SOME FUNCTIONS OF THE SWIMBLADDER AND ITS DUCTS IN
ATLANTIC AND PACIFIC HERRING

by

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ABSTRACT

The swimbladder of Atlantic and Pacific herring has a pneumatic duct arising from the stomach caecum and a direct posterior opening to the exterior. The thesis is advanced that these peculiarities are associated with differences in function which may be related to the life of the herring.

Herring obtain swimbladder gas by swallowing air at the surface but not by secretion or bacterial gas generation over one week. Gas release from the swimbladder through the posterior duct occurs in response to pressure reduction, sympathomimetic drugs and atropine and is inhibited by spinal section or brain removal suggesting a gas release mechanism involving the central nervous system. Gas loss through the pneumatic duct is prevented by the swimbladder valve which opens in response to adrenalin. The swimbladder responds to adrenalin by moving its contained gas anteriorly and to pilocarpine by increasing internal gas pressure. The pneumatic duct, normally fluid filled, controls the applied pressure at which gas flow in either direction starts and finishes. This duct mechanically prevents the entry of particulate matter from the stomach and is able to remove air bubbles
or foreign matter. The stomach caecum when closed anteriorly by sphincters may force ingested air through the pneumatic duct. Ingested air does not provide a source of oxygen permitting survival of herring under low oxygen conditions. The origin of the pneumatic duct from the stomach may be associated with the use of the stomach as a temporary gas storage organ. Gas uptake appears to be mediated by parasympathomimetic drugs; gas release by adrenalin.

The percentage volume of swimbladder gas of Atlantic and Pacific herring from 36 cm of water was correlated with percentage fat volume. At 3% fat the swimbladder volume of both groups is 4.2% and at 12% fat, 2.5 to 3.1%. The excess pressure of swimbladder gas varied from 0 to 2.7 cm Hg independently of volume. Sinking factors lay between 1001 and 1005 for fat contents below 6% and were slightly higher in fatter fish.

Analysis of the body components of 13 Pacific herring gave the following mean values: swimbladder gas volume 4.1%, density .0013 g/ml; fat 3.5%, 0.926 g/ml; scales 0.5%, 1.966 g/ml; skeleton 1.2%, 1.993 g/ml; remainder of the fish 90.6%, 1.057 g/ml. The mean force in dynes/ml of fish acting upwards on these fish due to the swimbladder would be 41.4 and to fat 3.3 while downward forces due to the scales, skeleton and rest would be 4.6, 11.2 and 32.1 dynes respectively,
leaving a mean net force of 3.2 dynes/ml downwards to be compensated for by movements of the fish.

As the herring swimbladder functions as a hydrostatic organ the low skeletal body content and high fat content results in a low swimbladder volume, so reducing the change in density with depth, an advantage to a fish undergoing diurnal vertical migrations. It was calculated that herring of Passamaquoddy Bay, N.B. can descend to their median daytime depth of 10 metres in August and 35 metres in February for sinking factors of $10^{16}$ and $10^{18}$ respectively. Predation may be reduced by the ability of herring to complete air uptake rapidly, to move upward without restriction by expelling any excess gas through the posterior duct and to liberate gas in times of stress in response to adrenaline so increasing body density and permitting rapid downward movement. Thus in many ways the herring because of its anatomical modifications has been able to adapt the physostome condition successfully to its marine environment.
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CHAPTER I.
INTRODUCTION

The swimbladder of herring (Clupea harengus and C. pallasii) differs from that of most teleosts in having a pneumatic duct which in the adult fish arises at the posterior end of a large stomach caecum and in having a direct posterior opening to the exterior. The thesis is advanced that the anatomical peculiarities of the herring swimbladder and ducts are associated with differences in function which may be related to the life of the herring.

The herring occur in schools which are subject to heavy predation from above (gulls and diving birds), from the sides (whales, seals and many fish) and from below (dogfish, eels). Thus imposed on their daily cycle of vertical movements, which bring them towards the surface at night and in deeper water by day, may be the necessity of rapid flight horizontally or vertically. Any modification which increases the ability of the herring to move swiftly upwards without rupture of the swimbladder or downwards without an extreme increase in density or which enables the herring to leave a curtain of air bubbles which might confuse predators could be of advantage to the species. Because of predation pressure from above, modifications which
decrease the time at the surface needed to swallow air for the swimbladder could also be of use. Other less obvious functions might have necessitated the posterior movement of the pneumatic duct; studied here is the possibility that the vascular stomach wall can absorb enough oxygen from ingested air to allow herring to survive in water of low oxygen content and that the stomach caecum is the site of bacteria-generated gas which passes to the swimbladder.

Basic to all these topics is a thorough understanding of the physical, anatomical and functional properties of the herring swimbladder and its pneumatic and posterior ducts. The pneumatic duct, by its length and translucent walls which permit the movement of gas and fluid in the duct to be observed in living preparations, provides excellent material for study and suggests functions for this organ far exceeding that of a mere conduit for gas. The hydrostatic function of the swimbladder was studied here as this is most closely linked with the functions of the pneumatic and posterior ducts. The swimbladder also has sensory functions which were not studied here but which may impose some restrictions on its physical properties. The posterior duct in comparison is simple in structure and function.
The study is based on herring of the Atlantic and Pacific coasts of Canada. These may either be regarded as belonging to separate species (Clupea harengus L. and C. pallasii Valenciennes) or following the classification of Svetovidov (1952a) as subspecies, Clupea harengus harengus and C. harengus pallasi. The immature Atlantic herring and mature and immature Pacific herring used here were both obtained from coastal waters and in the aspects of swimbladder physiology studied here gave similar results. Greater differences between the groups might have appeared had it been possible to compare deep spawning Atlantic herring with shallow spawning Pacific fish.

The Atlantic herring were obtained from weirs in Passamaquoddy Bay, New Brunswick and the Pacific herring by seining shallow water along the southern coast of British Columbia between Horseshoe Bay and Squamish. In both instances the fish after being confined in a dip net were lifted from the sea in buckets of water and were transported to the holding tanks without removing the fish from water. Pacific herring were kept at the University of British Columbia in tanks with a recirculating and filtering system and were fed lightly on Mytilus. Atlantic herring were held in circular fibreglass tanks at the Atlantic Biological
Station, St. Andrews, New Brunswick with running sea water and were fed on scraped herring flash. Aerators were present in all stock tanks. All transfers of fish between tanks or into experimental surroundings were made without lifting the fish out of water and with the minimum of disturbance. These precautions were required to prevent accidental and unrecorded release of swimbladder gas and to prevent damage to the skin and mucus coat of the fish.
CHAPTER II

ANATOMY OF THE STRUCTURES INVOLVED IN THE UPTAKE AND RELEASE OF SWIMBLADDER GAS

Introduction.

The anatomy and histology of the swimbladder and the pneumatic duct of Atlantic herring, including the ontogeny of these structures, has been described in detail by Beaufort (1909) and later by Maier and Scheuring (1923). The swimbladder of herring including its connection with the ear and direct opening to the exterior was first described by Weber (1820). The work of this author and others of the nineteenth century on the herring swimbladder have not been consulted here; their work is reviewed by Beaufort and by Maier and Scheuring.

Much of the interest in the swimbladder of the herring arose from its connection with the ear. As described by Maier and Scheuring the swimbladder is connected to the ear by means of an anterior prolongation of the swimbladder which divides into two fine canals and after a second division ends in two bullae on each side of the head. The bulla in the prootic bone on each side is partly filled by the termination of the swimbladder canal and partly by the perilymph of the ear, so that changes in the volume of the swimbladder end
can be transmitted to the ear through the perilymph. This relationship of the swimbladder to the ear of herring is not studied here but it may impose certain restrictions on the physical properties of the swimbladder gas, especially its internal pressure.

Also described by Maier and Scheuring and not repeated here is a study of the ontogony of the pneumatic duct and swimbladder. These authors suggest that, although in the adult herring the pneumatic duct arises at the hind end of the stomach caecum, this is a secondary condition and the pneumatic duct develops as an outgrowth of the oesophagus. To support this view they point out that the pneumatic duct has no gastric glands and bears internal folds like those of the oesophagus.

In the present investigation the structure of the swimbladder and associated structures were studied in transverse and longitudinal sections of the Pacific herring so that the results could be compared with the published descriptions based on Atlantic herring. The interpretation of these sections was made easier by the examination of intact structures cut longitudinally by hand and the function of these structures was examined in living tissue in both Atlantic and Pacific herring.
General Description, Atlantic and Pacific herring.

The oesophagus of the herring is a short straight tube which posteriorly discharges through an oesophageal sphincter into an elongated horizontal stomach caecum (Figure 2.1). From the ventral side of this caecum close to the entrance of the oesophagus the pyloric part of the stomach descends ventrally and then runs forward. A sphincter guards the entrance of the pyloric part from the caecum of the stomach. The pneumatic duct arises from the posterior end of the stomach caecum, runs backward and then bends forward and finally opens into the swimbladder on its left ventral side. The swimbladder of the Pacific herring as of the Atlantic herring described by Maier and Scheuring narrows to a duct anteriorly which after a double bifurcation terminates in four bulla close to the inner ear. Within the body cavity the swimbladder is enlarged, silver in colour and delicately constructed. Posteriorly it curves along the posterior wall of the body cavity, loses its silver layer and finally discharges to the exterior by a pore located between the anal and urinogenital apertures on the left side.

Detailed description, Pacific herring.

Oesophagus.

Throughout most of the length of the oesophagus the only muscle coat in evidence is the layer of
circular striped muscle, covered exteriorly by the serosa. The submucosa consists of loose connective tissue with blood vessels except in the region closest to the mucosa where the connective tissue strands are close together and arranged parallel to the inner epithelium. The mucosa and submucosa are deeply folded and in parts in surface view show a honey comb structure. The mucosa consists of tall columnar epithelium with goblet cells (Figure 2.2).

Oesophageal sphincter.

Posteriorly the numerous folds of the oesophageal mucosa are replaced by the few longitudinal ridges of the stomach caecum and gastric glands appear in the submucosa. The oesophageal sphincter lies where these changes in the wall take place, and a section of this region is shown in figure 2.3. It is chiefly remarkable for the appearance of a band of tangential striped muscle which replaces the circular muscles along the ventral side of the oesophagus. Presumably the sphincter is closed by the contraction of the well-developed circular and tangential muscles in this area. As the lumen in the relaxed preparation is nearly filled by its internal ridges little contraction of these muscles would be required to close the sphincter (Figure 2.4).
Figure 2.1 Diagram of the herring swimbladder and its connections
Figure 2.2  Transverse section of oesophagus of Pacific herring, midway between pharynx and stomach.

Figure 2.3  Transverse section of oesophageal sphincter of Pacific herring.
Figure 2.4  Sphincter region of the stomach cut lengthwise.

From a 22 cm Pacific herring.
Figure 2.5  Transverse section of the stomach caecum at the level of the sphincter guarding the pyloric part of the stomach of Pacific herring.
Figure 2.6 Transverse section of the stomach midway along its length, Pacific herring.

Figure 2.7 Transverse section of the pneumatic duct at the level of the first internal pocket in Pacific herring.
Figure 2.8 Longitudinal section of the hind end of the stomach caecum and first part of the pneumatic duct in Pacific herring.
Figure 2.9  Part I of the pneumatic duct of Pacific herring cut lengthwise and drawn as a solid.
Sphincter at the entrance to the pyloric part of
the stomach.

The band of tangential muscle continues
posteriorly, lying in the acute angle between the oesophagus
and the pyloric part, but now it consists of smooth instead
of striped muscle fibres. A thin layer of longitudinal
muscle lies outside the circular and tangential muscle zone.
The pyloric part of the stomach leaves the stomach caecum on
its ventral side and can be constricted at the junction
partly by the tangential and partly by the circular muscles
which are thickened at this level. As with the oesophageal
sphincter, the lumen in the relaxed state is almost filled
by ridges running into it from the stomach so that little
constriction is required to close it (Figures 2.4 and 2.5)

Stomach caecum.

In the body of the caecum the gastric
glands lie in a distinct layer outside the columnar epithelium
lining the stomach. The area occupied by these glands is
increased by the folding of the mucosa into longitudinal
ridges. The circular smooth muscle coat is better developed
than the longitudinal muscle layer (Figure 2.6). At the
level of the sphincter guarding the pyloric part of the
stomach some of the gastric glands on the dorsal surface
become forced away from the interior of the stomach and form
a large glandular mass covered by distinct tangential striped muscle (Figure 2.5). These tangential muscles presumably are not part of the sphincter mechanism of this region but squeeze the glandular secretions into the lumen of the stomach.

Pneumatic duct.

Even externally, three regions of the pneumatic duct can be distinguished and are designated as parts I, II and III in order from the stomach caecum to the swimbladder.

PART I. The first region tapers posteriorly from the diameter of the stomach to that of the rest of the duct and consists of four forward facing pouches (Figures 2.7, 2.8 and 2.9). In addition to the main folds of the internal wall which form these pouches, a system of secondary folds running longitudinally is well developed and in the relaxed duct nearly obscure the lumen. The duct is normally liquid filled. As gas is forced into the duct from the stomach these pouches inflate in turn, the resistance to the passage of gas occurring at the lip of each pouch. The first pouch of the system is morphologically the hind end of the stomach and contains gastric glands in its walls. The true pneumatic duct in its histological structure resembles the oesophagus from which it is derived as shown by Maier and Scheuring (1923). The mucosa is greatly folded and consists
Figure 2.10 Transverse sections of Part II of the pneumatic duct of Pacific herring. A, B, C form a series of which A is most anterior.
of columnar epithelium. External to this is a connective tissue submucosa followed by a layer of circular muscle. A thin layer of longitudinal muscle is on the outside. The circular muscle layer extends into the walls of the pouches so that the size of the passage above each fold can be regulated. In herring with food in their stomachs food particles have been found as far in as the second pouch where they were caught between the secondary folds, but the rest of the duct was food free.

PART II. The second region extends from Part I to the bend in the pneumatic duct. Primary folds still divide the lumen into pouches but the regular arrangement of Part I is lost and the pouches are much smaller and tend to be spherical in shape (Figures 2.9 and 2.10). There is a tendency for the through channel to pass successively through dorsal and ventral spherical pouches but studies of longitudinal sections show that this is complicated and obscured by the development of blind pouches both dorsally and ventrally. Longitudinal secondary folds fill most of the lumen in the relaxed duct. Each pouch is surrounded by circular muscle and in the living duct the pouches can be seen to contract vigorously and successively expelling most of their contents. This region can move gas or liquid either toward or away from the swimbladder. It has a thin layer of longitudinal muscle on the outside.
PART III. The last region of the pneumatic duct reaches from the bend to the swimbladder valve. It is thin walled without internal divisions and with a wall consisting mainly of circular muscle fibres. Its epithelial cells are cubical rather than columnar (Figure 2.11).

Swimbladder valve.

The pneumatic duct enters the swimbladder after running parallel to the swimbladder wall. The entrance, as seen from inside the swimbladder appears like the entrance to an oval tunnel, bridged over by the fused walls of the ventral swimbladder and dorsal pneumatic duct. Anterior to this opening the swimbladder wall is thinner and so more transparent over an area which is circular in herring up to about 20 cm long but becomes elongated in larger fish. When this transparent area is flattened its posterior end lies against the lip of the valve, the space between them is filled with liquid from the pneumatic duct and the valve is closed. The valve is opened when the transparent area is permitted to rise outward from the swimbladder surface under the pressure exerted by the swimbladder gas. This gas inflates and distends the area into a dome and when the roof of the dome is forced away from the lip of the swimbladder the valve is opened. Gas passage down the pneumatic duct will now occur if the pressure of gas in the swimbladder is great enough to overcome the forces tending to retain fluid in the duct.
Microscopic examination of serial sections of the swimbladder valve fixed both while in its opened and in its closed state show how its structure permits these functions. In figure 2.12 is shown a transverse section through the transparent area of a closed swimbladder valve. The collapse of the dome and the tension that will prevent it being inflated by the pressure of the swimbladder gas is exerted by the elastic tissue coat of the tunica externa. The elastic fibres of this layer form a network which passes over the roof of the dome but does not arch over the pneumatic duct proper although it runs a short way up the side of this duct posterior to the valve. Some of the fibres of this layer are attached to the lateral wall of the dome so that tension in the tunica externa not only flattens the dome but pulls the walls out laterally so that they fold on themselves. The roof of the dome retains a layer of circular muscle continuous with that of the pneumatic duct. Contraction of this muscle layer would reduce the width of the dome and would resist the pressure from the swimbladder gas tending to inflate it. In sections of inflated domes the inner epithelium is stretched out into a thin layer but where the dome is flattened this same epithelium becomes thickened and the cells assume a columnar form being several times taller than they are broad. The lip of the valve is composed of a
dense layer of circular muscle, most of it of swimbladder origin from the tunica interna but also including the circular muscle of the pneumatic duct. At this level the tunica externa is excluded although posteriorly it separates the two layers of circular muscle.

From an examination of its structure and observation of the movement of the valve in living tissue it is assumed that the swimbladder valve is closed by the relaxation of the circular muscles of the swimbladder tunica interna in this region. As these muscles relax the outward pressure due to the swimbladder gas is taken up by the tunica externa. The resulting tension in the fibres of this layer then flattens the dome of the valve, pulling it down on the lip of the valve so that the valve is closed. Contraction of the circular muscles derived from the pneumatic duct may assist in flattening the dome and may provide a means of control of the valve less dependent of the excess pressure of the swimbladder gas than the control by means of the circular muscles of the swimbladder.

Sections of the open swimbladder valve show that the dome becomes thinner as it is stretched and loses the small longitudinal folds in its inner epithelium and the deep folds in its side walls (Figure 2.13). It is assumed
that the valve is opened by the contraction of the circular muscles of the tunica interna which take up the outward force due to the swimbladder gas leaving the tunica externa free from strain. The outward pressure due to swimbladder gas is then able to stretch outwards and inflate the dome, so lifting the dome away from the lip and opening the valve. This is probably accompanied by a relaxation in the circular muscles of the pneumatic duct. Indeed it has been shown in living tissue that a relaxation of the circular muscles of part III of the duct is necessary for the passage of gas even when the valve is open unless the swimbladder gas pressure is high.

This interpretation of the function of the swimbladder valve is supported by the effect of drugs on the valve, which is reported in chapter IV. It was found that adrenalin caused contraction of the circular muscles of the posterior half of the swimbladder including the region of the swimbladder valve. Adrenalin also invariably caused the dome to rise the the valve to open suggesting that contraction of the circular muscle of the tunica interna was responsible for opening the valve. Adrenalin had the opposite effect on the circular muscle of Part III of the pneumatic duct, causing it to relax and thus permitting gas passage once the valve was open.
Swimbladder.

In the swimbladder wall the following layers can be distinguished from the interior outwards. Firstly an epithelium continuous with that of the pneumatic duct which is stretched into a thin layer except where the circular muscles are contracted, when it becomes columnar in form. Beneath the epithelium is the tunica interna which appeared everywhere to be composed of smooth circular muscles in a compact layer. Next is a layer of blood vessels which are poorly represented. The concentration of blood vessels described by Maier and Scheuring for the narrow anterior part of Atlantic herring could not be found in these preparations of the Pacific herring.

The next layer, the tunica externa, consists of a trellis work of elastic fibres. When free from tension this layer appears as a very loose network of fibres but in the distended swimbladder wall it is closely applied to the tunica interna. A further layer of infrequently occurring blood vessels lies to the outside. On the ventral surface peritoneum separated this layer from the body cavity. On the lateral and dorsal sides of the swimbladder it was covered by a layer of connective tissue instead.
Figure 2.11 Transverse section of Part III of the pneumatic duct midway along its length, Pacific herring.

Figure 2.12 Transverse section through dome of swimbladder valve in the closed condition, Pacific herring.
Figure 2.13 Transverse section through the anterior margin of the lip of the swimbladder valve in the open condition, Pacific herring.
Posterior duct of the swimbladder.

The swimbladder at the hind end of the body cavity bends ventrally and running beneath the urinary duct and to the left of the genital duct discharges to the exterior on the excretory papilla. The final part of the swimbladder loses the tunica externa and with it the guanin which gives this layer its silvery appearance, but retains a well developed layer of circular muscle. The inner wall of the duct is folded longitudinally when the duct is closed but stretches and loses these folds during gas passage. The circular muscles of the duct wall allow the duct to be closed independently of the other ducts of this region. No sphincter muscle closing all the ducts was found.

Comparison of the swimbladder and pneumatic duct of Atlantic and Pacific herring.

In the present investigation the following structures of both species have been examined under the binocular microscope; longitudinal sections of the stomach system to display both sphincters, longitudinal sections of the hind end of the stomach and Part I of the pneumatic duct, the internal passages of the rest of the pneumatic duct displayed by filling them with coloured oil, the swimbladder valve viewed externally in living preparations, and the relationship of the posterior swimbladder duct to other ducts of the excretory papilla. In no instance was a difference in structure of these preparations discovered.
Figure 2.14 Horizontal section through the anal/urinogenital papilla of Pacific herring.

Figure 2.15 Vertical section through the anal/urinogenital papilla of Pacific herring at the level of the opening of the posterior swimbladder duct.
In the paper by Maier and Scheuring (1923) the figures given illustrating the anatomy of the Atlantic herring agree in all respects with the anatomy seen in the Pacific herring except that their figures for Part I of the pneumatic duct suggest that this had been reversed dorsal side for ventral and the vascular layer of the anterior swimbladder wall of Pacific herring was not as well developed as in the swimbladder as shown in their drawing. When differences appear between the present description of the Pacific herring and their description of the Atlantic herring these differences appear to be differences in interpretation rather than true differences between the species.

Maier and Scheuring describe tangential muscles bands at the junction of oesophagus and stomach caecum like those found in the Pacific herring but they do not relate these to the two sphincters or to the gastric gland. They assume that these muscles are for the movement of food. Describing the origin of the pneumatic duct, as mentioned, their preparation appears to be reversed as they describe the first pouch as dorsal rather than ventral. The presence of food in this pouch, they assume, closes the entrance to the pneumatic duct thus preventing the entry of food particles. If this were the true mechanism the pressure of gas in this pouch would also inflate it and close the duct. Based on observations on living tissue here it is
suggested instead that the gas pressure increases until the opening of the duct is stretched enough to allow the gas to displace the fluid in the duct. Maier and Scheuring agree with the interpretation here that the folds of Part I prevent food particles passing down the duct. These authors found no valve at the swimbladder end of the pneumatic duct although studies on Atlantic herring in this investigation show a swimbladder valve identical in appearance and function to that of the Pacific herring is present. They also describe the entrance of the pneumatic duct as medio-ventral although in both Atlantic and Pacific herring this opening lies to the left of the midline, close to the left gonad.

Concerning the swimbladder of Atlantic herring, Maier and Scheuring describe the tunica externa as being composed of circular fibres. However these fibres were found to form a trellis work and they had exactly the same appearance in transverse as in longitudinal section.

In describing the posterior duct of the swimbladder these authors describe it as lying on the left side of the genital duct, its true position. However, they state that it retains the silver coating which was seen here, in both Atlantic and Pacific herring, to disappear from the last muscular part of the duct, thus agreeing with the observations of Beaufort (1909). Maier and Scheuring could find no
closing device on the intestine, ureter or posterior swimbladder duct although dissections of Atlantic herring and sections of Pacific herring show that these ducts have well developed circular muscles walls which could act as sphincters. Instead, these authors assume that these ducts are surrounded and closed by one large ring muscle. This they show in transverse section in one of their figures. The corresponding muscle is seen in sections of Pacific herring but when followed anteriorly and posteriorly it is seen not to form a ring but to consist of two strands of parallel longitudinal skeletal muscle which run to the base of the anal fin.
CHAPTER III

SOURCES OF SWIMBLADDER GAS

Introduction.

The source of swimbladder gas in all physoclists and in some physostomes such as the eel is the gas gland in the wall of the swimbladder. Leading to the gland, the arterial capillaries come into close contact with the venous capillaries and by secretion from venous to arterial vessels in this rete mirabile may build up high concentrations of some substances at the gas gland (Denton 1961). The cellular mechanism by which the gas gland adds gas to the swimbladder has not yet been proven but possible mechanisms are discussed in detail by Denton in the same paper.

The herring swimbladder is supplied with a small number of blood vessels lying either between the tunica interna and externa or outside the tunica externa. Serial sections of the entire swimbladder of Pacific herring failed to disclose any concentration of these capillaries so anatomically the herring shows none of the expected modifications for gas secretion (Chapter II). It has been shown for the Atlantic herring that gas secretion over 24 hours is negligible (Brawn 1962).

Atlantic herring have been shown to take air from the surface when the swimbladder contains less that its normal
amount of gas but they appear to have no behavioural pattern to induce them to chase and capture rising bubbles of air in the water under similar circumstances (Brawn 1962).

As herring contain gas producing bacteria in their stomach caecum when this is filled with food (Obst 1919; Sadler, Mounce and Shanly 1919) these bacteria provide another possible source of swimbladder gas.

In this chapter the ability of Atlantic herring to secrete gas over periods longer than 24 hours is investigated and further information is presented concerning the relationship of gas producing bacteria to the gas supply of herring. Recovery of buoyancy by Pacific herring is also studied.

A. Conditions permitting recovery of buoyancy after gas loss from the swimbladder.

Methods, Atlantic herring.

Groups of Atlantic herring from one large group of stock fish were subjected to a pressure decrease of 48 cm Hg for 5 minutes using the apparatus shown in photograph 5.1, page 121. This pressure reduction caused loss of swimbladder gas through the posterior duct so that when the fish were placed in circular fibreglass tanks with water 50 cm deep to recover the gas lost, their density was greater than normal.
A measure of this greater density is that these fish have to be subjected to a greater than usual reduction in pressure before they have neutral buoyancy and will float. A control group A was allowed no time after removal of swimbladder gas to replace this gas but was anesthetised and the reduced pressure causing neutral buoyancy determined for each fish. Group B was kept in running bubble-free sea water 50 cm deep with access to the surface for one week and then the neutral buoyancy of its members was determined. Group C was held under the same conditions as group B but a horizontal circle of netting just beneath the surface prevented the herring taking in air. Both Groups B and C were not fed during the test. Group D was kept in an identical tank but because the fish were to be exposed to high concentrations of a gas forming bacterium an external recirculation and aeration system was substituted for the running sea water supply and temperatures were controlled by keeping the apparatus in a cool room at 11°C (Figure 3.1). The oxygen content of this tank was 5.8 ppm at the conclusion of the test. These fish were fed daily on plankton, Gammarus and scraped herring flesh and cultures of the gas forming bacteria isolated from herring gills were added to the tank. These fish were left one week before testing.
At the end of the recovery period all test fish were tranquillised by MS222 (tricaine methane sulphonate), placed in a water filled flask 36 cm deep and the reduced pressure causing neutral buoyancy for each fish noted. Using MS222 to quieten the fish before testing was a departure from the method used previously (Brawn 1962) necessitated by the difficulty of catching herring held beneath netting without giving them access to the surface. Previously herring were held in a carboy and their neutral buoyancy determined without removing them but this restricted the movements of the fish and was not suitable for the experiments lasting a week reported here. Once treated with MS222 the test herring although still slowly swimming were easily steered into the neck of the water filled flask immersed in the recovery tank. A control group of herring placed in the same concentration of MS222 showed no gas release due to the drug during a 15 minute observation period.

Results.

The mean pressure reductions necessary to cause neutral buoyancy in these groups of fish when anesthetised in the flask are given in Table 3.1. The results for each group are compared with those of Group A which was given no time to recover the gas lost and which may therefore be taken to represent the condition of all groups at the beginning
Figure 3.1 Apparatus used to investigate the restoration of buoyancy in herring denied access to the surface but given food and gas-forming bacteria culture.
of the recovery period. It is evident from this table that herring allowed access to the surface were able to replace the gas lost as only a slight reduction in pressure was needed to cause them to float. The mean reduced pressure causing neutral buoyancy in these fish showed a highly significant reduction from the control mean. The fish of Group C which had no access to the surface and were not fed were not able to secrete gas into the swimbladder over one week; in fact it appears that even more gas was lost from the fish during this period. When tested these fish were still swimming normally and were not resting on the bottom although it can be calculated that their mean sinking factor was then 1026 and they had been compensating for such a greater than usual body density for one week. Fish of Group D in which conditions were made optimum for the generation of gas by bacteria inside the stomach caecum of herring showed no recovery of buoyancy by the end of the week. Rather these fish, like those of Group C apparently lost further gas from the swimbladder during this period.

Conclusions.

From these results it can be concluded that Atlantic herring, when gas is removed from the swimbladder, will replace this gas if allowed to reach the surface. If denied access to the surface, the gas secreting ability of
the herring swimbladder is so poor that it cannot replace
the gas originally lost nor gas loss due to absorption or
liberation even when one week is allowed for this secretion
to take place. Atlantic herring given a culture of a gas
forming bacteria isolated from herring in weirs and a
suitable substrate for the growth of this organism as food
gave no evidence of bacterial gas production contributing
to the volume of the swimbladder gas during the test period
of one week. This throws doubt on the occurrence of bacterial
gas generation inside the herring unless this is due to a
species of gas producing bacteria which was not isolated
in the many cultures prepared from freshly caught herring.
<table>
<thead>
<tr>
<th>Group Recovery conditions</th>
<th>No. of fish</th>
<th>Pressure reduction at neutral buoyancy from mean of A (cm Hg)</th>
<th>Difference</th>
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</thead>
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<td></td>
<td></td>
<td>mean s t p</td>
<td></td>
</tr>
<tr>
<td>A  No recovery period</td>
<td>16</td>
<td>36.4 4.07 - -</td>
<td></td>
</tr>
<tr>
<td>B  Access to surface for 1 week, unfed</td>
<td>5</td>
<td>13.0 7.28 8.65 .001 HS</td>
<td></td>
</tr>
<tr>
<td>C  No access to surface for 1 week, unfed.</td>
<td>6</td>
<td>34.9 1.88 .83 .01 NS</td>
<td></td>
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<tr>
<td>D  No access to surface for 1 week, given food plus bacteria</td>
<td>4</td>
<td>30.5 8.20 1.94 .05 NS</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1 Pressure reduction causing neutral buoyancy in groups of Atlantic herring which, after removal of some swim-bladder gas, were kept under various conditions in water 50 cm deep to recover the gas lost.

<table>
<thead>
<tr>
<th>Group Recovery conditions</th>
<th>No. of fish</th>
<th>Pressure reduction at neutral buoyancy from mean of A (cm Hg)</th>
<th>Difference</th>
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<td></td>
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<td>mean s t p</td>
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</tr>
<tr>
<td>A  No recovery period</td>
<td>16</td>
<td>36.4 4.07 - -</td>
<td></td>
</tr>
<tr>
<td>B  Access to surface for 24 hours, unfed</td>
<td>5</td>
<td>13.0 7.28 8.65 .001 HS</td>
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<tr>
<td>C  No access to surface, fed brine shrimp, 48 hour period.</td>
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<td>34.9 1.88 .83 .01 NS</td>
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<tr>
<td>D  No access to surface, fed brine shrimp and euphausids, 48 hr.</td>
<td>4</td>
<td>30.5 8.20 1.94 .05 NS</td>
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</tbody>
</table>

Table 3.2 Pressure reduction causing neutral buoyancy in groups of Pacific herring which, after removal of some swim-bladder gas, were kept under various conditions in water 36 to 43 cm deep to recover gas lost.

HS - highly significant  PS - probably significant
S - significant          NS - not significant
Method, Pacific herring.

Groups of Pacific herring were placed in a seawater filled carboy 48 cm deep and the pressure reduced by 40 cm Hg for half an hour, then returned to atmospheric pressure. Group A were immediately anaesthetised and their neutral buoyancies determined with the fish still in the carboy. Group B was kept 24 hours in the carboy with the water depth reduced to 36 cm to provide an air surface. Groups C and D were kept for 48 hours in the carboy with water 43 cm deep and a grid to prevent fish reaching the surface. Group C was fed on brine shrimp and D on brine shrimp and Euphausids, both frozen. All fish of Group C had fed when killed at the end of the experiment but those of group D had not fed. An external recirculation and aeration system provided all recovering fish with a supply of bubble-free aerated seawater.

Results and conclusions.

The results are summarised in Table 3.2. Group B shows that Pacific herring like Atlantic herring can replace lost swimbladder gas if allowed access to the surface. Herring of Groups C and D given crustacean food, which is a suitable substrate for gas producing bacteria, and perhaps still carrying spores of gas producing bacteria in their gills were not able to generate gas by bacterial means during a 48 hour period. As these groups had mean neutral buoyancy pressures which did not differ significantly from those of Group A it may also be concluded that the Pacific herring is
unable to secrete significant amounts of gas into its swim-bladder over a 48 hour period.

B. The occurrence and characteristics of gas producing bacteria of herring.

Introduction.

At the time of the first World War interest was aroused in the bacterial flora of herring as a result of deterioration of canned herring, sold as sardines, through bacterial contamination. Two groups worked on this problem simultaneously; one group (Obst and Weber) on the American side and another (Sadler, Mounce, Shanly) for the Canadian industry although both conducted their investigations in or near Passamaquoddy Bay which supplies fish to both the Maine and New Brunswick "sardine" industries. Because of the practical application of their work both groups were interested in all gas forming bacteria which might be present in herring being processed whether these were the normal herring flora or had been picked up as contaminants in the weirs or boats. Thus the species described, especially by Sadler (1919) contain species which are normally found in sewage or milk.

Obst (1919) isolated and described two species of gas producing bacteria from the gills and stomachs of herring and also from their euphausid and copepod food. One of these organisms was identified as *Bacillus walfischrauschbrand* after
an organism briefly described by Nielsen (1890, not 1880 as stated) as occurring on diseased whales. The second organism was called merely Bacillus B and this was renamed *Eubacterium obsti* by Prevot (1938) without the addition of any new information. In addition to these two bacteria Obst found *Bacillus welchii* in water used to wash the fish indicating sewage contamination. In the stomachs of herring an aerobic micrococcus often accompanied Bacillus B. *Bacillus coli* was present in one stomach presumably originating in sewage. Bacillus B generated gas when incubated for 5 hours at $37^\circ$ C on the stomach contents of herring. *B. walfischrauschbrand* produced gas on 1% dextrose agar after 18 hours incubation at $37^\circ$ C and also on lactose agar. Reports of these organisms producing gas inside the stomach of well fed herring taken from the weir must be viewed with suspicion as evidence in this thesis has shown that gas readily passes from the swim-bladder to the stomach in dead herring. The presence of food may merely have retained this gas in the stomach.

Chemical changes which occurred in a fish media with 0.2% dextrose after inoculation with the organisms isolated by Obst were studied by Weber and Wilson (1920). Both ammonia and amines were formed when these organisms were grown in pure culture on this fish media at $37^\circ$ C and positive test for indole and skatole were obtained. The evolved gases were not analysed.
Sadler (1918a and 1918b) describes 8 strains of gas producing bacteria from swelled cans of sardines and one non gas producing rod from herring excreta. In the following year a paper was published in which these organisms had been sought in the intestine and gills of herring (Sadler, Mounce and Shanly 1919). From the intestine of herring were isolated strains of Bacillus vulgaris and B. acidi-lactici and from the gills B. aerogenes. Another organism was isolated from the gills and intestine of herring caught in a weir subject to sewage contamination and was provisionally identified as belonging to the "Para-Gaertner bacilli, after Savage". In addition B. coli and B. communior were isolated from sea water and might contaminate herring used for canning. Of all these bacteria the ones of interest here, which could be normal members of the intestinal flora of herring are Bacillus vulgaris, B. acidi-lactici and B. aerogenes and, from Obst's investigation, B. walfischrausbrand and Eubacterium obsti (Bacillus B.).

In the present investigation attempts were made to isolate gas producing bacteria from the gills, intestine and food of herring so that their gas producing ability at the normal temperatures of the herring stomach could be investigated. No gas forming bacteria were isolated from the Pacific herring bacteria cultured so that all work is based on bacteria isolated from Atlantic herring of the Passamaquoddy region.
The Occurrence of Gas Forming Bacteria on Atlantic Herring and on their Food.

Methods.

Four culture media were used; thioglycollate with 2% agar (B.B.L.), dextrose broth (Difco), stock culture agar (Difco), and a cooked fish medium containing 500 g of haddock fillet, 10 g peptone, 1 g K$_2$HPO$_4$ and 2 g dextrose per litre based on a recipe for beef liver given in the Manual of Microbiological Methods, Society of American Bacteriologists. A small amount of CaCO$_3$ was added to each tube of fish media unless stated otherwise. All the media used contained dextrose; the thioglycollate and cooked fish media provided anaerobic conditions beneath an aerobic upper zone. These media were placed in culture tubes and sterilised.

Obst attributed the tissue breakdown of herring gorged with food to the presence of gas forming bacteria which multiplied with gas formation when the herring taken from the weir warmed up to air temperature. She also isolated these bacteria from the euphausid and copepod food of the herring. For this reason the search for these bacteria began with the examination of herring some time after their removal from the weir. Fish were used which had been kept out of water to reduce the possibility of contamination of the
stomach caecum from outside. Typically these "feedy" fish which had started to show decomposition showed this first in the body wall overlying the pyloric caecae, bacteria were not numerous in the body fluid and in only 6 of 16 fish contained rods, and the stomach caecum was invariably intact. This supports the view of Almy (1926) who attributed the visible signs of decomposition to the action of trysin from the pyloric caecae and is inconsistent with Obst's view that this was facilitated by the gas producing bacteria. The stomach of these fish was cut across with sterile scissors and either the contents squeezed out or a sample taken with a sterile probe. Stomach contents were squeezed into sterile tubes and covered with stock agar while the probe was used to make stab cultures on thioglycollate agar. Euphausids both from fresh plankton tows and from tows with sterile nets and jars were placed whole in fish media or dextrose broth and incubated at 37°C. When these cultures failed to show the presence of gas formers two series of cultures were made from the gills of herring caught the same day. The first series, Series V used a sample of herring taken from a tank inside a herring factory. The possibility of contamination of such a sample by bacteria foreign to the herring is high. Series V did show the presence of gas forming bacteria on fish media so these were then sought on fresh herring which were brought from the weir in separate new plastic bags and
formed Series VIII. From each of these fish a slide was made from the gills and stomach and cultures made on fish medium and dextrose broth of bacteria from the gills. Details of the cultures made, omitting the subcultures, are given in table 3.3.

Results.

Of the 11 cultures made on thioglycollate agar, 14 on stock culture agar and 40 on dextrose broth from herring gills, stomachs and food as shown in table 3.3 none contained bacteria which were capable of producing gas from these media. There were two apparent exceptions, one each from Series IV and VII on dextrose broth which microscopic examination showed was associated with the presence of fungal hyphae which were absent from cultures on dextrose not showing fermentation. This scarcity of bacteria able to produce gas from dextrose broth was strikingly shown in series IV and VII where from the gills of each herring cultures were made on both fish media and dextrose broth. When all tubes were incubated at 37° C 18 out of 20 cultures on fish media produced gas while no gas was formed from dextrose broth except in one tube with a fungal contaminant.

Series IV from the gills of herring from the factory showed the presence of gas formers in 9 out of 10 cultures on fish media. The gas former from this source was
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<th>Media</th>
<th>No. of Cultures</th>
<th>No. producing gas</th>
<th>Temp. °C</th>
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<td></td>
<td>bags</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct.1</td>
<td>VIII</td>
<td>Euphausids,</td>
<td>whole</td>
<td>fish</td>
<td>12</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sterile Jar</td>
<td>euphausid</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Table 3.3. Summary of cultures made of material from herring gills, stomach and food.

thio - thiglycollate agar  
stock - stock culture agar  
fish - fish media  
dex - dextrose broth  

* fungal hyphae or yeast cells present.
later shown to be a rod. Two cotton swab samples were taken of the walls of the tank in the factory, brought back in sterile containers and when examined showed the presence of numerous bacterial spores or micrococci but no rods. It is possible that the gas forming rod was nonetheless a contaminant of the herring. Of the herring of Series VII brought directly from the weir in sterile bags 9 out of 10 showed the presence of organisms able to produce gas from fish media but not from dextrose broth. Of the slides made from gill smears from these fish, two showed bacteria morphologically similar to the gas former isolated from series IV. Slides of the stomach fluid of these fish, all of which contained no food, did not show the presence of rods. Cultures from the gills of these fish on fish media showed the presence of rods which could be the same as those of Series IV in four out of ten cultures two weeks old. Unfortunately the gas producing organism died out of Series VII so that all further work had to be done on the gas former of Series IV. However, the occurrence of a gas former similar in size and in its ability to produce gas from the fish medium but not from dextrose broth in the gills of herring brought directly from the weir suggests that the gas former from the gills of herring in the factory may be normally present on herring gills. Cultures of the gas former of Series IV on fish media were the bacteria given to the living herring in the experiments described at the beginning of this chapter.
The negative results obtained suggest that the two gas producing bacteria described by Obst may not be as plentiful now on the herring or its food as they were at the time of her investigation. Both Bacillus B and B. walfischausbrand are capable of producing abundant gas from media containing dextrose, which all media used in this investigation contained, yet 116 cultures from sources said to harbour these bacteria failed to demonstrate the presence of bacteria which could ferment dextrose. Even if B. walfischausbrand had failed to show up in the cultures it would have been recognisable in slides by the large spore which Obst says was invariably present at one end of the rod giving it a tennis racket shape. No rods with enlarged ends were found on 62 slides prepared of material direct from herring gills, stomach, body cavity or faeces, from cultures from these sources or from euphausids.

This leaves the possibility that herring carry the gas forming bacteria described by Sadler or perhaps gas formers not yet identified.

Characteristics of the gas forming bacteria isolated from the gills of Atlantic herring.
1) Influence of buffers on gas formation, change in pH during fermentation, and analysis of evolved gas.
Methods.

To investigate the effect of the CaCO\textsubscript{3} used to buffer the fish media, three series of fish media tubes were prepared, one with CaCO\textsubscript{3}, one without CaCO\textsubscript{3}, and one with a phosphate buffer to replace the CaCO\textsubscript{3}. The phosphate buffer was a mixture of sodium phosphate and potassium dihydrogen orthophosphate mixed in such proportions as to buffer tap water of pH 7 to 8 at 6.45. The cooked fish medium was placed in 8 x 2 cm tubes containing a small inverted tube 3.4 x 0.8 cm resting on the plug of fish solids (Figure 3.2). It was necessary to fill completely the small tube with medium before autoclaving as otherwise it still contained gas after sterilising. The pH of the cooked fish media without additives was 6.52 as determined by a pH meter.

Series IV tubes B1 to B10 on fish media with B4 replaced by B4 on dextrose were used to inoculate one tube each of the three series and one tube of each was left uninoculated as a control. All cultures were incubated at 37\textdegree C. After 23 hours the depth of gas retained in each inverted tube was measured by placing a scale against the outer tube close to the inner one. The tubes were replaced in the incubator. After a total of 3 days incubation the pH of tubes B6 to B10 of each series was determined and of the controls. The remaining tubes were used for gas analysis.
To test the evolved gas for oxygen a sample was drawn in a syringe filled with CO$_2$ absorber which after 5 minutes was replaced with Oxsorbent, the volume in each instance being noted. To test for the presence of hydrogen the inner tubes were removed from the culture tubes under water in the apparatus shown in figure 3.3. The depth of the gas column when the water surface inside and outside the tube coincides is proportional to the volume of the gas at atmospheric pressure. Oxygen slightly in excess of twice the volume of bacterial gas was added as shown and the mixture sparked using two electrodes embedded in plasticene which shed water as soon as it pierced the water surface. Unfortunately the resulting explosions were so violent that some gas was always lost from the open end of the tube so that the exact amount of hydrogen originally present could not be determined. The residual gas was drawn into a syringe containing Oxsorbent.

Results.

All cultures made on fish media, whether this contained CaCO$_3$ or phosphate or no additive, had produced gas after 23 hours at 37° C except all those innoculated from tubes B7 and B4 dextrose which were non gas forming and gas forming due to fungi in the original culture respectively. The greatest generation of gas occurred in the fish media from
Figure 3.2 Culture tube used to investigate effect of CaCO$_3$ on gas generation, shift of pH during fermentation and composition of evolved gases.
Figure 3.3 Apparatus used to test bacteria-generated gas for the presence of hydrogen.
which CaCO$_3$ had been omitted (table 3.4). This is of interest as it shows that the observed gas was not the result of acid produced by the bacteria acting on the CaCO$_3$ to liberate CO$_2$ but had come directly from the bacteria. Tests on the fish media alone with HCl had shown that it did not liberate gas when acidified. Addition of the phosphate mixture lessened the amount of gas evolved, which is not surprising as the mixture was chosen only for its buffering characteristics and not for its suitability for bacteria.

After this 23 hour incubation period all the cultures had a pleasant smell like dilute beef broth and sharp odours which could be due to ammonia or foul odours associated with indole or skatole were absent. Thus gas production can apparently occur without production of large quantities of these substances.

Two days later tubes B6, B8, B9, B10 and the control of each series were analysed for hydrogen ion concentration (table 3.5). All cultures showed a slight shift towards the alkaline side compared with their control, the shift being least in the presence of CaCO$_3$ and greatest with the Phosphate mixture. It is not possible to interpret these results in terms of the metabolism of the gas producing bacterium as the tubes were inoculated with a mixture of bacteria from the herring gills. They show that gas generation
can occur at a pH of 6.5 to 6.9 and that it continues for more than 23 hours at 37° C without renewal of medium. A foul odour was now present in all tubes and the medium gave a negative result when tested for indoles but was positive for the presence of ammonia.

The evolved gas after standing in contact with CO₂ absorber showed no change in volume over two minutes in the presence of Oxsorbent showing that the evolved gas did not contain oxygen. The presence of a considerable amount of hydrogen was demonstrated by the explosion of the gas when mixed with oxygen and sparked. The residual gas was exposed to Oxsorbent which would absorb any oxygen, CO₂ or NH₃. If we assume that all hydrogen was converted to water in the presence of excess oxygen than the residual gas which remained and was not absorbed by Oxsorbent suggests the presence of another gas in the original mixture.

Gas from the swimbladder of herring was tested for the presence of hydrogen in the same apparatus. There was no explosion and no decrease in volume although the gas mixture was sparked repeatedly. This suggests that gas due to a hydrogen forming bacterium is not finding its way in appreciable quantities into the swimbladder of captive herring.
Innoculated Amount of gas in inner tube after 23 hours at 37° C on
from Fish media Fish media Fish media
Fish media with CaCO3 without CaCO3 with phosphates

<table>
<thead>
<tr>
<th></th>
<th>Fish media with CaCO3</th>
<th>Fish media without CaCO3</th>
<th>Fish media with phosphates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm</td>
<td>mm</td>
<td>mm</td>
</tr>
<tr>
<td>B1</td>
<td>2</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>B2</td>
<td>2</td>
<td>4</td>
<td>½</td>
</tr>
<tr>
<td>B3</td>
<td>5</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>B4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B5</td>
<td>4</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>B6</td>
<td>½</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>B7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B8</td>
<td>1</td>
<td>3</td>
<td>½</td>
</tr>
<tr>
<td>B9</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>B10</td>
<td>8</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Mean 2.6 3.8 1.8

Control 0 0 0

Table 3.4. Generation of gas on fish media with various additives

Innoculated Fish media Fish media Fish media with with CaCO3 without CaCO3 with phosphates
from mm gas pH mm gas pH mm gas pH

<table>
<thead>
<tr>
<th></th>
<th>Fish media with CaCO3</th>
<th>Fish media without CaCO3</th>
<th>Fish media with phosphates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm</td>
<td>mm</td>
<td>mm</td>
</tr>
<tr>
<td>Control, not infiltrated</td>
<td>0 6.57</td>
<td>0 6.53</td>
<td>0 6.50</td>
</tr>
<tr>
<td>B6</td>
<td>4 6.41</td>
<td>7 6.82</td>
<td>4 6.80</td>
</tr>
<tr>
<td>B8</td>
<td>2 6.80</td>
<td>3 6.77</td>
<td>½ 7.00</td>
</tr>
<tr>
<td>B9</td>
<td>7 6.80</td>
<td>6 6.76</td>
<td>3 6.78</td>
</tr>
<tr>
<td>B10</td>
<td>14 6.91</td>
<td>12 6.80</td>
<td>8 6.75</td>
</tr>
</tbody>
</table>

Mean change in pH from that of control + .11 + .26 + .33

Table 3.5. Hydrogen ion concentrations of cultures shown in table 4 after three days incubation at 37° C.
2) Temperature relationships of the gas forming bacteria.

Methods.

A series of tubes of fish media without CaCO\(_3\) inoculated from tube B3 of the last test were immediately immersed in boiling water for 1, 2\(\frac{1}{2}\), 5, 7\(\frac{1}{2}\), 10, 15, 20, and 30 minutes each and then placed in water at 20\(^\circ\) C to cool. A dummy tube containing water of the same depth and original temperature as the culture tubes was provided with a thermometer to follow changes in the internal temperature of the tubes. After exposure to boiling water for these various periods all tubes were incubated at 37\(^\circ\) C.

This first heat kill was a preliminary step in purifying the culture. The tube (one minute) in which the gas producing organism survived provided material for a second heat kill at a lower temperature. A series of tubes was again set up on fish media without CaCO\(_3\) and immediately after inoculation the tubes were placed in a water bath at 64\(^\circ\) C for the same periods as before, with a dummy to give the culture temperature, and then were incubated at 37\(^\circ\) C.

Tubes of fish media without CaCO\(_3\) were inoculated from the same source as those of the first heat kill series and were immediately incubated at 2\(^\circ\), 10\(^\circ\), 23\(^\circ\), and 37\(^\circ\) C to test the effect of temperature on gas production. A similar series using the 2\(\frac{1}{2}\) minute tube of the second heat kill series as inoculating material was incubated at 37\(^\circ\), 20\(^\circ\), 15\(^\circ\), 10\(^\circ\), and 2\(^\circ\) C.
Results.

The results of the first heat kill show that the gas producing organism could survive 1 minute's immersion in boiling water giving a final temperature of 71°C but was killed by $2\frac{1}{2}$ minute's exposure with a final temperature of 83°C. The second heat kill showed that the gas producing organism was killed by 5 minutes exposure to 64°C (final temperature 64°C) but survived $2\frac{1}{2}$ minute's exposure (62°C). Thus the temperature tolerance of the organism over a 10 minute period is less than 64°C.

The first test of the effect of temperature on gas production showed that the organism produces gas from a fish medium in less than 16 hours at 37°C, is just beginning to show gas bubbles at 23°C at 23 hours and produces no gas at 10°C or 2°C during 4 days. After 4 days the tubes at 10°C and 2°C were transferred to 37°C where the subsequent generation of gas showed that the gas producing organism was present.

The second temperature test showed gas production in one of two tubes at 20°C after 22 hours and gas in both these tubes at 27 hours. No gas was produced at 10°C or 2°C in three days, but these tubes produced gas at 37°C. No results were obtained from the two tubes at 15°C as these were non gas forming at both 15 and 37°C and thus presumably lacked the gas producing organism.
Conclusions.

The temperatures at which gas production by this organism takes place make it unlikely that it could contribute gas to the swimbladder of the herring which swims for most of the year at temperatures around 10° C or below. Herrings have been caught in surface gill nets in water of 18° C at the height of summer (Leim, Tibbo and Day 1957) but under these conditions although gas formation by the bacteria may be possible it is of least use to the fish which can easily obtain air from the surface.

Another factor that makes it unlikely that this gas former or any gas former of similar reaction to temperature can contribute gas to the herring is the rapidity at which food passes through the gut of herring at elevated temperatures and the comparatively long period required for gas production. As the stomachs of herring without food contain very few or no bacteria (Obst 1919) we may assume that these are swept out mechanically with the food, reinfection occurring from the gills or on the food. Thus, as Battle (1934) has shown that herring take only 8 hours at 20° C to clear food from their stomachs and the bacterium tested here took about 24 hours to begin gas production at this temperature, it seems that the bacteria could be removed from the stomach long before they could produce gas.
3) Morphological characteristics of the gas forming bacterium.

Methods.

To isolate the gas forming organism the one minute exposure tube of first heat kill series was used to innoculate a small amount of sterile tap water in a sterile petri dish. This water was then used to innoculate a second similar dish and from the second a third was made. All three dishes were then filled with sterile semisolid (0.5\% agar) fish medium without fish solids and incubated at 37° C.

Results.

Plates 1 and 2 after 23 hours showed surface growth and numerous bacterial colonies in the body of the agar. Gas was present near the bottom. Plate 3 had no surface growth and two clusters of colonies whose centers were 7 mm below the agar surface. Under the microscope each cluster was seen to be composed of small spherical, cream coloured colonies which became more densely packed towards the center of the cluster but did not coalesce. The spherical colonies were granular with lobate edges. Cultures from these colonies on fish media produced gas.

Slides made from these colonies showed the presence of rods 0.7 \( \mu \) m wide and 2.1 to 4.2 \( \mu \) m long with blunt ends. As stained with crystal violet some of the rods showed a colourless area at one end which did not swell the tip of the rod. The rods occasionally occurred in pairs but did not form chains.
The absence of gas forming colonies from the agar surface suggests that the gas forming rod is an anaerobe or can tolerate oxygen only in low concentrations.

Summary of the investigation and conclusions.

A rod 0.7 μ by 2.1 to 4.2 μ is present in the gills of some herring and is capable of producing gas containing hydrogen on fish media. It is not able to ferment dextrose and is anaerobic. It does not produce gas on fish media at 10°C and takes 24 hours to produce gas at 20°C. It is killed by 5 minutes exposure to 64°C but survived 2½ minute's exposure. The inability of the organism to produce gas at 10°C or below suggest that it could not contribute gas to the herring swimbladder during most of the year and especially during January to April when the herring are deep in the water by day and may not reach the surface at night (Brawn 1960a) and when a supplementary source of swimbladder gas would be most needed. The fast clearing time of the herring at high temperatures (8 hours at 20°C) compared with the long time needed to generate gas at these temperatures (24 hours) makes it unlikely that gas could be generated before the gas forming organisms were swept through the herring.

This investigation suggested that Bacillus walfisch-rausbrand and Bacillus B of Obst are no longer prevalent in the gills, stomach or food of herring of the Passamaquoddy
region. It does not eliminate the possibility that bacteria other than the organism isolated are present but it seems likely that such bacteria are unable to ferment dextrose and have a lower temperature tolerance than that of the organism isolated.

Further tests on the gas producing organism isolated are required before this can be compared with the bacteria described by Sadler.
NERVOUS AND PHARMACOLOGICAL CONTROL OF THE PASSAGE OF GAS TO AND FROM THE SWIMBLADDER THROUGH THE PNEUMATIC DUCT.

Introduction.

It has been shown in Chapter III that herring apparently have only one source of gas for the swimbladder and this is air taken in through the mouth while the fish is at the surface. The mechanism of uptake can be broken down into the movement of air through the mouth, oesophagus, stomach caecum, the three morphological regions of the pneumatic duct and the swimbladder valve. Obviously this movement of gas must involve a coordination of muscle movements; peristaltic waves, for example, must run in a direction that forces air towards the swimbladder and sphincters controlling gas passage must relax at the right time. This coordination proved the most difficult barrier to duplicating the normal sequence of events in the living animal by means of nerve stimulation and the application of drugs to selected parts of the stomach or pneumatic duct for, while it was comparatively easy to obtain a response to these stimuli, it was seldom possible to obtain coordinated movements. In the absence of directional movements of the contents of the stomach or duct an observed response of one part of the system may be a response which normally occurs during gas gain or gas loss or perhaps during both
gain and loss. For this reason all evidence relating to the control of gas passage through the pneumatic duct is presented here regardless of the direction of gas flow and from this evidence the mechanisms governing both gas loss and gain are later suggested.

In fish the parasympathetic system apparently is represented only by fibres in the oculomotor and vagal nerves. Branches of the vagus serve the oesophagus, stomach and swimbladder (Nichol, 1952). In herring two branches, one from the right and one from the left vagus nerve, run along the oesophagus, stomach caecum and pneumatic duct to the swimbladder. These organs also receive sympathetic innervation through the splanchnic nerve, branches of which anastomose with vagal branches to form a plexus in the swimbladder wall (Nichol 1952).

In mammals nerves of the parasympathetic system release acetylcholine at their terminals and the structures they innervate respond to applied acetylcholine and parasympathomimetic drugs. The sympathetic nerves, with a few exceptions, are adrenergic and the effector organs they serve respond to sympathomimetic drugs such as adrenalin and ephedrine (Young, 1957). In fish, such as trout and Lophius, the response to drugs does not always parallel the known autonomic innervation so that according to Young (1950)."it is
not possible to divide up the autonomic system into sympathetic and parasympathetic divisions by either anatomical, physiological or pharmacological criteria." While this may prove to be too pessimistic a view it does invite caution in assuming that response of a fish organ to parasympathomimetic drugs shows parasympathetic innervation or to sympathomimetic drugs, sympathetic innervation. Fänge (1953) suggests that the intestinal branch of the vagus may contain adrenergic as well as cholinergic fibres so that some autonomic nerves of fish may be mixed in character. For these reasons, when interpreting the results obtained with drugs in this and subsequent chapters, innervation by a particular nerve is only suggested when electrical stimulation of this nerve was followed by effector response. When discussing the responses to drugs it has been assumed that response to parasympathomimetic drugs implies cholinergic nerve fibre innervation and response to sympathomimetic drugs, adrenergic innervation but even this limited interpretation may not be invariably justified as a tissue might have the ability to respond to the drug without possessing the equivalent innervation. Until more is known of the distribution of cholinergic and adrenergic nerve fibres in the autonomic nervous system of fish it seems unwise to assign these assumed adrenergic or cholinergic nerve fibres to specific nerves.
Response of the pneumatic duct and stomach to drugs applied to the intact living fish.

In Chapter V an experiment is described in which living herring were held in various concentrations of autonomic drugs for five minutes and then subjected to a decrease in pressure. Although designed to determine the influence of these drugs on gas release through the posterior duct this experiment also shows the effect of these drugs on the release of gas through the pneumatic duct in the intact fish. After the experiment the herring were immediately killed by brain destruction, opened ventrally and the internal organs examined. The drugs tested were adrenalin, atropine, pilocarpine, eserine and acetylcholine in concentrations of 1:10,000 and eserine and pilocarpine at 1:100,000 each being used on six herring.

Results.

Control fish subjected to the experimental procedure without the presence of drugs had no gas in the stomach or pneumatic duct and a full swimbladder when opened at the end of the experiment. The stomach was contracted in half of the control fish examined and partially so in the remainder, sometimes containing water. One stomach showed peristalsis directed toward the pneumatic duct.
Adrenalin 1:10,000 (National Biochemicals Corp. in acidified sea water), alone of the drugs tested, favoured release of gas through the pneumatic duct to the stomach, though under the conditions of the test this always occurred later than gas release through the posterior duct. Three of the six test fish showed coughing or head shaking while swimming and one of these lost gas from the mouth. When opened four of the six fish had gas in the pneumatic duct or stomach.

Pilocarpine 1:10,000 and 1:100,000 caused the stomach of the twelve test fish to contract, sometimes strongly. No gas was found in the pneumatic duct or stomach of these fish.

Eserine 1:10,000 and 1:100,000 similarly caused contraction of the circular muscles of the stomach in all but one of the twelve fish tested and there was no gas in the pneumatic duct or stomach of these fish. Two of the six fish from eserine 1:10,000 showed constrictions of the stomach. In one instance, the constriction was so tight that considerable force was required to push water contained in the stomach past the constriction. One other stomach, also water filled, showed peristaltic waves directed towards the pneumatic duct.
When acetylcholine 1:10,000 was added to eserine 1:100,000 results were similar to eserine alone in that five of the six test fish had contracted stomachs and no gas in the pneumatic duct or stomach. The other fish had a water filled stomach and numerous small bubbles in the intestine which presumably came from the swimbladder as this was almost empty, but no gas remained in the pneumatic duct or stomach.

Atropine 1:10,000 did not cause gas to leave the swimbladder through the pneumatic duct in any of the six fish tested. In five of these fish the stomach was relaxed and two of these had water filled stomachs. The pneumatic duct and stomach were free from gas.

Conclusions.

These results show that adrenalin permits the passage of gas through the swimbladder valve to the pneumatic duct and along the duct to the stomach. A study of the anatomy of the swimbladder valve presented in Chapter II has shown that the valve will open if the circular muscles of the tunica interna of the swimbladder contract. Thus it may be concluded that contraction of the circular smooth muscle of the swimbladder in this region occurs in response to applied adrenalin suggesting control by adrenergic nerve fibres and adrenaline from the chromaffin tissue. The
anatomy of the valve also suggested that it is closed by tension in the tunica externa fibres resulting from the excess internal gas pressure when the circular muscle fibres are relaxed. Thus the closing mechanism is a mechanical one and only opening the valve requires active muscle contraction. This provides an explanation for the failure of atropine, which blocks acetylcholine, to cause the valve to open. In contrast it will be shown that the posterior duct is closed actively under the influence of cholinergic nerve fibres and is opened by atropine as well as by adrenalin. This is not to suggest that cholinergic fibres play no part in the control of gas release through the swimbladder valve. It will be shown later that acetylcholine affects the diameter of the pneumatic duct and may under certain conditions prevent gas loss from the swimbladder even if the valve is open.

The results also suggest that contraction of the circular muscles of the stomach caecum is controlled by cholinergic nerve fibres as acetylcholine, eserine which intensifies the action of acetylcholine, and pilocarpine all caused strong contractions of these muscles. This conclusion is supported by the action of atropine which caused these muscles to relax by inhibiting the muscarinic action of acetylcholine. Contraction of the circular muscles of the stomach, especially in the form of peristalsis toward the
pneumatic duct may be an essential part of the mechanism responsible for driving ingested air toward the swimbladder.

The effect of drugs injected into the body cavity of living fish.

Adrenalin and ephedrine were injected into the body cavity of lightly anaesthetised herring which were subsequently observed in a glass fronted aquarium, then killed by brain destruction and immediately examined internally. Five control fish were injected with 0.5 ml of Young's marine teleost saline and similarly examined.

Results.

The control fish injected with saline released gas through the posterior duct only after a severe decrease in pressure (-37 cm Hg). Herring injected with a sufficient amount of adrenalin (Parke Davis Co., adrenalin chloride 1:1000) gave a consistant response. After a delay of several minutes these herring lost copious amounts of gas from the mouth. They tended to stay near or on the bottom, became light in colour, unusually quiescent and frequently showed haemorrhages at the fin bases. When killed and opened, the swimbladder was found to have collapsed, the pneumatic duct was gas filled and the stomach, if the oesophageal sphincter has not relaxed, was distended with gas. This response is illustrated in photographs 4.1 and 4.2 which show
4.1  Herring with body cavity opened

partly deflated swimbladder  dome of swimbladder valve

4.2  Body cavity of herring after injection of adrenalin
4.2 Body cavity of herring after injection of adrenalin
an untreated fish and one injected with 0.2 ml of 1:1000 adrenalin while still alive and photographed to show the internal organs immediately after death.

The amount of adrenalin necessary to cause the response in the 12 to 28 gm fish tested appeared to be greater than 0.2 ml of 1:5000 solution as one fish given this amount and one fish given 0.2 ml of 1:10,000 solution lost no gas from the swimbladder. Eight fish injected with 0.1 ml of 1:1000 adrenalin and five fish injected with 0.2 ml of this solution released swimbladder gas through the mouth and showed the pattern of internal changes described above.

A different response was obtained after injecting a solution of adrenalin made by dissolving National Biochemicals Corporation adrenalin in acidified saline to give a concentration of 1:1000. This when injected into 10 fish in amounts of 0.2 to 0.4 ml did not cause gas liberation by mouth and when opened only one of these fish had gas in the pneumatic duct or stomach. Two or more fish when alive lost gas through the posterior duct. The "NBC" adrenalin in acidified sea water was used in the experiments in Chapter V when living herring swam in a 1:10,000 solution of this drug. Here also adrenalin from this source favoured release of gas through the posterior duct but the difference in response was not absolute as after longer exposure to the drug gas was also lost through the pneumatic duct in four of the six test
fish. The observed differences in responsiveness of the posterior duct and pneumatic duct to adrenalin from these two sources may be either a true reflection of differences in drug composition or may be due to differences in acidity between the two solutions. In making the solution in sea water dilute HCl was slowly added until all adrenalin went into solution. The solution in saline was made by dissolving adrenalin in acidified saline and then reducing the acidity with NaOH until a faint pink tinge was observed. The Parke Davis adrenalin was bought as a solution.

Ephedrine 1:1000 (my solution in saline) injected in 0.2 ml amounts was not as effective in causing gas release through the pneumatic duct as a similar solution of adrenalin. Of the four fish injected only two had gas in the stomach or pneumatic duct when opened while injection of this amount of adrenalin (Parke Davis) was invariably followed by gas loss.

Conclusions.

These results suggest that the loss of swimbladder gas through the pneumatic duct is controlled by the amount of adrenaline present either from the terminals of adrenergic nerve fibres or released from the chromaffin cells which in fish appear to be the physiological equivalents of the adrenal medulla of higher vertebrates (see Hoar, 1957).
Electrical stimulation of the visceral branches of the vagus nerve and of the swimbladder wall.

Six herring with brain destroyed were opened ventrally and the visceral branch of the vagus nerve where it runs along the oesophagus was lifted and placed over twin electrodes. The nerve was stimulated electrically and the effect on the stomach or pneumatic duct observed. The wall of the swimbladder was stimulated directly.

Results.

Stimulating the right vagus caused movement and shortening in length of the stomach, drawing forward parts I and II of the pneumatic duct. No effect was seen on part III of the duct when this was gas filled and showing spontaneous movements but in another preparation peristaltic movements of part III were initiated.

Stimulating the left vagus caused a pronounced decrease in the diameter of the oesophagus especially just anterior to the oesophageal valve. It caused the oesophageal valve to constrict and also caused constriction of the anterior half of the stomach. In two of the four preparations examined stimulation of the left vagus was followed by doming of the swimbladder valve but this may have occurred spontaneously.

Direct electrical stimulation of the ventral swimbladder wall caused a slight contraction which moved the
wall out of contact with the electrodes. The contraction was
greater just anterior to the swimbladder valve than in the
mid body region. The response of the muscular elements in
the swimbladder wall was slow and sustained and thus was
probably due to smooth muscle.

Conclusions.

The visceral branch of the right vagus is motor
to the stomach and appears to affect mainly the longitudinal
muscles. The visceral branch of the left vagus is motor to
the oesophagus, oesophageal valve and the circular muscles
of the anterior half of the stomach. These two nerves may
also serve the pneumatic duct but as the responses here were
not invariable and as this region is subject to spontaneous
movements it was not possible to show this conclusively.

The swimbladder wall appears to contain circular
muscular elements, probably smooth muscle, which are capable
of very slight contraction. Anatomical studies of Pacific
herring described in Chapter II have shown that circular
smooth muscle is present in the tunica interna of the swim-
bladder.

The influence of drugs on the passage of gas through an
isolated stomach/pneumatic duct system.

Air under pressure can be forced from the stomach,
through the pneumatic duct and swimbladder valve to the
swimbladder. Drugs which affect the pneumatic duct would
be expected to change the applied pressure required to
initiate gas flow and alter the pressure at which gas flow ceased. As applied pressures and the flow of gas through the duct are easily recorded advantage was taken of this relationship to investigate the effect of drugs on the pneumatic duct.

Method.

The preparation, consisting of stomach caecum, pyloric stomach, oesophagus, pneumatic duct and a small amount of swimbladder wall bearing the swimbladder valve was removed immediately after killing the fish and placed in saline. A hypodermic needle #21 connected to the apparatus shown in figure 4.1 was inserted through the cut end of the oesophagus into the stomach so that the collar of wax passed through the oesophageal valve. A ligature round the stomach just posterior to the descending limb and behind the wax collar then made a gas tight connection between the preparation and hypodermic needle. The collar of wax also served to hold the point of the needle clear of the stomach wall. The preparation was placed in Young's marine teleost saline and the pressure inside the apparatus slowly increased until gas started to leave the swimbladder end of the preparation. The start of gas flow was recorded on the kymograph by means of a signal lever. The pressure was then slowly reduced by the escape of gas through a capillary tube and through the preparation with the pump turned off. The point at which gas flow through the preparation ceased was recorded. A
tambour activating a writing lever recorded this cycle of pressure change on the drum and in addition note was made of the manometer reading at the start and end of gas flow. Irrespective of the point at which gas flow ceased the pressure was always reduced to 1 cm Hg before beginning the next cycle to allow the pneumatic duct to return to its normal fluid filled condition. Three cycles in saline preceded three cycles in the test drug. The drug was replaced by saline and after a five minute recovery period three more cycles were recorded. In early experiments there was no temperature control other than keeping the solutions refrigerated until use and the room cool. Later the test container and reservoir were chilled with ice. The rate of pressure increase was 0.1 cm Hg/sec; of decrease 0.06 cm Hg/sec.

Results.

A sample of the record obtained with this apparatus is shown in figure 4.2. To permit statistical treatment, the applied pressure at which gas flow started and finished was read from each cycle on the record and mean values for the preparations in saline and in the test drug were calculated (table 4.1). Where gas flow was intermittent the lowest pressure at which gas still left the preparation was taken as the applied pressure at which gas flow ceased. Table 4.1 shows that acetylcholine raised the pressure needed to start gas flow and eserine lowered it. Eserine also lowered the
Figure 4.1 Apparatus used to record the pressure at the beginning and end of gas flow through a stomach/pneumatic duct preparation.
Figure 4.2  Effect of eserine 1:1000 on the applied pressure at beginning and end of gas flow through a stomach/pneumatic duct preparation.
pressure at which gas continued to pass through the preparation as did adrenalin. Other drugs did not significantly alter the pressures governing gas flow from those in saline.

The passage of gas through the preparation in saline was observed under a microscope. Increasing applied pressure caused the stomach caecum to expand laterally and to lengthen as it became distended with air. The main resistance to the flow of gas through the preparation occurred in the first part of the pneumatic duct, which has a succession of inner chambers of decreasing size which are liquid filled until the advancing air surface pushes in, displaces the liquid and distends the chamber. Under increasing pressure the chambers of part I slowly fill until part II is reached, when gas passage through the rest of the duct occurs rapidly and air leaves the preparation through the swimbladder valve. In these preparations part II of the pneumatic duct was sometimes wound on itself, up to one and a half spirals being seen. This occurred only at low applied pressures and straightened out as the pressure rose. Part III took no part in this winding but was quite straight. Waves of contraction of the circular muscles of the stomach wall were seen which even under conditions of falling applied pressure were able to force gas towards the swimbladder. Of the 93 cycles recorded with the preparation in saline 29 showed an intermittent gas flow when the pressure was falling which may
may have been related to these waves of contraction of the stomach. When the applied pressure fell below the value necessary to cause gas flow the entire pneumatic duct returned to its fluid filled condition and the distension of the stomach was lost.

Conclusions.

The results given in table 4.1 show that 5-hydroxytryptamine and ephedrine in concentrations of 1:1000 have no significant effect on the pressures governing gas flow through the preparation. Ephedrine is a sympathomimetic drug but its properties differ somewhat from adrenalin especially in being not nearly as active as adrenalin on isolated tissues (Goodman and Gilman 1956), hence the failure of the isolated pneumatic duct to respond to ephedrine does not necessarily denote a lack of responsiveness to adrenergic stimulation. The preparation showed a sensitivity to adrenalin, not in a change in the pressure needed to initiate gas flow but by significantly lowering the pressure at which gas could still flow through the duct. This may have been caused by a relaxation of the circular muscles in the wall of the pneumatic duct so that less pressure was needed to keep the duct filled with air.

Acetylcholine almost doubled the mean applied pressure necessary to initiate gas flow through the duct, but did not
<table>
<thead>
<tr>
<th>Drug</th>
<th>Pressure causing gas flow, cm Hg</th>
<th>Test of significance of difference in means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in saline n mean s</td>
<td>in drug n mean s</td>
</tr>
<tr>
<td>Acetylcholine 1:1000</td>
<td>30 5.6 2.76</td>
<td>11 11.0 2.72</td>
</tr>
<tr>
<td>Eserine 1:1000</td>
<td>19 6.1 1.71</td>
<td>14 2.9 1.23</td>
</tr>
<tr>
<td>5-Hydroxytryptamine, 1:1000</td>
<td>18 5.4 1.56</td>
<td>14 4.5 1.57</td>
</tr>
<tr>
<td>Adrenalin 1:1000</td>
<td>12 6.1 2.05</td>
<td>7 6.0 2.57</td>
</tr>
<tr>
<td>Ephedrine 1:1000</td>
<td>14 5.4 1.93</td>
<td>8 6.4 1.35</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>Pressure when gas flow ceased, cm, Hg</th>
<th>Test of significance of difference in means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in saline n mean s</td>
<td>in drug n mean s</td>
</tr>
<tr>
<td>Acetylcholine 1:1000</td>
<td>30 3.0 1.76</td>
<td>11 5.0 2.05</td>
</tr>
<tr>
<td>Eserine 1:1000</td>
<td>19 4.0 1.42</td>
<td>14 2.1 .58</td>
</tr>
<tr>
<td>5-Hydroxytryptamine, 1:1000</td>
<td>18 3.3 1.19</td>
<td>14 2.5 1.27</td>
</tr>
<tr>
<td>Adrenalin 1:1000</td>
<td>12 4.2 1.45</td>
<td>7 2.3 .58</td>
</tr>
<tr>
<td>Ephedrine 1:1000</td>
<td>14 2.3 .74</td>
<td>8 1.9 .42</td>
</tr>
</tbody>
</table>

Combined saline results

<table>
<thead>
<tr>
<th>Start of gas flow, cm H.</th>
<th>End of gas flow, cm Hg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>n mean</td>
<td>n mean</td>
</tr>
<tr>
<td>93 5.7</td>
<td>93 3.3</td>
</tr>
</tbody>
</table>

Table 4.1. Applied pressures at which gas flow through isolated stomach/pneumatic duct preparations began and ceased in saline and various drug solutions.

HS - highly significant  S - significant  NS - not significant
significantly change the pressure at which gas flow ceased. This suggests that the action of acetylcholine is not merely to tighten the circular muscles in a sphincter somewhere in the stomach pneumatic duct system as this would raise the pressure at which gas flow ceased as well as raising the pressure needed to initiate flow. The mechanism suggested here assumes that gas flow through the pneumatic duct is analogous to the movement of gas down a narrow liquid filled tube. If the angle of contact is zero in such a tube the liquid surface forms part of a sphere of radius equal to the radius of the tube. The pressure over the curved surface equals the gas pressure but at a point just below the surface the pressure is less than the gas pressure by an amount \( \frac{2\gamma}{r} \), where \( r \) = radius, \( \gamma \) = surface tension of the liquid. Liquid from the rest of the column will flow into this region of low pressure and the liquid surface will advance along the tube unless the gas pressure is high enough to oppose this movement. For gas to replace the liquid in the tube it must exert a force on the liquid greater than the force drawing the liquid along the tube. This force, as can be seen from the formula is inversely proportional to the radius of the tube. In the pneumatic duct the walls and pockets of the inside of the tube remain wetted with liquid even when the center is air filled. Thus the liquid surface at its circumference makes contact with the liquid film and the angle of contact will be approximately zero. Thus it is
to be expected that the applied pressure needed to force air through the fluid filled duct will be inversely proportional to the radius of the duct at the liquid/air interface. This radius and hence the pressure will vary along the length of the tube and at any one position it will vary according to the degree of contraction of the circular muscles. Acetylcholine may cause the observed rise in the pressure needed to initiate gas flow by causing these muscles to contract and so decreasing the radius of the curved liquid surface. Once gas flow starts the liquid surface across the duct is destroyed and the damp walls of the duct are held apart by the pressure of the gas in the center. The more relaxed the muscles of the duct wall the greater will be the separation of the duct walls for a given internal pressure. As the pressure falls the walls of the duct move inwards until the liquid lining the walls again occludes the duct and a new curved surface is formed. At this low pressure forces due to this surface cause the whole duct to fill with liquid and air is excluded. Drugs which increase the tension in the circular muscles would be expected to favour this occlusion at a higher pressure but not necessarily at a pressure any where as high as the pressure needed to initiate gas flow through the same duct. Acetylcholine in these experiments did cause a rise in the pressure at which gas flow stopped but the rise was not great enough to
differ significantly from normal. This increased pressure was well below the pressure needed to start gas flow in the same ducts.

Eserine was the only drug tested which had a highly significant effect on both the pressure needed to initiate gas flow and the pressure at which gas flow ceased, lowering both these pressures below the saline levels. The reason for this result is unknown as in the denervated, isolated preparation in the absence of acetylcholine, eserine which acts by inhibiting acetylcholinesterase would be expected to have no effect.

From the 93 records of the preparations in saline it was found that the mean applied pressures at the beginning and end of gas flow were 5.7 and 3.3 cm Hg respectively. As the mean excess pressure in the herring swimbladder is 1.1 cm Hg (Brawn 1962) it is clear that gas under sufficient pressure to pass through the pneumatic duct will enter a region of lower pressure on entering the swimbladder. Thus if air contained in the herring stomach can be raised to an excess pressure of an average of 5.7 cm Hg this gas would be able to pass from the stomach to the swimbladder via the pneumatic duct.
Direct observations on the structures involved in the passage of gas to and from the swimbladder through the pneumatic duct and on the effect of drugs on these structures.

Methods.

The uptake of air from the surface could be seen in herring made dense by the loss of gas from the swimbladder under reduced pressure. Observations on these fish were supplemented by studies on herring rendered permanently denser than normal by bringing either the anterior or posterior end of the swimbladder out through an incision in the body wall so that it had a direct opening to the exterior. Six fish were operated on in this manner and it was found that the posterior cut was most satisfactory as herring after this operation still swam repeatedly to the surface two days later.

To observe other structures the herring were killed by destroying the brain, held on their backs and the wall of the body cavity on the left side removed. The stomach, pneumatic duct, swimbladder valve and all but the extreme anterior and posterior ends of the swimbladder could be seen by displacing the intestine and one gonad. The oesophagus was mainly hidden by the liver which was too fragile to displace. These structures were examined under a dissecting microscope for spontaneous movements and for their response to autonomic drugs administered one drop at a time in concentrations of 1:1000.
Observations were also made on excised stomach/oesophagus preparations opened longitudinally to show the movements and structure of the oesophageal and pyloric sphincters and on swimbladders ligatured to retain their original gas content and removed from the body cavity.

Results.
a) The uptake and swallowing of air.

After loss of gas from the swimbladder intact herring made sudden infrequent rushes to the surface, broke the surface with their lips and immediately swam down again. This response, probably evolved to expose these fish to predaceous sea gulls for as short a time as possible, made it difficult to observe or film the actual uptake of gas. Herring which had the swimbladder opened to the exterior through the body wall spent long periods at the surface in the inclined position of herring taking in air, although they were capable of normal, though tail heavy, swimming movements below the surface. These fish inclined their bodies at approximately 45° to the surface, an angle which brought both lips through the surface film. The water appeared to fall away from the lips on either side so that air entered the buccal cavity during each inspiration. A film of water across the lips was formed momentarily each time the mouth closed. Thus herring were able to take air into the mouth without thrusting the head through the surface. Occasional gulping movements were seen which were probably associated with the swallowing of air.
It was possible to induce gas uptake and release through the mouth by procedures other than opening the swimbladder or reducing the pressure. Fish injected with 0.2 ml of 1:1000 acetylcholine showed the same attraction to the surface as herring with an open swimbladder. Herring injected with adrenalin showed no surface seeking behaviour but remained near the bottom and after a delay lost gas through the mouth. Although these fish after gas loss were denser than normal and had partially collapsed swimbladders they did not attempt to take gas from the surface. Thus adrenalin not only facilitates gas loss but also appears to inhibit gas uptake. Injecting 1 ml of air into the body cavity of a 12 gm fish caused gas to be lost through the mouth in large bubbles after preliminary choking movements and almost half of the gas in the swimbladder was lost. Injections of 0.2 and 0.6 ml of air into other fish were not sufficient to cause this response. This suggests that increase in buoyancy without increase in swimbladder volume is sufficient to cause gas loss.

No observations were made on the passage of gas through the oesophagus to the stomach.

(b) Oesophageal and pyloric sphincters.

Between the oesophagus and the stomach and between the stomach caecum and its descending limb are sphincters of similar construction which can be completely closed. Their
properties were investigated in isolated stomach/descending limb/oesophagus preparations by splitting the stomach longitudinally through one of the two sphincters leaving the other intact and open to observation. In saline both sphincters presented an identical picture internally. Pale coloured ridges running longitudinally along the interior of the stomach converged at the sphincter, filling the lumen so that it appeared closed. The area surrounding the sphincter was flat. On adding pilocarpine the surrounding area assumed a funnel shape and the sphincter became firmly shut. Air introduced into the oesophagus after the oesophageal sphincter had been treated with pilocarpine could only pass to the stomach after raising the pressure so high that it distended the oesophagus to the diameter of the stomach. Even at such a high applied pressure gas was only able to pass through the sphincter in small bubbles. Pilocarpine touching the inner stomach wall posterior to the pyloric sphincter caused dilation of the blood vessels turning the interior a rosy red.

When air and pilocarpine were injected together into the intact stomach the drug caused the two sphincters to close strongly enough to hold air in the stomach under considerable pressure. These sphincters may play an important part in the movement of gas to the swimbladder if the driving force for this movement comes from a contraction of the whole stomach musculature. Such a contraction could not raise the pressure of gas contained in the stomach unless the sphincters prevented the loss of gas from the stomach.
Adrenalin so relaxed the oesophageal valve that air could pass through it due to its buoyancy alone. Thus it appears that the sphincters are under the control of adrenergic and cholinergic nerve fibres working antagonistically.

(c) Stomach caecum.

Spontaneous peristaltic movements of the stomach directed toward the pneumatic duct were occasionally seen. Contractions of the stomach were obtained by applying parasympathomimetic drugs. Acetylcholine caused immediate contraction of the stomach caecum both in diameter and in length, drawing the pneumatic duct forward. Pilocarpine caused strong rhythmic contractions of the circular muscles, or in greater amounts a contraction of the entire stomach so pronounced that the diameter became less than that of the first part of the pneumatic duct. Pilocarpine was also effective in initiating pulsating movements of the pyloric caecae causing them to shorten and lengthen repeatedly and in causing peristalsis of the intestine.

Variable effects were observed after the application of adrenalin to the stomach. Often there was no visible change in appearance but the drug allowed the stomach to be easily distended by gas suggesting that a relaxation of the circular muscles had occurred. Occasionally contraction of the longitudinal muscles was observed and increase in
diameter was accompanied by decrease in length. The effect of ephedrine was even more variable. Of three stomachs tested one did not respond, one shortened in length and the third became thinner and longer moving the pneumatic duct backward.

These observed movements of the stomach suggest that air in the stomach could be driven through the pneumatic duct either by contraction of its entire musculature with the sphincters closed or by strong peristalsis directed towards the duct. That the stomach is capable of strong peristaltic movements was shown by a stomach treated with eserine which was strongly constricted in the middle into an hour glass shape. When one of the relaxed portions was cut through to reduce its contents to atmospheric pressure the other bulge resisted considerable pressure and only when relaxation began was it possible to force the liquid in the stomach through the constriction. Eserine caused peristalsis towards the pneumatic duct showing that the stomach can exert a force on its contents in this direction.

(d) Pneumatic duct, part I.

This region of the duct has a few large internal pockets arranged with their open ends facing towards the stomach. This structure influenced the flow of gas through this region according to its direction. Gas entering the duct from the stomach under slowly increasing pressure flooded
these pockets slowly in turn, the resistance to gas flow occurring as the gas occupying one pocket began to pass over the lip of that pocket into the next one. Once the fluid in this region had been displaced by gas and gas pressure distended the duct walls giving a rigidity to the whole structure gas flow continued unimpeded. There were no active movements of the duct wall associated with the initiation or maintenance of gas flow. Gas passing in the opposite direction under pressure from the swimbladder tended to flatten the pockets as it advanced rather than fill them successively so that the gas passed through with little resistance.

Both acetylcholine and pilocarpine caused this region to contract strongly and sometimes initiated slow, rhythmic peristaltic movements which were seen to follow a gas bubble moving it to the stomach. Adrenalin inhibited all movements of the pneumatic duct and relaxed the muscles in its wall so that the lumen of the duct became more than usually enlarged during the passage of gas from the swimbladder. There was usually a delay of about one minute before the duct responded to adrenalin although it responded almost immediately to acetylcholine or pilocarpine.

(e) Pneumatic duct, part II.

This region with its numerous small internal pouches frequently showed spontaneous contractions at a rate of about
three per minute during which the pouches and the wall of the duct contracted in order along the duct driving gas bubbles either towards or away from the swimbladder. Acetylcholine caused a strong contraction in length and diameter of this part of the duct driving out the contained air and inhibiting regular peristaltic movements momentarily. As the initial effect of acetylcholine wore off regular coordinated peristaltic movements of the duct were reestablished. In the resting duct peristalsis could be initiated by applying acetylcholine, pilocarpine or 5-hydroxytryptamine. In a duct treated with pilocarpine and severed closed to the swimbladder the movement of a gas bubble from part III to the stomach was observed showing that passage of gas through the duct can occur due to movement of its wall in the absence of pressure from the gas in the swimbladder.

The initial effect of adrenalin on part II was often to narrow the duct or initiate peristalsis but this effect was short lived and soon all movements were lost and the duct walls relaxed. Ephedrine also caused contraction and peristalsis directed toward the swimbladder but this was not seen to be followed by relaxation.

Part II of the pneumatic duct was the most motile of the three parts and most responsive to drugs. It appears to play a passive rôle during the passage of gas under pressure in either direction but it is capable of moving gas bubbles
left in the duct after gas passage to the stomach or swim-bladder to restore the duct to its fluid filled resting condition. In Atlantic herring as in Pacific herring described later this part of the duct responded to introduced mineral oil with peristalsis which removed mineral oil from the duct after a few minutes. Thus a further function of this region appears to be the removal of foreign material from the duct. (f) Pneumatic duct, part III.

This portion of the duct with straight internal walls also showed spontaneous peristaltic movements at a rate of about three per minute which originated either at the swimbladder valve or at the bend in the duct and moved towards or away from the swimbladder. These movements were only shown when the duct contained gas in bubbles and sometimes seemed to be initiated by peristalsis in part II. When part III was greatly distended with gas the application of acetylcholine was sometimes followed by further enlargement. Usually acetylcholine caused a pronounced narrowing of the duct, sometimes expelling all contained gas, later followed by peristalsis. An apparent relaxation of the wall without inhibiting rhythmic movements was observed after the application of eserine. Acetylcholine after eserine increased the rate of peristalsis. Pilocarpine also initiated or increased the rate of peristalsis of part III and increased the diameter of the duct.
Adrenalin relaxed the walls of part III, sometimes after an initial brief narrowing, and inhibited peristalsis. Ephedrine appeared to have no effect.

Part III appeared to have two functions other than that of passively conveying gas; by peristalsis it was able to move gas bubbles along the duct and by changes in diameter it was able to govern the applied pressure needed to drive gas through the fluid filled duct once the swimbladder valve was open. This last function is discussed more fully in the section on Pacific herring but was also shown by Atlantic fish.

(g) Swimbladder valve.

The swimbladder valve was opened by adrenalin or ephedrine and the dome of the valve responds to acetylcholine or eserine. The action of these drugs showed how the valve was controlled. A description of the function of the swimbladder valve in terms of its anatomy has been given in Chapter II. The valve prevented gas flow in one direction only, from the swimbladder, and was never seen to impede the flow of gas from the pneumatic duct.

In the absence of drugs the last part of the pneumatic duct ran like a flattened ribbon along the ventral surface of the swimbladder finally merging with the swimbladder surface a little to the left of the mid line. In
this condition the valve was closed and the area just anterior
to the lip of the valve was flush with the general swimbladder
surface being held thus by tension in the lattice of fibres
of the tunica externa of the swimbladder which flattened the
dome of the valve and collapsed its side walls (fig. 4.3).
The flattened dome came in contact with the lip of the
valve so preventing the passage of gas into the pneumatic duct.
Flattening of the dome may also have been aided by contraction
of its transverse muscle layer which is a continuation
of the circular muscle of the pneumatic duct. The decrease
in surface area of the dome caused a slight wrinkling of
its internal surface which could be seen in some preparations
as fine lines apparently continuing the lines along the duct.
The tension in the tunica externa perhaps aided by the
contraction of muscles in the dome resisted the pressure of
the swimbladder gas which tended to push the dome outwards
pulling it away from the valve lip and so permitting the
passage of gas into the pneumatic duct. The opening of this
valve occurred spontaneously after death in some fish, the
area in front of the lip becoming transparent, rounded and
visible to the unaided eye as a bulge in the swimbladder
surface (Fig. 4.4).

Adrenalin acting on the closed swimbladder valve
caused the valve to open and showed that there were slight
differences between herring of different sizes in the shape
Figure 4.3 Sketches illustrating the closed swimbladder valve in the absence of drugs.
Figure 4.4  Open swimbladder valve without drugs.
Figure 4.5 Effect of adrenalin on the swimbladder valve.
Figure 4.6  Effect of acetylcholine on the swimbladder valve.
of the dome. In a herring about 15 cm long adrenalin caused a doming of a circular area anterior to the lip. This dome became inflated with gas from the swimbladder as one unit and if the walls of part III of the pneumatic duct were relaxed gas flow into the duct occurred as soon as the dome was formed. In herring larger than about 20 cm an elongated instead of a circular area in front of the lip became raised and was inflated with gas from its anterior margin backwards but passage of gas across the lip of the valve occurred as in the smaller fish (Fig. 4.5). These observations and a knowledge of the anatomy of the valve suggest that three changes accompany gas release; contraction of the circular muscles of the swimbladder to remove the tension in the tunica externa, relaxation of the circular muscles of the dome and relaxation of the circular muscles of the pneumatic duct. All these changes are known to occur in response to adrenalin and hence may be presumed to be under control of adrenergic nerve fibres.

Under acetylcholine a closed swimbladder valve remained closed. If the dome had already been formed and part III was distended with gas the muscles across the dome showed rhythmic contractions at a rate of about two per minute which flattened the domed area from its anterior edge backwards, but were unable to close the valve. Thus while gas distends part III the valve cannot be closed and so could not prevent gas flow from the duct to the swimbladder.
Under normal conditions the duct responds to the presence of gas bubbles by peristalsis which removes the bubbles from the duct and permits the valve to function. As the muscles of the dome contract in response to acetylcholine in the open valve it is likely that they also respond in this way in the closed valve although it is not visible in this condition. Such dual control of the raising of the dome, by the degree of contraction of these muscles as well as of the circular muscles of the swimbladder should permit greater precision. It also seems likely that in the absence of gas in part III of the duct the observed rhythmic contractions of the dome under acetylcholine would succeed in closing the valve.

(h) Swimbladder.

The swimbladder showed a slight response to drugs. In the decerebrate fish opened ventrally adrenalin added to the whole body cavity caused almost immediate contraction in diameter of the swimbladder posterior to the swimbladder valve. As this valve did not open until two minutes later this decrease in diameter was not a collapse due to gas loss but caused a redistribution of swimbladder gas, a function which could be important in altering the position of the center of gravity in the living fish. Adrenalin also caused a slight concavity to form anterior to the dome
of the swimbladder valve. The delay in opening of the swimbladder valve suggests that the circular muscles of this region take longer to respond to adrenalin, perhaps allowing a certain degree of independence between the two. That adrenalin will cause contraction of the circular muscles of the swimbladder valve region as well as over this greater area was shown by observations on excised, gas filled swimbladders whose entire silvered length was removed from the fish after ligaturing both ends and the pneumatic duct. While floating on saline slow spontaneous repetitive movements were observed in one preparation which increased and decreased the slight curvature of the swimbladder towards its ventral side. The initial response of an excised swimbladder to adrenalin was a transverse wrinkling of its surface at intervals of about 3 mm which was more pronounced in the posterior half. With time the lumen of the posterior half became progressively smaller until it measured only 3 mm across in a swimbladder 5.5 cm long. In contrast the anterior half enlarged to a diameter of 1 cm (Fig. 4.7). In the excised swimbladder the contracted region extended anterior to the swimbladder valve and so included the region that became concave when adrenalin was placed on swimbladders in the body cavity.

Pilocarpine on the swimbladder in situ also caused a slight concavity of the area anterior to the valve, an
area which became hollow after electrical stimulation of the visceral branch of the left vagus, but had no other obvious effect. On the excised swimbladder pilocarpine again had no apparent effect, the wall remaining tight and smooth. It was evident, however, when the swimbladder was lifted that an even contraction of the circular muscles must have taken place increasing the internal gas pressure as the rigidity of the swimbladder sharply increased. It was possible to hold the swimbladder by one end with very little bending of the preparation. After a return to saline the preparation became flaccid and bent sharply in two places when lifted by one end. Pilocarpine was also able to reverse the uneven gas distribution caused by adrenalin in an excised swimbladder suggesting that the two drugs work antagonistically.

The ability of the swimbladder to contract slightly in diameter may allow the internal gas pressure to be modified and so affect the discharge of gas from the swimbladder. This ability may be important in two other connections not associated with gas discharge. The redistribution of gas may allow the fish to adjust its center of gravity and so compensate for any tendency to become head or tail heavy. It was seen that herring with their swimbladders opened to the exterior were noticeably tail heavy. Another function may be to adjust the internal gas pressure
to a value which is optimum for the functioning of the anterior prolongations of the swimbladder into the ear of herring.

Observations on the pneumatic duct of Pacific Herring.

As with the Atlantic fish, freshly killed Pacific herring were opened ventrally and the pneumatic duct examined with the aid of a binocular microscope. The passage of air through the duct was studied by supplying air under increasing pressure through a hypodermic needle to the ligatured stomach until it passed through the duct. To display the lumen of the duct mineral oil stained with Sudan Black was injected into the stomach and forced into the duct by the applied air pressure in some of the preparations. The mineral oil acted as an irritant and allowed the response of the duct to foreign matter to be studied. Artemia eggs suspended in saline were also forced into the duct in some preparations to show the response of the duct to small particulate matter.

Results.

In gross appearance the pneumatic duct of Pacific herring did not seem to differ from that of the Atlantic herring. Likewise as the applied air pressure rose the stomach slowly increased in length and diameter and air gradually entered the pneumatic duct until a point was reached at which air flow through the rest of the duct occurred very rapidly. As with Atlantic fish the swimbladder
Figure 4.7 Diagrams showing the effect of adrenalin on excised swimbladders. Dimensions without drug obtained from a photograph; with drug from measurements at maximum response.
valve offered no resistance to the passage of gas into the swimbladder and when passage of gas ceased the duct immediately became fluid filled with the fluid coming apparently from the sides of the duct as it did not flow in from one end. The applied pressure needed to force gas through the duct in situ showed a mean of 3.0 cm Hg, a lower value than that obtained for excised ducts of Atlantic herring under quite different conditions but quite comparable to the value of 2.3 cm Hg which kept gas passing intermittently through the duct in one Atlantic herring where gas passage was studied in situ. In this fish and in the Pacific herring where the duct was studied in the body cavity the gas slowly filled the pockets of part II as well as part I before rapid gas flow occurred. Gas passage through the duct appeared to be a passive process as the duct walls showed no motility and were merely stretched by the advancing air column.

Flow of swimbladder gas through the swimbladder valve to the pneumatic duct often occurred spontaneously a short time after the death of the fish. It could be induced by increasing the pressure of the swimbladder gas until the roof of the dome immediately anterior to the lip of the swimbladder valve stretched and moved outward from the swimbladder surface lifting the duct wall away from the lip and so opening the entrance to the pneumatic duct. As the pneumatic duct is fluid filled the gas once it has passed
over the lip of the valve has to exert a force on the surface
of the fluid sufficient to overcome the force on the film
due to surface tension tending to drive it in the opposite
direction. In fresh preparations when gas was forced over
the lip into part III of the duct this gas returned to the
swimbladder when the pressure in the swimbladder was released
and the fluid edge moved along the duct to the lip of the
valve. A preparation kept two hours in saline showed
passage of gas over the lip at a swimbladder gas pressure
of 0.9 cm Hg but a pressure of 1.1 cm Hg was needed to
displace the fluid in the duct and permit gas flow. The
pressure needed to open the valve and allow gas passage
along the duct would vary according to the degree of muscle
contraction in the circular swimbladder muscles controlling
the tension in the wall of the duct over the lip of the
valve and in the circular muscles of the duct controlling
the diameter of the duct and so the force on the surface
of the fluid. In Atlantic herring this control of gas flow
from the swimbladder through the duct being dependant both
on the opening of the valve and on the diameter of the duct
governing the driving force needed for gas flow was well
shown in preparations treated with adrenalin. When adrenalin
was placed on the valve only the usual doming of the area
in front of the lip occurred and although the valve was open
gas did not enter the duct. When forced into the duct by
increasing the swimbladder gas pressure it flowed back to the swimbladder when this tension was reduced. When both duct and valve were treated with adrenalin thus relaxing the circular muscles of the duct gas flow to the stomach occurred.

Introducing mineral oil or Artemia eggs into the pneumatic duct of Pacific herring showed clearly how the pneumatic duct is able to free itself of foreign matter accidentally brought in with the flow of air from the stomach. Both substances were effective stimuli initiating pulsating movements of the pouches in the wall of part II and peristalsis of parts I and II directed towards the stomach. These movements were so strong that a pouch could be completely emptied of oil either by the peristaltic wave or by the vigorous contraction of the musculature round an individual pouch. All movements of the duct were inhibited by passing air along its length only to be resumed as soon as the applied pressure was reduced and air flow ceased. During air flow the lumen of the duct enlarged to about twice its fluid filled diameter and the resulting stretch of the wall may have been the stimulus inhibiting contraction. Peristalsis was resumed when this distension was lost. In preparations where both the stomach and swimbladder were split to reduce their internal gaseous contents to atmospheric pressure peristalsis of the oil filled duct
continued vigorously showing that this was not dependant on applied pressure at either end of the duct.

These investigations also showed that the pneumatic duct can to a great extent prevent particulate matter entering the duct from the stomach. While mineral oil could be forced along the duct easily from the stomach it was not easy to introduce Artemia eggs suspended in saline into the duct. Most of the eggs were retained in the posterior end of the stomach held back by the wall of the first internal pocket of part I of the duct. A smaller number of eggs were trapped in the second and third pockets of this region and few (32 out of several hundreds in one instance) managed to pass along the duct beyond part I. Thus the pneumatic duct not only can remove foreign matter from its lumen but can also prevent most matter from entering the duct.

Suggested control of gas uptake.

In most of its aspects the movement of air to the swimbladder appears predominantly controlled by the cholinergic nerve fibres. Ingested air in the oesophagus can be moved towards the stomach by peristalsis controlled by the visceral branch of the left vagus nerve. Once in the stomach, if the oesophageal and pyloric sphincters are closed and the stomach musculature contracts the pressure exerted on the contained gas could force it down the pneumatic duct. Both
closure of these sphincters and contraction of the stomach muscles could be elicited by applying parasympathomimetic drugs.

Observations on the passage of gas down isolated pneumatic ducts showed that the resistance to gas flow became significantly greater in preparations treated with acetylcholine. While this may seem a response to a parasympathomimetic drug unfavourable to the passage of gas to the swimbladder it may form part of a protective mechanism excluding particulate matter from the duct during gas flow. Stimuli arising from the presence of food particles at the entrance to the pneumatic duct may initiate a reflex closing of part I of the duct through the sensory and motor fibres of the vagus nerve. The resulting increase in the resistance of part I to the flow of gas could either raise the applied pressure required above the maximum pressure which the stomach could produce, thus preventing gas flow until the food particles had been moved away, or by decreasing the aperture form an effective sieve which mechanically separates gas from solid matter. It has already been shown that the pockets in the interior of part I of the duct can prevent much solid matter from entering the duct even in its uncontracted state.

The passage of gas through the duct appeared to be a passive process as distension of the duct walls by the
gas inhibited all contractions. Peristalsis of the duct, which could be initiated or strengthened by acetylcholine or pilocarpine and which normally developed in response to the presence of gas bubbles in the duct, may be important in restoring the duct to its fluid filled condition after gas uptake and also in removing foreign matter carried in by the gas flow. The swimbladder valve does not impede the flow of gas into the swimbladder but depends for its successful functioning in preventing gas loss on the filling of part III of the duct with fluid after gas passage.

As cholinergic nerve fibres appear to govern all the movements associated with the passage of air to the swimbladder it is interesting that the injection of acetylcholine into the body cavity of living fish initiated the entire sequence of activities resulting in the swallowing of air from the surface.

Suggested control of gas release through the pneumatic duct.

The release of gas through the pneumatic duct in its many aspects was mediated by a single drug, adrenalin, suggesting that the chief control of this release is through adrenergic nerve fibres.

The passage of gas from the swimbladder is controlled by the valve and by the relaxation of the duct. Gas flow through the valve follows contraction of the circular muscles
of the swimbladder in response to adrenalin so removing tension from the tunica externa fibres. Removal of this tension allows the dome to rise and fill with gas and the mouth of the duct to open. Gas passage along the duct then depends on a high enough gas pressure or a sufficient relaxation of the duct. The released gas accumulates in the stomach until the oesophageal sphincter relaxes and it is possible that if conditions causing gas release are short lived that this gas might be returned to the swimbladder instead of being lost. Relaxation of the oesophageal valve is also controlled by adrenergic nerve fibres working against the discharge from cholinergic fibres.

Although gas release through the pneumatic duct is easily induced by the injection of adrenalin and occurs spontaneously after the death of the fish it is not the normal route of gas loss in living herring subjected to a decrease in pressure. Its main function in the herring may be to prevent an excessive rise in the internal gas pressure of the swimbladder if the posterior duct becomes blocked.

The presence of excessive amounts of adrenalin not only led to the loss of gas through the pneumatic duct but inhibited the complex series of actions which normally cause a herring with a depleted swimbladder gas volume to seek the surface and swallow air. Thus at the behavioural level as well as at the physiological, adrenalin seems to favour decrease in swimbladder volume.
CHAPTER V

RELEASE OF SWIMBLADDER GAS THROUGH THE POSTERIOR DUCT.

Introduction.

The release of swimbladder gas through the posterior duct of herring in response to a reduction in pressure was reported, without details, by Verheijen (1956). That the posterior duct is the usual channel of gas release in Atlantic herring subjected to a decrease in pressure was shown by Brawn (1962). Gas release in these fish occurred at a mean pressure reduction of 6% from the pressure to which they were adjusted in rapidly swimming fish and at a mean reduction of 32% in moderately swimming fish. This suggests that gas release is influenced by the state of excitement of the fish. The gas release brought the herring to within 19% of perfect adjustment to the new reduced pressure within half an hour when the pressure had been reduced 49 to 52% below the pressure to which the fish were adjusted. Most of this adjustment probably occurred in the first six minutes, the time at which bubbling from the fish ceased. By swimming movements the herring were able to compensate easily for their increased buoyancy during pressure decrease at a rate of 10% per minute until their buoyancy was reduced by gas release. Thus it was calculated that herring rising at a rate of 11 m/min at 100 m depth; at 6 m/min at 50 m or at 2 m/min at 10 m would have no limit placed on the extent of their
upward movement by too great an increase in buoyancy or through rupture of the swimbladder. The direct, functional connection between the swimbladder and the exterior thus gives the herring an advantage in this respect over physoclist species which have to absorb excess swimbladder gas and probably over other physostomes which liberate gas by more devious routes.

The release of gas from herring may also be observed at sea but there appears to be only one mention of this in scientific literature. Verheijen (1956) reports that gas bubbles are used as a sign of ascending clupeoids on the European coast but does not specifically name the herring. The scarcity of reports may be due, not to the infrequency of gas release by herring, but to the difficulty in observing this at sea. Dr. J. L. Hart and Mr. S. N. Tibbo of the Biological Station, St. Andrews, N.B., who have long been acquainted with the herring fisheries of the Pacific and Atlantic coasts of Canada, confirmed that fishermen in these areas can detect the presence of herring schools by the presence of fine bubbles rising to the surface. However, since the general adoption of echo-sounders by the herring fleet, skippers are relying more on fish traces on the sounder than on herring "put-up". To document the release of gas by herring at sea, Dr. Hart wrote to various people on the Pacific coast who has first hand knowledge of the phenomenon or who questioned the fishermen on its occurrence. Excerpts from their replies are given below:
Capt. C. Clarke, Nelson Brothers Fisheries, Vancouver

"Put-up" is breathing of herring. When they come to the surface, if there is no wind, it looks like a white sheet. As you get closer, you can actually hear it breaking. In the olden days the Japanese used to listen to locate the schools. Also, when you purse your seine and have any fish, this put-up appears white all over the net. Salmon have the same characteristic—but only when they are in the net. Outside the net you never see any put-up from salmon. Herring and salmon are the only fish I know of that have this characteristic—pilchards don't have it, and I have never seen it in anchovies. On a clear day you can see put-up from herring for half a mile. When they are really solid you can actually hear it if you are, say, 20 feet over the top. When you see herring putting-up there is really a solid school swimming in the water. This is seldom seen in the morning, mostly late afternoon on a sunny day. It looks white, bubbles are about the size of pinheads, and seem to burst as they come up, real close together."

Capt. H. C. Auchterlonie, British Columbia Packers Vancouver

"Herring 'put-up' can be sighted throughout the year, but is is more in evidence when the fish are schooled up before spawning. It consists of small bubbles rising to the surface producing a froth-like effect. One description likens 'put-up' to the bubbles and froth accompanying an underwater bilge pump discharge, while at a distance it appears as a fine smoke on the water.

"Herring of average size put-up bubbles about the size of a pinhead, but with small or under-size herring the bubble is large, approximately the area of a hat pin head.

"'Put-ups' can be observed any time during the day or night, although a calm, sunny afternoon seems to be a more favourable time. The direction of the school can be judged by the path of the bubbles and the depth ascertained by the surfacing force. If the bubbles jump from the water with considerable force, the fish are deep, if they surface gently, the fish are shallow."
"Prior to the advent of the paper sounding machines, herring were caught in part by the observation of 'put-up'. The practiced eye of the fisherman could evaluate the size, depth and direction of the school by the 'put-up' it produced.

"After the seine had been set and pursed, the presence of 'put-up' inside the net assured the fishermen that their efforts were successful. This still applies to present day fishing, a large catch will turn the water inside the seine white with 'put-up'.

"If, however, the purse lines are drawn completely under the fish without touching them, there could be no sign of 'put-up' until later when the net is being hauled aboard. On the other hand, if the purse lines in closing are dragged through the fish, the 'put-up' can be seen immediately. With the use of mercury-vapour lamps to gather the fish under the anchored seine boat, the fish are more apt to show themselves by surface flipping and little or no 'put-up' is seen until the fish are inside the net.

"This information has been garnered from fishermen involved in both past and present herring fisheries. They cannot advance any explanations of this phenomena, other than the fact it is more noticeable when fish are confined in a school or caught in a seine."

Mr. J. Dale, The Canadian Fishing Co. Ltd., Vancouver

"Herring usually put-up in the late afternoon just before dark and they usually put-up most in cold, clear weather. Put-up shows in bubbles about the size of a pea. The bubbles burst open when they reach the surface of the sea. Put-up shows in patches, usually the size of the herring school. If this is what we call a heavy school of herring, the put-up or air bubbles are very plentiful and, when they burst, a light grey patch is formed on the water - sometimes it looks like a patch of drifting snow. When herring are putting up they are usually swimming very fast and, like most fish, they are swimming against the tide. Put-up can be seen to a depth of 15 to 20 fathoms."
"With the large herring seines they are using here in British Columbia, 300 fathoms long and about 45 fathoms deep, they can catch herring very easily when the school is putting up if the school stays at the same depth and does not sound. Herring are often very hard to catch when putting up."

These reports show that the most usual time for gas release by free living herring is in the afternoon, though not confined to this time. Such release may be associated with the upward movement of herring toward the surface at night resulting in a decrease of hydrostatic pressure. The lack of gas release shown by herring drawn to mercury lamps at night, mentioned by Capt. Auchterlonie, similarly may be due partly to the presence of herring in the upper water layers at that time so that they do not undergo any great change in depth. Another feature is also evident from these reports; that herring release gas when swimming very fast, perhaps as a response to strong tidal currents, predators, the stimulus of spawning or the advancing wall of the seine. This is in accord with the observation that captive herring release gas much more readily when swimming in a fast, panicky manner than when moderately swimming. This also provides a second explanation for the lack of gas release by herring gathering round a light at night as here the situation is one of attraction rather than flight until the net is drawn round the fish.
The behaviour of Atlantic herring while releasing gas in response to a decrease in hydrostatic pressure has been described previously (Brawn 1962). These herring in a 51 litre carboy responded to a pressure decrease by changing from random swimming at all depths to swimming in the lower half of the container. Here the fish compensated for their excess buoyancy until gas release while still swimming predominantly horizontally and did not show any change in behaviour associated with the release. In the present investigation Atlantic herring released gas following pressure reduction when tested under more crowded conditions and their behaviour was consequently modified. Photographs 5.1 to 5.5 show these herring in a 6 litre flask before, during and after a pressure decrease of 42 cm Hg lasting five minutes. Here the rate of pressure decrease (> 0.35 cm Hg/sec) is more than three times faster than in the experiment with the carboy and the fish have less room to swim horizontally. Under these conditions the herring swim side by side with their heads towards the bottom of the flask until the pressure is returned to its initial value. The head down response occurred as soon as the pressure was reduced but there was a lag of between one and two minutes before gas was released in significant amounts. These photographs also show the appearance of a copious release of gas by herring.
This review of literature on gas release by captive herring and of personal communications concerning the release of gas by herring in nature, covers most aspects of this phenomenon readily observed in living fish. Still to be described is the mechanism of gas release related to the anatomy of the posterior duct. The susceptibility of this mechanism to drug and operational interference was investigated in the following experiments.

The influence of drugs and nerve section on the gas release mechanism.

Method.

Single herring adjusted for several days to 36 cm depth of water were induced to swim into a 6.5 litre test flask of the same depth by immersing the fluid-filled flask in the stock tank and directing the neck towards the fish. Fish undergoing operative procedures before testing were gently grasped by the head underwater after confining them in a net, the body was then placed along a groove lined with absorbent paper and the operative procedure completed as quickly as possible with the fish still under water and held only by the head. After the operation the herring was placed in the test flask. Operated and control fish were tested in sea water while fish subjected to various concentrations of physiological drugs were exposed to these solutions on
5.1 Group of Atlantic herring before pressure reduction
5.2 Same herring 30 seconds after a pressure reduction of 42 cm Hg
5.3 Close-up of herring after 4 minutes at -42 cm Hg to show appearance of released gas.
5.4  Herring after 5 minutes at -42 cm Hg
5.5  Herring 2 minutes after return to atmospheric pressure
swimming into the flask. Netting was placed in the neck of the flask to exclude the herring and the flask attached to the vacuum pump, pressure chamber and manometer system shown in photograph 5.1. The fish was observed for 5 minutes without pressure change then the pressure was reduced slowly (0.12 to 0.08 cm Hg/sec) and the pressure at which gas was released was recorded. After the test, all fish were immediately examined for the presence of gas in the pneumatic duct or stomach and for the presence of any response of the abdominal organs to the drug. Herring whose spinal cord had been cut were lightly steamed after the test and then dissected to ascertain that the cord had been truly severed.

The test fish all came from one group and had a mean length of 15.3 cm. The test solutions used ranged in temperature from 11.0 to 14.3 °C. All solutions were made up in sea water.

Results.

The results obtained are summarised in table 5.1 and are considered in turn below. One mean pressure reduction had to be recorded as >43.3 cm Hg as this was the lowest pressure possible with this apparatus. Of the group of six spinal fish tested the results in this table refer to only three as the remainder liberated gas by mouth. All fish except spinal fish lost gas through the posterior duct.
a) Controls. These fish swam moderately and mainly horizontally before and during pressure reduction. Gas was released through the posterior duct when the pressure had been sufficiently reduced. No gas was present in the pneumatic ducts or stomachs of these fish after testing. The stomach was typically contracted although in one fish it was half filled with water and showed contractions passing towards the pneumatic duct.

b) Eserine 1:10,000. This drug caused 5 of the 6 test fish to liberate gas from the posterior duct without pressure reduction, the sixth liberating it at -10.3 cm Hg. The time to first gas release varied from 1/2 to 6 minutes with a mean of 3 1/2 minutes thus taking slightly longer on the average than when treated with adrenalin (2 min, 20 sec) or with atropine (40 sec) at the same concentration. The behaviour of the fish and type of gas release appeared normal except that the amount of gas in each burst was often large. When opened the fish had no gas in the pneumatic duct, stomach or intestine. The stomach often showed strong contractions either as stationary constrictions or as peristaltic waves towards the pneumatic duct. Eserine 1:100,000 was too dilute to cause a significant change in the gas release pattern.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Fish</th>
<th>Mean pressure reduction cm Hg</th>
<th>Sample variance</th>
<th>Diff. in means, control/test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>20.1</td>
<td>85.40</td>
<td>-</td>
</tr>
<tr>
<td>Eserine 1:100,000</td>
<td>6</td>
<td>20.2</td>
<td>58.81</td>
<td>.02 &gt;0.5 NS</td>
</tr>
<tr>
<td>Eserine 1:10,000</td>
<td>6</td>
<td>1.8</td>
<td>14.74</td>
<td>4.39 &lt;0.001 HS</td>
</tr>
<tr>
<td>Acetylcholine 1:10,000 plus eserine 1:100,000</td>
<td>6</td>
<td>23.4</td>
<td>*175.45</td>
<td>0.58 &gt;0.5 NS</td>
</tr>
<tr>
<td>Pilocarpine 1:100,000</td>
<td>6</td>
<td>18.0</td>
<td>87.37</td>
<td>0.43 &gt;0.5 NS</td>
</tr>
<tr>
<td>Pilocarpine 1:10,000</td>
<td>6</td>
<td>14.6</td>
<td>*110.00</td>
<td>1.07 &gt;0.1 NS</td>
</tr>
<tr>
<td>Atropine 1:10,000</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>5.03 &lt;0.001 HS</td>
</tr>
<tr>
<td>Adrenalin 1:10,000</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>5.03 &lt;0.001 HS</td>
</tr>
<tr>
<td>Brain removed</td>
<td>6</td>
<td>32.9</td>
<td>61.45</td>
<td>2.73 &lt;0.05 PS</td>
</tr>
<tr>
<td>Spinal cord cut at skull</td>
<td>3</td>
<td>&gt;43.3</td>
<td>0</td>
<td>4.06 &lt;0.01 S</td>
</tr>
</tbody>
</table>

Table 5.1. The effect of drugs and operative procedures on the mean pressure reduction necessary to cause gas release through the posterior duct.

NS, PS, S, and HS stand for not significant, probably significant, significant and highly significant respectively.

* An F test showed that these sample variances did not differ significantly from the sample variance of the control group.
c) Acetylcholine 1:10,000 plus eserine 1:100,000. In these fish the presence of eserine should have prevented the destruction of acetylcholine and intensified its effects. These drugs had no significant effect on gas release. The herring swam normally and when opened typically had no gas in the pneumatic duct or stomach. The stomach was contracted. One fish had many small bubbles in the intestine which moved rapidly towards the anus and probably came initially from the swimbladder. A more than normal liberation of bile from the gall bladder coloured the intestinal contents of these fish bright green and the intestine was abnormally motile. During gas release no faecal matter was voided showing that gas loss was through the posterior duct and not via the anus.

d) Pilocarpine 1:10,000. Pilocarpine had no significant effect on the release of gas at this concentration. In behaviour, the herring treated with this drug were normal and liberated gas under reduced pressure in the usual burst of bubbles. The fish when opened had no gas in the pneumatic duct or stomach. The stomach in all fish was contracted.

e) Atropine 1:10,000. The six fish tested liberated gas through the posterior duct without pressure reduction. The fish responded very rapidly to the drug, within an estimated 15 to 50 seconds, and released large amounts of gas in prolonged bursts during vigorous swimming. No gas was present in the pneumatic duct or stomach when the fish were opened.
f) Adrenalin 1:10,000. All six fish like those in atropine liberated gas through the posterior duct without a reduction in pressure but the nature of the release and the behaviour of the fish were quite different. These fish responded to the drug more slowly, taking from one to four minutes before the first release was seen. All the fish were unusually quiescent, often ceasing to swim and three fish showed sideways shakes of the head or coughing movements. The release of gas was unusual as it trickled from the posterior duct of the fish bubble by bubble over considerable periods rather than being liberated in short bursts as in the control fish. When opened four of the test fish had gas in either the pneumatic duct or stomach but only one of these while living had lost gas through the mouth and this occurred after gas loss through the posterior duct. These results suggest that adrenalin taken up by the gills acts first on the posterior duct and only later on the pneumatic duct and swim-bladder valve. Half of a group of six herring given adrenalin at a concentration of 1:100,000 also liberated gas from the posterior duct without pressure reduction.

g) Brain removed. The six fish in this group showed an increase in the pressure necessary to cause gas release which is probably significantly different from that of the control group. Respiratory movements in these fish ceased so that with time these fish would suffer increasingly from oxygen
lack. All observations were completed before trembling of the skeletal musculature was seen. No gas was present in the pneumatic duct or stomach of these fish when opened.

h) Spinal cord severed. The results with this group of six fish differed from all those previously mentioned in having three fish which liberated gas through the mouth. The complete results for this group are as follows:

<table>
<thead>
<tr>
<th>Fish</th>
<th>Pressure reduction causing gas release (cm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish by mouth</td>
<td>by posterior duct</td>
</tr>
<tr>
<td>1</td>
<td>&gt;43.3</td>
</tr>
<tr>
<td>2</td>
<td>&gt;43.3</td>
</tr>
<tr>
<td>3</td>
<td>24.7*</td>
</tr>
<tr>
<td>4</td>
<td>9.4*</td>
</tr>
<tr>
<td>5</td>
<td>15.8*</td>
</tr>
<tr>
<td>6</td>
<td>&gt;43.3</td>
</tr>
</tbody>
</table>

*gas in the pneumatic duct or stomach when opened.

Herring which first liberate gas from the mouth cannot truly be compared for gas release through the posterior duct with those that do not, as the expanding gas inside the swimbladder will no longer exert a comparable pressure on the swimbladder wall or posterior duct. For this reason only the three fish which did not liberate gas from the mouth were entered in table 1 and these fish showed that the pressure reduction necessary to cause gas release from the posterior duct was significantly greater than that for the control fish. There can be no control group with which to compare the three
fish liberating gas by mouth as under normal conditions this does not usually occur. Here it is the change of method of gas release which is of interest rather than the pressure reduction necessary to cause the release.

i) Spinal fish in 1:10,000 eserine. Two spinal fish placed in eserine liberated gas through the posterior duct without pressure decrease giving a mean value of 0 which is probably significantly different from the control mean (p < 0.05). Both fish started to lose gas 2 minutes after being placed in eserine and continued to do so over a period of time.

j) Spinal fish in 1:10,000 atropine. Three fish were tested. Two of these lost gas through the posterior duct after 2 minutes, without a change in pressure. The third fish did not release gas although held 10 minutes without pressure change and then subjected to a decrease of 38.8 cm Hg. There was no obvious reason for the difference in these results. All three fish when opened had no gas in the pneumatic duct or stomach. The stomach was relaxed and filled with water.

Conclusions.

From the results obtained it is possible to suggest tentatively a mechanism for the control of gas loss in herring. However as the interpretation relies heavily on the known action of the drugs on mammals, especially man, the proposed mechanism must remain uncertain until more is known of the nervous and endocrinological relationships.
in teleosts, especially as these affect the chromaffin (adrenaline producing) tissue. Most of the information on the effect of drugs on man has been taken from Goodman and Gilman (1956).

Atropine is known to specifically inhibit the muscarinic actions of acetylcholine both in man and in teleosts (Frey 1928) but except under special conditions leaves the nicotinic responses unblocked (Goodman and Gilman, 1956). In the herring atropine caused the release of gas through the posterior duct without a change in the external pressure, suggesting that gas release is normally prevented by smooth muscles under the control of cholinergic nerve fibres. The circular smooth muscles in the wall of the posterior duct are most likely to control this release. They may be innervated by the vagus as branches from the nerve ramify throughout the swimbladder wall in teleosts (Terio 1948; Abraham and Stammer 1954; Qutob 1962).

Atropine acts directly on the effector and this may be the reason for the herring's rapid response to it. As it acts directly it is not affected by nerve section and spinal fish should also respond by gas release without pressure change, as two of the three fish tested did.

In man atropine also affects the central nervous system causing restlessness. A similar effect was observed
in herring as unusually vigourous swimming.

The influence of atropine on gas release suggested that this is normally prevented by smooth muscles under the control of cholinergic nerve fibres. As adrenalin was found to cause gas release it seems that acetylcholine and adrenaline work antagonistically in the control of gas release through the posterior duct. Adrenalin also permits the passage of gas through the pneumatic duct but under the conditions of the test this occurred later than through the posterior duct. If naturally produced adrenaline also elicits a response from the posterior duct more readily than from the pneumatic duct, either because of different thresholds or differences in the blood supply, this could explain why herring normally liberate gas only through the posterior duct.

As adrenalin causes the release of gas it is to be expected that in times of stress when the production of adrenaline by the chromaffin tissue may be assumed to rise, herring will release gas more readily than under normal conditions. This increased activity of the chromaffin tissue would be expected in fish chased by predators, confined in nets, swimming hard against currents or while spawning. This has been shown to be true for the skate as recently trawled specimens already have so much adrenaline in their blood that they show no pressor response to adrenalin artificially
administered although rested fish respond (MacKay 1931). In herring the presence of a high level of adrenaline in the blood in conditions of stress could explain the observed readiness of excited fish to release gas both under laboratory conditions (Brawn 1962) and when confined by nets at sea, swimming fast or aggregating before spawning (Auchterlonie and Dale, personal communications quoted in the introduction to this chapter).

The balance between adrenaline and acetylcholine which appears to control gas release would be expected to be upset by the addition of acetylcholine or pilocarpine, which exhibits most of the muscarinic properties of acetylcholine, to the system. As these drugs did not significantly alter the point of gas release it may be concluded either that enough acetylcholine is normally present to evoke a full response from the closing muscles or that the applied drugs also stimulated the chromaffin tissue to liberate enough adrenaline so that a new balance was struck between increased adrenaline and increased acetylcholine close enough to the original balance that it could not be distinguished from it. The third possibility, that the system did not respond to these drugs was ruled out because of the pronounced sensitivity of the system to the acetylcholine blocking agent, atropine.
Although the addition of acetylcholine or pilocarpine did not decrease the pressure necessary to cause gas release below its normal value, this could be accomplished by removing the brain or sectioning the spinal cord at the skull. Thus, in the normal fish, there must be a gas release mechanism acting through the central nervous system that in the intact fish picks up sensory stimuli as the external pressure is reduced, passes the information to the brain and spinal cord and expedites the release of gas. When this release mechanism is interrupted by removing the brain or sectioning the spinal cord, the fish although otherwise intact needs a much greater reduction in pressure before gas release will occur. This release mechanism presumably operates at the level of the posterior duct by increasing the amount of adrenaline either directly at the terminals of the adrenergic nerve fibres or less directly via the chromaffin tissue.

The manner in which fish detect pressure changes in their environment has long been in doubt, the suggested mechanisms being exteroceptive stimuli, pressure receptors in the swimbladder wall, pressure receptors in the surrounding tissues that respond to changes in swimbladder volume and reception of pressure changes in the swimbladder by means of the ear, either through the Weberian ossicles or by means of anterior prolongations of the swimbladder into the ear, such
as are found in herring. All these possible means of pressure reception have recently been critically reviewed by Qutob (1962) who shows by his electrophysiological work on cyprinids that the swimbladder wall has a sensory function, as considerable change in the rate of impulse discharge occurs in the afferent fibres of the vago-sympathicus nerve which innervates the swimbladder when a pressure change occurs in the environment of the fish. Qutob concludes that a fish may use sensory stimuli from the swimbladder wall, from the labyrinth or from exteroceptors to evoke the many different reflexes regulating hydrostatic equilibrium, perhaps using one stimulus for one reflex and another for another. He quotes Kuiper, Franz and Dijkgraaf to show that the swimbladder wall plays the major sensory rôle in the "gas spitting reflex." Thus there are many pathways by which sensory stimuli may reach the brain of the herring either directly or through the spinal cord. All that may be deduced of the mechanism at this level is that it must involve the brain and that either the afferent or efferent pathways or both pass between the spinal cord and the brain at the level of the posterior margin of the skull.

Three herring with the spinal cord severed at the skull liberated gas from the mouth instead of through the posterior duct and while in one of these fish this might have been an artifact, in the other two it was certain that
the gas had come from the swimbladder as this was partially empty and large amounts of gas were seen to leave the mouth. This release was not due merely to a rise in internal swimbladder gas pressure above normal due to the non-functioning of the gas release mechanism as it took place in one fish at -9.4 cm Hg and in the other at -24.7 cm Hg, which do not usually cause gas release by the posterior duct. It also seems unlikely that release through the mouth was caused by circulating adrenaline in the blood as this usually favours release through the posterior duct first. An interpretation must take into account that gas release by mouth was seen only in spinal fish and not in fish with brains destroyed. The spinal fish, alone of all fish tested lost their equilibrium and lay belly up on the bottom of the flask and were presumably "aware of" this unusual posture, unlike fish with brain removed, since ears, eyes and brain were still intact. Thus there is the possibility that these fish were subjected to a stimulus unlike that of other fish which may have led to the unusual response. The means by which gas release was accomplished, however, is uncertain as communication between the brain and the sympathetic nervous system was severed. Anatomically the most likely nerve which could mediate gas release through the swimbladder valve under these conditions would seem to be the vagus which passes directly from the brain to the stomach and along the pneumatic duct. At this
time no firm conclusions can be drawn concerning this release of gas by mouth in spinal fish.

There is one result which is apparently at variance with the suggested control of gas release and this is that eserine, which prevents the destruction of acetylcholine at the nerve endings and so intensifies its action would be expected to impede gas release; yet in concentrations of 1:10,000 it facilitated release. A possible explanation may be that the effect of eserine at the chromaffin cells leading to the production of large amounts of adrenaline outweighed its effect on acetylcholine levels at the posterior duct. Thus the balance between adrenaline and acetylcholine was upset in a direction favouring gas release.

The mechanism controlling gas release in herring suggested here is shown in diagrammatic form in figure 5.1.
SENSORY STIMULI WHEN EXTERNAL PRESSURE REDUCED

GAS RELEASE MECHANISM

Destroy gas release mechanism

BRAIN

SPINAL CORD

ACETYLCHOLINE
Pilocarpine (effects cancel out)

CHROMAFFIN TISSUE

ADRENERGIC NERVE FIBRES

ADRENALINE IN THE BLOOD

ADRENALINE

NORMAL BALANCE

MUSCARINIC ACTION OF ACETYLCHOLINE

GAS RETENTION

Normal components – solid lines, capitals
Operative procedures or applied drugs – dotted lines, small letters

GAS RELEASE

Figure 5.1 Diagrammatic representation of suggested method of control of gas release through the posterior duct.
CHAPTER VI
THE SWIMBLADDER AS A HYDROSTATIC ORGAN

Introduction.

Herring given access to the surface and held in water of constant shallow depth adjust their swimbladder gas volume until their density is slightly greater than the density of the water (Brawn 1962). Using an apparatus devised by Alexander (1959 a and b) it is possible to determine the volume of gas in the swimbladder, the excess internal gas pressure, the pressure reduction necessary to cause neutral buoyancy and the density of the fish using living fish tested at the water depth to which they had become adjusted. These values have previously been obtained for immature Atlantic herring caught in Passamaquoddy Bay, N.B. (Brawn 1962). For comparison similar tests were made during this investigation on herring from the Pacific. The same apparatus was then taken to the Atlantic coast and further determinations made on Clupea harengus.

In the present investigation the analysis of the relationship of the density of the fish to that of its environment was carried beyond that described for Atlantic herring in the 1962 paper. After the determination of the physical properties of the swimbladder and of the density of the living fish, the fish was analysed to determine the amount and density of its components which might differ from
the average density of the fish. Such information allows a comparison of the problems of maintaining nearly neutral buoyancy in herring of different sizes, degrees of maturity or fatness and also, where such information is available, with fish of other species. Particular attention was given to the relationship between fat content and swimbladder volume as fat, being less dense than sea water, should contribute an upthrust to the fish which, if the swimbladder is truly a hydrostatic organ, should be reflected in a decrease in the swimbladder gas volume.

In this chapter these two topics; comparison of physical properties of the swimbladder of Atlantic and Pacific herring, and the analysis of the components of the fish, especially the relationship between fat content and swimbladder volume will be presented under two subheadings although the second is based on the same groups of test fish as the first.

A. Comparison of the physical properties of the swimbladder of Atlantic and Pacific herring.

Method.

Alexander's elegant method of determining the size of the swimbladder in living physostomes uses the measured change in volume of a gas with change in pressure to determine the total gas volume. This calculation requires that the gas was free to expand and was at constant temperature during the determination. To determine the change in volume
of the swimbladder gas of herring with changes in applied pressure the apparatus shown in figure 6.1 was used.

Herring were held two days or more before testing in water 36 cm deep, the same depth as the test flask, and it was assumed as consistent results were obtained thereafter that this period was sufficient for the herring to become adjusted to the pressure of atmospheric pressure plus 2.6 cm Hg at the bottom of the tank. Individual fish were allowed to swim into the 6.5 litre test flask filled with sea water from the holding tank. The flask was placed in a water bath of the same temperature as the tank and one litre of boiled sea water at tank temperature was added to reduce the gaseous content below the level at which gas would come out of solution at the reduced pressures used. To lightly anaesthetise the fish 0.34 g of MS 222 (tricaine methane sulphonate) was added to the flask which was then connected to the rest of the apparatus taking care to exclude all air bubbles. By operating the stopcock to add water from the funnel to the system the water meniscus was brought approximately halfway along the capillary tube whose capacity of 0.07378 ml/cm had been determined previously.

As soon as the herring ceased to swim and lay on the bottom of the flask the pressure inside the apparatus was increased by 2, 4, 6, 12, and 18 cm Hg. The position of the water meniscus was recorded at atmospheric pressure
before and after the run and at each of these applied pressures. After return to atmospheric pressure the pressure was reduced in steps of 2 cm Hg and the meniscus positions at each pressure including the pressure at which the fish had neutral buoyancy were recorded. It was not possible to determine neutral buoyancy for all fish as some released gas before this condition was reached.

In this first run the change in volume of the swimbladder gas was measured but as this may have been compressed by the swimbladder walls it cannot be assumed to have expanded freely. Thus a second run was made after extracting some gas from the swimbladder by reducing the pressure to -36 cm Hg. The extracted gas remained inside the flask so that the total mass of gas inside the system remained unchanged. Previous work with Atlantic herring had shown that pressure reduction to -36 was sufficient to remove enough gas so that the swimbladder wall was left slack and for this reason only a first and second run were made with these fish. Pacific herring after the second cycle of pressure changes were subjected to a pressure reduction of -42 cm Hg which removed more gas from the swimbladder before making a third run.

After the second or third run had been completed the density and temperature of the flask water and atmospheric
to pressure or vacuum pump

Figure 6.1 Diagram of apparatus used to determine the physical properties of the swimbladder of living, anaesthetised herring.
pressure were recorded. The fish was subjected to a standard drying procedure before weighing. This procedure was adopted after observing the change of weight of fish after various manipulations and represented the point at which excess water in the mouth and gills were removed and surface evaporation reached a constant low value (figure 6.2). The fish was blotted with absorbent paper until the towel came away dry. A roll of absorbent paper was placed in the mouth and the gills were wet with alcohol. Air was blown on the gills until the gill filaments separated easily but were not reduced in size, a process which took 3 to 5 minutes according to the size of the fish. The herring was hung head downwards at room temperature until 20 minutes from the beginning of the drying period had elapsed and then was weighed. Herring were frozen to await subsequent analysis of their fat and skeletal components.

As the apparatus itself is subject to change in volume with pressure blank runs were made with the flask filled with sea water left to attain temperature equilibrium. The mean result of these blank runs provided a distortion correction which was applied to all results obtained with the apparatus. The same apparatus was used in determinations with both Atlantic and Pacific herring.

Another group of Pacific herring were subjected to a simpler determination using the same apparatus. With these
fish the pressure was reduced in -2 cm Hg steps until neutral buoyancy was reached. The meniscus position at manometer zero and at neutral buoyancy was recorded and corrected for temperature change and distortion. After each run the density of the sea water and atmospheric pressure were recorded and the fish weighed after the standard drying procedure. The information from this group allowed the neutral buoyancy, density and sinking factor of each fish to be calculated but was not sufficient to give the swimbladder volume.

Calculations.

The meniscus positions recorded were corrected for slight temperature changes by apportioning the difference between the two readings at manometer zero, corrected for distortion and then expressed as movements of the meniscus with change in pressure below an applied pressure of 18 cm Hg. It was found that the results following reduction to -36 and -42 cm Hg agreed within 0.1 cm so that in both instances the gas was not constrained by the swimbladder wall and a mean value for the determination could be used in further calculations with the Pacific herring. As the movement of the meniscus between 12 and 18 cm Hg was similar both when the gas was entirely within the swimbladder and after part of it had been removed, it is assumed that at
Figure 6.2  Graph used to determine standard drying period and procedure. Initial weight of herring 14.6455 g.
these pressures the swimbladder gas is no longer compressed by the swimbladder wall and will obey Boyle's Law. Thus the results of these two runs can be graphed with a common origin at 18 cm Hg, taking the meniscus position at 18 cm Hg as zero. Such a graph is shown in figure 6.3. On this graph 0 cm Hg represents the pressure to which the fish had become adjusted and it can be seen that at this pressure the swimbladder gas has a volume less than the unconstrained gas by an amount represented by the difference in the two meniscus positions. This difference multiplied by the capacity of the capillary gives the difference in volume between the swimbladder and unconstrained gas. The volume of unconstrained gas can be calculated directly from the change in volume, shown as meniscus displacement, between atmospheric pressure and atmospheric pressure plus 18 cm Hg. Thus the volume of the swimbladder at the pressure of adjustment can be found.

The pressure exerted on the swimbladder gas by the walls can be read directly from the graph as it equals that pressure at which the unconstrained gas has the same volume as the swimbladder gas at manometer zero.

The density of the fish can be determined as follows. At neutral buoyancy the density of the fish equals the density of the sea water. As the pressure is returned to manometer zero the volume of the fish decreases by an amount represented by the movement of the meniscus between neutral buoyancy and
zero while the mass of the fish remains constant. Thus:

\[
\text{Volume of fish at } 0 = \frac{\text{mass of fish}}{\text{density of sea water}} - \text{change in volume,}
\]

N.B. to 0

From the volume of the fish at manometer zero its density at this pressure can be calculated as its mass is known. The sinking factor, a term introduced by Lowndes (1942) for the density of the fish divided by the density of its environment times 1000 can then be calculated.

These calculations are more exact than those presented in the 1962 paper in that the density for each fish has been calculated individually using the neutral buoyancy determined for each fish. In the previous paper the density of herring was calculated for the whole group using a mean mass, a mean change of volume with pressure below manometer zero and a mean value for neutral buoyancy obtained from a separate group.

Results.

The results obtained for Atlantic herring are given in tables 6.1 and 6.2, those for Pacific herring in tables 6.3 and 6.4 and in table 6.5 the mean values for each group are presented together to permit comparison.
Figure 6.3  Graph of meniscus displacement against pressure for swimbladder gas and unconstrained gas for Pacific herring P2.
1) Neutral buoyancy. With one exception all groups showed a mean pressure reduction causing neutral buoyancy below -6 cm Hg. The exception was a group of Atlantic herring selected for fatness, where only two values for neutral buoyancy were obtained giving a mean of -11.2. These abnormally high neutral buoyancy figures were supported by two other fish of this group A7 and A8 which were taken to -14 and -15 cm Hg without floating and which therefore must have had a neutral buoyancy above this value. These fish and the remaining two of the group of six fat fish released gas before neutral buoyancy was reached. The difference between the neutral buoyancies of fat and thin Atlantic herring is much greater than the difference between the two herring species.

2) Density of the fish and sinking factors. The Pacific fish tested had a lower density than the Atlantic herring; individual values for the Pacific fish lying almost entirely below 1.0260 g/ml while those of the Atlantic herring lay almost entirely above this figure. The mean value for Pacific herring was 1.0243 and for Atlantic herring, 1.0268. This difference may not be a true difference between species as the Pacific herring were held and tested in sea water less dense (1.0188-220) than the Atlantic sea water (1.0238-247). When values of sinking factor for individual fish are considered it is evident that there is a complete overlap of values for the Atlantic and Pacific herring which, as sinking
factors are automatically corrected for differences in the density of the environment, suggests that the observed differences in density were a response to the environment rather than a between species difference. Both species appear to adjust their densities to bear the same relationship to their environment density.

The group of fat Atlantic herring had densities above those shown by any individual of any of the other groups and this difference is not abolished when sinking factors are compared. The lowest sinking factor obtained for the four fat Atlantic herring was 1006.2 which is above the highest sinking factors obtained for thin Atlantic herring (1004.7) and Pacific herring (1005.4).

3) Swimbladder volume and internal pressure. The range of % volume of the swimbladder values obtained for Atlantic (2.65 - 5.23%) and Pacific (2.58 - 5.43%) herring were similar. The fat Atlantic herring had percentage swimbladder volumes generally below those of thin Atlantic fish resulting in mean values of 3.5 and 4.5% respectively.

The excess internal pressure of the swimbladder gas of herring was never very high, the maximum value being 2.7 cm Hg, the minimum 0. Individual values for Atlantic and Pacific herring completely overlapped.
### Table 6.1. Fish densities and physical properties of the swimbladder of Atlantic herring kept in captivity to lose the fat deposits around the intestinal organs (mean fat volume 0.87%).

<table>
<thead>
<tr>
<th>Fish Period for Mass Adjustment (days)</th>
<th>Mass (g)</th>
<th>Neutral Buoyancy (cm Hg)</th>
<th>Density of fish (g/ml)</th>
<th>Density Sea Water (g/ml)</th>
<th>Sinking Swimbladder Factor</th>
<th>% Vol Excess Pressure (cm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>34</td>
<td>11.622</td>
<td>-1.2</td>
<td>1.0295</td>
<td>1.0247</td>
<td>1004.7</td>
</tr>
<tr>
<td>A2</td>
<td>34</td>
<td>14.287</td>
<td>-2.0</td>
<td>1.0256</td>
<td>1.0243</td>
<td>1001.3</td>
</tr>
<tr>
<td>A3</td>
<td>34</td>
<td>11.580</td>
<td>-4.6</td>
<td>1.0286</td>
<td>1.0242</td>
<td>1004.3</td>
</tr>
<tr>
<td>A4</td>
<td>34</td>
<td>8.987</td>
<td>-4.0</td>
<td>1.0266</td>
<td>1.0240</td>
<td>1002.5</td>
</tr>
<tr>
<td>A5</td>
<td>35</td>
<td>16.429</td>
<td>-4.5</td>
<td>1.0279</td>
<td>1.0238</td>
<td>1004.0</td>
</tr>
<tr>
<td>A6</td>
<td>35</td>
<td>23.076</td>
<td>-3.2</td>
<td>1.0263</td>
<td>1.0240</td>
<td>1002.3</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>-3.2</td>
<td>1.0274</td>
<td></td>
<td></td>
<td>1003.2</td>
</tr>
</tbody>
</table>

### Table 6.2. Fish densities and physical properties of the swimbladder of Atlantic herring with large fat reserves (mean fat volume 9.76%).

<table>
<thead>
<tr>
<th>Fish Period for Mass Adjustment (days)</th>
<th>Mass (g)</th>
<th>Neutral Buoyancy (cm Hg)</th>
<th>Density of fish (g/ml)</th>
<th>Density Sea Water (g/ml)</th>
<th>Sinking Swimbladder Factor</th>
<th>% Vol Excess Pressure (cm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A7</td>
<td>2</td>
<td>37.64</td>
<td>&gt;-14.0</td>
<td>&gt;1.0307</td>
<td>1.0240</td>
<td>&gt;1006.6</td>
</tr>
<tr>
<td>A8</td>
<td>2</td>
<td>30.51</td>
<td>&gt;-15.0</td>
<td>&gt;1.0317</td>
<td>1.0238</td>
<td>&gt;1007.7</td>
</tr>
<tr>
<td>A9</td>
<td>3</td>
<td>30.00 unknown</td>
<td>a</td>
<td>1.0243</td>
<td>-</td>
<td>3.93 2.7</td>
</tr>
<tr>
<td>A10</td>
<td>3</td>
<td>26.42</td>
<td>-12.5</td>
<td>1.0330</td>
<td>1.0246</td>
<td>1008.3</td>
</tr>
<tr>
<td>A11</td>
<td>3</td>
<td>24.67</td>
<td>-10.0</td>
<td>1.0308</td>
<td>1.0245</td>
<td>1006.2</td>
</tr>
<tr>
<td>A12</td>
<td>3</td>
<td>29.17</td>
<td>&gt;= 4.0</td>
<td>a</td>
<td>1.0242</td>
<td>-</td>
</tr>
<tr>
<td>Mean of fish A10 and All</td>
<td></td>
<td>-11.2</td>
<td>1.0319</td>
<td></td>
<td></td>
<td>1007.2</td>
</tr>
<tr>
<td>Fish Period for Mass Neutral Density Density Sinking Swimbladder adjustment days g cm Hg g/ml g.ml %vol excess pressure cm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>-------</td>
<td>--------</td>
<td>--------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>---</td>
</tr>
<tr>
<td><em>P1</em> 25</td>
<td>34.622</td>
<td>-12.2</td>
<td>1.0268</td>
<td>1.0213</td>
<td>1005.4</td>
<td>2.58 1.0</td>
</tr>
<tr>
<td><em>P2</em> 10</td>
<td>52.627</td>
<td>-4.8</td>
<td>1.0245</td>
<td>1.0213</td>
<td>1003.1</td>
<td>4.63 0.8</td>
</tr>
<tr>
<td><em>P3</em> 12</td>
<td>22.974</td>
<td>-4.6</td>
<td>1.0249</td>
<td>1.0216</td>
<td>1003.2</td>
<td>4.34 0</td>
</tr>
<tr>
<td><em>P4</em> 5</td>
<td>15.108</td>
<td>-2.8</td>
<td>1.0213</td>
<td>1.0193</td>
<td>1002.0</td>
<td>4.96 0.3</td>
</tr>
<tr>
<td><em>P5</em> 31</td>
<td>18.164</td>
<td>-6.1</td>
<td>1.0265</td>
<td>1.0216</td>
<td>1004.8</td>
<td>4.20 0</td>
</tr>
<tr>
<td><em>P6</em> 28</td>
<td>25.792</td>
<td>unknown</td>
<td>a</td>
<td>1.0213</td>
<td>1001.4</td>
<td>3.65 1.1</td>
</tr>
<tr>
<td><em>P7</em> 3</td>
<td>7.934</td>
<td>2.0</td>
<td>1.0230</td>
<td>1.0216</td>
<td>1002.0</td>
<td>3.72 0.7</td>
</tr>
<tr>
<td><em>P8</em> 19</td>
<td>14.220</td>
<td>-6.0</td>
<td>1.0257</td>
<td>1.0216</td>
<td>1004.0</td>
<td>4.32 0.5</td>
</tr>
<tr>
<td><em>P9</em> 11</td>
<td>28.988</td>
<td>-3.8</td>
<td>1.0246</td>
<td>1.0213</td>
<td>1003.2</td>
<td>4.49 1.3</td>
</tr>
<tr>
<td><em>P10</em> 31</td>
<td>17.095</td>
<td>-5.6</td>
<td>1.0248</td>
<td>1.0216</td>
<td>1003.1</td>
<td>3.87 2.5</td>
</tr>
<tr>
<td><em>P11</em> 7</td>
<td>21.090</td>
<td>-3.7</td>
<td>1.0215</td>
<td>1.0188</td>
<td>1002.7</td>
<td>5.43 0.7</td>
</tr>
<tr>
<td><em>P12</em> 4</td>
<td>51.759</td>
<td>-7.5</td>
<td>1.0258</td>
<td>1.0220</td>
<td>1003.7</td>
<td>3.74 0.9</td>
</tr>
<tr>
<td><em>P13</em> 5</td>
<td>27.753</td>
<td>-3.2</td>
<td>1.0216</td>
<td>1.0193</td>
<td>1002.2</td>
<td>4.91 1.0</td>
</tr>
</tbody>
</table>

Mean _P1-P4_ males   - 6.1  1.0244  1003.4  4.12  0.5
Mean _P5-P6_ females - 6.1  1.0265  1004.8  3.46  0.3
Mean _P7-P11_ immature - 4.2  1.0239  1002.9  4.35  1.2
Mean _P12-P13_ spent  - 5.3  1.0237  1002.9  4.32  0.9

Mean for whole group  - 6.0  1.0243  1.0210  1003.2  4.14  0.8

Table 6.3. Fish densities and physical properties of the swimbladder of Pacific herring of mean fat volume of 3.5%.

_a_ - density of this fish could not be determined as it released gas before reaching neutral buoyancy. Its density was assumed to equal 1.0265, the density of the other female fish tested.
<table>
<thead>
<tr>
<th>Fish</th>
<th>Period for adjustment</th>
<th>Mass</th>
<th>Neutral buoyancy</th>
<th>Density of fish</th>
<th>Density sea water</th>
<th>Sinking factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days</td>
<td>g</td>
<td>cm Hg</td>
<td>g/ml</td>
<td>g/ml</td>
<td></td>
</tr>
<tr>
<td>P14</td>
<td>4</td>
<td>7.480</td>
<td>-5.5</td>
<td>1.0248</td>
<td>1.0214</td>
<td>1003.4</td>
</tr>
<tr>
<td>P15</td>
<td>4</td>
<td>6.907</td>
<td>-7.0</td>
<td>1.0232</td>
<td>1.0213</td>
<td>1001.9</td>
</tr>
<tr>
<td>P16</td>
<td>5</td>
<td>4.616</td>
<td>-2.6</td>
<td>1.0225</td>
<td>1.0213</td>
<td>1001.2</td>
</tr>
<tr>
<td>P17</td>
<td>6</td>
<td>6.9022</td>
<td>0</td>
<td>1.0216</td>
<td>1.0216</td>
<td>1000.0</td>
</tr>
<tr>
<td>P18</td>
<td>6</td>
<td>5.8532</td>
<td>-3.0</td>
<td>1.0261</td>
<td>1.0216</td>
<td>1004.4</td>
</tr>
<tr>
<td>P19</td>
<td>7</td>
<td>23.2415</td>
<td>-5.0</td>
<td>1.0251</td>
<td>1.0214</td>
<td>1003.7</td>
</tr>
<tr>
<td>P20</td>
<td>7</td>
<td>5.7120</td>
<td>-4.1</td>
<td>1.0237</td>
<td>1.0215</td>
<td>1002.1</td>
</tr>
<tr>
<td>P21</td>
<td>7</td>
<td>5.1475</td>
<td>-1.0</td>
<td>1.0235</td>
<td>1.0214</td>
<td>1002.0</td>
</tr>
<tr>
<td>P22</td>
<td>7</td>
<td>6.4745</td>
<td>-2.0</td>
<td>1.0231</td>
<td>1.0213</td>
<td>1001.7</td>
</tr>
<tr>
<td>P23</td>
<td>7</td>
<td>12.7460</td>
<td>-3.6</td>
<td>1.0239</td>
<td>1.0213</td>
<td>1002.6</td>
</tr>
<tr>
<td>P24</td>
<td>8</td>
<td>4.6055</td>
<td>-3.7</td>
<td>1.0258</td>
<td>1.0214</td>
<td>1004.3</td>
</tr>
<tr>
<td>P25</td>
<td>8</td>
<td>4.2455</td>
<td>-5.0</td>
<td>1.0242</td>
<td>1.0214</td>
<td>1002.8</td>
</tr>
<tr>
<td>P26</td>
<td>8</td>
<td>4.4993</td>
<td>-3.0</td>
<td>1.0238</td>
<td>1.0214</td>
<td>1002.3</td>
</tr>
<tr>
<td>P27</td>
<td>8</td>
<td>7.0205</td>
<td>-5.5</td>
<td>1.0225</td>
<td>1.0214</td>
<td>1001.1</td>
</tr>
</tbody>
</table>

Mean   | -3.6 | 1.0238 | 1002.4 |

Table 6.4. Fish densities of Pacific herring tested by the shorter method.
Herring tested | Number | Neutral buoyancy of fish | Fat factor bladder | % vol excess pressure | cm Hg | g/ml | cm Hg
--- | --- | --- | --- | --- | --- | --- | ---
Atlantic (1962 paper) | 16 | -4.3 | 1.026 | 1002.9 | 4.2 | 1.1 | -
Atlantic (1963 thin fish) | 6 | -3.2 | 1.0274 | 1003.2 | 4.54 | 1.4 | 0.87
Atlantic (1963 fat fish) | 2 | -11.2 | 1.0319 | 1007.2 | 3.52 | 0 | 9.76
Pacific (1963 full run) | 13 | -6.0 | 1.0243 | 1003.2 | 4.14 | 0.8 | 3.52
Pacific (1963 short run) | 14 | -3.6 | 1.0238 | 1002.4 | - | - | -
Atlantic (mean all results) | 24 | -4.6 | 1.0268 | 1003.3 | 4.23 | 1.1 | -
Pacific (mean all results) | 27 | -4.8 | 1.0240 | 1002.8 | 4.14 | 0.8 | -

Table 6.5. Summary of mean values obtained for fish densities and physical properties of the swimbladder of Atlantic and Pacific herring.
Conclusions.

A consideration of the results has already shown that the values considered in this section differ less between species than between groups, notably between fat and thin Atlantic herring, of the same species. It has already been shown that the difference in density of Atlantic and Pacific herring is abolished when this is considered in relationship to the density of the sea water. The relationship between the various physical properties of a herring and its fat content will be discussed in the last division of this chapter. This leaves individual variation to be considered here.

1) Variation with size. To study the effect of size on fish density it is desirable to remove the influence of variations in sea water density by considering instead changes in the sinking factor with size. Sinking factor has been plotted against size in figure 6.3 for the Atlantic and Pacific herring. It is evident that variation in sinking factor does not depend on fish size as expressed by its mass but may be influenced by fat content.

The effect of size on percentage swimbladder volume is examined in a plot of these two factors against each other in figure 6.4. It appears from this graph that variations in the proportion of the body occupied by the swimbladder are independent of the size of the fish.
2) Relationship of percentage swimbladder volume to other physical factors. If the walls of the swimbladder are relatively inelastic it would be expected that the force exerted by these walls on the contained gas would rise as the volume of the swimbladder gas increased. A plot of percentage swimbladder volume against excess internal gas pressure however fails to show such a relationship (figure 6.5). This suggests that the excess internal pressure is not a result of a passive stretching of the elastic fibres of the swimbladder wall but is a result of an active process independent of the swimbladder volume over the range investigated.

Another relationship which might be expected is one between fish density and percentage volume of the swimbladder as the greater the relative volume of gas inside the fish the lower would its density be provided all other components contributing to its density remained constant. The graph of percentage swimbladder volume against density shown in figure 6.6 suggests that this relationship may be obscured by other factors. As the density of swimbladder gas at the pressure of adjustment may be taken as constant for all the test fish the observed variability in density must be affected by variations in the volume and/or density of other components of the fish body. This possibility is examined in the next subsection of this chapter.
Figure 6.3a Graph of sinking factor against size for Pacific and Atlantic herring
Figure 6.4  Graph of percentage swimbladder volume against size for Atlantic and Pacific herring.
Figure 6.5  Graph of excess swimbladder gas pressure against percentage volume for Atlantic and Pacific herring.
Figure 6.6  Graph of fish density against percentage swimbladder volume for Atlantic and Pacific herring
This section has shown the great similarity in the physical properties of the swimbladder and in the relationship of fish density to sea water density in the two species of herring. It has eliminated some of the possible causes of between individual variation and has suggested that one of the most important causes of this variation may be differences in fat content.

B. Analysis of the amounts and densities of some herring components.

In addition to the swimbladder the components of the herring body which would appear likely to influence the density of the fish most are the skeleton, scales, oil and perhaps in the mature fish, the gonads. When the density and amount of each of these components have been determined it is possible by subtraction from the density and volume of the entire fish to determine the mean density and volume of the remaining parts of the fish, which are referred to simply as "the rest".

Methods.

All the Atlantic herring and those Pacific herring for which percentage swimbladder volumes were determined were analysed to find the volume of ether-extractable oil in their bodies. In addition the volume and density of the scales, skeleton and oil was determined for all the Pacific
fish. All fish were analysed separately. When only the fat content was required the frozen fish was sliced and ground in a mortar with 3 parts of Na$_2$SO$_4$ to each part of herring by weight as a drying agent. Oil was extracted from the resulting powder by three changes of ether over a period of three hours. The filtered ether extract was evaporated and the remaining oil dried to constant weight in a drying oven.

A more complex method was required for the analysis of Pacific herring into oil, scales and skeletal components. The frozen fish was wrapped in filter paper and placed in a 20 x 3 cm test tube closed by a rubber bung bearing a curved glass tube. The test tube was supported in the neck of a flask so that the curved glass tube opened beyond the steam which was forced through the flask. The fish was cooked from 7 to 15 minutes according to size, which allowed the easy separation of the skin, skeleton and flesh. Cooking by this method resulted in very little change in weight. One fish of 5.236 gm uncooked weight lost only 0.002 gm after 7 minutes cooking. Any oil which left the fish during cooking was absorbed by the filter paper and as both the filter paper and the interior of the test tube were extracted with ether this oil was not lost from the total.

After cooking, the fish was placed on layers of filter paper in an enamel dish and dissected into skeleton,
scales and "the rest". The skeleton was placed in a test tube and the scales plus skin in another tube and both were covered with ether. "The rest" was placed in a mortar with 3 parts of Na₂SO₄ to each part by weight of herring and ground to a dry uniform powder. This powder was placed in a flask with ether. The enamel dish used for the dissection and the pestle and mortar were wiped clean with filter paper which together with the filter paper under the fish during the dissection was dried by infra red light, cut into strips and added to "the rest" in the flask. The ether in the two test tubes and in the flask was renewed after 1 1/2, 2 and 2 1/2 hours, the ether extracts being filtered into a single weighed flask. The flask was reweighed to give the mass of ether present then two 25 ml samples were removed, placed in separated weighed 250 ml Florence flasks and the ether evaporated in an air flow while the flasks were in a water bath at 35° C. After all ether appeared to have evaporated these flasks were placed in a vacuum oven at 50° C and 29 lb reduced pressure until constant weight was attained. The mass of oil in the 25 ml sample could then be found by subtracting the weight of the empty flask.

The skeleton and scales after extraction with ether in the determination above were rinsed well with distilled water while still in their respective test tubes and then soaked for one day in 20 volume hydrogen peroxide (6%).
Any fine bones overlooked in the initial dissection and present in the dried residue of "the rest" were added to the skeleton fraction at this stage. After 24 hours hydrogen peroxide in the tubes was replaced by several changes of distilled water. The hydrogen peroxide caused disintegration of the skin freeing the scales and removed any flesh adhering to the skeleton. Both fractions were carefully picked over to remove any extraneous material. To remove any gas adhering to the scales or skeleton these were placed, while still in distilled water, in the vacuum oven at 50° C and minus 29 lb pressure for half an hour and then cooled to room temperature.

To determine the density and volume of the scales and skeleton it was necessary to find their weight in distilled water of known temperature and their dry weight. For each fraction an aluminium weighing dish was prepared with a light wire frame which would enable it to be hung from the hook of a balance and with narrow slits in the bottom to allow water drainage after the weighing. The weight of this pan when completely immersed in distilled water was found and the scales or skeleton added by pushing them through the surface film above the pan. The weight of the pan plus scales or skeleton and the water temperature were recorded. The pan was removed with its wire frame from the water, retaining the scales or skeleton inside and the water
inside the pan allowed to drain away through the slits. The pan with its contents underwent preliminary drying in the vacuum oven, then was subjected to one minute exposures to infra red light in an Anco moisture balance followed by cooling in a dessicator until constant weight was reached. The scales and skeleton were then removed, the pans cleaned with acetone and their weight determined so that by subtraction the dry weight of the scales or skeleton could be found.

To determine the density of herring oil at various temperatures a weight thermometer was made by blowing a small bulb in the sealed end of capillary tube and tapering the neck of the thermometer to a point. First it was necessary to calibrate the weight thermometer by finding the mass of a substance of known density and thermal expansion which completely filled the thermometer at various temperatures. Ethyl alcohol was used as it can be removed completely from the thermometer by evaporation after the determination and its density at various temperatures is given in the Handbook of Chemistry and Physics. To check that the ethyl alcohol used resembled that of the table its density was determined using a 50 ml volumetric flask which gave a calculated density of 0.7836 g/ml at 26.5°C compared with a value of 0.7839 g/ml for this temperature in the table.
The weight thermometer was weighed empty with its supporting wire and then was completely filled with ethyl alcohol by alternately heating and cooling it with the neck below the alcohol surface. The final filling was made in the freezing compartment of a refrigerator at -11\(^\circ\) C. The beakers and water shown in figure 6.7 were left in the refrigerator overnight and the weight thermometer removed from the freezer and placed in position in the small beaker while this was still in the refrigerator. The only purpose of starting with the weight thermometer at freezer temperature was to overfill it so that the apparatus could be set up at refrigerature temperature without losing too much alcohol from the thermometer. After setting up, time was allowed for the thermometer to come to the same temperature as the small beaker. The apparatus was transferred to the glass case of a balance and the supporting wire of the weight thermometer attached to the hook of a Mettler H5 balance. After blotting excess alcohol from the end of the weight thermometer its weight was recorded at a series of temperatures as the apparatus slowly warmed up to room temperature. The rise from 6\(^\circ\) C to 19\(^\circ\) C took 2 hours. At the end of the run the weight thermometer was emptied of alcohol, dried in a vacuum oven and refilled with herring oil.

All the oil extracted from the test group of Pacific herring was pooled, filtered and dried to constant weight in the vacuum oven before being used in the density
Figure 6.7 Diagram of apparatus used to find the mass of ethyl alcohol filling the weight thermometer at various temperatures.
determination. The oil proved more difficult to handle than alcohol requiring some modification of the method used previously. At temperatures below $0^\circ C$ so much stearine condensed from the oil that with further cooling air was drawn into the thermometer instead of oil. Thus it was necessary to work at temperatures above $0^\circ C$. Another difficulty was to keep the outside of the thermometer completely free of oil as oil unlike alcohol did not evaporate completely. To overcome these difficulties the following method was used. The 100 ml beaker of the original apparatus was filled with ether and both beakers and their contents refrigerated. The filled weight thermometer was placed in the small beaker with its end above the ether surface. After ten minutes the apparatus was taken to the balance but the weight thermometer was not connected to the balance hook. Instead at a series of known temperatures all excess oil was blotted from the end of the capillary tube and the weight thermometer immediately removed from the ether. The evaporation of ether momentarily reduced the temperature of the oil (but not its mass) causing a movement of the oil column down the capillary neck, thus permitting the outside to be cleaned if necessary without oil loss or contamination. The weight thermometer was quickly weighed on filter paper of known weight on a balance pan. This could usually be accomplished before the oil column again reached the top of the capillary but if any
oil were lost it was caught and weighed with the filter paper. The weight thermometer was then returned to the ether bath. As 10 minutes or more usually elapsed between one weighing and the next there was time for the weight thermometer to regain the temperature of the ether bath. The ether served a further purpose of removing any oil which accidentally clung to the outside of the thermometer. A series of readings were taken until the apparatus reached room temperature. This apparatus took 2 hours to warm up from \(9^\circ\) to \(19^\circ\) C.

After the determination the mercury thermometer used was calibrated against a standard thermometer and a correction applied to the recorded temperatures. From the data collected a graph was constructed of mass of alcohol against temperature (figure 6.8). From this graph the mass of alcohol in the thermometer at temperatures corresponding to those at which the mass of oil was determined can be read.

Lastly a determination of the density of ovaries and testis was made. The mass of the gonad was found in air and then in distilled water of known temperature and from this information its density could be calculated. The density of the gonad so obtained was its density in the normal liquid containing condition while for the scales and skeleton the density of the dry material was determined.
Calculations.

1) Oil content and density.

The total volume of oil in the fish can be calculated as follows:

\[
\text{Total volume of oil} = \frac{\text{mass of all ether fractions}}{\text{density of ether}} \times \frac{\text{mass of oil in 25 ml}}{25}
\]

The density of herring oil was determined for a range of temperatures from the data obtained with the weight thermometer. The graph in figure 6.8 was used to find the mass of alcohol corresponding to the observed mass of oil at the same temperature. Then for any one temperature

\[
\text{Density of oil} = \frac{\text{mass of oil}}{\text{mass of alcohol}} \times \text{density of alcohol}
\]

2) Volume and density of scales and skeleton.

The weight of scales or skeleton in water was less than their dry weight by an amount which is equal to the mass of the water displaced. Thus:

\[
\text{Volume of scales/skeleton} = \frac{\text{dry weight} - \text{weight in water}}{\text{density of water at test temperature}}
\]

As the volume and dry weight are now known the density of the scales or skeleton can be calculated.
Figure 6.8 Graphs of mass of ethyl alcohol and of herring oil which exactly filled the same weight thermometer at different temperatures.
Figure 6.9 Graph of density of ether-extracted oil from Pacific herring against temperature.
3) Density of gonads.

The density of the gonads was determined for their normal wet state.

\[
\text{Volume of gonad} = \frac{\text{wet weight} - \text{weight in water}}{\text{density of water at test temperature}}
\]

As the volume and wet weight are known then density can be calculated.

4) Density and volume of "the rest".

\[
\text{Density of "the rest"} = \frac{\text{mass of fish} - (\text{total mass of gas, oil, scales, skeleton})}{\text{volume of fish} - (\text{total volume of gas, oil, scales, skeleton})}
\]

The value on the lower right hand side of this expression gives the volume of "the rest".

5) Percentage volume.

All component volumes were also expressed as percentages of the volume of the fish.

Results.

Pacific herring P1 to P13 were analysed to find the percentage volume and density of the swimbladder, scales, skeleton, oil and "the rest" and the results obtained are given in table 6.6. Atlantic herring A1 to A12 were analysed only for the percentage volumes of swimbladder and oil and their results are given in table 6.7. In these tables...
density of swimbladder gas has been assumed to equal the density of air at the same temperature and pressure, which can be obtained from tables in the Handbook of Chemistry and Physics. The density of herring oil was determined experimentally for a pooled sample of ether-extracted oil from Pacific herring. The results of this determination are shown graphically in figure 6.9. In constructing the tables for Atlantic and Pacific herring the value for oil density presented is taken from this graph at temperatures corresponding to the temperature of the water in which the living fish was tested.

In the Atlantic and Pacific herring the component with the lowest density was the swimbladder gas of density 0.0013 g/ml followed by oil at a mean density of 0.9258 g/ml. All other components examined for the Pacific herring had densities greater than the sea water density (1.0188 to 1.0220 g/ml). In order of increasing density these components were: "the rest" 1.0568 g/ml, scales 1.9658 g/ml, and skeleton 1.9930 g/ml. With the exception of oil which reached 12% in one Pacific herring and 16% in one Atlantic herring, the percentage volume of the fish occupied by these components was low. The swimbladder occupied about 4%, scales about 0.5% and skeleton 1% of the total volume of the fish.
### Table 6.6

Percentage volume and density of some components of the body of Pacific herring whose density and physical properties of the swimbladder had been determined in the living state (table 6.3).

<table>
<thead>
<tr>
<th>Fish sex</th>
<th>fish vol.ml.</th>
<th>swimbladder %vol. density</th>
<th>scales %vol. density</th>
<th>skeleton %vol. density</th>
<th>oil %vol. density</th>
<th>&quot;the rest&quot; %vol. density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/ml</td>
<td>g/ml</td>
<td>g/ml</td>
<td>g/ml</td>
<td>g/ml</td>
<td>g/ml</td>
</tr>
<tr>
<td>P1 male</td>
<td>33.817</td>
<td>1.0268</td>
<td>2.58 .0013</td>
<td>0.19 *</td>
<td>2.2632</td>
<td>0.95 1.9166</td>
</tr>
<tr>
<td>P2 male</td>
<td>51.370</td>
<td>1.0245</td>
<td>4.63 .0013</td>
<td>0.46</td>
<td>1.8422</td>
<td>0.90 1.9969</td>
</tr>
<tr>
<td>P3 male</td>
<td>22.416</td>
<td>1.0249</td>
<td>4.34 .0013</td>
<td>0.63</td>
<td>1.8662</td>
<td>1.09 2.2352</td>
</tr>
<tr>
<td>P4 male</td>
<td>14.792</td>
<td>1.0213</td>
<td>4.96 .0013</td>
<td>0.84</td>
<td>1.9224</td>
<td>1.55 1.8825</td>
</tr>
<tr>
<td>P5 female</td>
<td>17.694</td>
<td>1.0265</td>
<td>4.20 .0013</td>
<td>0.63</td>
<td>1.9678</td>
<td>1.37 2.1260</td>
</tr>
<tr>
<td>P6 female</td>
<td>25.125</td>
<td>a</td>
<td>2.72 .0013</td>
<td>0.42</td>
<td>1.8566</td>
<td>1.26 1.6471</td>
</tr>
<tr>
<td>P7 immature</td>
<td>7.7552</td>
<td>1.0230</td>
<td>3.65 .0013</td>
<td>0.41</td>
<td>1.8201</td>
<td>1.18 1.8660</td>
</tr>
<tr>
<td>P8 immature</td>
<td>13.863</td>
<td>1.0257</td>
<td>4.32 .0013</td>
<td>0.58</td>
<td>2.0232</td>
<td>1.20 2.1532</td>
</tr>
<tr>
<td>P9 immature</td>
<td>28.292</td>
<td>1.0246</td>
<td>4.49 .0013</td>
<td>0.44</td>
<td>1.9110</td>
<td>1.08 2.0450</td>
</tr>
<tr>
<td>P10 immature</td>
<td>16.681</td>
<td>1.0248</td>
<td>3.87 .0013</td>
<td>0.45</td>
<td>1.9739</td>
<td>1.04 2.2648</td>
</tr>
<tr>
<td>P11 immature</td>
<td>20.646</td>
<td>1.0215</td>
<td>5.43 .0013</td>
<td>0.62</td>
<td>1.9219</td>
<td>1.49 1.9333</td>
</tr>
<tr>
<td>P12 spent</td>
<td>50.532</td>
<td>1.0258</td>
<td>3.74 .0013</td>
<td>0.33</td>
<td>2.3477</td>
<td>1.09 2.0005</td>
</tr>
<tr>
<td>P13 spent</td>
<td>27.167</td>
<td>1.0216</td>
<td>4.91 .0013</td>
<td>0.63</td>
<td>1.8397</td>
<td>1.24 1.8424</td>
</tr>
</tbody>
</table>

| Mean values | male | 1.0244 | 4.12 | .0013 | 0.53 | 1.9735 | 1.12 | 2.0078 | 4.63 | .9266 | 89.56 | 1.0582 |
| female     | 1.0265 | 3.46 | .0013 | 0.52 | 1.9122 | 1.31 | 1.8865 | 4.82 | .9254 | 89.88 | 1.0535 |
| immature   | 1.0239 | 4.35 | .0013 | 0.50 | 1.9300 | 1.20 | 2.0524 | 2.16 | .9257 | 91.79 | 1.0564 |
| spent      | 1.0237 | 4.32 | .0013 | 0.48 | 2.0937 | 1.16 | 1.9214 | 3.41 | .9248 | 90.63 | 1.0585 |

| Mean of all fish | male | 1.0239 | 4.14 | .0013 | 0.51 | 1.9658 | 1.19 | 1.9930 | 3.52 | .9258 | 90.63 | 1.0568 |

Table 6.6. Percentage volume and density of some components of the body of Pacific herring whose density and physical properties of the swimbladder had been determined in the living state (table 6.3).

- a - No neutral buoyancy was obtained for this fish. Its density was assumed to be 1.0265 g/ml
- * - some scales may have been lost from P1.
### Table 6.7

Percentage volume and density of swimbladder and oil in the body of Atlantic herring whose density and physical properties of the swimbladder were determined in the living state (tables 6.1 and 6.2)

<table>
<thead>
<tr>
<th>Fish Condition</th>
<th>Fish vol. density g/ml</th>
<th>Swimbladder vol. density g/ml</th>
<th>Oil vol. density g/ml</th>
<th>Density of sea water g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 thin</td>
<td>11.289</td>
<td>1.0295</td>
<td>5.23</td>
<td>0.67</td>
</tr>
<tr>
<td>A2 thin</td>
<td>13.930</td>
<td>1.0256</td>
<td>3.09</td>
<td>0.54</td>
</tr>
<tr>
<td>A3 thin</td>
<td>11.258</td>
<td>1.0286</td>
<td>4.72</td>
<td>0.58</td>
</tr>
<tr>
<td>A4 thin</td>
<td>8.754</td>
<td>1.0266</td>
<td>4.82</td>
<td>1.85</td>
</tr>
<tr>
<td>A5 thin</td>
<td>15.983</td>
<td>1.0279</td>
<td>4.73</td>
<td>0.74</td>
</tr>
<tr>
<td>A6 thin</td>
<td>22.484</td>
<td>1.0263</td>
<td>4.67</td>
<td>0.82</td>
</tr>
<tr>
<td>A7 fat</td>
<td>36.476</td>
<td>a</td>
<td>2.65</td>
<td>10.99</td>
</tr>
<tr>
<td>A8 fat</td>
<td>29.567</td>
<td>a</td>
<td>3.04</td>
<td>16.59</td>
</tr>
<tr>
<td>A9 fat</td>
<td>29.072</td>
<td>a</td>
<td>3.93</td>
<td>6.64</td>
</tr>
<tr>
<td>A10 fat</td>
<td>25.573</td>
<td>1.0330</td>
<td>4.01</td>
<td>7.30</td>
</tr>
<tr>
<td>A11 fat</td>
<td>23.933</td>
<td>1.0308</td>
<td>3.04</td>
<td>7.13</td>
</tr>
<tr>
<td>A12 fat</td>
<td>28.268</td>
<td>a</td>
<td>3.02</td>
<td>9.90</td>
</tr>
</tbody>
</table>

**Mean values**

- **thin fish**: 1.0274 4.54 .0013 0.87 .9257
- **fat fish**: 1.0319 3.28 .0013 9.76 .9259

**Mean of all fish**: 1.0285 3.91 .0013 5.31 .9258 1.0242

- **a**: density of these fish was taken as 1.0319 g/ml
The density of the ovaries and testes in their normal wet state was determined separately. A mature female Pacific herring of 19.2 cm fork length (approximately 60 g) yielded ovaries weighing 7.927 g of density 1.1022 g/ml. A ripe male (P2 in tables 6.3 and 6.6) contained testes weighing 5.9208 g of density 1.0674. The percentage volume occupied by the testes in this fish was 10.80%. Thus in density the gonads are more dense than sea water but less dense than scales or skeleton.

Conclusions.

a) The means by which herring attain a density close to that of their environment.

1) The inclusion of gas. It has been shown that herring which have been made considerably denser than their environment by the removal of gas from the swimbladder come to the surface and swallow air until a fairly constant relationship between their density and that of the sea water has been reestablished. Thus the attaining of this relationship is an active process. It is also evident that it is this relationship between these densities that is being established not a process which ceases when a certain fish density or swimbladder volume is reached. This is shown by considering the Atlantic and Pacific herring (neglecting for the moment the "fat" Atlantic fish) which have completed this adjustment and have overlapping sinking factors between 1000 and 1004.8 but quite distinct densities.
The percentage of the total volume of the herring occupied by the swimbladder showed a mean of 4.14 for Pacific herring and 4.23 for Atlantic herring (table 6.5). This is a little lower than the values obtained for some other free swimming marine fish such as Gadus luscus 4.9% and Zeus faber 5.6% (Plattner 1941) or Crenilabrus melops 4.9% (Jones 1951) or the theoretical value of 5% calculated by Jones in the same paper. This suggests that the herring has been able to use some other flotation device or has reduced some of the heavy components of its body so that compared with these other marine fish it is able to achieve a density close to its environment with a lower swimbladder volume. Reduction of the swimbladder volume lowers the change in fish density with change in depth. For example if we consider Pacific herring of density 1.0239 g/ml (the mean for the group) at the depth at which they were tested and swimbladder volumes of 3, 4, and 5% (which occurred in this group) their density and sinking factor would change with depth as follows:

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Total Pres. cm Hg</th>
<th>3% Swimbladder g/ml</th>
<th>4% Swimbladder g/ml</th>
<th>5% Swimbladder g/ml</th>
<th>S.F.</th>
<th>3% Swimbladder</th>
<th>4% Swimbladder</th>
<th>5% Swimbladder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Density g/ml</td>
<td>Density g/ml</td>
<td>Density g/ml</td>
<td></td>
<td>Density S.F.</td>
<td>Density S.F.</td>
<td>Density S.F.</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>1.0229</td>
<td>1.0229</td>
<td>1.0229</td>
<td>1001</td>
<td>1.0222</td>
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<tr>
<td>0.36</td>
<td>78.6</td>
<td>1.0239</td>
<td>1.0239</td>
<td>1.0239</td>
<td>1003</td>
<td>1.0239</td>
<td>1.0239</td>
<td>1.0239</td>
</tr>
<tr>
<td>(test depth)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>142</td>
<td>1.0378</td>
<td>1.0425</td>
<td>1.0473</td>
<td>1026</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>284</td>
<td>1.0466</td>
<td>1.0544</td>
<td>1.0623</td>
<td>1040</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>426</td>
<td>1.0496</td>
<td>1.0584</td>
<td>1.0674</td>
<td>1045</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

assuming an increase in pressure of one atmosphere for each 10 m depth and a uniform sea water density of 1.0211 g/ml.
The lower the change of density with depth the greater are the vertical movements which can be made for the same expenditure of energy in compensatory movements at the new depth. This can be seen as a lower sinking factor at a given depth when the swimbladder volume and thus the change of density with depth are lower. Thus fish starting at 36 cm depth with a swimbladder volume of 3% could live at 30 metres for the same expenditure of energy that an identical fish with a 5% swimbladder volume would need to live at 10 metres. Thus there is an advantage in reducing the swimbladder volume but this can only be accomplished without increase in density if another component assists in counteracting the pull of gravity and heavy structures are minimised.

2) Reduction of heavy structures. The scales and skeleton, which have the highest density of the components analysed, have been reduced in the herring so that they occupy on the average only 0.5 and 1.2% respectively of the volume of the fish giving a total bony content of 1.7%. This reduction is evident when comparing these values with those given by Alexander (1959b) for roach whose total bony content was 4.3; dace 2.8 and carp 4.4 expressed on a volume per 100 gm basis which approximates to a true percentage volume basis as the density of these freshwater fish was close to 1.
The mean density of herring skeleton was found to be 1.99 and of scales 1.97 g/ml. Thus like the fish above studied by Alexander there was little difference in the density of scales and skeleton obtained from the same fish species. The herring shows no modification in the density of its bony structures compared with these other fish as its mean density of 1.97 is similar to the specific gravity of bone of 1.9 for roach, 2.0 for dace and 1.6 for carp.

3) Inclusion of oil. The density of Pacific herring oil at the temperatures (13° C) at which the test fish were living was found to have a mean value of 0.9258 g/ml. This may be compared with the density of 0.933 calculated from the data given by Brocklesby (1941) for herring oil at 13° C after warming up from lower temperatures. This was determined for commercial herring oil presumably extracted by pressure from cooked fish. As the specific gravities of four teleost oils given by Spector (1956) averaged 0.91 and that of cod liver oil is 0.93 (Denton 1963) there is no modification of the oil of herring to give it a lower than normal density. Such a modification of the density of oil by the inclusion of squalene has been shown by Corner, Denton and Forster (quoted by Denton 1963) to increase the effectiveness of oil as a buoyancy device in some species of shark of the family Squalidae.
The herring is unusual in the extreme variability of its fat content. In the herring analysed here the percentage volume of fat varied from less than 1 to over 16. *Clupea pallasii* can contain up to 30% oil by weight but this falls to 5% after spawning (Brocklesby 1941). Leim (1943) shows the decrease in percentage oil by weight in immature Atlantic herring from 20 in January to 8 in June, increasing to 15 in December. According to Brocklesby most of the oil is stored in the muscle and only small amounts in the liver. When dissecting herring of high fat content it was evident that fat was also stored under the skin and along the mesenteries.

It is self evident that the inclusion of large amounts of oil of low density will make the fish more buoyant if all other factors remain the same. The analysis of individual herring into their components allows the results of different oil contents to be determined. When percentage swimbladder volume is plotted against percentage oil content for Pacific (figure 6.10) and Atlantic (figure 6.11) herring it is seen that the percentage swimbladder volume decreases as the percentage of oil increases. This is the relationship expected if the swimbladder volume is adjusted to maintain the density of the fish near the surface almost constant with respect to the density of sea water. However the replacement is not perfect as from the densities of swimbladder
gas and oil previously calculated it can be shown that 1 ml of gas provides the same upthrust as 10.7 ml of oil under the test conditions of the Pacific herring and as 10.4 ml of oil under those of the Atlantic herring. From figure 6.10 for Pacific herring it can be seen that 1 ml of gas is replaced by 5.1 ml of oil. For Atlantic fish in figure 6.11, 1 ml of gas is replaced by 8.0 ml of oil. As a result of this, which may be regarded as the swimbladder becoming reduced in volume more than is justified by the amount of oil present as the proportion of oil increases, the fish becomes denser as the fat content increases. This is shown in figure 6.12 where a plot of sinking factor against percentage volume of oil shows that when the oil content exceeds 7% the sinking factor for both Atlantic and Pacific herring rises.

A feature of the calculation which might be thought to distort the relationship of oil to gas is that both have been expressed as percentages of the total fish volume. Considering a thin fish which increases its fat content it is evident that the total volume of the fish is increased as the fat is deposited so that if the swimbladder volume remained constant it would appear as a lower percentage volume of the fat fish than the thin one. To investigate this factor the oil and gas contents of Pacific fish were expressed in terms of the volume of a fat and gas free fish. As can be seen in figure 6.13 the relationship between volumes of gas
Figure 6.10 Graph of swimbladder volume against oil volume for Pacific herring. Line fitted by method of least squares.
Figure 6.11 Graph of swimbladder volume against oil volume for Atlantic herring. Line fitted by method of least squares.
and oil remains and the slope of the line is similar giving 1 ml of gas replacing 6 ml (instead of 5.1 ml) of oil. Thus it seems valid to consider these volumes as originally calculated.

The increase in sinking factor with increase in fat content was an unexpected feature of the gas/fat relationship in herring as the herring which by its high fat content and low swimbladder volume has a decreased change of density with depth seems to throw away this advantage by starting at a greater density near the surface than herring of low fat content. We may calculate the density changes involved by considering mean values based on the fat and thin Atlantic herring. Let the thin herring have a density at the surface of 1.027 g/ml and swimbladder volume of 4.5% while the fat herring has a density of 1.032 and gas volume of 3.3%. Then:

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Total Pressure (cm Hg)</th>
<th>Density of thin fish (g/ml)</th>
<th>Density of fat fish (g/ml)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>76</td>
<td>1.027</td>
<td>1.032</td>
</tr>
<tr>
<td>25</td>
<td>266</td>
<td>1.057</td>
<td>1.057</td>
</tr>
<tr>
<td>50</td>
<td>456</td>
<td>1.063</td>
<td>1.061</td>
</tr>
</tbody>
</table>

Thus at 25 metres the lower change in density with depth shown by the fat fish exactly compensates for its greater initial density so that both fat and thin herring have the same density.
Figure 6.12 Graph of sinking factor against volume of oil for Atlantic and Pacific herring.
Figure 6.13 Oil and swimbladder volumes of Pacific herring expressed as percentage volumes of the fat and gas free fish.
At depths below this the thin fish becomes slightly more
dense than the fat one. The ecological significance of this
phenomenon is unknown but it does lead to greater uniformity
in sinking factor of fat and thin fish when the fish are
deep in the water by day. Atlantic herring of this region
are at a median depth of 9 to 13 metres by day from May
until December and from 25 to 38 metres by day from January
to April (Brawn 1960) thus for most of the year they are
deep enough for the initial density difference between such
fat and thin fish to be decreased but not eliminated.

b) An analysis of the forces acting on a herring living at
a depth of 36 cm.

From the percentage volumes and densities of the
components of the Pacific herring shown in table 6.6 it is
possible to calculate the force in dynes acting in the verti­
cal plane on the fish due to each of these components (table
6.8). All forces in this table have been expressed in terms
of a 100 ml fish. To find the true force in dynes acting on
a fish the values given should be multiplied by
\[
\frac{\text{volume of fish, ml}}{100}
\]
. The values in the table have been
given in order of increasing lift due to oil.

Among components lifting these fish in the water
the force due to the swimbladder gas contributes more to the
buoyancy of the fish than the upward force due to the oil
even when the oil content reaches 12%. Of components which
exert a downwards force the most important is "the rest". The scales and skeleton together exert a force downwards equal to about one third the force due to "the rest" although their respective volumes are 1:63. This emphasises the importance of skeletal reduction in reducing the downward force on the fish.

The forces exerted downwards by scales, skeleton and "the rest" appear to vary independently of the amount of oil present and give a mean downward force of 47.9 dynes/ml of fish. The upward forces are complementary as an increase in buoyancy due to oil is compensated for by a decrease in the upward force due to the swimbladder gas. At the two highest oil contents overcompensation occurs and the force due to the swimbladder gas decreases more than the force due to the oil increases. As a result the total upward force is reduced and the compensating force which the fish must exert on the water increases from a mean of 3 dynes/ml to 5 dynes/ml.

In table 6.6 where the percentages and densities of the components of Pacific herring were shown grouped according to the sexual condition of the fish no consistent differences were evident between the mature male or female and the immature or spent fish. In nature differences in oil content would be expected to cause differences between these groups. The effect of gonads on the forces acting on the fish can be calculated for fish P2 which contained testes
Fish Oil Force in dynes acting on 100 ml fish at 36 cm depth

<table>
<thead>
<tr>
<th></th>
<th>UP</th>
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<th>DOWN</th>
<th>DOWN</th>
<th>DOWN</th>
<th>UP</th>
<th>DOWN</th>
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</thead>
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<tr>
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<td>52</td>
<td>384</td>
<td>1084</td>
<td>3391</td>
<td>4545</td>
<td>4860</td>
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<td>78</td>
<td>549</td>
<td>1338</td>
<td>3864</td>
<td>5498</td>
<td>5750</td>
</tr>
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</tr>
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<td>1.31</td>
<td>4204</td>
<td>124</td>
<td>585</td>
<td>1484</td>
<td>2749</td>
<td>4328</td>
<td>4818</td>
</tr>
<tr>
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<td>507</td>
<td>1001</td>
<td>3759</td>
<td>5046</td>
<td>5267</td>
</tr>
<tr>
<td>P3</td>
<td>2.51</td>
<td>4344</td>
<td>233</td>
<td>522</td>
<td>1298</td>
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<td>311</td>
<td>570</td>
<td>1332</td>
<td>3130</td>
<td>4635</td>
<td>5032</td>
</tr>
<tr>
<td>P12</td>
<td>5.25</td>
<td>3745</td>
<td>508</td>
<td>429</td>
<td>1046</td>
<td>3146</td>
<td>4253</td>
<td>4622</td>
</tr>
<tr>
<td>P10</td>
<td>5.68</td>
<td>3873</td>
<td>540</td>
<td>420</td>
<td>1268</td>
<td>3037</td>
<td>4414</td>
<td>4726</td>
</tr>
<tr>
<td>P6</td>
<td>8.32</td>
<td>2722</td>
<td>778</td>
<td>344</td>
<td>774</td>
<td>2902</td>
<td>3499</td>
<td>4020</td>
</tr>
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<td>P1</td>
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<td>1144</td>
<td>231</td>
<td>834</td>
<td>3191</td>
<td>3726</td>
<td>4256</td>
</tr>
</tbody>
</table>

Mean herring 4143 331 460 1124 3206 4474 4790 316

Sinking Factor 1003

Swimbladder 4.1%

Mean Roach 9700 70 1801 1872 5806 9770 9479 291

Sinking Factor 1003

Swimbladder 9.9%

Pollock 4097 98\(\text{ (scales included with rest)}\) 1626 2623 4195 4249 54

Sinking Factor 1002

Swimbladder 4.1%

Table 6.8. Force in dynes acting on a 100 ml fish due to each of its components. The values in the main body of the table are based on volumes and densities of the components of Pacific herring given in table 6.6. These values are presented in order of increasing force due to oil. The forces acting on a roach, a freshwater fish, have been calculated using information given by Alexander (1959 b). Values for a single pollock were obtained in the same manner as for the herring (see appendix). The force of gravity "g" was taken as 981 cm sec\(^{-2}\).
occupying 10.8% of its volume and of density 1.0674 g/ml. Of the downwards force of 3932 dynes/100 ml due to "the rest" calculated for this fish 488 dynes/100 ml were due to the testes. The volume of the ovary was not determined for any of the fish of table 6.8 but if we assume it to occupy the same volume as the testes and have a density of 1.1022 g/ml the ripe female P5 would experience a downwards force of 854 dynes/100 ml due to the ovary out of a total of 2749 dynes for "the rest". The downward force due to "the rest" was not consistently greater in mature than immature fish. If the entire contents of the testes and ovaries in P2 and P5 were voided during spawning at 36 cm water depth both fish would become less dense than the sea water. However as spawning usually occurs at depths below this where the swimbladder gas is compressed and offers less buoyancy it is unlikely that this occurs in nature.

The comparison of the forces acting on the herring due to its various components with those acting on other fish is handicapped by the scarcity of comparable data. Alexander (1959 b) gives data for the roach, a fresh water fish, which can be recalculated to give the forces acting on the fish (Table 6.8). In the roach the upward force due to oil is small compared with that of the herring and the swimbladder contributes most of the lift to the fish. The bony structures exert a greater downward force on the roach than on the herring, due in part to the supporting medium being fresh water
instead of sea water but mainly to the reduction of the bony parts, especially the scales, in herring. The sum of all these forces leaves a compensating force to be exerted by the fish of about 3 dynes/ml for both fish.

The pollock from the Atlantic coast gives values roughly comparable to the herring. It relies less on oil as an upward force than does the herring but gets similar support from the swimbladder. The skeleton of the pollock contributes a downwards force greater than that of the herring. The lower sum of upward forces and the increased downward force due to the skeleton in the pollock are balanced by a lower downward force due to "the rest" (even though this included minute scales) so that the pollock has to exert a lower compensating force than the herring. The reduction in the downward force of "the rest" may be accomplished by the inclusion of relatively greater amounts of body fluid of lower density than the sea water. In the herring the higher the fat content, the lower the amount of water in the muscles which constitute 55% of its weight. The relationship is given by \( \% \text{water} = 80.1 - 0.94 \times \% \text{fat} \) (Reay et al, 1943) so that with no fat the muscles of herring would contain 80.1% water; with 15% fat only 66% water resulting in a loss of upthrust due to water of 157 dynes/100 ml.

As a result of this study it is shown that the herring is well adapted for vertical movements at sea. Its
high fat content and reduced scales and skeleton allow a reduction in the percentage volume occupied by the swimbladder resulting in a lower change in sinking factor and in density with depth when compared with more typical marine fish with a 5% swimbladder volume. That the fat content of herring muscle is unusually high is shown by the values given by Reay et al (1943) for the muscles of 27 other edible marine fish, which with 3 exceptions, are all below 5% fat. As a consequence of its high fat content the muscle of herring may have a lower water content than in other fish. The cod, for example, has 80 to 83% water in the muscle and under 1% fat while a herring may have 15% fat and 66% water. As fat provides more upthrust in sea water than does water this does not result in a net loss of buoyancy in fat herring, only a change in the proportions of the components providing lift.

In addition to these advantages of low change in density with depth, the herring has an advantage over all physoclistous in its ability to make unlimited upward movements. Whatever the initial volume of gas in the swimbladder of herring, gas expansion will not injure the fish as excess gas can be liberated by posterior or pneumatic duct. Thus vertical movement as a means of escape from physoclistous predators is a possibility.
Appendix. Modification of Alexander's method to determine the swimbladder volume and density of fish with closed swimbladders.

Alexander's apparatus was designed for use with fish with functional pneumatic ducts so that after a run with the swimbladder gas in situ, part of the gas could be removed to eliminate the pressure of the swimbladder walls on the gas and a further run to determine the change in volume with change in pressure made. As the unconstrained gas then obeyed Boyle's Law the total volume of gas at manometer zero could be calculated. In the following modifications the pressure during the first run is increased considerably above the pressure at which the swimbladder walls cease to compress the gas. At these pressures the product of total pressure and volume is a constant and the total volume of gas at any pressure can be calculated from the known change in volume with change in pressure.

Method.

To find the swimbladder volume of the "harbour" pollock, Pollachius virens, O class fish were held in tanks to adjust to water 36 cm deep. They were then placed in the same apparatus used for the tests on herring and subjected to a series of increasing applied pressures as follows: 0, 2, 4, 6, 12, 18, 24, 30, 36, 42 cm Hg. After return to manometer zero the pressure was reduced until the fish had
neutral buoyancy. The meniscus position at all these pressures was recorded and later corrected for temperature change and apparatus distortion. Only this single run was made. The fish was then weighed after a standard drying period and frozen to await analysis.

Other pollock adjusted to the same water depth were stunned, opened ventrally and the effect of increased applied pressure on their swimbladders recorded. By 24 cm Hg increased pressure their swimbladders had so decreased in volume that the posterior end of the swimbladder wall crumbled inwards. Thus at applied pressures above this level it is safe to assume that there was no pressure on the gas due to the swimbladder walls.

Now for an unconstrained gas at constant temperature a plot of volume against the reciprocal of the total pressure will yield a straight line. For the pollock we may plot the meniscus movements from the maximum applied pressure of 42 cm Hg, assigning a value of 0 at this pressure, against the reciprocal of total pressure (figure 6.14). As expected the meniscus positions for the applied pressures above 24 cm Hg lie on a straight line showing that the gas was unconstrained. The point where this straight line cuts the axis may be calculated, as in figure 6.14, which is more accurate than extending the line graphically. The volume of the free gas at manometer zero can be calculated and the
Figure 6.14 Graph of volume change against the reciprocal of total pressure for pollock C.
volume of the swimbladder gas found by subtracting its difference in volume represented by the difference in the two intercepts at manometer zero. From the volume change between neutral buoyancy and manometer zero the density of the fish can be found as the density of sea water is known.

Results.

Three pollock were tested for their densities and swimbladder volumes. Only one pollock was analysed for its oil and skeletal components. The scales in this fish were too fine to be separated so are included in "the rest". In calculating the forces due to these components the skeleton was assumed to have a density of 2.0 g/ml and the oil a density of .93. Thus although the determinations in which the three test fish are involved should be up to the standard of the determinations on herring, the calculations of the forces due to the oil and skeleton are less accurate as they depend on these assumptions. The results are given below:
Pollock Mass of Neutral Density Density of Sinking Swimbladder
Fish Buoyancy of fish sea water Factor

<table>
<thead>
<tr>
<th></th>
<th>g</th>
<th>cm Hg</th>
<th>g/ml</th>
<th>g/ml</th>
<th>% vol</th>
</tr>
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<tr>
<td>A</td>
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<td>-9.7</td>
<td>1.0305</td>
<td>1.0246</td>
<td>1005.8</td>
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<tr>
<td>B</td>
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<td>-9.8</td>
<td>1.0321</td>
<td>1.0250</td>
<td>1007.0</td>
</tr>
<tr>
<td>C</td>
<td>16.82</td>
<td>-1.1</td>
<td>1.0271</td>
<td>1.0250</td>
<td>1002.1</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>-6.9</td>
<td></td>
<td>1.0299</td>
<td></td>
</tr>
</tbody>
</table>

Pollock Swimbladder Oil Skeleton "the rest"

\%vol density \%vol density \%vol density \%vol density

<p>| | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<tr>
<td>C</td>
<td>4.08</td>
<td>.0013</td>
<td>1.05</td>
<td>.93</td>
<td>1.7</td>
<td>2.0</td>
<td>93.17</td>
</tr>
<tr>
<td></td>
<td>(assumed)</td>
<td>(assumed)</td>
<td>(assumed)</td>
<td>(assumed)</td>
<td>(assumed)</td>
<td>(assumed)</td>
<td>(assumed)</td>
</tr>
</tbody>
</table>

Force in dynes/100 ml fish for pollock C

<table>
<thead>
<tr>
<th>Swimbladder</th>
<th>Oil</th>
<th>Skeletal</th>
<th>&quot;the rest&quot;</th>
<th>Total</th>
<th>Total</th>
<th>Total</th>
<th>Compensation</th>
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</thead>
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<tr>
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<tr>
<td>4097</td>
<td>98</td>
<td>1626</td>
<td>2623</td>
<td>4195</td>
<td>4249</td>
<td>54</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER VII
AIR UPTAKE AT THE SURFACE AS A SOURCE OF OXYGEN FOR RESPIRATION

Introduction.

The herring appears to satisfy most of the anatomical criteria for an air breathing fish. It is able to pass air from the mouth through stomach and swimbladder and then to the exterior through the posterior duct. This one direction flow in fact would make a gas exchange mechanism in the herring more efficient than in air breathing fish previously described by reducing the contamination of incoming air with expired gases. Although the swimbladder of the herring is poorly supplied with blood vessels and thus an unlikely site for gas exchange, the inspired air passes the whole length of the stomach caecum which is well vascularised and might serve as a respiratory organ. Furthermore some analyses of herring swimbladder gas show a lower \( \text{O}_2 \) content than the air (table 7.1), thus if all swimbladder gas has ultimately been derived from air there must be some \( \text{O}_2 \) uptake, though not necessarily by the fish, once the gas is inside the body of the fish.

A consideration of the habitat of the herring, however, makes it seem unlikely that such a mechanism would be functional in this fish. The herring is marine, although it may enter brackish water, and the upper waters of the sea
where air breathing alone could occur, are usually well supplied with oxygen. For this reason air breathing marine fish are rare (Carter 1957). In the range of salinities and temperatures where the herring is usually found $O_2$ concentrations of between 13 ppm (at the lowest temperature and salinity) and 7 ppm would normally be present; while present experimental evidence suggests that $O_2$ concentrations have to fall below 3 ppm to cause respiratory difficulties. Nevertheless the possibility that under low $O_2$ conditions air breathing might occur was tested experimentally.

Method.

The two compartments of the apparatus shown in figure (7.1) were used to expose simultaneously two groups of six herring to a lowering of $O_2$ concentration in the sea water. The herring, 11 to 17 cm long, were in excellent condition and had been held for about a week previous to the test in tanks of running sea water at 7 to 8 ppm $O_2$. The fish in compartment A were allowed access to the surface, but not those in group B. The water was continuously circulated through the system, its $O_2$ content being controlled by the $N_2$ washing tower and its temperature by the water bath. Samples of water from the bottom of both compartments were taken at intervals and analysed for $O_2$ content by means of a Southern Analytical Dissolved Oxygen Meter. The behaviour and mortality of the herring were recorded.
Pre-test Conditions Composition of swimbladder gas

<table>
<thead>
<tr>
<th>Period</th>
<th>Water depth</th>
<th>CO₂</th>
<th>O₂</th>
<th>Remainder, presumed N₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>days</td>
<td>cm.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>90</td>
<td>x</td>
<td>x</td>
<td>98</td>
</tr>
<tr>
<td>30</td>
<td>90</td>
<td>x</td>
<td>no value</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>x</td>
<td>x</td>
<td>98</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>3.0</td>
<td>2.7</td>
<td>94</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>11.9</td>
<td>3.9</td>
<td>84</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>5.1</td>
<td>8.3</td>
<td>87</td>
</tr>
<tr>
<td>5</td>
<td>36 (no access to surface)</td>
<td>x</td>
<td>3.3</td>
<td>97</td>
</tr>
</tbody>
</table>

x - values below 2%, which is the limit of accuracy for this method of gas analysis using the Krogh gas analyzer.

Table 7.1. Composition of the swimbladder gas of herring.

Results and Conclusions.

Curves of O₂ concentrations and mortality of fish for the four runs are shown in figures 7.2 to 7.5. In the first three runs deaths occurred fairly rapidly after the oxygen concentration had been reduced to 2 ppm. The time to death of half of the fish group for these three runs is given below, the time being counted from the point at which O₂ concentration first reached 2.0 ppm.
Run  Time to 50% mortality in minutes since $O_2$ reached 2.0 ppm

<table>
<thead>
<tr>
<th>Access group A</th>
<th>Non-access group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
</tr>
</tbody>
</table>

Thus in these runs where oxygen deprivation was quite severe the fish having access to the surface actually survived for a shorter period than those in compartment B.

In the fourth run, except for a short dip of compartment A below 2.0 ppm, the $O_2$ concentration was held between 2.2 and 2.6 ppm. At these concentrations the herring mortality was spread over a greater interval of time than in the first three runs. Here death of half the access group occurred after 2 hr. 22 min. and of the non-access group in 1 hr. 40 min., the time being recorded from the point when $O_2$ concentration in a compartment first fell below 2.4 ppm. Time to death of five sixths of each group was similar for the two compartments (2 hr. 46 min. for A; 2 hr. 27 min. for B).

If air was being taken from the surface by the fish in compartment A when the $O_2$ level decreased below a certain value this should have been evident from the behaviour of the fish. It was found that the behaviour of the fish with respect to the surface was influenced by several factors. Firstly, as the compartment was rather shallow, fish newly put into the tank often came to the surface and broke it with
head or back during normal swimming. As time passed the frequency of breaking the surface decreased as the fish settled down in their new surroundings. This can be followed in run 3 where the fish were held at about 4.0 ppm O₂ for more than 6 hours. Here the frequency of breaking the surface in a 5 minute period decreased progressively from 23 or more to 2 after 6 hours. Secondly, the external signs of stress resulting from low O₂ concentration sometimes included a period of violent swimming either before or after the fish lost their equilibrium. This resulted in more breaks of the surface. This swimming appeared to be quite at random and did not include the rush to the surface, gulp and quick swimming down shown by herring taking in air to restore their normal density. In run 3 this violent swimming began when the O₂ concentration had dropped to 2.7 ppm. Thirdly, herring before death frequently became quite heavy and spent most of their time resting on the bottom right side or later belly up. Some of these fish were seen to lose gas from the posterior duct, which could explain their increased density. These fish often swam to the surface and may have taken in air but they were not able to restore their normal buoyancy. These dense fish were the only ones to give the impression of breaking the surface to obtain air but they still died before fish of the same group that swam normally. In none of the runs were fish observed to come frequently to the
surface, take air and expel it later in a regular sequence from the posterior duct, anus or mouth. Thus the expected behaviour of an air breathing fish was not shown by the herring.

The supposition that herring could not use $O_2$ derived from the air in significant amounts under low $O_2$ conditions was borne out by the mortality figures for the two groups. In runs 1 to 3 where there was a rapid exposure to low $O_2$ concentrations, the group given access to the surface actually showed 50% mortality before those denied access to the surface. In the fourth run where there was a longer exposure to less severe $O_2$ deprivation the group given access to the surface took longer to reach 50% mortality but were not able to obtain enough $O_2$ from the air to ensure their survival. Thus it seems that the herring, although possessing anatomical structures which might permit air breathing and able to take and swallow air from the surface, does not use this mechanism to ensure survival in low $O_2$ conditions.
Figure 7.1 Apparatus used to investigate air breathing in herring under low O\textsubscript{2} conditions.
Figure 7.2 Graphs of oxygen levels and herring mortalities in first run of air breathing experiment.

Temperature 13 to 13.5° C. Fish lengths 12 to 15 cm.
Figure 7.3 Graphs of oxygen levels and herring mortalities in second run of air breathing experiment. Temperature 12.9 to 13.4° C.
Figure 7.4 Graphs of oxygen levels and herring mortalities in the third run of air breathing experiment. Temperature 14.1 to 14.5° C. Fish lengths 11 to 16 cm.
Figure 7.5  Graphs of oxygen levels and herring mortalities in the fourth run of air breathing experiment.

Temperature 13 to 14°C. Fish lengths 11 to 17 cm.
CHAPTER VIII
DISCUSSION

Introduction.

Considerable discussion of the results obtained has been presented in previous chapters. To avoid duplication these are not repeated here but instead an attempt is made to apply the findings to the biology of the herring using examples based on conditions in Passamaquoddy Bay, N.B. This restricted area was selected because it was the source from which the Atlantic herring were obtained and because the seasonal variation of many of its physical properties and the properties of its herring stock are known (Figure 8.1). This permits the problems faced by the herring during its vertical migrations and in escape from predators to be stated in exact terms. The probable functions of the swimbladder and its ducts are then deduced from the experimental results and the biology of the herring. Although examples are drawn from a restricted area, herring from other regions show similar diurnal vertical migrations, are subject to intense predation, contain relatively high amounts of fat and, as the comparison of Atlantic and Pacific herring showed, have similar swimbladder and density characteristics. Thus the modifications shown by the herring of the Passamaquoddy region to overcome the problems of its habitat should be applicable in general terms to all herring stocks.
Modifications assisting the herring in its daily vertical migration.

1) Pressure changes and their effect on swimbladder volume.

Because of the compressibility of gas, fish which rely on gas spaces for part of their buoyancy have the uplift provided by this gas halved each time the total pressure is doubled and conversely when rising in the water experience a doubling of swimbladder volume each time the pressure is halved if the swimbladder walls do not compress the gas. Thus two problems are presented to a fish undergoing vertical movements; the increase in density with depth as the swimbladder gas is compressed and the removal of excess gas when a fish with a full swimbladder below the surface begins to rise. Both problems are most severe in movements to or from the surface as the rate of doubling of total pressure decreases with increasing depth below the surface.

Physoclists and physostomes capable of gas secretion and absorption solve these problems by adding or removing gas from the swimbladder until after each vertical change the original swimbladder volume is restored. Although imposing some restrictions on the rate of vertical migration such regulation of swimbladder volume always tends to produce approximate neutral buoyancy and so reduces the energy expenditure required to maintain constant depth. It is a mechanism especially advantageous to species with restricted vertical ranges.
Figure 8.1 Seasonal variations in properties of Passamaquoddy Bay, N.B. and of its herring stock.
As the herring is unable to secrete gas into the swimbladder and is unlikely to receive gas from stomach bacteria the mass of gas present below the surface can never exceed the amount carried down by the fish and some different method than that shown by physoclist has to be used to reduce density change with depth. Modifications to reduce this density change are needed as apparently all herring stocks show diurnal changes in depth, sometimes descending far below the surface. In the Passamaquoddy region the herring move from the surface at night to median depths by day of 25 to 38 m from January to April and to 9 to 13 m by day for the rest of the year (Brawn 1960). In the North Sea feeding shoals of herring were at 30 fathoms (55m) by day and at 10 to 16 fathoms (18-29 m) at night (Richardson 1952). Herring off the Norwegian coast go as deep as 75 fathoms (137 m) by day, rising towards the surface at night (Balls 1951). Pacific herring also show daily changes in depth, juveniles being at 1 to 6 m at dawn and dusk descending to 3 to 15 m by day (Hourston 1959). At 137 m, the greatest depth given above, any gas carried down from the surface would have only one fifteenth of its surface volume and the upward force that it could contribute to the herring would be negligible. Thus it is not surprising that modifications are found in the herring which reduce its dependence on the swimbladder as a hydrostatic organ.
2. The reduction of body components of high density.

The two components of greatest density in a fish are its skeleton and scales. Reduction either in the amount or density of these components would reduce the density of the fish and so its need for buoyancy devices. It has been shown here that the density of the herring skeleton, 1.99 g/ml are comparable to the densities of bone in the freshwater roach, dace and carp studied by Alexander (1959 b). However the amount of both scales and skeleton are reduced in the herring so that together they occupy only 1.7% by volume compared to 4.3, 2.8 and 4.4% for the fish above. Such a reduction in scales and skeleton allows the swimbladder volume of herring to be reduced by about 0.2% of the volume of the fish.

3. The increase in fat content.

The occurrence of large amounts of fat in the herring appears to be the most important modification reducing fish density and hence lowering the volume of gas required for neutral buoyancy. Unlike swimbladder gas the upthrust provided by body fat is hardly affected by pressure and temperature changes, attributes which are valuable in a buoyancy device required to function throughout the herring's vertical range.

In nature the fat content of herring as a percentage by weight varies from 4.1% to 19.4% in Pacific coast herring
(Hart et al, 1940) and may rise as high as 30% (Brocklesby 1941). Atlantic herring of the Passamaquoddy region show seasonal fluctuations in fat content being fattest in January (20%) and thinnest in May (6%) based on mean values (Leim 1943 and figure 8.1). In mature fish seasonal variation in fat content depends on the spawning time, thus herring of the west coast of Newfoundland which in December and January have 13% fat decrease this to 9% by May when spawning commences (Leim et al, 1957). The average fat content of North Sea herring in winter and spring is 4.3% and 16% in autumn (Mygind 1949, quoted by Leim et al, 1957). These percentages are mean values and we may take the lowest fat content present under normal conditions to be about 3%, the lowest individual value given by Leim (1943).

To demonstrate the effect of fat content on the variation of herring density with depth the sinking factors of a herring with a swimbladder volume of 4.2% at 36 cm depth, living in sea water of 1.0242 g/ml have been calculated for various depths assuming a fat content of 3% and 23% (figure 8.2, upper and lower solid curves). The additional fat greatly decreases the density and hence the sinking factor of the fat fish. This example is not a true representation of the sinking factors shown by a herring with 23% fat as it makes no allowance for the decrease in swimbladder volume which accompanies increase in fat content in these fish. To obtain true values for such a fish the swimbladder
volume should have been reduced from 4.2% to 2.5%. The two lower solid curves of figure 8.2 represent the sinking factors of herring having only 3% fat in the low salinity water of Passamaquoddy Bay and in the more saline water of the North Atlantic. As 3% fat is probably the lowest fat content that herring reach in nature these curves may be taken to represent the most extreme increase in sinking factor with depth that herring ever normally encounter.

Changes in sea water density between Passamaquoddy water (roughly 30% salinity) and North Atlantic water (35%) with North Pacific water in between (34%) have only a slight effect on the sinking factors of herring in these regions.

In figure 3 are given curves showing the change in sinking factor with depth of Passamaquoddy herring in February and August when the greatest differences in water temperature, fat content and extent of diurnal vertical migration occur. The calculations take into account fat content using Leim's data, related swimbladder volume, the change in sea water density calculated from the mean monthly salinities and temperatures, using Trites' data and the change in fat density with temperature. Thus these curves show as truly as possible the sinking factor changes with depth that these herring experience in these months. The major cause of the difference between these two curves is the difference in fat content of 18.4% in February and 10.9% in August as the other two variables; sea water density
Figure 8.2 Theoretical values of sinking factor with depth all based on herring with a swimbladder volume of 4.2%, gas free body density of 1.0718 g/ml.
Figure 8.3  Theoretical curves showing change in sinking factor with depth for herring of Passamaquoddy Bay based on actual mean values of relevant variables.
Figure 8.4  Relationship of swimbladder volume to fat volume.
and the slight difference in swimbladder volume tend to cancel each other out. The increase of fat density at the low February temperature increases the sinking factor by about 2. A noteworthy feature of these curves is that the herring in February, although it is much deeper in the water by day, has almost the same sinking factor (1018) at its median daytime depth as it has in August (1016). In spite of these seasonal differences in subsurface density the herring in both months has almost neutral buoyancy at the surface.

As it is known from the literature that herring such as those off the Norwegian coast can descend almost to 200 metres it is interesting to calculate what fat content of density 0.9340 g/ml at 5° C would be required to allow them to descend to this depth with a sinking factor of 1018 at 200 m. For this calculation, since the herring are in Atlantic water, a water density of 1.027 g/ml has been assumed. The swimbladder volume at the surface was taken as 2.5% and the fat and gas free herring density as 1.0765 g/ml. Under these conditions it is calculated that a fat content of 21.4% of the total volume is all that would be required. Such a fat content is physiologically possible for the herring and only slightly exceeds the mean content of herring taken from Passamaquoddy Bay in January. Thus at seasons of the year when the fat content of herring is highest they are able to descend even to 200 m without exceeding the moderate sinking factor of 1018. As herring
under experimental conditions were able to compensate while swimming for a sinking factor of 1026 for at least a week there is no doubt that herring with these high fat contents could remain at depths of 200 m for long periods without undue fatigue.

4. The swimbladder as a hydrostatic organ.
   a) changes in gas volume.

   A desirable characteristic of a hydrostatic organ is that it should be capable of adjusting the uplift it provides fairly rapidly according to the changing requirements of the organism. Although fat content has been shown to be of great importance in providing buoyancy to the fish it suffers the disadvantage of being changed only by slow metabolic processes. In contrast, experimental fish have shown that herring living close to the surface regulate their swimbladder volumes by taking in air from the surface or releasing gas from the posterior duct to maintain their body densities a little higher than the density of their environment. To accomplish this, the swimbladder volume is varied between 2.6 and 5.4% of the fish volume. The swimbladder volume was shown to be adjusted to compensate for varying fat contents, for changes in water density, for increase in fish density following gas loss and for increase in positive buoyancy following gas expansion due to pressure reduction.
It has been shown that the percentage swimbladder volume of Atlantic and Pacific herring is negatively correlated with percentage fat volume between 0.5 and 12% fat and above 12% the curve appears to flatten out above a suggested minimum swimbladder volume of 2.5% (figure 9.4). Herring under natural conditions usually have 3 to 20% fat so their swimbladder volume will vary from about 4.2 to 2.5 which is a reduction of 16 to 50% of the 5% swimbladder volume suggested as typical of marine fish by Jones (1951). This reduction is the cause of the low increase in sinking factor with depth shown by the herring.

b) Swimbladder extensibility and excess internal pressure.

The swimbladder volume of herring varies from 2.6 to 5.4% and yet the excess internal pressure of the gas remains between 0 and 2.7 cm Hg and shows no consistent relationship to the volume. This suggests that the herring swimbladder is freely extensible over this range and that the observed variation in excess pressure was a result of changes in contraction of the muscular layer of the swimbladder.

An extensible swimbladder wall is required to permit the wide variation of swimbladder volume needed if the herring swimbladder is to function as a hydrostatic organ. In a closed swimbladder secretion and absorption tend always to restore the gas volume to approximately the same
value and swimbladder extensibility is of greatest importance in permitting some vertical movement above the plane of adjustment without excessive increases in internal pressure. In herring if no gas is added below the surface the swimbladder volume will never exceed its volume at the surface; if gas is added from the stomach it can be removed through the posterior duct so in both instances the upward movement of herring does not require an extensible swimbladder. Rather the extensible swimbladder wall is required because the herring, unlike the physoclist, adjusts the swimbladder volume to amounts which may vary greatly from one time to another according to the fat content of the fish. This ability to make the necessary adjustment in swimbladder volume permits herring of varying gas free body densities to approach neutral buoyancy during the period spent in surface waters at night.

5. The possible importance of accessory gas spaces.

The stomach caecum of the herring by its dorsal and central position, large capacity and position between the oesophagus and pneumatic duct is well suited to serve as an accessory gas space which could be filled with air at the surface and slowly passed to the swimbladder as the gas volumes of both were compressed with increase in depth. Such a mechanism as far as buoyancy is concerned would have no advantage over carrying the additional amount of gas in the
swimbladder which would probably by physically possible even at surface pressures if it did not exceed about 1%. However such a mechanism might have some or all of the following advantages. It has the advantage of quickness so that the stomach could be filled with air immediately before descent and the slower process of passing it to the swimbladder delayed. This would reduce to a minimum the length of time that the herring had to compensate for the positive buoyancy resulting from the combined upthrust of stomach and swimbladder gas. It is possible that the stomach caecum walls may be able to compress gas more strongly than the weaker swimbladder walls, again reducing positive buoyancy until the fish had left the surface. It could be an advantage if the sensory functions of the swimbladder required a moderate swimbladder volume by reducing the necessity of filling the swimbladder to capacity at the surface and by providing a source of subsurface gas which could raise the swimbladder volume after compression. In figure 9.2 the effects of 1% volume of stomach gas on the change of sinking factor with depth have been given as dotted curves above the corresponding solid curve representing the same fish without stomach gas. A 1% stomach gas volume was selected as a possible value on the basis of the dimensions of a moderately extended stomach caecum.
Evidence from herring at sea is scanty but tends to support the suggestion that the stomach may sometimes act as a gas storage organ. In 28 herring taken from a weir 25 feet deep in the afternoon 17 had gas in the stomach caecum although 6 to 9 hours must have elapsed since these fish were at the surface (Brawn 1962). These herring were dipped in water from the weir and immediately anesthetised and then examined, a procedure which would not cause swimbladder gas to pass to the stomach in captive herring. Evidence of a different type is given in the communications from Capt. Clarke, Capt. Auchterlonie and Mr. Dale quoted in Chapter V which all mention that the most usual time for gas bubbles to be seen from herring shoals along the Pacific coast is in the afternoon or late afternoon. Verheijen (1956) reports that bubbles are a sign of ascending clupeoids although he has only seen this from anchovies and sardines in the Mediterranean. Such a release of gas is hard to explain unless gas had been added to the swimbladder as return to surface pressures at dusk would otherwise only restore the swimbladder gas to its original volume. In addition herring would be aided in their upward movement by their buoyancy and release of gas would only increase the expenditure of energy needed to reach the surface.
Gas is not found in the stomach caecum of herring in captivity maintained in water of constant depth but in these fish vertical migration has been completely suppressed. Obviously more information from free living herring is required to settle this question. If substantiated it may be one function of the buoyancy system requiring the origin of the pneumatic duct from the stomach caecum and the presence of the posterior swimbladder opening.

6. The ability of herring to compensate for positive or negative buoyancy.

Herring can compensate for positive buoyancies produced experimentally by pressure reductions until these are reduced by liberation of swimbladder gas through the posterior duct. Thus there is no buoyancy limit placed on the upward movement of herring whatever their initial swimbladder volume.

Herring in captivity compensated for one week for sinking factors of 1026 without signs of fatigue. In terms of the Passamaquoddy herring (figure 9.3) this is equivalent to a stay of one week at 33 m for August fish and for February fish this sinking factor would never be reached at any depth by compression of swimbladder gas alone. The February fish could thus remain in water of any depth for at least a week and would be free to move vertically at any speed in either direction between the surface and the bottom.
Summary.

The herring in its natural state appears to have attained freedom of vertical movement without the necessity of expending large amounts of energy in compensatory movements. The most important modification permitting this achievement is the possession of 3 to 20% of its body volume as fat. This fat adds buoyancy to the fish which is almost unaffected by the normal changes in pressure and permits the herring to reduce its swimbladder volume to between 2.6 and 4.2% of the total volume. A small reduction of the swimbladder volume can also occur because of the reduction of the scales and skeleton of the herring to 1.7% of the total volume. As a result of this reduction in gas volume the herring as a whole is much less compressible and so shows a low increase of density with depth which has the double advantage of reducing the energy expenditure needed for compensation at any given depth and allowing the herring to descend far from the surface for only moderate density increases. Upward movement of the herring is unrestricted because of the possession of a direct opening of the swimbladder to the exterior through which excess gas if present can be expelled. Thus the herring has attained a freedom of vertical movement impossible for a physoclistous fish while minimising the disadvantages of the lack of a subsurface means of regulating its density.
Modifications possibly related to predation.

1) Air intake at the surface.

Herring in captivity which need to replace lost swimbladder gas make infrequent and very swift movements to the surface and down again. Such behaviour will minimise exposure to predation by gulls which can only descend two or three feet below the surface in a clumsy dive. The anatomical modifications which allow air ingestion to be accomplished so rapidly are the upward directed gape of the herring mouth, which permits the herring to follow a curving path bringing only the mouth above the surface, and the possession of a large extensible stomach caecum into which the air can immediately pass. The stomach caecum also allows large quantities of gas to be taken during one excursion to the surface and allows the slower transfer of gas through the pneumatic duct to be postponed. Predation pressure from above may have played a part in the evolution of the pneumatic duct from the stomach caecum.

2) The adrenergic control of gas release.

It has been shown that gas release through the pneumatic duct and through the posterior swimbladder duct is facilitated by adrenalin. As stressful situations cause the liberation of adrenaline in vertebrates it is possible that prolonged stress due to predation might lead to gas release by herring. A similar effect was observed in captive
herring which if they showed agitated swimming in the test flask released gas at a significantly lower pressure reduction than herring which swam moderately (Brawn 1962).

Gas release in the presence of predators may account for the reported release of gas by Pacific herring at hours other than when the herring are rising in the water (Chapter V). These reports all mention the production of gas bubbles by herring when pursed in the seine and Capt. Auchterlonie notes that gas is seen if the purse lines are drawn through the fish but is not seen until later in the operation if the lines are drawn below the fish without touching them. The unnatural conditions of seining may be compared to those experienced by a herring school subjected to predation from many sides. Gas release under these conditions could have the important consequences of quickly reducing the fish density to expedite their downward escape movement and of leaving behind a cloud of ascending bubbles to partly blind and perhaps confuse the predators.

Atlantic herring will sound if startled by a sudden vibration such as dropping an oar in a rowboat. On the one occasion this was observed in herring enclosed in a seine sounding was preceded by concerted violent horizontal swimming which whipped the surface into a foam. After the fish went down bubbles continued to rise but it was impossible to determine if these were released from the posterior duct or
were part of the surface foam carried down in the mouths of the fish. The violent swimming which preceded sounding may be an important part of the response, tending to bring all fish to the same physiological state so that gas release and sounding may occur in unison.

3) Freedom of vertical movement.

The possible escape of herring from physoclist predators such as the cod by their greater freedom in upward movement was suggested by Jones (1952). Based on his data for perch and wrasse he estimated that marine physoclists could move freely upward in a zone extending above their plane of equilibrium for a distance equivalent to a 22% reduction in pressure. Thus a cod adjusted to the pressure at 50 m could rise to 37 m; adjusted to 20 m it could rise to 13 m. As herring can swim vertically upwards without restriction and their maximum rate of swimming varies from 0.9 to 1.4 m/sec for lengths 15 to 27 cm (Brawn 1960b) herring swimming upwards could theoretically move out of the range of cod or similar fish in 9 to 15 seconds at 50 m and in 5 to 7 seconds at 20 m.

The limit to rapid downward movement of physoclists, although not discussed in detail, is attributed by Jones (1951) to the resultant increase in density and that too large a vertical movement below the plane of equilibrium might result
in the fish being carried down to the bottom. A consideration of the increases in sinking factor involved during downward movement and the ability of experimental fish to swim in midwater when all gas has been removed from the swimbladder cast doubt on the suggestion that compression of swimbladder gas alone would ever cause a density greater than an active physoclist could compensate for by fin movements, especially during descents of short duration. For example if a marine physoclist with a 5% swimbladder volume in sea water of 1.026 g/ml had this gas compressed to vanishing point its density would become 1.080 g/ml and its sinking factor 1053, which while high is not much higher than the sinking factor of 1043 which mackerel apparently compensate for throughout their lives (Jones and Marshall, 1953). Even freshwater fish with the swimbladder gas completely removed can compensate for their increased density until secretion restores the normal volume (Copeland 1952) although if we assume they had a 7% swimbladder volume this would require an initial compensation for a sinking factor of 1075.

It is not suggested that there is no limit to the downward excursions of physoclists below their plane of adjustment, only that the limit seems unlikely to be absolute and physical acting through an increase in density due to gas compression greater than that for which the fish can compensate. Rather a behavioural limit is suggested which would operate whenever negative stimuli such as increasing
discomfort from the collapsing swimbladder, stimuli associated
with increasing body density and perhaps decreases in light
intensity outweigh the attraction to further downward movement.
Such a limit would be very flexible and would vary greatly
according to the physiological condition of the fish, nature
of the downward attraction, past conditioning of the fish
and the immediate physical conditions of the environment.

In discussing the modifications of herring in
relation to downward escape from such physoclist predators
no actual limits below which the herring would be safe can
therefore be given. However at all depths below the surface
the herring will have a greater sinking factor than a physoclist
adjusted to that depth and the herring density may be
immediately increased further by the release of gas through
the posterior duct. This increased density may give the
herring an initial advantage in the speed of downward movement.

4) Summary.

Some of the modifications shown by the herring in
connection with the functioning of its swimbladder may be
related to predation. The upward gaping mouth and large
stomach caecum in which gas may be temporarily stored mini-
mise the period required for air intake at the surface. The
small volume of swimbladder gas decreases the increase in
sinking factor with depth so that the herring has almost
completely freedom of downward movement to escape predators. The posterior duct not only permits the release of bubbles which may confuse predators but may enable herring to descend rapidly by increasing their density. The lack of subsurface gas secretion coupled with the possession of the direct swimbladder opening removes all restrictions on the rapid upward escape movement which could take the herring above the range of physoclist predators.

Possible functional significance of the origin of the pneumatic duct from the stomach caecum.

The pneumatic duct of herring, in spite of its unusual adult position, is formed during development as an outgrowth of the oesophagus (Maier and Scheuring 1923). If its subsequent shift to the posterior end of the stomach caecum has functional significance it is probably associated with the function of this large, muscular and vascular organ.

The vascular nature of the stomach caecum suggested that it might function as a lung when dissolved oxygen levels were low. Such a function would have required the insertion of the caecum in the line of gas flow if the air was to be continuously renewed by uptake at the surface and release through the posterior duct. However experiments showed that herring could not use such a mechanism to survive in water of low oxygen content.
Another possible function of the stomach caecum, which would require the presence of the pneumatic duct, is as a site of gas generation by bacteria living on the contents of the stomach. Although a gas generating bacterium was isolated from the herring its inability to generate gas at the temperatures normally experienced by the fish suggest that it is not a source of swimbladder gas under natural conditions.

The main advantage of the position of the stomach caecum in the air passage to the swimbladder appears to be the possibility of temporary storage of air in this organ. This increases the amount of air that can be taken and carried down from the surface in a short time and because of the position of the pneumatic duct gas transfer to the swimbladder can occur without regurgitation.

A further function of the caecum is to provide the force required to drive gas through the pneumatic duct since this lacks an "oesophageal bulb". The position of the pneumatic duct in relation to the stomach allows the well developed stomach musculature to be used for this purpose.

Functions of the pneumatic duct.

The pneumatic duct of herring passes between the highly extensible stomach caecum and a fixed point on the swimbladder surface. For this reason it is of much greater
proportional length than is usual in physostomes and a bend
in the duct supported only by mesentery further permits
movement of the posterior end of the stomach relative to
the swimbladder without placing a strain on the pneumatic
duct. The great length and translucent walls of the duct
in herring make it excellent material for the study of
pneumatic duct functions.

1) The passage of gas.

The pneumatic duct can convey gas in either direc-
tion between the stomach caecum and the swimbladder. Except
at the time of gas passage the lumen of the duct is of small
size, being reduced by numerous internal primary and secondary
folds, and is filled with fluid. During gas passage the
walls of the duct are distended to twice the diameter of the
resting duct. This distension inhibits all peristaltic
contractions of the duct wall so that gas flow once initiated
is not due to active movements of the duct but to the
pressure difference across its ends.

2) Control of the initiation and cessation of gas flow.

While the pneumatic duct does not appear to control
gas flow once this is initiated, it exercises considerable
control over the initiation of flow in both directions.
The physical basis of this control is discussed in Chapter IV.
Briefly it appears to operate through changes in cross section of the fluid filled duct which alter the curvature of the fluid/gas interface. The applied pressure required to displace fluid in tubes of small bore is very sensitive to changes in radius and so regulation of the initiation of gas flow can be controlled by moderate changes in the degree of contraction of the circular muscles of the duct. Control of gas flow from the stomach caecum appeared to be exercised in first third of the pneumatic duct, each of its four or five internal sections requiring a slightly higher applied pressure to displace the fluid with gas. Control of gas flow from the swimbladder even with the swimbladder valve fully open could be exercised by the last third of the pneumatic duct provided the excess swimbladder pressure was low. Contraction of the circular muscles increasing the applied pressure required to initiate gas flow in either direction occurred in response to cholinergic drugs. Relaxation of the swimbladder end of the duct in response to adrenergic drugs permitted gas loss from the swimbladder if the valve was open but in tests where air was being driven from the stomach adrenalin did not reduce the pressure required for gas passage below that shown by preparations in saline.

Once gas fills the duct the fluid surface is broken leaving the fluid in the folds and pouches of the duct. Regulation of gas flow by a fluid surface can only occur
after collapse of the duct and reestablishment of this surface. For this reason the pressure needed to initiate gas flow through the duct from the stomach was almost twice the pressure at which gas flow ceased even when application of drugs altered the absolute pressures at which this occurred. Application of acetylcholine raised the pressure at which gas flow ceased while adrenalin decreased it suggesting that even during gas flow the tension in the duct walls can be regulated and by this means the applied pressure at which gas flow ceases may be controlled.

The anatomical structure of the first part of the pneumatic duct and of the swimbladder valve are such that while controlling gas flow from the stomach and swimbladder respectively neither hinders the flow of gas from the duct into these structures.

3) Exclusion of particulate matter.

The first two thirds of the pneumatic duct display an intricate system of internal folds so that any substance passing from the stomach towards the swimbladder goes through a succession of wide and narrow spaces. The first part contains forward opening pockets which extend up the lateral walls of the duct as well as across its floor. Particulate matter such as Artemia eggs suspended in saline were trapped in the first three pockets and very few managed to reach the central
part of the duct. Under natural conditions semidigested food has been found in the second pocket but no further along the duct. Thus the anatomy of the duct is such that almost all particulate matter can be prevented from entering the duct by mechanical means.

4) Removal of gas bubbles and foreign matter.

In addition to mechanical means of excluding food from the duct a further function of the duct is the active removal of foreign matter. Except during gas passage the walls of the duct respond strongly to the presence of gas bubbles, *Artemia* eggs or mineral oil by coordinated peristaltic contractions vigorous enough to squeeze oil completely out of one internal pouch into the next along the duct. Besides its obvious importance in removing foreign matter from the duct the ability of the duct to respond to the presence of such matter can be expected to add an active component to the mechanical one excluding solids from the duct.

The duct also responds to the presence of air bubbles by driving them either towards the stomach or the swimbladder and so restores the duct to its normal fluid filled condition.

Peristaltic movements on which these functions of the duct depend are initiated by cholinergic drugs and inhibited by adrenergic agents.
Functions of the swimbladder.

1) Retention and regulation of gas volume.

The swimbladder both retains and regulates the amount of its contained gas at the valve at the entrance to the pneumatic duct and at the final muscular part of the posterior duct. Normally removal of excess gas occurs through the posterior duct and the swimbladder valve is primarily concerned with gas retention. Both the swimbladder valve and the posterior duct are opened by adrenalin but as the posterior duct responds first to this blood borne drug, release through the posterior duct is favoured.

The swimbladder valve operates by changing the aperture of the pneumatic duct from a narrow slit in the closed condition to a rounded shape through which gas may pass in the open condition. The valve is closed by tension in the tunica externa fibres resulting from the excess gas pressure and opens when contraction of the circular muscles of the tunica interna removes this tension. If the gas pressure of the swimbladder is excessively raised the area of the swimbladder just in front of the duct bulges outward and opens the valve suggesting it functions as a safety device if the posterior duct is blocked.

Gas release through the posterior duct of the swimbladder is prevented by contraction of its coat of circular
smooth muscles under cholinergic control and occurs in response to adrenalin, suggesting that these agents work antagonistically in the control of gas release. As the lumen of the contracted duct partly fills up with longitudinal folds only moderate contraction of the circular muscles is required to close it completely. It was shown that the gas release mechanism causing the posterior duct to open acts through the central nervous system. Gas release occurs in response to pressure decrease and occurs more readily under conditions of stress. Thus gas release is expected to be normally associated with an upward movement of herring except in times of stress when it may accompany sounding. This contradicts the theory of the function of the herring swimbladder advanced by Svetovidov (1952 b).

2) Regulation of excess internal gas pressure.

It was shown that pilocarpine increased the internal gas pressure of isolated ligatured swimbladders without altering their shape noticeably. This suggests that the internal gas pressure of the swimbladder is capable of a certain amount of regulation presumably by changes in the tension of the entire circular muscle coat in response to cholinergic stimulation. A limited control of the excess pressure may be necessary for the successful functioning of the swimbladder valve, for the liberation of gas through the posterior duct, for sensory functions of the swimbladder wall and for its sensory function in connection with the ear.
3) Regulation of posture.

Isolated swimbladders were also found to respond to adrenalin but in this instance the response was markedly uneven, beginning with a slow contraction of the posterior half of the swimbladder and enlargement of a region which normally lies anterior to the dorsal fin until the posterior region had only one third the diameter of the anterior enlargement. The contraction of the posterior half of the swimbladder in response to adrenalin was also seen in situ and in such instances occurred several minutes before the swimbladder valve opened suggesting a certain degree of independence between the two responses. The action of adrenalin on the swimbladder was reversed by pilocarpine.

It appears therefore that the herring is able to regulate the horizontal distribution of gas within its swimbladder from a uniform distribution to one in which most gas is anterior to the dorsal fin thus tending to lift the head upward. Thus the herring may be able to regulate its posture by use of its intrinsic swimbladder muscles in a similar way to that shown by Peters (1951) for the sea horse.

Observations on herring raised and lowered in a cage at sea showed that the herring were able to swim straight for the surface with their bodies held vertically but they descended by sinking with their bodies mainly in a horizontal position even when an increase in the rate of descent to
18 cm/sec carried them to the top of the cage (Brawn 1960). The ability of the herring to move the swimbladder gas from an even distribution to a predominantly anterior position may account for the vertical posture when rising. The apparent inability to move the swimbladder gas to the posterior part of the swimbladder and the negative buoyancy shown by herring below the surface may explain the descent of herring by sinking instead of by active downward directed movements.

Conclusions.

It has been shown that many of the modifications of the swimbladder and its ducts may be associated with the diurnal vertical migrations of the herring and with predation avoidance. The swimbladder, pneumatic duct and stomach caecum have many functions in addition to their primary hydrostatic, gas passage and digestive functions respectively giving further explanations for the unusual anatomical features displayed by the herring.
CHAPTER IX
SUMMARY AND CONCLUSIONS

Summary.
1. The herring swimbladder is unusual in having a pneumatic duct which in the adult arises from the posterior end of a long stomach caecum instead of directly from the oesophagus and in having a direct functional opening to the exterior close to the anus.

2. No anatomical differences were found between Pacific herring and published descriptions of Atlantic herring in respect to their swimbladders and its ducts. The stomach caecum is a long cylindrical muscular organ lying dorsally in the body cavity and capable of isolation from the oesophagus and pyloric stomach by the action of sphincters. The pneumatic duct is morphologically divisible into three sections, the region nearest to the stomach bearing anteriorly directed internal pockets, the center section bearing smaller internal spherical pouches and the last section being without internal divisions and opening through a valve to the swimbladder. The swimbladder is thin walled with a tunica externa of trellis like elastic fibres and a tunica interna of circular smooth muscle. Anteriorly it bears narrow prolongations terminating close to the ear and posteriorly it discharges to the exterior through a posterior duct with well developed circular muscle walls.
3. Herring obtain gas for the swimbladder by swallowing air at the surface. Herring denied access to the surface are unable to obtain swimbladder gas by secretion or by bacterial gas generation over one week. Such herring show a further gas loss over this period but are able to compensate while swimming for their high sinking factor of 1026 for one week. A gas forming bacterial rod was isolated from herring gills but it required 24 hours to generate gas at 20° C and did not generate gas at 10° C.

4. Living herring held in 1:10,000 concentrations of autonomic drugs released gas through the posterior swimbladder duct and later through the pneumatic duct in response to adrenalin and through the posterior duct in response to atropine and eserine. Removal of the brain or section of the spinal cord increased the pressure reduction required for gas release through the posterior duct above that of the controls. Spinal section caused gas release through the pneumatic duct in half of the fish tested. Injected acetylcholine favoured gas uptake behaviour, injected adrenalin inhibited such behaviour.

5. Application of autonomic drugs to the internal organs of decerebrate herring gave the following results. The sphincters anterior to the stomach caecum were closed by pilocarpine and opened by adrenalin. Parasympathomimetic
drugs caused contraction of the longitudinal and circular muscles of the stomach caecum and in low concentrations initiated peristaltic contractions. The pneumatic duct responded by constriction or peristalsis to acetylcholine or pilocarpine and was relaxed by adrenalin. Adrenalin caused the swimbladder valve to open permitting gas flow from the swimbladder to the stomach. The swimbladder valve does not control gas flow into the swimbladder. The pneumatic duct was shown to control the applied pressure at which gas flow through the duct began and ceased. The pressure of gas inside the swimbladder was raised by the application of pilocarpine to isolated swimbladders. Adrenalin caused redistribution of swimbladder gas so that most gas came to lie anterior to the dorsal fin. This effect was reversed by pilocarpine.

6. The percentage volume of swimbladder gas of Atlantic and Pacific herring was negatively correlated with percentage fat volume between 0.5 and at least 12% fat. At 3% fat content the swimbladder volume of both groups is 4.2% and at 12% fat 2.5 to 3.1%. The excess pressure of the swimbladder gas varied from 0 to 2.7 cm Hg and was not related to swimbladder volume over the range 2.6 to 5.4%. The sinking factors of Atlantic and Pacific herring 36 cm below the surface lay between 1001 and 1005 for fat contents up to 6% and were below 1009 for higher fat contents.
Analysis of the body components of 13 Pacific herring gave the following mean values: swimbladder gas 4.1%, density .0013 g/ml; fat 3.5%, density 0.926 g/ml; scales 0.5%, 1.966 g/ml; skeleton 1.2%, 1.993 g/ml; remainder of fish 90.6%, 1.057 g/ml. It was calculated that the mean force in dynes/ml acting on these fish due to swimbladder gas was 41.4 and to fat 3.3 both acting upwards and 4.6, 11.2, and 32.1 due to the scales, skeleton and rest of the fish respectively acting downwards leaving a mean net force of 3.2 dynes/ml downwards for which the herring had to compensate while swimming.

7. Herring were shown to be unable to use air taken from the surface as a source of oxygen which would permit survival in water of low oxygen content.

8. The results were used to calculate the sinking factors of herring of the Passamaquoddy Bay, N.B. region for February and August when mean fat contents, median herring depths by day and mean sea water densities diverge most widely. These calculations gave a sinking factor of 1016 at the median daytime depth of 10 m in August and 1018 at the median depth of 35 m in February.

Conclusions.

It is concluded that the anatomical modifications of the herring swimbladder and its ducts have functional significance. The stomach caecum can function as a pump
supplying the pressure required to drive ingested air to the swimbladder through the pneumatic duct. The caecum may also function as an accessory gas space temporarily storing ingested air. The stomach caecum although highly vascular is not used as a lung in low oxygen conditions and neither does it function as a site of bacterial gas generation.

The posterior position of the pneumatic duct may be related to the function of the caecum as a gas storage organ. The duct is normally liquid filled and controls the pressure needed to force gas through the duct by changes in the radius of the liquid/gas interface. The complex internal anatomy of the pneumatic duct is associated with its function of mechanically excluding food from the duct and with the necessity of retaining duct fluid within the duct during gas passage so that control of gas flow is restored immediately when this ceases. The circular muscle layer of the pneumatic duct allows active expulsion of gas bubbles and foreign matter from the duct. Gas loss from the swimbladder through the pneumatic duct is controlled by the swimbladder valve.

The herring swimbladder volume can be regulated according to the fat content of the fish to give almost neutral buoyancy at the surface. The reduction of swimbladder volume permitted by the high fat content and low volume of bony
matter allows the herring to undergo diurnal vertical migrations with only a moderate increase in density even in the absence of subsurface sources of swimbladder gas. The swimbladder is able to regulate the excess pressure of its contained gas irrespective of the volume of the gas within the range of 2.6 to 5.4%. The swimbladder, through control of the distribution of its gas, influences the posture of the fish. The posterior swimbladder duct allows removal of excess gas if this has been added to the swimbladder from the stomach below the surface and allows gas release under conditions of stress. The swimbladder gas cannot be replaced by secretion.

The swimbladder and its ducts respond to autonomic drugs and the nervous control of the function of the swimbladder and its ducts may be deduced if we assume that response to cholinergic drugs indicates innervation by cholinergic nerve fibres and to adrenergic drugs innervation by adrenergic fibres.

The motor fibres of the vagus apparently cause closure of the anterior sphincters of the stomach caecum and cause contractions of the caecum wall driving ingested air into the pneumatic duct. Simultaneously cholinergic nerve fibres may cause constriction of the pneumatic duct which although raising the applied pressure required for gas passage
reduces the possibility of food particles accidentally entering the duct. Under control of cholinergic fibres is also the removal of foreign matter and gas bubbles from the duct. Cholinergic nerve fibres by maintaining the tone of the circular muscles of the duct prevent the backward passage of gas from the swimbladder. The vagus nerve also supplies the swimbladder of teleosts and in the herring may control the excess swimbladder pressure through its cholinergic fibres. The posterior swimbladder duct is closed by contraction of its circular muscle coat in response to cholinergic nerve impulses.

Adrenergic nerve fibres, probably augmented by blood borne adrenaline in conditions of stress, causes release of gas through the posterior duct and later through the swimbladder valve. They relax the circular muscles of the pneumatic duct permitting quick passage of gas from swimbladder to stomach. An increase in blood borne adrenaline inhibits the uptake of gas from the surface. Redistribution of gas within the swimbladder to a more anterior position is controlled by the adrenergic nerve fibres.

Finally it is concluded that many of the modifications of the swimbladder system of herring are related to its diurnal cycle of vertical migrations and to strong predation pressure. The energy expenditure during vertical migrations is reduced by the low sinking factor increase with increase in depth and perhaps by the addition of gas from
the stomach to the swimbladder below the surface. Modifications related to predation include the presence of a large stomach caecum which can temporarily store gas so allowing rapid uptake of air and minimising exposure to surface predation. The posterior duct may allow gas release under conditions of predator stress, perhaps confusing the predators and also permitting more rapid sounding by increasing fish density. The possession of a posterior duct also removes all restrictions on the upward movement of herring which may thus be able to escape from midwater physoclist predators whose upward movements are restricted. The herring demonstrates how successfully the physostome condition can be adapted to the demands of the environment.
LITERATURE CITED

(Abbreviations from the World List of Scientific Periodicals, Butterworths:London. 1952)


