 Hz signal (mains A.C. hum) could be detected using an oscilloscope. The tissue holder and measuring probe were both mounted on micromanipulators and in this way rapid estimates of thickness of the tissue (now the distance between the electrodes) could be made to within ± 5 μm. This system was considered to be a distinct improvement over most others as no deformation of the tissue takes place during measurement.

The oxygen permeability of tissues is expressed as $K(O_2)$, the diffusion constant of Krogh (1919). Lapennas and Schmidt-Nielsen (1977) in a study of oxygen permeability of some North American fish discussed the use of the diffusion constant, K, and argued that it is a composite quantity consisting of an integration of the solubility coefficient, α, and the diffusion coefficient, D, for the

Fig.19. Schematic diagram of the complete system used for measurement of oxygen permeability.

used

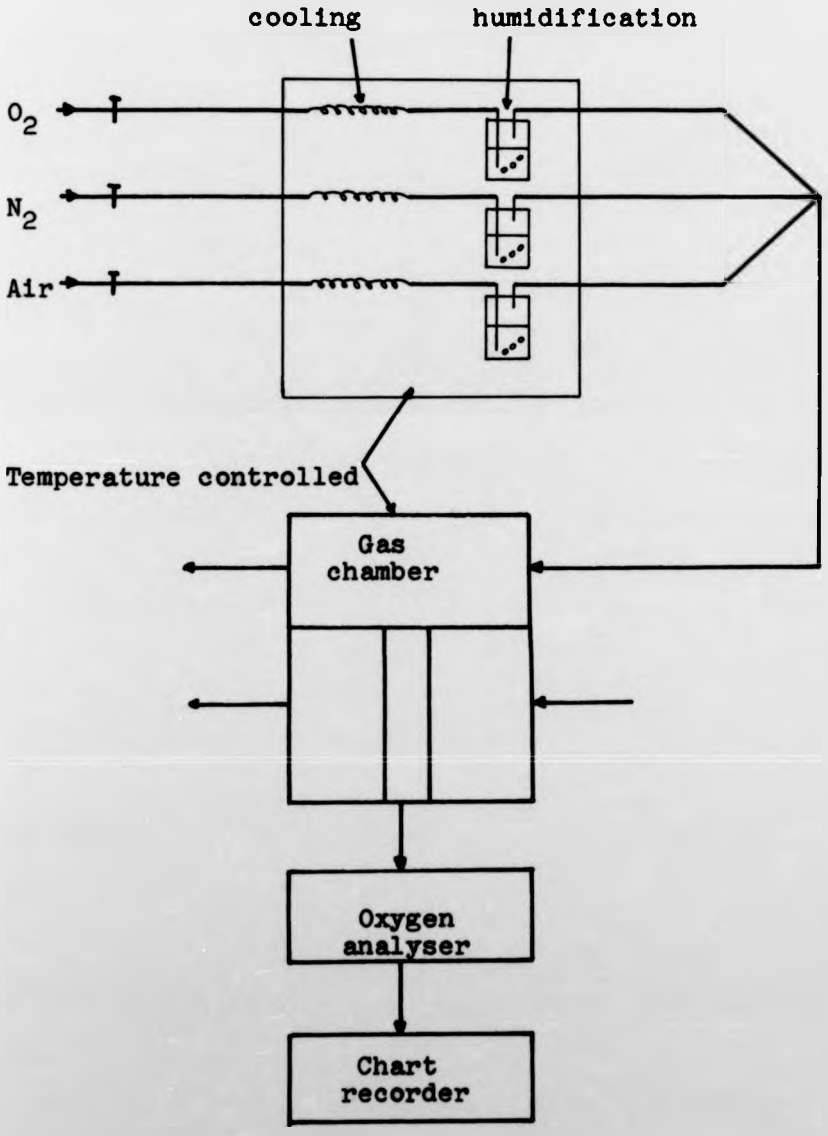


FIGURE 19

Fig.20. Thickness measuring equipment.

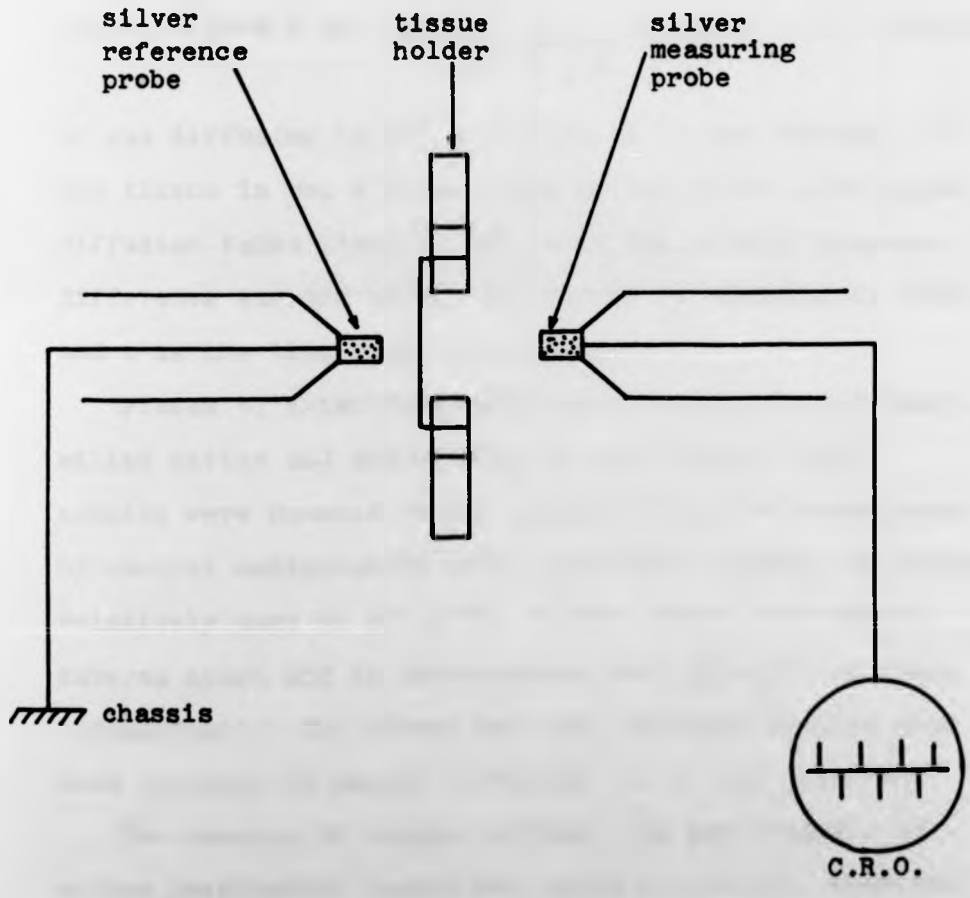

silver
reference
probe
tissue
holder
silver
measuring
probe
chassis
C.R.O.

FIGURE 20

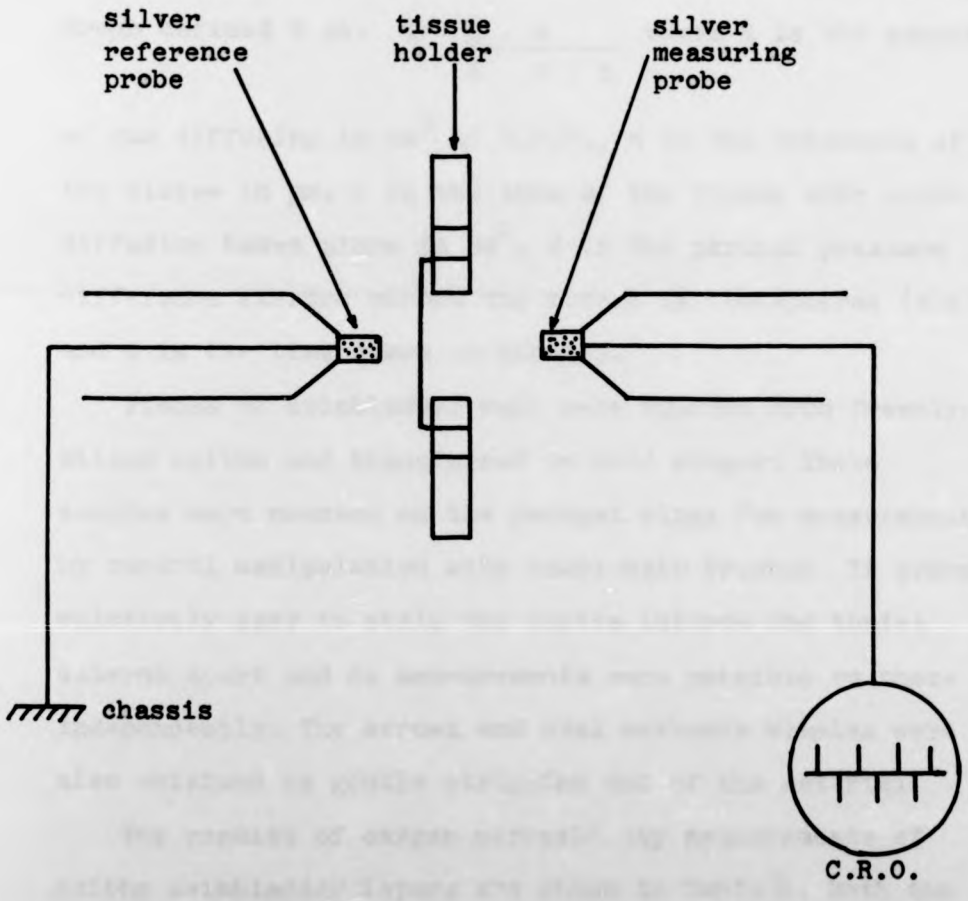

silver
reference
probe
tissue
holder
silver
measuring
probe
chassis
C.R.O.

FIGURE 20

material in question. These principles were understood by Krogh and other workers and the use of K enables the comparison of gas diffusion between tissues on an overall basis, obviating the measurement of D and α independently. Krogh defined K as; $K = \frac{Q \cdot d}{A \cdot P \cdot t}$ where Q is the quantity of gas diffusing in cm^3 at N.T.P., d is the thickness of the tissue in μm, A is the area of the tissue over which diffusion takes place in cm^2, P is the partial pressure difference exerted across the tissue in atmospheres (atm), and t is the time taken in minutes.

Pieces of swimbladder wall were removed from freshly-killed saithe and transferred to cold ringer. These samples were mounted on the perspex rings for measurement by careful manipulation with camel-hair brushes. It proved relatively easy to strip the tunica interna and tunica externa apart and so measurements were possible on these independently. The serosa and oval membrane samples were also obtained by gentle stripping out of the material.

The results of oxygen permeability measurements of saithe swimbladder layers are shown in Table 3. Both the serosa and tunica externa have a relatively high oxygen permeability, frog connective tissue has a $K(O_2)$ of 0.115 and so these results seem quite reasonable. The slightly lower permeability of the serosa may or may not be due to

Table 3. The oxygen permeability of component layers of the swimbladder of Pollachius virens.

TISSUE	$K(O_2)$	S.E.	n
Serosa	0.2276	0.0643	4
Tunica externa	0.6140	0.1540	4
Tunica interna	0.0254	0.0099	4
Swimbladder wall intact	0.0534	0.0173	3
Oval	0.0252	0.0115	3
Frog mesentery (Krogh, 1919)	0.1150	-	-

the presence in this tissue of melanophores (Denton et al, 1972; Lappenas and Schmidt-Nielsen, 1977). The intact swimbladder wall has a $K(O_2)$ between that of the tunica externa and the tunica interna, and it seems that the site of the permeability barrier must be located in the tunica interna. The oval membrane samples had essentially the same $K(O_2)$ as the tunica interna and this fact is of some structural and functional significance in true physoclists.

The location of the permeability barrier in the tunica interna in physoclists is of fundamental importance in the physiology of gas loss from the bladder. The possession of an oval derived from the tunica interna (see earlier) means that the oval membrane automatically forms an impermeable layer over the resorptive plexus. In this way gas retention in the bladder is achieved, and controlled, by a single layer. Assuming that fishes use the bladder to attain a neutrally buoyant state, then maintenance of that state will depend upon low leakage of contents into the general tissues. Once a gas has moved from the gas phase of the bladder into the liquid phase of the tissues, then even if the tissues are in the swimbladder wall itself, the buoyant effect of the gas is lost. The possession of an internal rather than an external permeability barrier helps to reduce this by reducing the "dead space" of the swimbladder tissues. This point may be particularly

important in deep-sea fish, such as morids, where the thickness of the tunica externa may be up to several millimetres.

The loss of gas through the swimbladder wall has been examined by Denton et al (1972) and Kutchai and Steen (1971), and the latter authors have described how diffusion losses may be a critical factor in determining the maximum depth of neutral buoyancy of a species. The hypothesis that deeper dwelling species may have a highly impermeable bladder has been suggested by these authors, and it proved possible to make a partial assessment of these ideas by measuring the permeability to oxygen of the bladder wall of species of fish from different environments. This work largely involved estimates at sea, working with trawled material in "field" conditions and this gave a high experimental failure rate. Several experiments gave poor results because of decompression damage to tissues, but these were recognisable by the rapid passage of oxygen through the tissue at the outset of the experiment. The results of work on six species of fish are shown in Table 4, and these are results in which the tissue samples were not obviously damaged.

Several specimens of the epipelagic fish Scomber japonicus (L) were taken off the Salvage islands in the eastern North Atlantic. These were kept on board ship for

Table 4. The permeability to oxygen of the swimbladder wall of certain fish.

FISH	$K(O_2)$	S.E.	n
Pollachius virens	0.0534	0.0173	3
Ceratoscopelus maderensis	0.0672	0.0111	8
Argyropelecus aculeatus	0.0437	0.0058	3
Scomber japonicus	0.2224	0.0447	4
Malacocephalus laevis	0.0685	-	1
Mora moro	very low.	-	3

several days before examination of the swimbladders. The $K(O_2)$ was high, and was similar to that found for saithe tunica externa. The bladder contains a series of small gas glands and a well-defined oval, however, the rate at which vertical excursions are made is probably high and there may be little value in attempting to maintain neutral buoyancy. A great deal of lift is probably generated by the continuous swimming which is a feature of scombroids. Gas efflux is, in any event, probably fairly low because of the low partial pressure differences experienced across the bladder wall in surface waters.

The coastal species Pollachius virens makes an extensive diel vertical migration (Schmidt, 1955), as does the mesopelagic fish Ceratoscopelus maderensis (Barham, 1971; Backus et al, 1968). Although the absolute depths between which these migrations occur are different, the percentage pressure change experienced by the two species is probably quite similar, of the order of an 80% pressure drop at night and between 650% and 850% increase by day. The problems for these fish in maintaining an inflated bladder are aggravated at their maximum depths by gas leakage through the bladder wall. Rearrangement of Krogh's equation to; $P = \frac{Q \cdot d}{A \cdot K \cdot t}$ will give an estimate of the maximum depth at which the swimbladder volume can be maintained, if the maximum rate of gas secretion is

known. Using measures of secretion rate from other authors, these can be substituted for Q in the equation, and this would be the situation where gas secretion and loss were exactly equal. The area, thickness and oxygen diffusion constants are all known, and so the maximum sustainable internal pressure can be calculated, and this in turn equated to depth. Maximum depth calculations for Ceratoscopelus maderensis based on the generous secretion rates of Pomatomus saltatrix (Wittenberg et al, 1964) give a balance point of about 85 m. Similar calculations for saithe give a maximum depth of about 65 m, and while this figure is probably reasonable for most saithe, the conclusion must be that Ceratoscopelus maderensis does not use its swimbladder alone to maintain neutral buoyancy (Bone, 1973; Ross, 1976).

The hatchetfish Argyropelecus aculeatus is a midwater fish with a similar depth range, but much less pronounced diel migration than most lantern fish (Badcock and Merrett, 1976). Oddly its oxygen diffusion coefficient is only slightly smaller than that of the migratory Ceratoscopelus maderensis, and with a generous gas secretion rate, its maximum depth of neutral buoyancy emerges as 81 m. This is embarassing for a fish with a mean depth of occurrence of 400 m, and it can only be concluded that they must be capable of very high gas secretion rates.

The demersal fish Mora moro has a swimbladder which is in general greater than 1mm thick in adult specimens. It thus proved impossible to measure oxygen diffusion in the few suitable specimens obtained. A single specimen of the shallower-dwelling macrourid Malacocephalus laevis was examined and the oxygen permeability was quite low, but, again, not sufficient to give a maximum depth greater than 100m. Specimens examined later had much thicker swimbladder walls (about 500 μm, compared to 90 μm) and it is possible that the tissue had been damaged.

Estimates of diffusion constants of deep-water fish swimbladders will, sadly, remain an unknown quantity until these animals can be raised alive from their environment. The estimates in this thesis are under constant suspicion because of the possibility of stretching of the tissue beyond a point where it is realistic to make measurements. There is also the possibility that there were actual holes in the tissue samples. The latter point can be discounted because all samples were checked visually, and any badly damaged tissue could be recognised by a rapid passage of oxygen into the cuvette. The former point is more complex, and aspects of it are discussed at a later stage.

THE PHYSICAL BASIS OF IMPERMEABILITY

The tissue of the swimbladder wall is at least an order of magnitude less permeable to gases than most other tissues. Scholander (1954) proposed the inclusion of solid materials in the tissue as a means of preventing gas loss, and there are two substances in swimbladder walls which could contribute to low permeability, collagen and purine crystals.

A form of collagen peculiar to fish, and known as ichthyocol, is found in swimbladders. Fänge (1966) suggested that this may contribute to reduction of gas loss. The tunica externa is, very largely, constructed of collagen fibres with small amounts of interwoven elastic and muscle fibres. The results of permeability studies in this work, and by Lappenas and Schmidt-Nielsen (1977) adequately demonstrate that the collagen has an oxygen permeability quite similar to other connective tissues. It is thus unlikely that the collagen in the tunica externa plays anything other than a minor role in gas retention.

Guanine and hypoxanthine are purines occurring as solid deposits in fish scales and swimbladders and also surrounding the choroid rete of the eye. The purines are deposited as crystals and are highly reflective (Denton and Land, 1971). Guanine is the major constituent and may

be between 75 and 97% of the material (Greenstein, 1966). The crystals can be seen clearly using polarised light microscopy, and the use of a rotating stage greatly facilitates these observations. The layers of the saithe swimbladder were gently stripped apart as described earlier and were examined as fresh squashes on a Wild geological polarising microscope. Several attempts were made to prepare sectioned material for polaroid examination, but both formalin and alcohol fixation failed to retain any recognisable crystalline material in thick sections. Denton et al (1972) refer to this apparent disappearance of crystals and the problem was resolved in an earlier section using frozen-sectioned material.

The results of crystal location by polarised light microscopy of separated swimbladder material from a range of anacanthines, and some other species of interest, are shown in Table 5. The crystals occur most frequently as needles between 40 and 100 μm in length and from 0.5 to 3 μm wide. Plate-like stacks were found in some fish, notably Coryphaenoides rupestris and these were hexagonal crystals of approximate dimensions 35 x 125 μm. It can be seen that the crystals consistently appear in the tunica interna, and only rarely are they seen in the tunica externa. Lappenas and Schmidt-Nielsen (1977) described crystals in the sub-mucosa and this was confirmed in this

Table 5. The location of purine crystals in the component layers of fish swimbladders.

(+ denotes birefringent crystals noted)
(o denotes no birefringence)
(- denotes no observation)

SPECIES	SEROSA	TUNICA EXTERNA	TUNICA INTERNA	OVAL
Pollachius virens	o	o	+++	+++
Molva dypterygia	o	o	+++	+++
Coryphaenoides rupestris	o	+	++	-
Nezumia aequalis	o	o	++	-
Lepidion eques	o	+	++	-
Halargyreus johnsonii	o	o	++	-
Mora moro	o	o	++	-
Synaphobranchus kaupi	o	+++	+	-
Ceratoscopelus maderensis	o	o	++	-

thesis. The occaisional appearance of crystals in the tunica externa was most probably due to inefficient separation of the tissue layers. The serosa never contained birefringent crystals, but the oval, in those species examined, had essentially the same crystalline content as the tunica interna. The swimbladders of the apodans Synaphobranchus kaupi and Anguilla anguilla contained dense crystal layers in the tunica externa. This arrangement could be related to the mechanisms of buoyancy control practised by apodans, and is discussed later.

The purine content of tissues can be determined by enzyme assays (Schuster, 1955; Nicol and Van Baalen, 1968). The technique involves the use of two enzymes of the purinolytic pathway acting on guanine and xanthine to produce uric acid, and involving a monitorable optical density change at 290 nm. The pathway of the reaction is shown in Fig.21. The assay system can measure hypoxanthine and xanthine independently of guanine, and these were measured separately in this work. The final results are expressed as total purine, however, as all these substances are crystalline in nature and contribute to the permeability barrier.

Tissue samples were obtained either by weighing out aliquots of fresh material or by punch biopsy. In many

Fig.21. Purinolytic pathway in fish.

(based on Florkin, 1949).

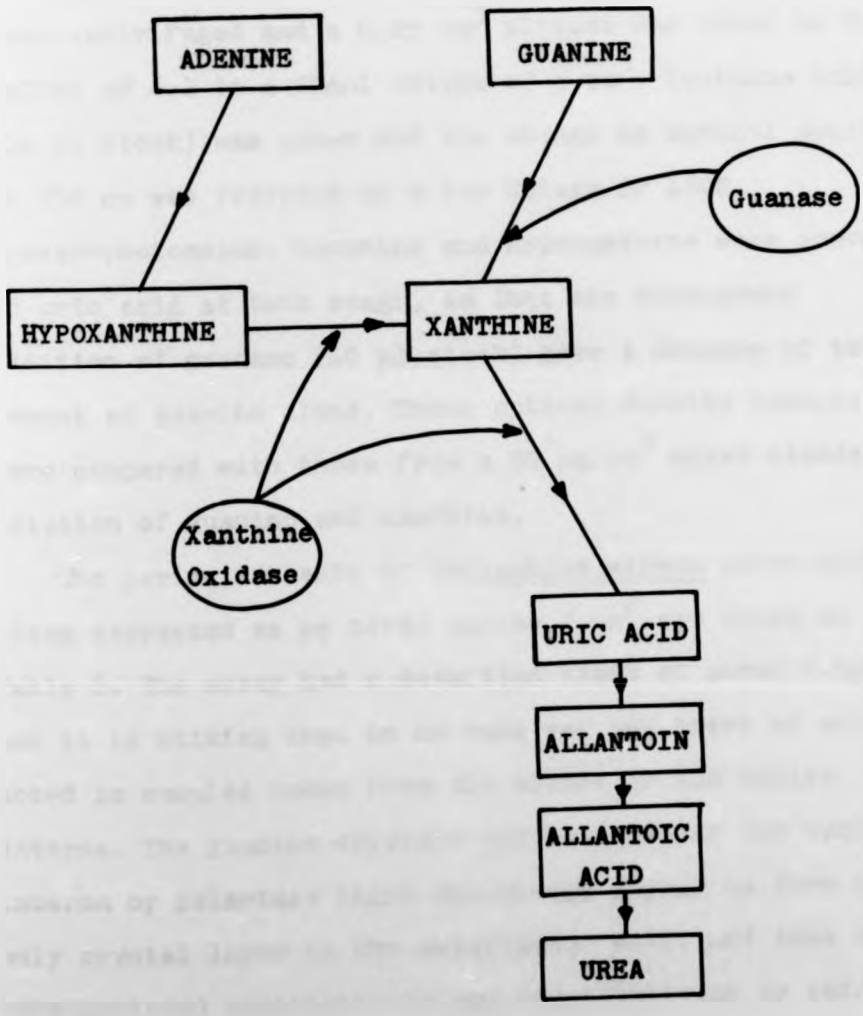

ADENINE
GUANINE
Guanase
HYPOXANTHINE
XANTHINE
Xanthine Oxidase
URIC ACID
ALLANTOIN
ALLANTOIC ACID
UREA

FIGURE 21

cases biopsy discs were separated into layers, i.e. tunica interna or externa or serosa, and these were soaked in 3 cm^3 0.1N NaOH for 48-72 h. The resultant solutions were centrifuged and a 0.25 cm^3 aliquot was added to Tris buffer pH 8.1 to a final volume of 3 cm^3. Xanthine oxidase (10 µl stock) was added and the change in optical density at 290 nm was recorded on a Pye Unicam SP 1800 spectrophotometer. Xanthine and hypoxanthine were converted to uric acid at this stage, so that the subsequent addition of guanase (10 µl stock) gave a measure of the amount of guanine alone. These optical density changes were compared with those from a 50 $\mu g/cm^3$ mixed standard solution of guanine and xanthine.

The purine contents of Pollachius virens punch biopsy discs expressed as µg total purine / cm^2 are shown in Table 6. The assay had a detection limit of about 0.5$\mu g/cm^2$ and it is stiking that in no case was any trace of activity noted in samples taken from the serosa or the tunica externa. The guanine crystals demonstrated in the tunica interna by polarised light microscopy appear to form the only crystal layer in the swimbladder wall, and this is substantiated qualitatively and quantitatively by this assay.

The idea that purine content and permeability to oxygen may be inversely related proved difficult to assess, as it

Table 6. The total purine content of punch biopsy discs from the swimbladder of Pollachius virens.

TISSUE	TOTAL PURINE ug / cm^2	±S.E.	N
Whole swimbladder wall	47.1	8.34	5
Serosa	0	-	5
Tunica externa	0	-	5
Tunica interna	51.8	8.07	5

was not possible to determine both of these parameters on many species. The results of observations where both values have been determined are shown in Table 7, along with results by other authors. There is a general suggestion of an inverse correlation between total purine content and oxygen diffusion constant, and this may also be related to the animals lifestyle. It must be conceded, however, that better estimates than those presently available are needed before any firm statement on this relationship could be made.

The interesting idea that purine content may increase in swimbladder walls as a function of depth or pressure-stress imposed by the animals lifestyle has been suggested by Denton et al (1972). Preliminary work on deep-sea species prompted a fuller investigation of this phenomenon, and with the help of Dr J.D.M.Gordon of the S.M.B.A. it was possible to pursue this further. The shallower-living species studied included rudd, trout and saithe, but the majority of the work was on demersal slope-dwellers caught by bottom-trawl from R.R.S.Challenger. The study area in this work was the Hebridean Terrace (56°-57°N, 9°-11°W), which ranges in depth from 500 m to 2000 m.

The depth-range and mean depth of occurrence of the species involved was extracted from trawling data based on work carried out over the last three years by Dr Gordon.

Table 7. The permeability to oxygen and total purine content of the swimbladders of some fish, with a note on their lifestyle.

(* this work)
(** Ross, 1976)
(*** Denton et al, 1972)

SPECIES	$K(O_2)$	TOTAL PURINE	HABITAT
Pollachius virens	0.0534	47.1 *	Coastal Vertical migrant 200m max
Ceratoscopelus maderensis	0.0672	73.2 **	Mesopelagic Vertical migrant 650m max
Anguilla anguilla	0.0106 0.0024	183 *** - *	Freshwater Spawning migrant 150m max?
Conger conger	0.0010	239 ***	Spawning migrant 2000/3000m max
Mora moro	very low	314 *	Deep-sea Demersal 1000m max

These pooled data do not take into account any known seasonal or sex differences which may occur in these fish (Gordon, pers comm). This information, together with the results of total purine assays, is shown in Table 8. Results of purine assays are expressed as ug/cm^2, or ug/mg as appropriate.

The mean depth of occurrence of these fish is plotted against total purine in Fig.22, and Fig.23. In both plots a positive correlation of purine content with depth is clearly discernable. The correlation coefficient of mean depth against total purine, by weight, is 0.63 (n = 11), and is significant at the 2% level. The correlation coefficient of mean depth against total purine by biopsy is 0.899 (n = 14) and is significant at the 0.1% level. It can be seen that there is a strong trend for the swimbladder to contain more deposited purines as the mean depth of the species increases. The relationship is not a perfect functional one, however, and accordingly the regressed data are shown as broken lines. In particular it should be noted that species from depths greater than 1750m have not yet been investigated, and so the shape of the curve from about 1500m is by no means clear.

Some species were examined at more than one depth, on a gravimetric basis, and the results of these assays are shown in Table 9. Four out of the five species show a

Table 8. The purine content of the swimbladder and the depths of occurrence of various fish.

Figures in parentheses denote number of purine assays performed.

SPECIES	DEPTH RANGE	MEAN DEPTH	PURINE ug/cm^2	PURINE ug/mg
Salmo trutta	0-15	8	24 (2)	-
Scardinius erythropthalmus	0-5	2	14 (2)	-
Cerastoscopelus maderensis	0-650	300	73 (6)	-
Coryphaenoides rupestris	540-1750	1125	393 (4)	5.0 (13)
Nezumia aequalis	670-1070	865	217 (3)	10.6 (10)
Trachyrhynchus murrayi	960-1500	1230	-	13.0 (10)
Coelorinchus caelorhinchus	460-760	630	-	6.2 (5)
Coelorinchus occa	960-2000	1480	-	7.0 (5)
Malacocephalus laevis	460-540	500	-	6.2 (2)
Chalinura mediterranea	1240-2000	1620	-	9.9 (4)
Pollachius virens	0-200	100	47 (5)	-
Molva dypterygia	480-1050	770	275 (3)	-
Brosme brosme	460-1020	750	236 (2)	1.1 (2)
Micromesistius poutassou	200-550	350	195 (3)	-
Merluccius merluccius	400-800	500	292 (1)	-
Lepidion eques	460-1280	870	466 (7)	3.9 (11)
Halargyreus johnsonii	540-1270	908	253 (2)	10.5 (8)
Mora moro	650-1050	850	314 (2)	-
Synaphobranchus kaupi	500-3000	1750	1097 (2)	19.5 (5)

Fig.22. Graph of total purine content of the swimbladder wall, determined by biopsy, against mean depth of occurrence.

(correlation coefficient r = 0.899)

(y = 0.33 x + 28.65)

swimbladder

ean depth

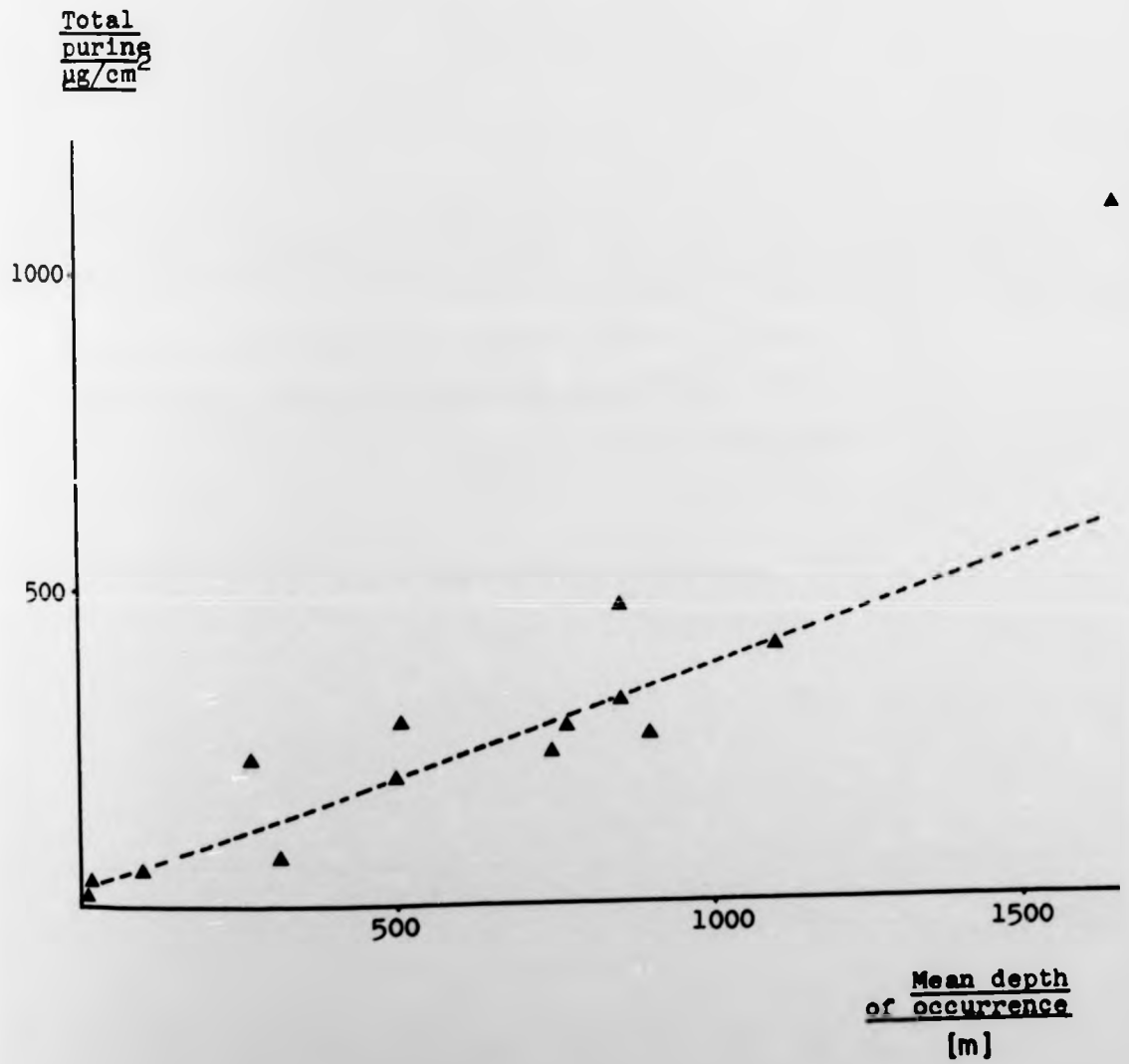


FIGURE 22

Fig.23. Graph of total purine content of the swimbladder wall, determined by weight, against mean depth of occurrence.

(correlation coefficient r = 0.63)

(y = 0.008 x + 0.237)

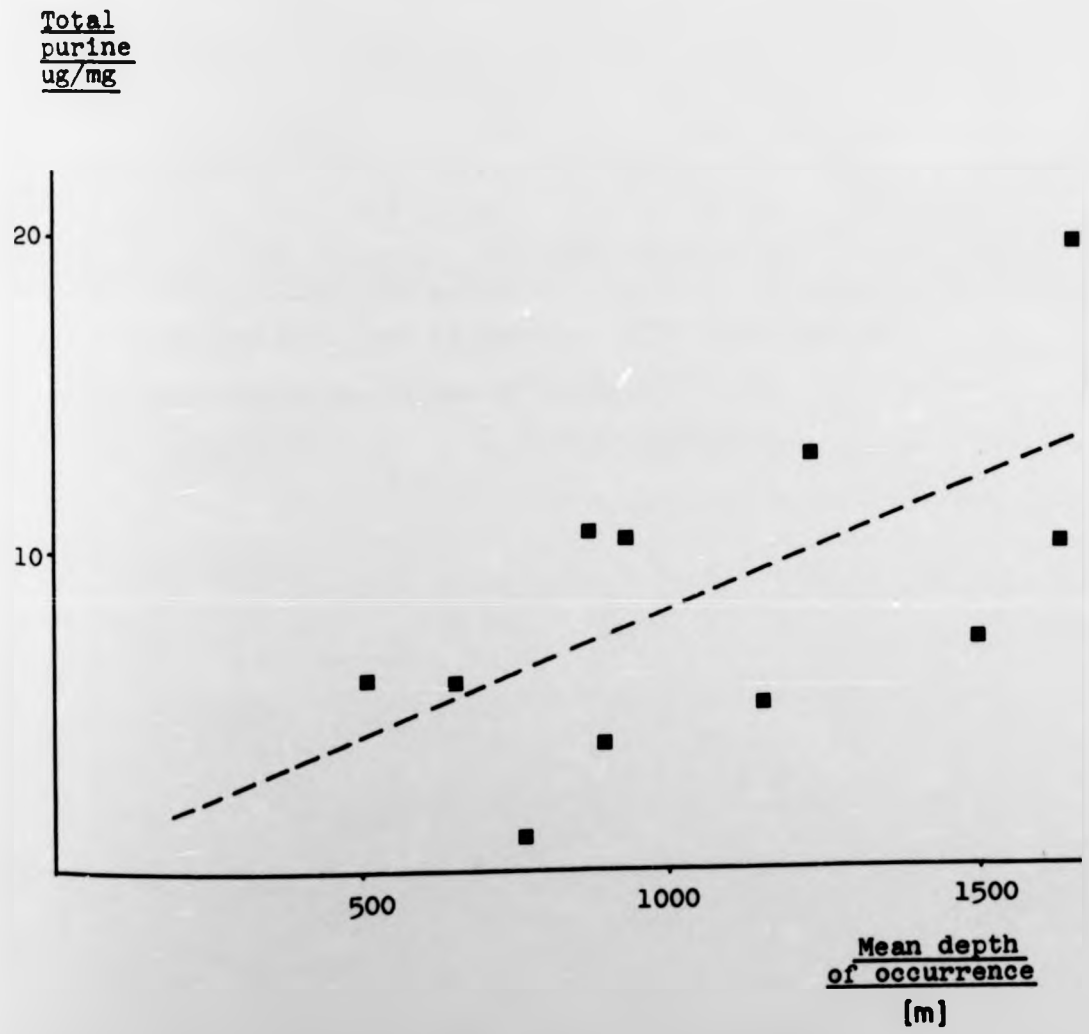

Total
purine
ug/mg
20
10
500
1000
1500
Mean depth
of occurrence
[m]

FIGURE 23

Table 9. The purine content of the swimbladder at different capture depths in some slope-dwelling fish.

SPECIES	TOTAL PURINE	n	DEPTH OF CAPTURE
Coryphaenoides rupestris	3.63	3	1750
	4.10	5	1250
	6.73	5	750
Trachyrhynchus murrayi	12.90	5	1250
	13.20	5	1000
Halargyreus johnsonii	8.01	3	1250
	11.92	5	1000
Lepidion eques	2.14	1	1250
	3.87	5	1000
	4.33	5	750
Nezumia aequalis	13.10	5	1000
	8.15	5	750

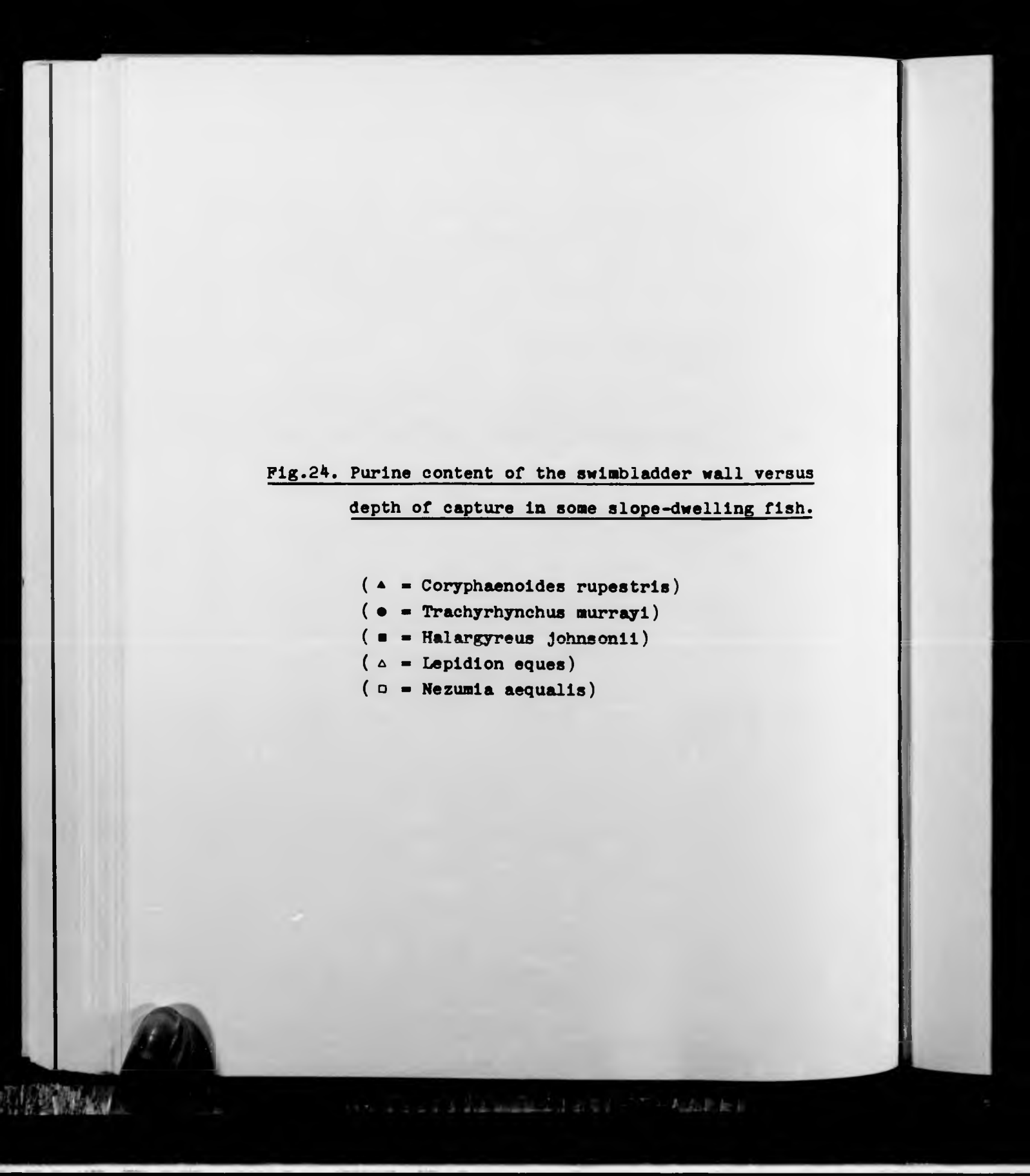


Fig.24. Purine content of the swimbladder wall versus depth of capture in some slope-dwelling fish.

(▲ = Coryphaenoides rupestris)
(● = Trachyrhynchus murrayi)
(■ = Halargyreus johnsonii)
(△ = Lepidion eques)
(□ = Nezumia aequalis)

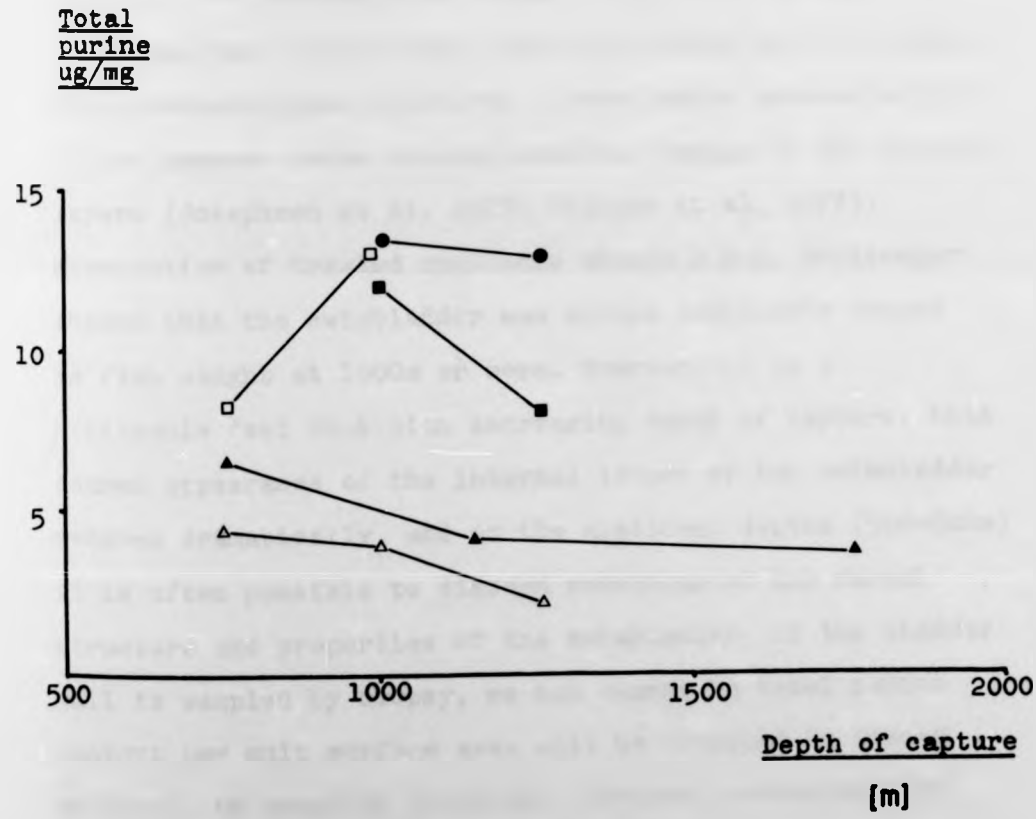

Total
purine
ug/mg
15
10
5
500
1000
1500
2000
Depth of capture
[m]

FIGURE 24

wall versus

lling fish.

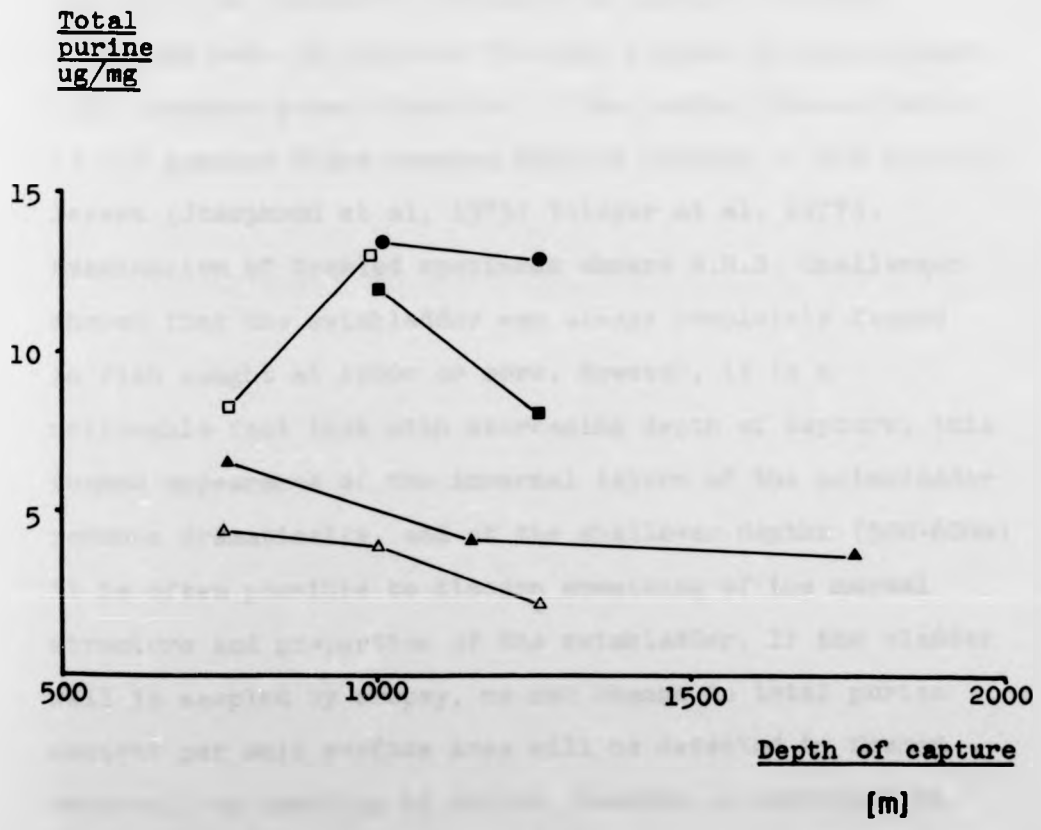


FIGURE 24

negative trend of decreasing purine content with depth, as shown in Fig.24. It should be borne in mind, however, that these results are expressed as total purine per unit fresh weight of tissue, and because of this the negative trend may still be indicative of depth of capture, in the following way. As deep-sea fish are brought to the surface, high pressure gases dissolved in the tunica interna revert to the gaseous phase causing massive foaming of the interior layers (Josephson et al, 1975; Phleger et al, 1977). Examination of trawled specimens aboard R.R.S. Challenger showed that the swimbladder was always completely foamed in fish caught at 1000m or more. However, it is a noticeable fact that with decreasing depth of capture, this foamed appearance of the internal layers of the swimbladder reduces dramatically, and at the shallower depths (500-600m) it is often possible to discern something of the normal structure and properties of the swimbladder. If the bladder wall is sampled by biopsy, no net change in total purine content per unit surface area will be detected in foamed material. On sampling by weight, however, a reduction in purine content per unit weight may be expected, as the same quantity of crystals are dispersed through a greater volume of tissue than normal. These observations fit together fairly well, and as a general statement it may be said that while purine content increases with mean depth of occurrence

of a species, both for biopsy and gravimetric estimates, the increase of depth of capture of individuals may cause a small net decrease in purine content estimated gravimetrically. It may be that if an index of degree of foaming were available, then the gravimetric results could be modified to give a more positive correlation with depth of occurrence.

OTHER FACTORS AFFECTING BUOYANCY

On the basis of currently available data it has been suggested that vertically migrating fishes may not be able to maintain a gas-filled swimbladder at depth (Alexander, 1971, 1972). The most important factors in this are the rate of gas secretion into the bladder, and of gas leakage from the bladder. Known gas secretion rates appear to be inadequate to keep the bladder fully inflated in a downward migration, and the very high rates of secretion needed may, in any case, be unattainable because of the low oxygen content of the water. The factors affecting the rate of gas loss from the swimbladder of physoclists have been assessed in this work and it appears that high rates of gas loss could be expected in vertical migrants.

The water content, lipid content and skeletal structure of fish all contribute to the buoyancy balance sheet and these factors have been described (Denton and Marshall, 1958; Denton, 1961; Bone, 1973). Three other factors contributing to the maintenance of constant depth are considered in this chapter, fin size, ventilation force and elasticity of the swimbladder wall. These are probably of greatest importance to mesopelagic vertical migrators, but may also in some small measure assist in the lives of demersal and epipelagic species.

Bone (1973) suggested that myctophids may use their fins to generate dynamic lift in a manner similer to that employed by selachians. If the swimbladder volume were reduced, as is the case in many vertical migrants, then those species possessing large pectoral fins would not only benefit from the lift produced on swimming, but could also use these as a braking mechanism to reduce the sinking rate. The manoeuvrability of the fins would mean that they could be used as a brake regardless of the orientation of the fish.

The braking power of the fins was tested on deep-frozen specimens of Ceratoscopelus maderensis. The swimbladder was emptied and the carcasses were degassed in a Gallenkamp vacuum oven for ten minutes. The sinking rates of the cadavers was then determined in sea-water by timing their passage between two points in a two litre measuring cylinder (Ehrlich, 1972). The pectoral fins were folded away throughout this procedure. The fish was then equipped with a pair of artificial pectoral fins of life size, made by tracing the expanded fin shape onto used 35mm film. These artificial fins gave the correct surface area and shape without adding any significant weight. The sinking rates were then redetermined.

The mean sinking rates in four fish without swimbladders or fins was 0.12 m/sec, and the addition of fins reduced this to 0.068 m/sec, a factor of almost two. One carcasse was

provided with a "swimbladder" of 4% body weight by injection of air into the body cavity. The sinking rate in this case was reduced considerably to 0.038 m/sec. The combined effect of fins and swimbladder can be predicted from these results, and the overall reduction in sinking rate would be about six times to 0.021 m/sec.

During deep dives in the submersible research vehicle "Alvin", Backus et al (1968) noted schools of the myctophid Ceratoscopelus maderensis hanging apparently motionless in the water at depths of 300-600m. Barham(1971), however, diving in "Deepstar", noted that myctophids were most often vertically orientated in the water. He suggested that opercular currents may assist in the maintenance of a position in the water column, or even that fish may breathe their way up or down. The respiratory currents in fish are of some propulsive significance. Goldfish, for example, can be observed "backing" water with their pectoral fins while maintaining a stable position in a tank. This is necessary to overcome the tendency to breathe their way forward. More recently Blake (1976) showed that the ventilatory behaviour of the sea horse, Hippocampus hudsonius, was modified to allow the fish to ascend in the water more efficiently.

In terms of the maintenance of a position in the water column, Barham's hypothesis is probably only of significance in mesopelagic and bathypelagic species and it is unfortunate

that these species cannot be maintained alive for any length of time to allow assessment of ventilatory force. It was possible to evaluate this force in saithe, however, and a semi-isometric lever was used with a lightly weighted spinally anaesthetised fish, as shown in Fig. 25. Injection of $0.1cm^3$ 1% procaine intravertebrally at about the level of the first dorsal fin stopped body movements but preserved the ventilatory rhythm. Typical records from this experimental system are shown in Fig. 26, and fish of about 50g generated a thrust of up to 30mg.f at each exhalation. This is relatively insignificant when compared to the seahorse (Blake, 1976) and a major factor is the area of the exhalant openings. In saithe, and other epipelagic fish, ventilatory forces are probably not used to decrease sinking rates, and with resting ventilation rates of 30-40/min little lift could be produced in this way. In myctophids, the conversion of exhalant water motion into useful thrust may be more efficient. Barham (1971) noted ventilation rates of about 30/min, but Tytler and Ross (unpublished data) observed ventilation rates of up to 160/min in specimens of Gonichthys dipnetted at the surface at night. In general, myctophids and other vertical migrants are of small size, and it is a well established fact that smaller animals have a higher metabolic rate (Prosser, 1973). It may be that these smaller species have a relatively

Fig.25. System used to determine the force generated during ventilation in Pollachius virens.

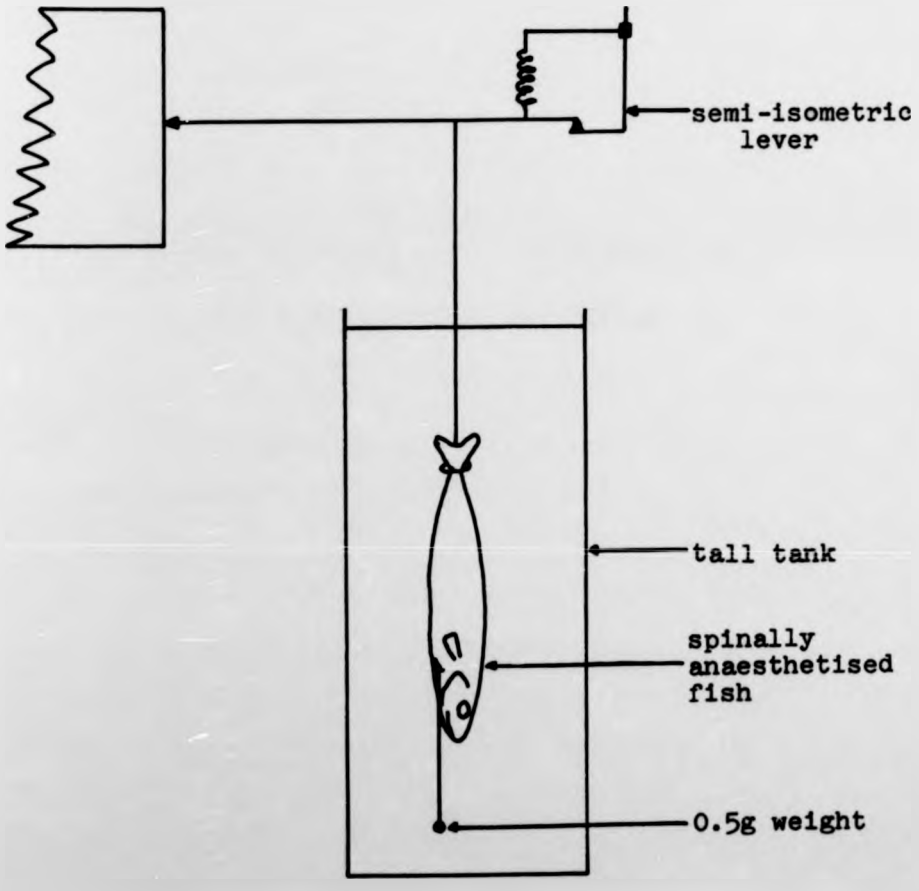

semi-isometric
lever
tall tank
spinally
anaesthetised
fish
0.5g weight

FIGURE 25

Fig.26. Records of force generated during ventilation in spinally anaesthetised Pollachius virens.

(v = ventilation only)
(s = swimming with pectoral fins)

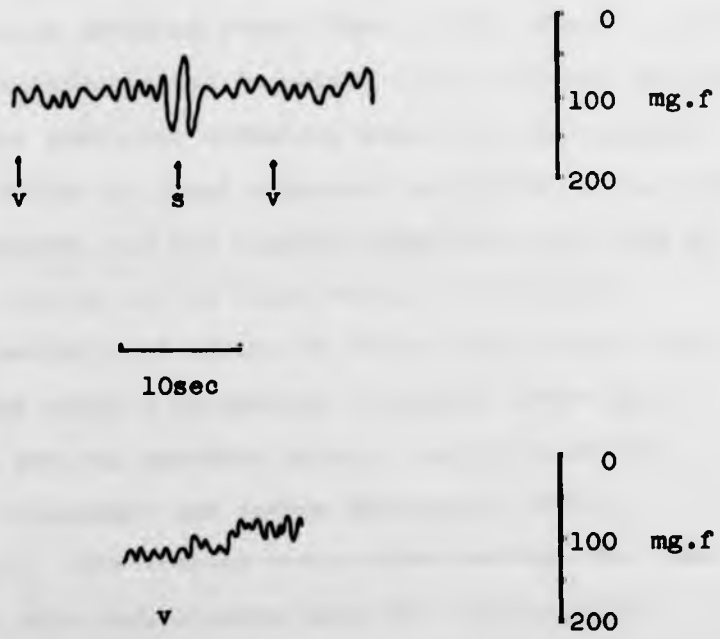


FIGURE 26

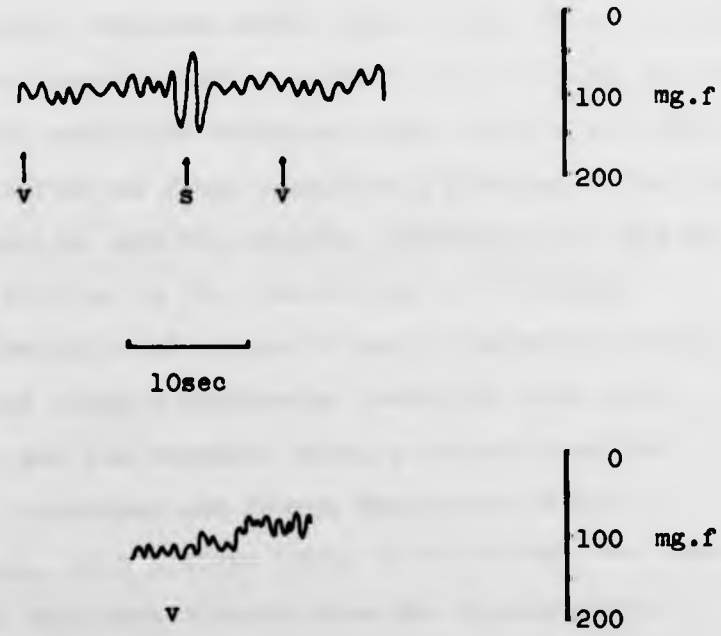


FIGURE 26

higher ventilation volume and can thus generate a more significant thrust.

It has been known for some time that the swimbladder has some elastic properties (Fänge, 1953; Alexander, 1961; Mohr, 1971), which could enable the bladder to decrease in volume without changing shape (Ross, 1976). These elastic fibres were demonstrated in saithe tunica externa in Plate 2b. From the available evidence, most vertical migrants appear to suffer at least a partial deflation of the bladder as they descend, and the elastic properties may have an important bearing on the functioning of the organ.

The elasticity of strips of saithe swimbladder wall was measured using a calibrated isometric lever and kymograph, and was repeated using a resistive bridge isometric transducer and George Washington MD400/2 oscillograph, both systems being shown in Fig. 27. The results of this work clearly show the visco-elastic properties of the tissue, and typical tension-length results are shown in Fig. 28. In several experiments of this kind it was consistently shown that the tissue could be stretched by 50% before irreversible damage occurred, and this is consistent with the work of Tytler and Blaxter (1973) on rupture pressures of saithe swimbladders. This property is not constant in all directions, however, as Fig.28 shows, and antero-posterior strips have a much

Fig.27. Systems used to measure the elasticity of swimbladder wall strips from Pollachius virens.

A. Isometric lever and kymograph.

B. Isometric bridge transducer.

(TX = transducer)
(O/P = output)

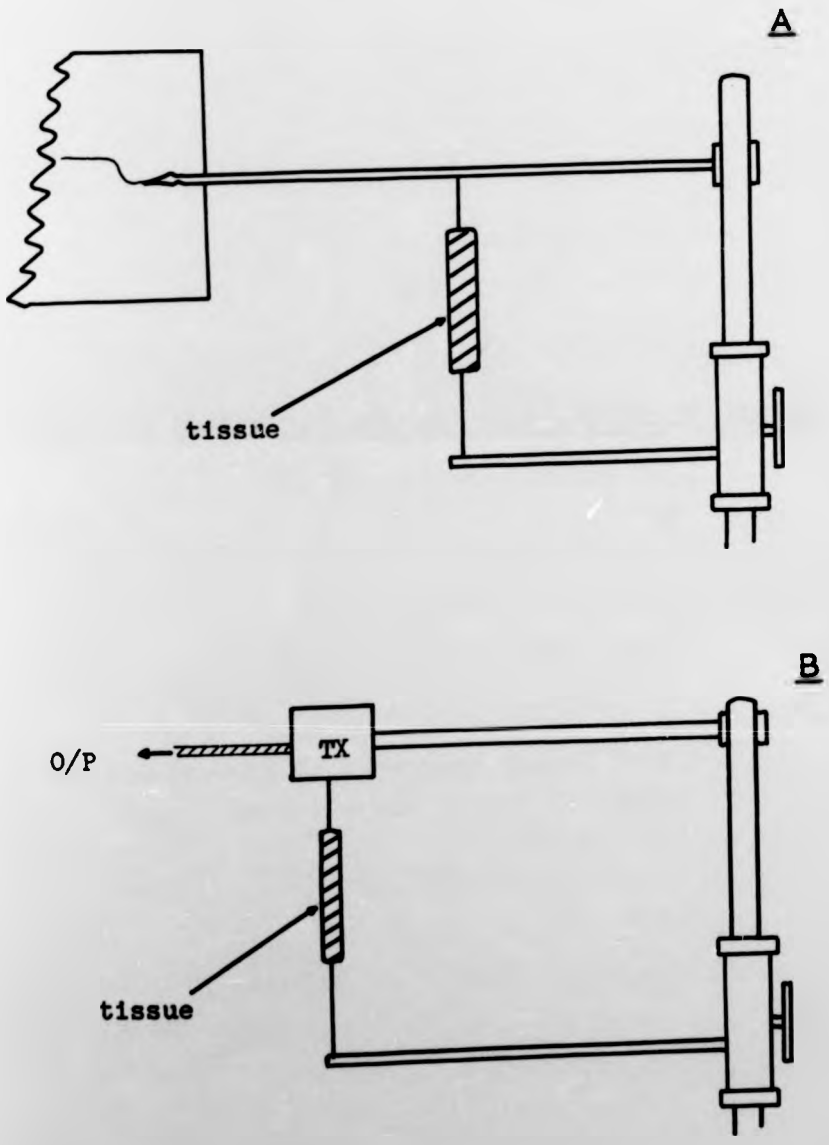


FIGURE 27

Fig.28. Tension-length records for strips of swimbladder wall from Pollachius virens.

A. Transverse strip.

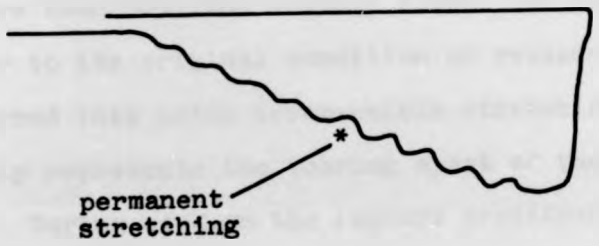
B. Antero-posterior strip.

(each length increment is approximately equal to 10% of the original length)

of swimbladder

imately
ngth)

A



B

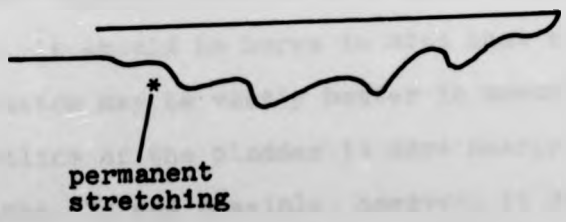


FIGURE 28

reduced strength. Splits in the tunica externa are often apparent in fish raised from depth as a series of ruptures along the more dorsal parts of the wall. It seems from this work that these splits are between the bundles of collagen fibres, which run in a predominantly transverse direction (see Fig. 9.). When the tissue is stretched by not more than 50%, the elastic fibres can restore the bladder to its original condition on release of tension, but beyond this point irreversible stretching occurs which probably represents the tearing apart of the collagen fibres. During work on the rupture pressures of swimbladders Tytler and Blaxter (1973) made similar observations on irreversible damage and were able to construct a safe decompression table to enable fish to be brought to the surface without harm.

These observations probably only apply to epipelagic fish and demersal species with dense connective tissue layers. It should be borne in mind that elastic shape restoration may be vastly better in mesopelagic fish where the outline of the bladder is more nearly spherical than in saithe. It was possible, however, to demonstrate something of the speed and extent of shape restoration in saithe, following overinflation of the bladder. The animals were killed by anaesthetic overdose, the bladder emptied, and the cadaver pinned to a cork board ventral side up.

A cannula was introduced into the bladder through the epaxial musculature, and changes in diameter caused by inflation and deflation were monitored using a simple balanced lever. The preparation is shown in Fig. 29, and results of this experiment in a 100g fish are shown in Fig. 30. Following overinflation of the bladder, the tissue initially sagged at the tip of the probe. The elastic fibres slowly restored normal tone, however, and this took about one minute following a 100% overinflation.

Tytler and Blaxter (1973) noted that when the swimbladder gas pressure was reduced below ambient in saithe, the bladder collapsed and did not appear to be elastic. This result made the estimation of shape restoration under these circumstances pointless, in saithe. Nevertheless, excised tissue contracts noticeably within a few seconds of removal from the animal and this lends credence to the idea that swimbladder wall thickness may not be a constant factor. Marshall (1960) showed the stretched and compressed states of the swimbladder of Vinciguerria sp, and he suggested that shape retention would help to prevent the internal structures from touching each other. The situation may be more complex than this, however, as increasing the wall thickness on deflation of the bladder would inevitably mean that any purine crystals present would be consolidated into a

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Fig.29. System used to examine the elastic properties of the whole swimbladder in Pollachius virens.

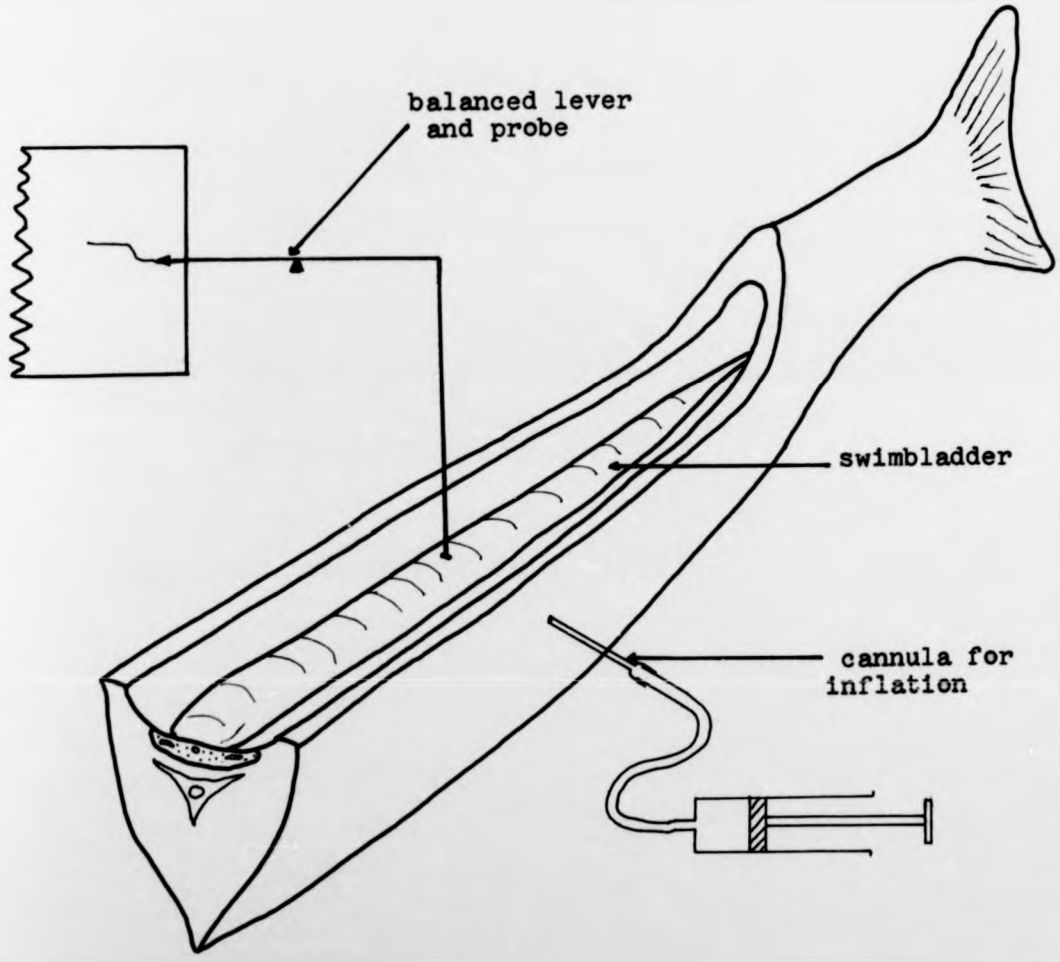
balanced lever
and probe
swimbladder
cannula for
inflation

FIGURE 29

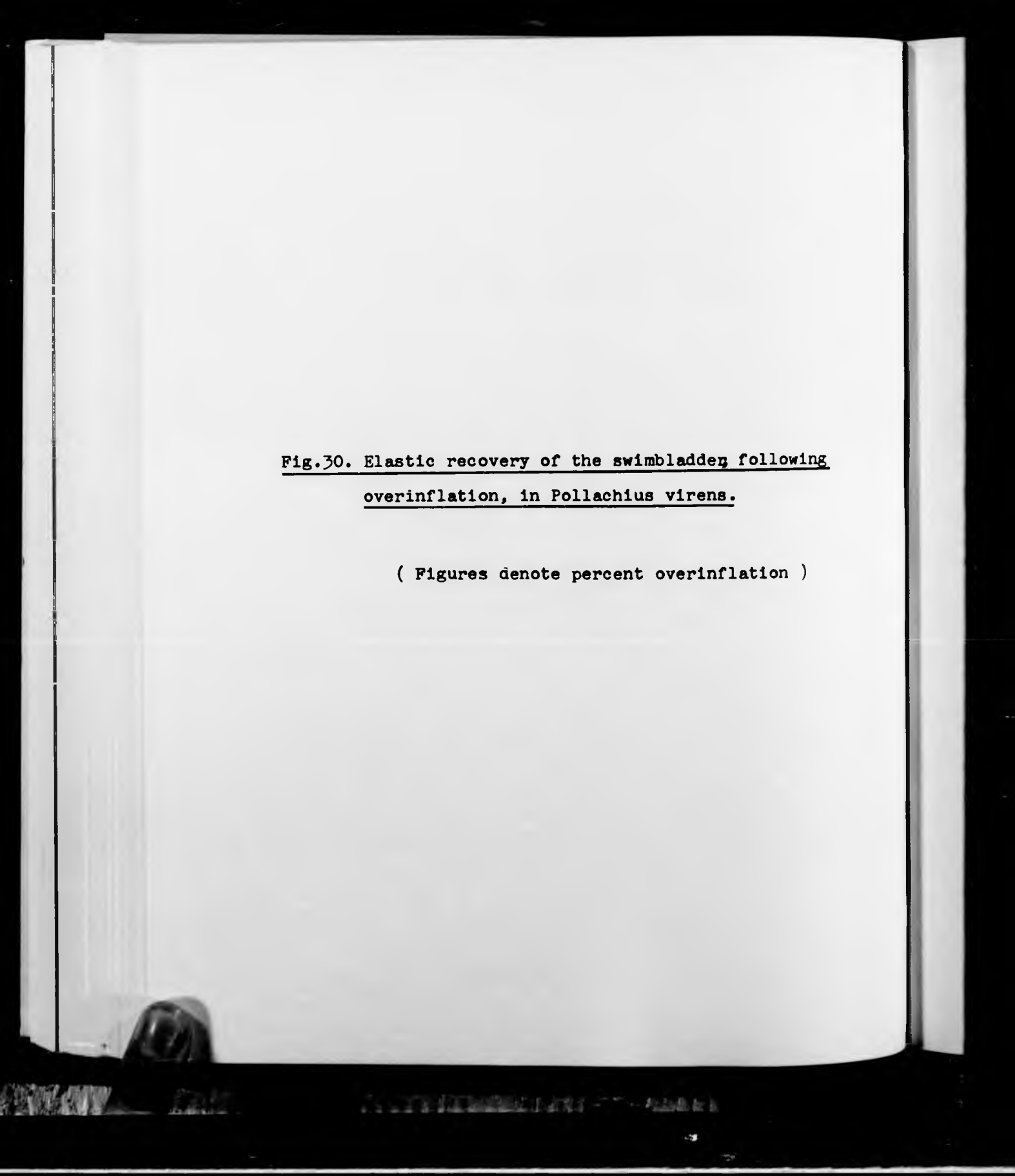

Fig.30. Elastic recovery of the swimbladder following overinflation, in Pollachius virens.

(Figures denote percent overinflation)

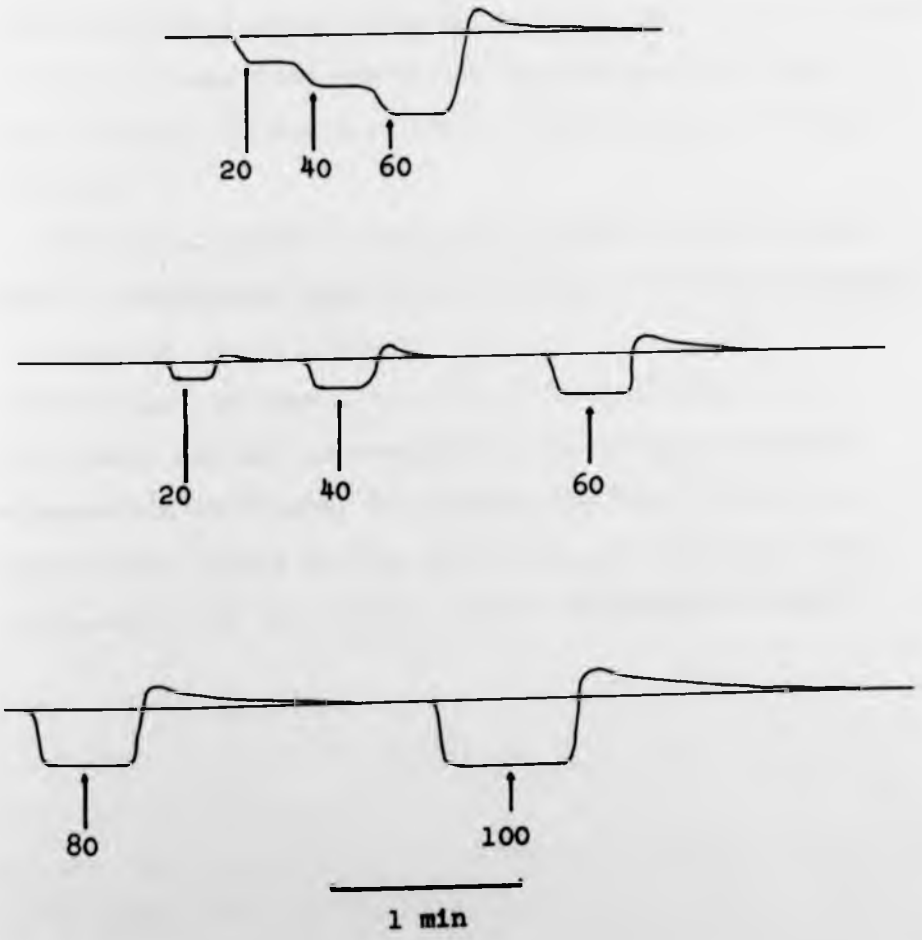

FIGURE 30

smaller tissue volume. This would have the effect of reducing the oxygen permeability of the bladder and hence the rate of gas loss. The situation may be envisaged where this phenomenon could act as a self-limiting system, ensuring that some lift was always provided by the bladder, and at the same time preventing the complete collapse of the bladder with the inevitable complications of tissue adhesion.

The three factors discussed in this section are not usually considered important in the hydrostatic mechanisms of teleosts. It is probable that they have little significance for most fish and it is unfortunate that they could not all be assessed in mesopelagic vertical migrants in this work. In combination they could have a significant effect on the sinking rate of migrants whose swimbladders do not provide neutral buoyancy at depth.

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DISCUSSION

The relative importance of the many functions of a gas-filled swimbladder varies in different groups of teleost. It is an incontrovertible fact, however, that whatever the function gas will be lost from the bladder by one of two routes. Gas loss in buoyancy adjustment is controlled by the oval, but uncontrolled loss will also occur continuously through the swimbladder wall at a rate dependent on the partial pressure difference experienced across the wall. This thesis reconsiders the factors influencing this gas loss, principally in terms of the hydrostatic function of the bladder in anacanthines.

The structure of the anacanthine swimbladder, and of the resorptive area in particular, follows a common basic plan. This has been noted by earlier workers (Hagman, 1921; Fänge, 1953), and was reaffirmed in this work by examination of several species of the order. With the exception of Moridae, which have a three-chambered bladder, it is a single-chambered structure with a gas-gland and oval formed from modified portions of the tunica interna. On a microanatomical level it was found that the histological arrangement of tissues in these fish was adequately described by the scheme outlined by Fänge (1953). The few differences are in thickness of the wall, of the

connective tissue layers, and in density and arrangement of the gas-gland. In some deeper-dwelling species the tunica externa is very substantial. The strength which this confers on the swimbladder is of doubtful significance, however, as the relative pressure changes experienced by Morids and Macrourids are probably small. Suspected vertical migrations appear to be linked with attainment of sexual maturity and are probably slow, at least in Coryphaenoides rupestris (Gordon, pers comm).

Controlled resorption of gas takes place at the oval. This loss is mediated by two distinct mechanisms operating together and probably coordinated by some higher nerve centre. The contribution that each of these makes in the resorption of gas, and its control, have been assessed in this work.

The oval membrane is operated by two opposing muscle systems which are most probably innervated by fibres from the left and right vagi. The deflatory response appears to be adrenergic and can be either nerve-mediated or hormonal depending upon the circumstances. There is no direct evidence that these muscles are innervated by the sympathetic nerves, and this is consistent with the findings of others (Nilsson, 1971).

In order to control gas loss from the bladder the oval membrane would need to be relatively impermeable to

gases. In view of its relatively insubstantial appearance in inshore gadoids, this property was examined in this thesis. The physical basis of low gas permeability was described by Denton et al (1972) and depends on the inclusion of solid materials of extremely low permeability in the tissues. The materials are purine crystals, mainly guanine, and they appear to be consistently located in the tunica interna in anacanthines. For reasons of functional economy this is probably the logical location in all physoclists possessing an oval. Lapennas and Schmidt-Nielsen (1977) recently described guanine crystals in the sub-mucosa of the tunica interna of a number of North American fishes based on electronmicrographs. Other workers have described structures similar to these in different layers, (Phleger and Holz, 1973; Morris and Albright, 1975), and some means of resolving this controversy was needed. Reliable location of the crystal layers was achieved in this work by using frozen-sectioned material and observing this by plane-polarised light microscopy. In this way it was possible to confirm the statements of Lapennas and Schmidt-Nielsen (1977) without involving the doubtful interpretation of electronmicrographs.

The general location of these crystals in the tunica interna was confirmed by polarised-light microscopy in seven anacanthine species, and by guanine assay in several

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The general location of these crystals in the tunica interna was confirmed by polarised-light microscopy in seven anacanthine species, and by guanine assay in several

more. This has some functional significance in physoclists. In two apodan species examined the bulk of the crystals appeared consistently in the tunica externa and this observation agreed with the earlier findings of Denton et al (1972). The consistency of this observation, and the morphology of the apodan bladder, suggests that there is a functional reason for location of the crystals in the tunica externa. Gas volume regulation in eels is achieved mostly by vascular adjustments in the two well-differentiated sections of the bladder and an adjustable internal layer of low gas permeability would be of little use in these species. In true physoclists, however, the oval membrane needs to have a low permeability to oxygen, and it seems logical that it should be formed from an extension of the tunica interna, the same layer providing low gas permeability for the bladder in general. The impermeable layer in conger eels was located in the submucosa by Lapennas and Schmidt-Nielsen (1977) and it is possible that splitting the bladder into two layers in eels tends to leave the submucosal crystal layer with the tunica externa.

The enzyme assay of purines in different layers of the saithe swimbladder has shown that the swimbladder wall and tunica interna contain very similar levels of these materials. This is consistent with the microscopical observations and it was possible to confirm that the serosa

and tunica externa contain no significant quantities of purine.

The low gas permeability of the swimbladder walls of certain fish was realised by earlier workers (Bohr, 1894; Scholander, 1954). The swimbladder gas often has a high oxygen content, and this is particularly so in deeper dwelling species (Scholander and Van Dam, 1953; Kanwisher and Ebeling, 1957). The oxygen permeability of sections of swimbladder wall assessed in this work clearly demonstrate that, in saithe, the major barrier to gas loss is the tunica interna. This finding is consistent with the microscopic location of the purine crystal layer and the theories of Scholander (1954) and Denton et al (1972). The suggestion by some workers that collagen may form a barrier to gas movement can now be discounted as the tunica externa has been shown to have an oxygen permeability similar to other connective tissues both in this work and by Lapennas and Schmidt-Nielsen (1977).

It is possible to estimate the amount of gas lost by leakage through the bladder wall generally and through the thin tunica interna overlying the oval plexus. Let us take the example of a saithe of say 100g, with a swimbladder volume of $5cm^3$. The internal surface area of the bladder would be $22.6cm^3$. The movements of the oval membrane are such that the plexus is probably never completely isolated

from the lumen of the bladder, leaving say 1% of the total internal area always exposed. Conversely, at maximum, the exposed plexus probably represents some 40% of the internal surface area. Certainly the extreme conditions indicated by Lapennas and Schmidt-Nielsen (1977) probably do not occur in anacanthines. The exposed plexus may thus occupy between 1% and 40% of the total internal surface area.

The extremes of gas loss by this route can be calculated by rearranging the equation of Krogh (1919) to $Q = \frac{K.A.P.t}{d}$. The $K(O_2)$ of the thin tunica interna in the plexus region is probably close to that of the tunica externa, 0.228, the thickness being about 10μm. At a pressure of Patm, the difference in partial pressure of oxygen between the swimbladder and the blood is usually about (P - 1)atm (Alexander, 1970). At a depth of 2m the partial pressure difference would be (1.2 - 1) = 0.2atm, and this can be substituted for P in the equation. The gas loss through this region would thus range from 0.062cm^3/h when the oval is closed, to 2.46cm^3/h when the oval is open.

The gas loss via the swimbladder wall under these conditions can also be calculated. In the case of the closed oval, 99% of the internal surface area could have the low oxygen permeability of 0.0534, a thickness of about 300μm, and a consequent gas loss of 0.045cm^3/h. When the oval opened the swimbladder wall would reduce to 60%

of the internal surface area, for the purpose of the calculation, and loss by this route would reduce to 0.027cm^3/h. Thus loss from both sources in a fish at a depth of two metres would be 0.107cm^3/h in the case of a closed oval and 2.485cm^3/h if the oval was open. Fänge (1953) suggested that there may be a steady turnover of gas in resting physoclists, and it is fair to say that near the surface, with a closed oval, the low rates of gas loss predicted in this work would be easily tolerable.

At depth, the rate of gas loss will increase. The percentage of oxygen in the bladder would be greater and the hydrostatic pressure would be much higher. These two factors in combination serve to greatly increase the partial pressure difference across the bladder wall, and this has a large effect on gas loss. At 100m depth, a freshly filled swimbladder could contain up to 90% oxygen and the external hydrostatic pressure would be 11atm. The oxygen partial pressure difference would thus be around 10atm and the consequent gas loss would be 5.32cm^3/h in the case of a closed oval, and a staggering 129.26cm^3/h if the oval were fully open. This calculation clearly demonstrates the effectiveness of the oval membrane as a barrier to gas loss. The potential of this adjustable window in the control of gas access to the vascular plexus is now obvious despite its insubstantial initial appearance.

Despite this demonstration of the effect of the oval membrane, a 100g fish with a closed oval could still lose some 5.32cm^3/h from the bladder at a depth of 100m. Even if the oval closed tight the loss through the bladder wall would be 2.26cm^3/h. This, of course, would appear to be intolerably high and must be considered in the light of known rates of gas secretion. Gas secretion in saithe has been measured fairly recently by Blaxter and Tytler (1973) and they reported a rate of 2.5cm^3/kg/h. Their estimates represent the excess of gas secretion over gas loss, however, as refilling of the bladder was observed as a buoyancy adjustment. Their maximum figure was obtained in a pressure change from 1 to 4 ATA, and at this pressure gas leakage with a fully closed oval would be about 5.20cm^3/kg/h. The total rate of secretion in these fish was thus about 5.20 + 2.50 = 7.70cm^3/kg/h and it can be seen that under these conditions the depth range of the fish would be severely limited. After complete evacuation of the swimbladder Wittenberg et al (1964) found a maximum secretion rate in the bluefish, Pomatomus saltatrix, of 15cm^3/kg/h. The opinion of many workers is that secretion rates are likely to be as high as this (Alexander, 1971). Using this generous secretion rate the maximum sustainable pressure in the bladder was calculated as 6.6 ATA, equivalent to a depth of about 56m.

Once the gas has gone into solution in the bladder wall, it cannot then provide buoyancy, and as the tunica interna is the first layer encountered by the gas it may be more relevant to consider only the $K(O_2)$ of this layer in calculations of total sustainable pressure. With a $K(O_2)$ of 0.025 and a thickness of say 200μm, the maximum sustainable pressure in the bladder becomes 8.5 ATA, equivalent to a depth of about 75m. The maximum depth of neutral buoyancy of other species examined in this work was generally less than 100m and although doubts can be raised concerning some permeability measurements, it is also certain that current estimates of maximum rates of gas secretion are not reliable.

The purine content of swimbladder walls in slope-dwelling fishes has been demonstrated to be proportional to their known depth of occurrence in a British sampling population. These animals generally do not experience large relative pressure differences at the depths at which they live, nevertheless the quantities of gas involved in secretory activity at these depths are large. A 100g fish with a swimbladder of $5cm^3$ in moving from 1000m to 1100m, would experience a small relative pressure change of about 8%. The volume of gas at N.T.P. that it would need to secrete in order to remain neutrally buoyant, however, would be $50cm^3$. The time taken to refill the swimbladder completely at surface pressures varies considerably but an average

period appears to be about one day. If the fish were able to secrete 5cm^3 of gas in one day at the surface, then ten days would be needed to regain neutral buoyancy in a move from 1000m to 1100m (Scholander, 1954). Thus what appears to be a small relative depth change involves a big secretory effort. Fish living at depth have been shown to possess retes which vary in number and length with depth of occurrence (Marshall, 1965). They thus appear well-adapted to secrete gas, and may be able to do so at extremely high rates. It is logical therefore, that having secreted gas at depth every effort should be made to retain it, and an increase in the purine content of the swimbladder wall is consequently quite predictable.

It would be very interesting to know to what extent the increase in purine content reduces the permeability to oxygen. From this work, and that of others, there is some, albeit insubstantial, indication that these two factors are inversely related. While it is possible to make reasonable estimates of purine content from trawled demersal fish, it is almost impossible to measure the permeability to oxygen. Tissues are either split, badly foamed, or as often happened in this work too thick for the measuring system. Large areas of tissue would need to be used before any useful results could be obtained from these fish. One of the next steps in deep-sea fish biology will probably be to decompress trapped fish, and land them intact aboard

ship. When this happens our knowledge of deep-sea swimbladder physiology can be expected to increase dramatically.

A further factor in reduction of gas loss from the bladder is the barrier function of th rete. This structure is not only fundamental to secretion of gas, but its layout also prevents high pressure gas from leaking away into the bloodstream (Scholander, 1954). This means that a variable proportion of the internal surface of the bladder is probably completely impermeable to oxygen. Some fish have a small button-like rete, as in the macrourid Chalinura mediterranea, while others, in particular the deep gadoids Molva dypterygia and Micromesistius poutassou (see Plate 5), have an extensive gas gland. In saithe the gas gland occupies at most 10% of the internal surface area of the bladder, and this will only slightly reduce gas loss. In deeper fish, the larger area may play a significant part in reducing gas loss, especially following any reduction in volume of the bladder as fish migrate downwards. In some mesopelagic fish the resorbent and secretory plexes are often very closely associated (Marshall, 1960), and the secretory mucosa could spread very widely over the inner surface of the bladder. In these species the barrier function of the rete may be extremely important in maintainance of an

inflated bladder.

It is extremely difficult to define the exact physical role that the oval plexus plays in the removal of gas from the bladder. The plexus is supplied richly with arterial blood, and the extent of this plexus is such as to produce a sheet of adjustable blood bearing capillaries. The haemoglobin in this blood will be almost fully saturated, estimates vary from 75% (Steen, 1963) to 85% (Brown, 1957) and chemical combination of oxygen will only be of importance in surface waters (Alexander, 1971). Most of the transport of oxygen away from the bladder must consequently be in physical solution in the plasma. The solubility of oxygen in plasma is low, about $4cm^3/100cm^3/atm$, and assuming a maximum flow-rate of say 60ul/min in the plexus of a 100g fish (Table 2), the maximum rate of gas removal at a depth of 2m would be only $0.0144cm^3/h$.

In most physoclists, the partial pressure of oxygen in the swimbladder at the surface is almost identical to that in the blood, and this will virtually abolish gas loss altogether via the plexus. At a depth of 100m, however, the solubility of oxygen in plasma is about $44cm^3/100cm^3$. Under these conditions the maximum rate of gas removal would be $1.58cm^3/h$, and this corresponds to a maximum ascent rate of about 3.2m/h. The inevitable conclusion from this calculation is that many physoclists cannot

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resorb gas at a rate consistent with their observed rates of vertical migration, although in demersal fish these rates are probably adequate for the minor adjustments commonly required (Schmidt, 1955; Beamish, 1966).

It seems, from these results, that in saithe at least the limiting factor in removal of gas from the bladder may be the rate at which it can be removed from the site by the vascular system. It should be borne in mind that if this oxygen is not removed then the tissues around the open oval will become saturated with oxygen at high partial pressure. This could leave large tissue volumes exposed to the toxic effects of hyperbaric oxygen and exposure for only a short period would result in cell death. The purine crystals and connective tissue layers of the bladder wall can probably withstand hyperbaric oxygen well, because of their largely extracellular nature. The major vulnerable areas are the lining epithelium of the bladder, and the tissue surrounding the oval. D'Aoust (1969, 1970) has shown that gas gland tissues are very insensitive to hyperbaric oxygen, and exhibit no Pasteur effect. Boström et al (1972) showed that the oxygen sensitive steps were shunted by non-sensitive systems, and it may be that the tissues of the oval region are similarly modified.

It is possible that blood flow rates could have been underestimated in this work. Blood clots and haemorrhagic

shock could reduce flow rates measured by the microsphere technique, and it is known that handling causes haematological changes in fish. The observation that the withdrawal cannulae remained patent even after 5 minutes of implantation helps to overcome this argument, although some doubt will remain. Steen (1963), by very indirect means, estimated blood-flow to the resorbent bladder in eels to be about 20% of the total cardiac output, and this is about ten times greater than that estimated in this work. The calculation of potential maximum flow-rate using the Poiseuille equation provides some reference point in this debate as the parameters used are purely physical ones. It is encouraging to note that the results obtained by these widely divergent methods in this thesis are quite similar.

In saithe, lantern fish, and other species, it seems that the bladder cannot be maintained at constant volume during diel vertical migrations. In recent years it has become apparent that known maximum secretion rates are not sufficient to maintain the bladder on descent of a vertical migrant. It is now apparent that for any substantial upward movement, the rate of removal of gas by the blood may be insufficient to compensate correctly. This lends further support to the increasingly widely held view that migrants may be neutrally buoyant only at, or near, the

top of their vertical range (Alexander, 1970; Tytler and Blaxter, 1973).

It is tempting to speculate on means other than the swimbladder whereby the maintenance of a stable position in the water column could be made less energetically expensive, and two factors have been suggested in this thesis. In inshore and demersal fish, finsize has little influence on sinking rate. Diel migrators, however, undoubtedly achieve some reduction in sinking rate by spreading their fins, and the large pectorals noted in some myctophids (Bone, 1973) will be useful in this context. A decrease in sinking rate of one half was observed in this work, in the large-finned fish Ceratoscopelus maderensis. In life a greater retardation may be achieved as the fish fin would certainly form a better hydrofoil shape than the artificial fins used in this work.

A small, but measureable, force is generated by the ventilatory current in saithe. This force is quite significant in the sea horse, which has been observed to depress its breathing rhythm in order to move more efficiently upwards (Blake, 1976). The force generated by saithe is probably of no significance, but in mesopelagic vertical migrants it may be put to good use. Barham (1971) described the vertical orientation of myctophids in

mid-water, and suggested that they could breathe their way up or down in the water column. It is possible that these fish use the ventilatory currents to assist in maintenance of a position in the water at depth.

The elastic collapse of the bladder seems to be a reasonable proposition, within limits, and Marshall (1960) described its possible occurence in a mesopelagic fish. Tytler and Blaxter (1973) found that ultimate collapse of the bladder always occurred in saithe, and shape retention is probably of no importance in these fish. Slow compression of the bladder of mesopelagic vertical migrants would, however, certainly result in some thickening of the swimbladder wall with a consequent concentration of purine crystals. The ensuing increase in gas retention would be of great benefit to many fish and it may mean that some static lift could be provided by the bladder over a greater vertical range.

In this thesis a concerted attempt has been made to assess gas leakage from the swimbladder of a gadoid, Pollachius virens, and to compare it as far as possible with other members of its own order, Anacanthini. The anacanthine swimbladder is, for three major reasons, a difficult subject for study. The fish, broadly, live at great depth, and intact material is extremely difficult to obtain except in carefully handled coastal species.

The bladder is closely bound to the vertebral processes and cannot be removed intact as in eels and some other species. Lastly, but most importantly, in all species it is located deep in the body and is not suitable for cannulation of blood vessels and the elegant surgery which can be achieved with apodans. In many respects eels are an easy option in swimbladder studies, they are extraordinarily hardy and have a very accessable bladder. It is obvious, however, that they have a very different anatomy from other important physoclists and in this respect they are not typical of the group. Nevertheless, a great deal of our knowledge of swimbladder physiology is based on pioneer work with eels, and indeed some information for calculations in this thesis was drawn from published work on eels. It is felt, however, that there is a strong case for avoiding generalisations in fish studies, and anacanthines, with their classic closed swimbladder and range of habitat, are worthy of more detailed study.

The nature and location of the barriers to gas movement have been demonstrated and quantified as far as possible in this thesis. The impermeable crystal layer is sited in the sub-mucosa of the tunica interna, and it is a neat point of design that the oval membrane should be formed from an extension of this layer. The amount of

purine in swimbladder walls has been measured in a number of species extending from coastal to deep-sea in habit, and a direct relationship with depth of occurrence was established. Some evidence for an inverse relationship of purine content and oxygen permeability was amassed although the evidence is scanty. This would be an important area for development in any future work. The induction of the deflatory reflex has a rapid and noticeable effect on blood-flow in the oval plexus. It appears, however, that removal of excess gas by the blood will be the limiting factor affecting the rate of resorption and of vertical ascent. In saithe, the swimbladder may be able to provide neutral buoyancy throughout the known vertical range, but at depth gas leakage would be high. This leakage is even greater in mesopelagic vertical migrants and finsize, ventilation force and swimbladder elasticity have been proposed as additional factors in the buoyancy balance sheet or contributing to maintenance of a station in the water column.

There are a number of areas for future work which have emerged in the evaluation of this study. Where does the excess gas go when buoyancy adjustments are made? Are the estimated blood-flow rates correct? The further refinement of the microsphere method would be of great interest in all aspects of fish physiology although it is

felt that it would adapt more easily to larger individuals. Is the tissue of the oval region immune from hyperbaric oxygen effects, or is the gas gland unique in this respect?

The effect of purine quantity on permeability to gases should be assessed by measurement in as many species as possible. Some estimate of the expected effects could be derived perhaps from studies of simulated purine-containing systems, in which crystals were deposited in varying amounts in supporting matrices. The correlation of the purine content of the swimbladder and depth of occurrence could be extended to include specimens from about 5000m due to the recent development of single-warp trawling techniques. This system has only recently been proved on R.R.S.Challenger by Dr Gordon, and it was unfortunately too late to extend the depth series in this work.

In mesopelagic fish it is apparent that studies of ventilatory force and fin size, and their effects on sinking rate would be of great interest. It is unfortunate that mesopelagic cruises are rare and that the fish do not survive well in trawls. The elastic collapse of the bladder would also be worth further study in fresh material.

Some of the more interesting aspects of swimbladder physiology are apparent only in deep-sea mesopelagic or

demersal fish and it is irritating that these fish cannot be brought to the surface intact. A great step forward would br the trapping and decompression of these species and it is probable that progress will be made in this field in the near future.

This discussion has shown that the main aims of the thesis have been achieved, in that it was possible to evaluate the major factors affecting the rate of gas loss from the physoclist swimbladder. In the course of the investigation, however, several areas for future investigation have inevitably been uncovered and it is apparent that gas loss should perhaps not have been taken for granted for so long.

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APPENDIX 1

Blood samples were obtained from six saithe by severing the tail and bleeding the anaesthetised fish into a heparinised tube. The blood was centrifuged at 5000 r.p.m. for 10mins and the resulting serum was drawn off for analysis. Sodium and potassium were determined by flame-emission spectroscopy and calcium and magnesium were determined by absorbance spectroscopy on a Pye Unicam SP 90 atomic absorption spectrophotometer. Serum chloride was determined using a Buchler-Cotlove chloridometer and osmotic pressure was monitored using a Fiske-QF-osmometer (330D). Blood glucose levels were determined using the glucose oxidase/o-dianisidine enzyme assay technique (Sigma). The results of these analyses are shown in Table 10.

Using the mean values of these analyses a saline formula was derived which could be used either with or without glucose or a phosphate buffer, according to the requirements of the experiment. The recipe is shown below.

NaCl	10.220g	to 1 litre.
KCl	0.460g	
$CaCl_2$	0.280g	
$MgCl_2$	0.087g	
$NaHCO_3$	0.050g	

If required, the salts can be dissolved in 975cm^3 of distilled water, and 25cm^3 of a pH 7.5 phosphate buffer added. This formulation was found to be suitable for tissue isolated from saithe kept in the Stirling aquarium in seawater of 32 ‰ NaCl. It was possible to maintain isolated, cleaned intestine in a stimulateable condition for seven days at 4°C.

Table 10. Results of analyses of saithe blood.

(- denotes no observation)

FISH	Na mM/l	K mM/l	Ca mEq/l	Mg mEq/l	Cl mM/l	pH	OP mOsm /Kg	Glucose mg%
1	178	4.8	3.54	1.15	133	7.62	285	68.8
2	183	7.5	4.14	2.63	162	7.55	333	81.5
3	185	6.7	3.74	1.73	144	7.40	295	68.7
4	176	6.9	5.59	4.80	144	-	305	-
5	178	2.2	5.04	1.98	144	-	-	-
6	165	2.2	5.24	4.28	147	-	-	-
$\bar{x}$	178	5.1	4.55	2.77	146	7.52	305	73.0