THE REGULATION OF BREATHING AND HEART BEAT IN TELEOST FISH

by

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Section I

GENERAL INTRODUCTION

The energy expended by the vast majority of living organisms is ultimately dependent on molecular oxygen. Respiration is the process by which oxygen is delivered to the tissues and carbon dioxide is removed from the body; also more recently it has come to include the series of chemical reactions that take place within the cell by which the energy of oxidation is made available for metabolic processes. The process of respiration is therefore vital to the organism, and must be able to supply oxygen at varying rates if the animal is to have a wide range of activity levels. The regulation of the rate at which oxygen is delivered for metabolism must inevitably be complex because of the diffuse nature of the respiratory system, but an important site of control must be at the site of oxygen and carbon dioxide exchange with the environment which, in teleost fish, is generally the gills.

Several reviews have been published covering various aspects of fish respiratory physiology. Krogh (1941) has reviewed respiration from a comparative standpoint, whereas Fry (1957) paid particular attention to oxygen consumption in fish, and stressed the importance of the effect of the environment in the measurement

of metabolic rate. Black (1951) excluded literature on oxygen consumption, but in a general review of respiration included papers on the respiratory functions of the blood. The regulation and mechanics of respiration have been extensively reviewed by Hughes and Shelton (1962).

The majority of fish extract oxygen from the water as it passes over the gills. In teleosts the water enters the mouth, is pumped over the gills and then passes out through the gill clefts. The branchial pump consists of the buccal cavity, the musculature of which acts as a force pump pushing water over the gills, and two lateral opercular suction pumps which draw water over the gills. The pressure relationships between the force and suction pumps are such that there is an almost continuous flow of water over the gills (Hughes, 1960, 1961; Hughes and Shelton 1957, 1958, 1962). The filaments and lamellae of the gills are arranged to produce a fine lattice dividing the buccal and opercular cavities (Bitjel, 1949).

The blood pumped by the heart passes through the gills, and comes into close contact with the respiratory epithelium, across which oxygen diffuses from water to blood, and carbon dioxide passes out from the body. The blood of fish has a high affinity for oxygen being fully loaded at quite low oxygen tensions, particularly in some freshwater forms (Black, 1940). The oxygen capacity of

the blood is generally of the order of 5 to 7 volumes percent, but the mackerel has an exceptionally high oxygen capacity of 15.77 volumes percent (Root, 1931).

The blood carries oxygen to, and carbon dioxide from the tissues. Little is known of the cytochrome system in fish, which is perhaps not surprising when it is realised that the regulation of respiration at the cellular level, although probably of great importance, is only, as yet, partially understood in mammals. Richardson et al (1962), however, have shown that although the cytochrome content of the mitochondria is slightly less, the cytochrome pattern in fish appears similar to that found in mammals.

Respiration in water as opposed to air will be largely influenced by the differing properties of the two media. Water is approximately 800 times as dense as air, contains only one thirtieth the amount of oxygen and the ratio of the diffusion coefficients for oxygen in air and water is $1{:}3 \times 10^{-5}$ (Krogh, 1941; Fry, 1957), and yet fish are able to maintain rates of oxygen consumption that are comparable with values for most air breathing poikilotherms (cf. Fry and Hart, 1948; Flemister, 1958). This must be largely due to the ability of the respiratory apparatus of fish to utilise up to 80% of the oxygen in the water that passes over the gills (van Dam, 1938) compared with an utilization of the order of 25% in air breathing forms. The high efficiency of the respiratory apparatus in removing oxygen from the water,

particularly necessary in so dense a medium, is related to four important characteristics of fish respiration:

(i) The anatomical arrangement of the gills allows all the water to come into close contact with the gill lamellae, reducing the necessity for the diffusion of oxygen in the water to a minimum.

(ii) The flow of water across the gills is almost continuous, allowing more time for gas exchange than if the same volume of water was passed over the gills intermittently (van Dam, 1938).

(iii) The flows of water and blood at the respiratory surface are in opposite directions. This is the counter current principle emphasised by van Dam (1938) and Hughes and Shelton (1962).

(iv) The high affinity of fish blood for oxygen.

Studies on the respiratory characteristics of different species of fish have indicated that certain adaptations of the basic respiratory plan enable higher activity levels in some species. Comparisons between active fish and more sluggish forms have shown that the more active species tend to have more haemoglobin (Hall and Gray, 1929), a higher blood sugar level (Gray and Hall, 1930), a larger gill area (Gray, 1947) and a higher rate of oxygen consumption of excised brain tissues (Vernberg and Gray, 1953). Peiss and Field (1950) have shown

that tissue respiration in the arctic cod is adapted to low temperatures showing a higher consumption of oxygen in the temperature range 0 - 10°C. than tissues from the golden orfe. These physiological adaptations have been correlated with high rates of oxygen consumption, and apparently enable a higher level of maximum activity in the particular species.

The most extensive and detailed work on the control and regulation of respiration in fish has been carried out on the mechanics and control of the breathing movements. The respiratory muscles of teleosts are innervated largely by the occipito-spinal nerves and branches of the V and VII cranial nerves. The respiratory movements are under the control of a rhythmically active respiratory centre situated mainly in the medulla (Shelton, 1959, 1961). This centre is envisaged as a loosely integrated group of neurones, with no distinct spacial grouping of the neurones in relation to phases of the breathing cycle. There is very little evidence to suggest that the generation of the rhythmic activity in the respiratory centre is dependent on extra-medullary stimulation (Hughes and Shelton, 1962). Powers and Clark (1942) however, considered that the IXth cranial nerves were of fundamental importance in the initiation of the respiratory rhythm. They found that bilateral sectioning of the IXth or IXth and Xth cranial nerves of the brook trout, rainbow trout and blue gill resulted in a cessation of the breathing movements. Breathing

however, although of a gasping nature, returned within a minute of the nervous section, and the transections themselves, carried out in the region of the gills, were always associated with a loss of blood. Shelton (1959) found that when the IXth and Xth cranial nerves of the tench were cut bilaterally as they emerge from the brain, respiration was not arrested, and therefore these nerves were not of fundamental importance in the initiation of respiratory activity in the tench. These sections however, did result in an increase in the amplitude of the respiratory movements, and therefore it appears that the IXth and Xth cranial nerves are of importance in the modification of rhythmic activity in the respiratory centre.

The regulation of respiration in fish to meet changes in either the activity level of the animal or in the environment have generally been restricted to the measurement of metabolic rate or the responses of the breathing apparatus. Activity raises the oxygen consumption of the animal and in common with excess carbon dioxide or decreased oxygen content in the ventilation stream, results in an increase in the volume of water ventilated by the breathing apparatus. The nervous pathways and location of the receptors involved in these changes in respiration are, as yet, unknown, except that it has been shown that there are chemosensitive areas in the medulla of teleosts which are sensitive to carbon dioxide, but the exact location of these has yet to be determined (Hughes and Shelton, 1962). The results obtained by

Shelton (see Hughes and Shelton, 1962) have demonstrated that the changes in the breathing movements in response to variations in gas tensions in the water passing over the gills are to some extent independent of receptors situated in the gills and innervated by the IXth and Xth cranial nerves.

The necessity for a relationship between cardiac output and ventilation volume, especially in fish where so much energy is required to ventilate the gills with water, has been stressed by Hughes and Shelton (1962). Some aspects of this relationship have already been demonstrated in both elasmobranchs and teleosts. In elasmobranchs a simple numerical ratio between heart and breathing rates has been recorded by Lyon (1926) and Satchell (1960). The relationship in teleosts appears more complex, a simple numerical ratio appearing rarely and then only under certain conditions (Shelton and Randall, 1962). In both elasmobranchs and teleosts the relationship between heart rate, cardiac output and blood flow through the gills is not clearly understood. The nervous control of the heart beat appears to be entirely inhibitory, affecting heart rate. Although acceleration of heart rate is a common occurrence in teleosts, tachycardial mechanisms are unknown. Increased cardiac output is possibly augmented mechanically by increased venous return, producing an increase in stroke volume rather than an increased heart rate (Johansen, 1962). Small doses of adrenaline have been shown to cause a rise in blood

pressure in the eel (Mott, 1951), constricting the systemic vessels (Keys and Bateman, 1932). This pressor effect is very prolonged.

The gills are undoubtedly an important site for the regulation of respiration, and the amount of oxygen crossing the respiratory epithelium from water to blood will be influenced by the relative flows of blood and water and the tensions of oxygen and carbon dioxide in the two media. The purpose of the present work was to study the regulation of the heart beat and breathing movements of teleost fish under varying environmental conditions, in the hope that the results would lead to a clearer understanding of the control of respiration at the site of oxygen and carbon dioxide exchange with the environment.

Section II

GENERAL METHODS

The majority of experiments were carried out on the tench, Tinca tinca, L., but several other species of freshwater fish were used, these included dace, Leuciscus vulgaris; chub, Leuciscus cephalus; roach, L. rutilus; eels, Anguilla anguilla; and goldfish, Carassius auratus.

Stocks of fish were kept in large slate tanks supplied with a continuous flow of aerated, sand filtered tap water. The tanks were cleaned and washed with a solution of potassium permanganate before the introduction of each new group of fish. Worms, bread and boiled salted liver mixed with baby food were fed to the fish at intervals according to the season and their particular needs; excess and decaying food was siphoned from the bottom of the tank. The health of the experimental animal is of primary importance (Fry, 1957), and only animals in a good condition were used in the experiments. To this end considerable attention was paid to the upkeep of the aquaria.

Seasonal as well as individual variations were noted in the stocks of fish, for example the heart rate was, on average, lower in winter than in summer, but as the experiments were designed to measure relative rather than absolute values in each fish, no

account of seasonal variation is included in the results. Attempts were made to stabilise the thermal history of the fish by performing the experiments at the same temperature as the stock tank, which in all cases was at tap water temperature.

When preparing for an experiment a fish was selected from the stock tank, anaesthetised, and placed in one of a series of clamps that were used to restrain the animal and fitted into the experimental tank.

The fish was anaesthetised by placing it in either an aqueous solution of 1% urethane or a 0.01% solution of MS 222 Sandoz. In a few experiments injections of nembutal (sodium pentobarbital) or a 1:1 mixture of paraldehyde and oleum arachis were used. The fish were anaesthetised to stage II plane 2 anaesthesia, as described by McFarland (1959), in which there is a total loss of equilibrium, and a lack of reaction to external stimuli, particularly, in this case, that there was no reaction to pinching of the pectoral fins with a pair of forceps. For light anaesthesia during experiments, stage I anaesthesia (McFarland, 1959) was used, in which there is a total or partial lack of response on the part of the fish to external stimuli, but equilibrium is undisturbed.

Urethane appears to be the most suitable of the four anaesthetics used for it "produces a rapid and profound narcosis with little change in circulation or respiration", Sollman, 1957, p. 931. Dosage levels for injection with this drug proved

difficult to determine, and large doses of up to 2 ml per 100 grm of fish of a saturated solution of urethane in saline were often needed to produce the required level of sedation. Bathing the gills or placing the fish in a bath of the anaesthetic was an easier method of inducing anaesthesia. In this case an 0.2% aqueous solution was used for light anaesthesia, and a 1% solution for deep anaesthesia. The higher concentration often produced a lengthening of the "mouth open" phase of the breathing cycle in the tench.

Nembutal, in common with other barbiturates, impairs the sensitivity of the respiratory centre of mammals to carbon dioxide (Sollman, 1957). On injection into fish, there is a delay of up to 30 minutes before the full action of the anaesthetic is seen, which can lead to overdosage by underestimating the effect of the drug. Once again dosage levels were difficult to determine; 0.5 ml of 2 mg/ml of nembutal in saline injected into the dorsal musculature had no effect on a 90 grm fish, whereas a 1 ml injection killed a 74 grm fish within 45 minutes. In general, because of its action on the respiratory centre of mammals and the difficulties inherent in injection, nembutal was thought to be unsuitable for these experiments.

Paraldehyde in a 1:1 mixture with oleum arachis injected into the rectum of a tench (0.6 ml per 100 grm of fish), produced prolonged anaesthesia in which skin reflexes and equilibrium were lost. Unfortunately operative procedures performed under this

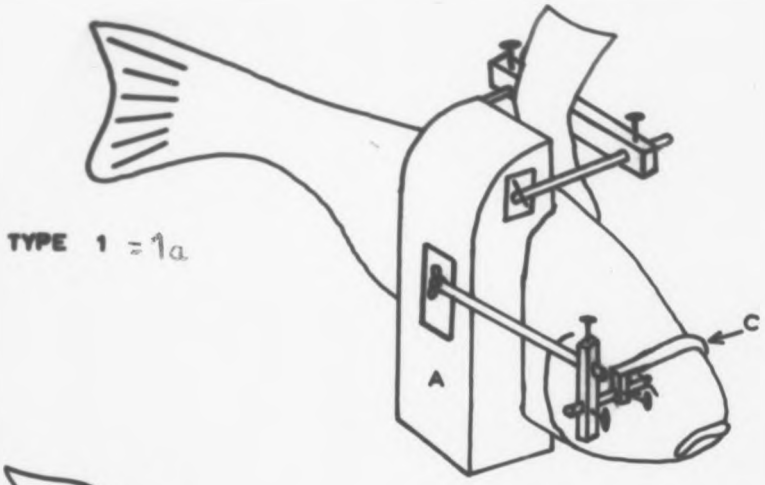
drug often resulted in excessive bleeding.

The effects of MS 222 Sandoz, a meta-amino-benzoic acid ethylester in the form of a soluble salt, as a general anaesthetic have been studied in some detail (Gilbert and Wood, 1957; Ball and Cowan, 1959; Serfaty et al, 1959; Randall, 1962; Campbell and Davies, 1963). The properties of this compound as an anaesthetic have led to its widespread use in physiology, and a more detailed account of the effects of MS 222 on some aspects of physiological function in the tench will be given in a separate section.

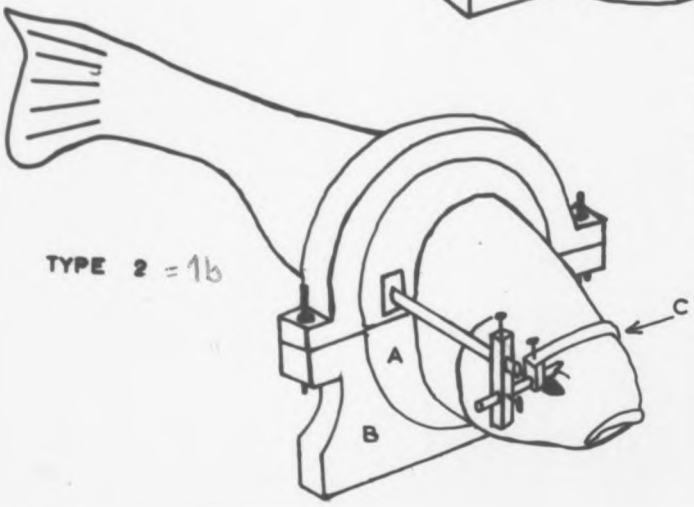
The fish were anaesthetised either by placing them in water containing MS 222, or by forcibly ventilating the gills with an aqueous solution of the compound. The concentrations of the anaesthetic required to produce a given level of sedation were variable, but, in general, bathing a tench in a solution of 20 mg per litre made the animal unresponsive to external stimuli, whereas a concentration of 100 mg per litre caused rapid loss of equilibrium, with no movements except those of breathing. Concentrations of 200 mg per litre were lethal if the fish remained in the anaesthetic for longer than 30-40 minutes.

The holders used for restraining the fish during experiments were of three types. The first two types were similar in design but varied in detail, and were used to restrain all the fish used except the eel. This fish, because of its shape, required a

Figure 1. The clamps used for holding the fish during the experiments.

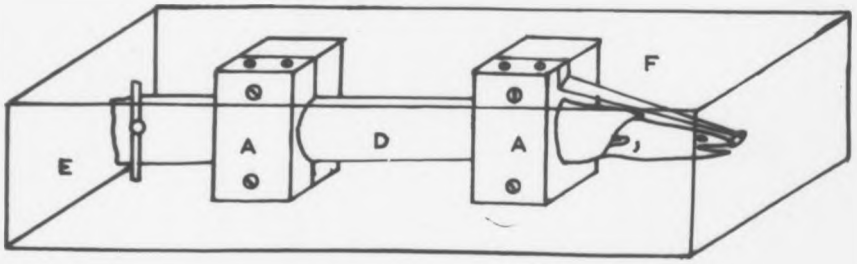


TYPE 1 = 1a



TYPE 2 = 1b

A. Trunk clamp
B. Perspex ring
C. Head clamp
D. Rubber tube
E. Mohr clip
F. Brass plate



EEL CLAMP

1c

special type of holder. (Fig. 1c)

The first type of holder consisted of a wooden clamp around the trunk, and a brass head clamp that fitted across the supra-orbital ridges of the fish. The trunk clamp was in two halves, each section being shaped on the inner surface to match the body of the fish, and the halves were held together by two adjustable bolts. The brass head clamp was fixed to one half of the wooden trunk clamp, which in turn was attached to a base plate that slotted into the bottom of the experimental tank. The head clamp was adjustable to fit varying sizes of fish. The free half of the trunk clamp was subsequently replaced by an adjustable cloth strap. (Fig. 1a)

The second type of holder was essentially similar to the first except that the trunk clamp was circular and enabled rotation of the fish around the long axis of the body. (Fig. 1b) The trunk clamp fitted inside a ring of perspex, again in two halves and held together by two adjustable bolts, one half of which was permanently fixed to the bottom of a tank. The head clamp resembled that described for the first type of holder.

The third type of holder, used to restrain eels, was based on different principles from the two already described. The eel was first placed inside a length of bicycle inner-tube, which was then sealed at the posterior end by a Mohr clip. The tube fitted closely over the body but left the head and pectoral fins free. The fish and tube were then placed in two wooden clamps shaped

to the body of the eel, and fixed to the lid of the experimental tank. These clamps surrounded the body anteriorly just behind the pectoral girdle and posteriorly in the region of the anus. The head was held by passing a cotton round the premaxilla and tying the upper jaw to a brass plate projecting from the most anterior of the two clamps.

The experimental tanks were of between $1\frac{1}{2}$ and 3 litres in volume, and were all made of perspex. Some tanks were sealed, others were open at the top, depending on the nature of the particular experiments. Although the experimental tanks were only between 15 to 20 times the volume of the fish, and experiments lasted for several hours, depletion of oxygen from the water in the tank by the fish was avoided by a continuous flow of water through the tank, either directly over the gills from a tube inserted into the mouth, or by a tail to head flow through the tank, which avoided forced ventilation of the gills. Water was passed through the tank at rates of a litre every three or four minutes, and the gills were perfused at rates of between 100 and 400 ml/ minute.

The experiments were carried out at tap water temperature, the total range for all experiments being between 10 and 18°C, the temperature never varying more than 1°C during any one experiment.

The heart rate and the rate and amplitude of the breathing movements were measured in many of the experiments under a variety of conditions. Three methods were used to determine the rate and amplitude of the breathing movements, each of which utilised

only a small percentage of the energy expended in performing the breathing movements. In all cases movements of a part of the respiratory apparatus (lower jaw or operculum) were taken as representative of the rate and amplitude of the breathing movements as a whole. A length of cotton, tied to either the lower jaw or an operculum relayed the breathing movements to the recording system. Originally the movements were recorded mechanically with a straw lever on a smoked drum, but later mechano-electric transducers were used, the output from these being conveyed either to an Ediswan pen recorder or an Oscilloscope (Tektronix 502 or Cossor 1049 Mk IIIA). Two types of transducer were used, firstly an RCA 5734 or latterly a glycerine trough transducer (Popple, see Donaldson, 1958, p. 487), made by D. R. Jones of this department.

A perspex extension arm was attached to the anode of the RCA 5734 mechano-electric transducer in order that the movements of the lower jaw or operculum could be reduced to a level suitable for recording with this instrument. The response of the second transducer was approximately linear for deflections of up to 1 cm at the tip of the recording arm. The force required to move the arm was small, and again approximately linear for deflections of up to 0.6 cms, but above this the force required plotted against deflection tended to become asymptotic. Movements of between 0 and 0.6 cms at the tip of the recording arm were adequate, however, even when recording directly from the operculum. The transducer

was capable of recording movements at frequencies of up to 400 cycles per minute.

The heart rate was determined from continuous records of the electrocardiogram (E.C.G.). This was recorded using a unipolar system. A steel needle electrode insulated, except at the tip, with araldite or varnish, pierced the skin of the fish mid ventrally in front of the pectoral girdle, being pushed forward to come to lie ventral to the heart. An indifferent electrode was placed in the water of the tank. The signal recorded was amplified by an Ediswan E.P.A. amplifier or with a Tektronix preamplifier type 122, and the output connected to the second pen on the Ediswan pen recorder (the first pen recording the breathing movements), or alternatively displayed on an oscilloscope. To avoid damage to the heart when exposed the relatively large steel electrode was not used, but was replaced by either a length of .010" gauge silver wire or a short length of saline soaked cotton, which could be placed on or near the heart without risk of injury.

Section III

THE EFFECT OF MS 222 SANDOZ ON THE HEART RATE AND BREATHING OF TELEOST FISH

A. INTRODUCTION

MS 222 Sandoz was used as a general anaesthetic during many of the experiments. It was found that bathing the fish in an aqueous solution of this compound induced anaesthesia quickly and efficiently, and that the process was easily reversed, for even after respiratory failure the animal could be revived without noticeably affecting its health, by perfusing the gills with oxygenated water.

The effects of several anaesthetics on fish have been studied by McFarland (1959, 1960). The changes produced by anaesthetics when used in sublethal concentrations during physiological experiments are of considerable importance (Wilber, 1962), but because anaesthesia is usually a means to an end in experimental zoology very little other information has accumulated on the effects of various narcotics on fish. In these experiments it was particularly important to know if MS 222 Sandoz was having any effect on respiration and heart rate, and therefore a series of experiments was designed to record the effects of various concentrations of this anaesthetic on the heart rate and the parameters of the

breathing movements in these particular animals.

B. THE EFFECT OF MS 222 SANDOZ ON THE HEART RATE AND BREATHING OF THE INTACT FISH

(i) Method

The experiments were carried out on 13 tench, weighing between 34 and 144 grms. A known concentration of an aqueous solution of MS 222 Sandoz was passed through a sealed experimental tank of 1750 ml volume for periods of up to 90 minutes. The animal breathed the water passing through the tank, and the breathing rate and amplitude, recorded on an RCA 5734 transducer, and the heart rate were measured at intervals of five minutes before, during and after bathing the fish in the anaesthetic. Six concentrations of the anaesthetic were used from 25 to 200 mg per litre. The solution of the anaesthetic in the tank was completely changed every 3 to 5 minutes, and was aerated before entering the tank, so that the oxygen level in the tank remained at air saturation values. In high concentrations respiratory and cardiac collapse was common within 20 or 30 minutes of the introduction of the anaesthetic into the tank, in which case the flow of anaesthetic through the tank was stopped, and the gills were forcibly perfused with oxygenated tap water to revive the fish.

(ii) Results

(a) The Electrocardiogram

The primary object of recording the E.C.G. in these

Figure 2.

Variations in the configuration of the E.C.G. of the tench with changes in the position of the recording electrode.

Position of recording electrode.

a) Auricular-ventricular junction

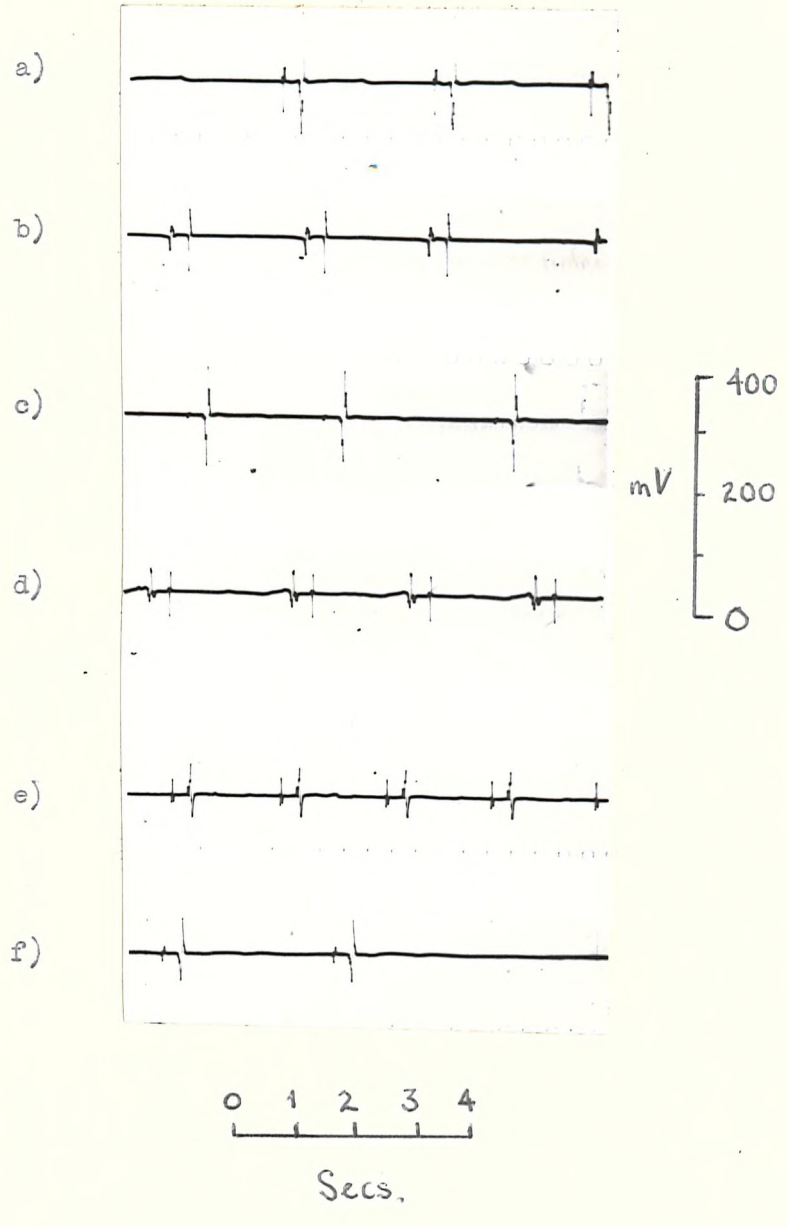
b) Ventral surface of auricle.

c) Ventral surface of ventricle.

d) Between auricle and ventricle.

e) Ventral to sinus venosus.

f) Ventral surface of bulbus.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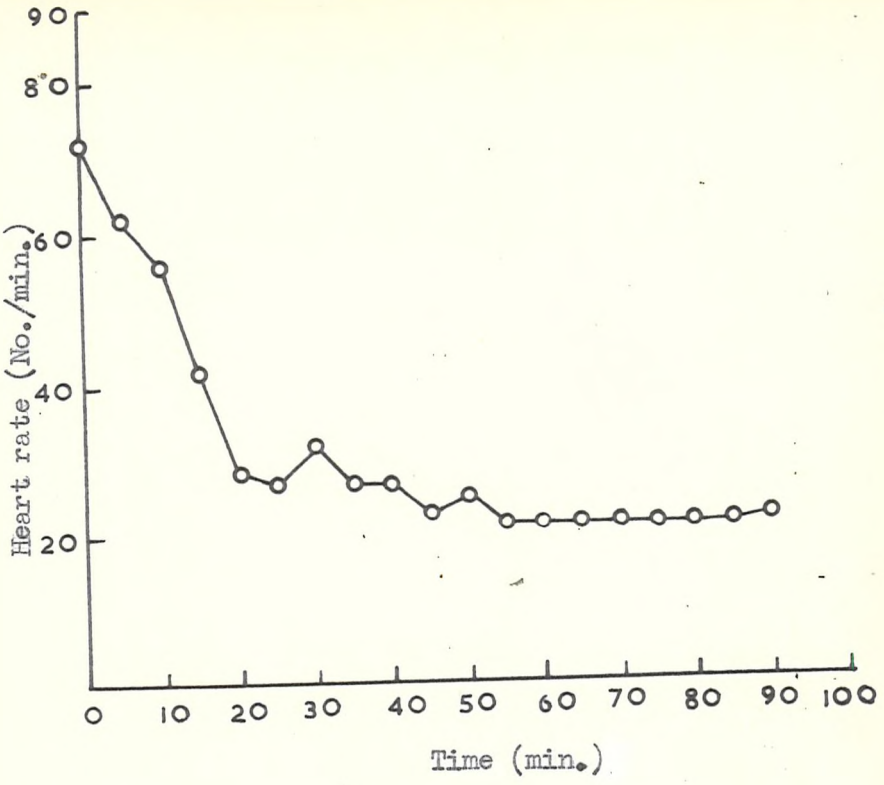


Figure 3. The effect of the experimental procedure on the heart rate of the tench. The fish was placed in the experimental tank at zero time and then left undisturbed while the heart rate was recorded at intervals of 5 minutes.

experiments was to determine heart rate, and not to study the E.C.G. itself. Adequate descriptions of the E.C.G. of variety of teleosts have been given by Kisch (1948), Oets (1950), Serfaty and Raynaud (1956, 1957). In some of the later experiments, however, the time relationships between the P and QRS waves were determined, as well as heart rate, from records of the E.C.G.

The time relationships between the various component waves of the E.C.G. are not affected by the position of the recording electrode, but the size and shape of these waves does alter with the position of the recording electrode. (Fig. 2)

(b) The effect of the experimental procedure on the fish

It has been shown (Fry, 1957) that handling, changes in temperature, seasonal variations, visual and auditory stimulation and the health of the experimental animal can all affect metabolic rate, and therefore the whole of respiration. For this reason it was necessary to have some knowledge of the effects of the experimental procedure on the fish. The heart and breathing rates and the breathing amplitude were always extremely high if recorded immediately after setting up the animal for the experiment. If, however, the animal was left undisturbed in the tank, through which a constant flow of oxygenated water was passing, the heart rate and the parameters of the breathing movements would fall to a low and constant value within an hour. Figure 3 shows the changes in heart rate that occur during the first 90 minutes after the fish has been prepared for an experiment.

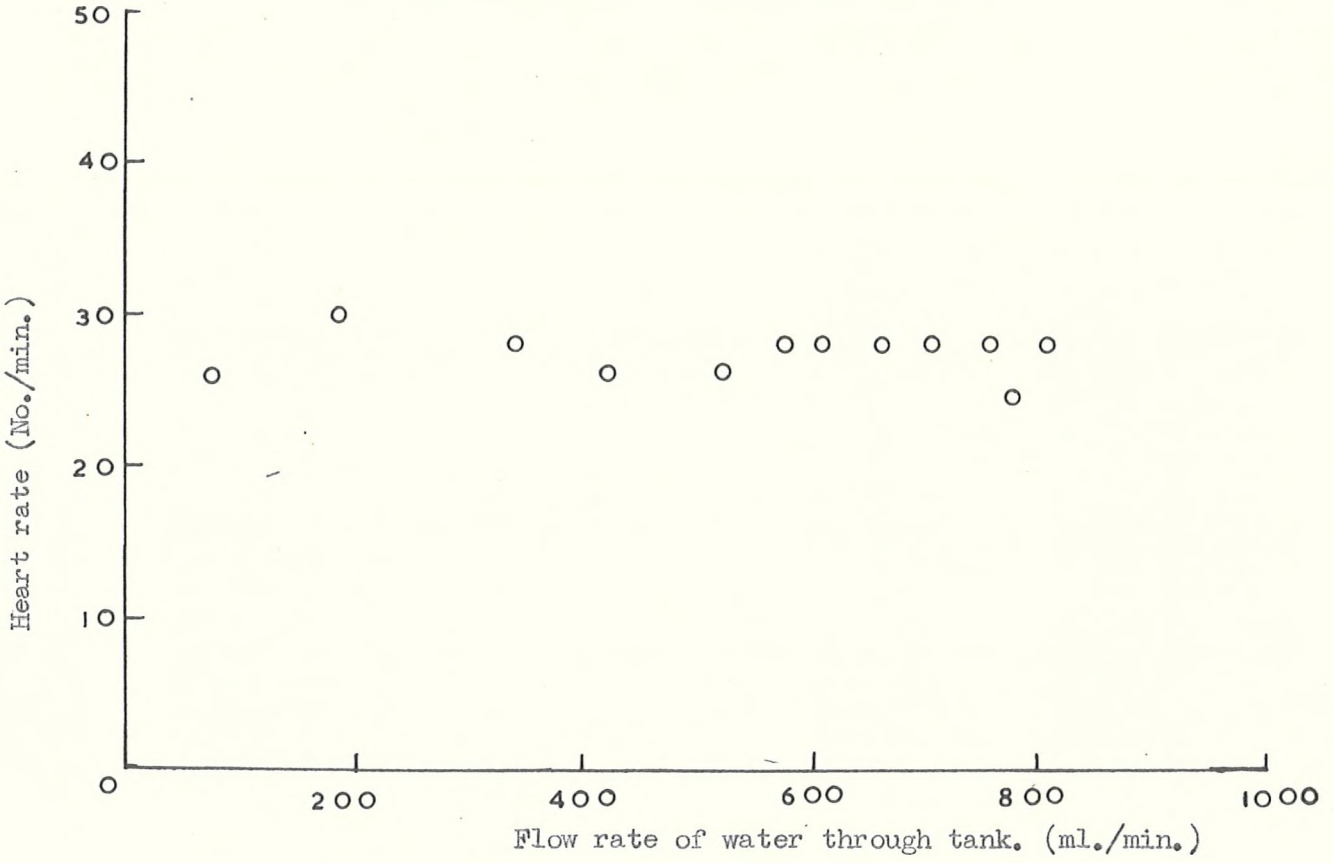


Figure 4. The effect of variations in the flow rate of water through the experimental tank on the heart rate of the tench.

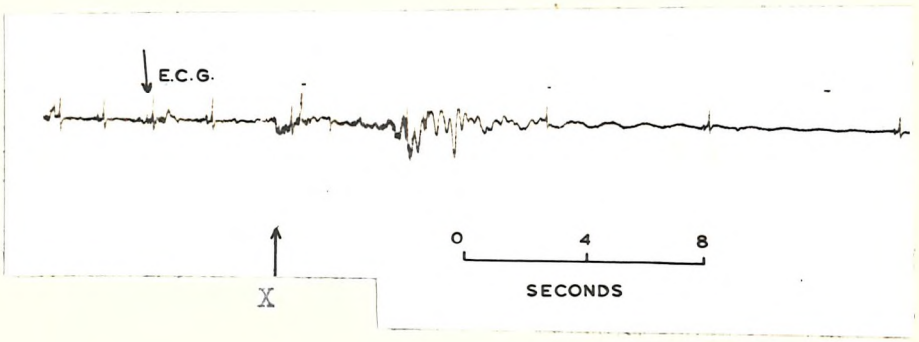


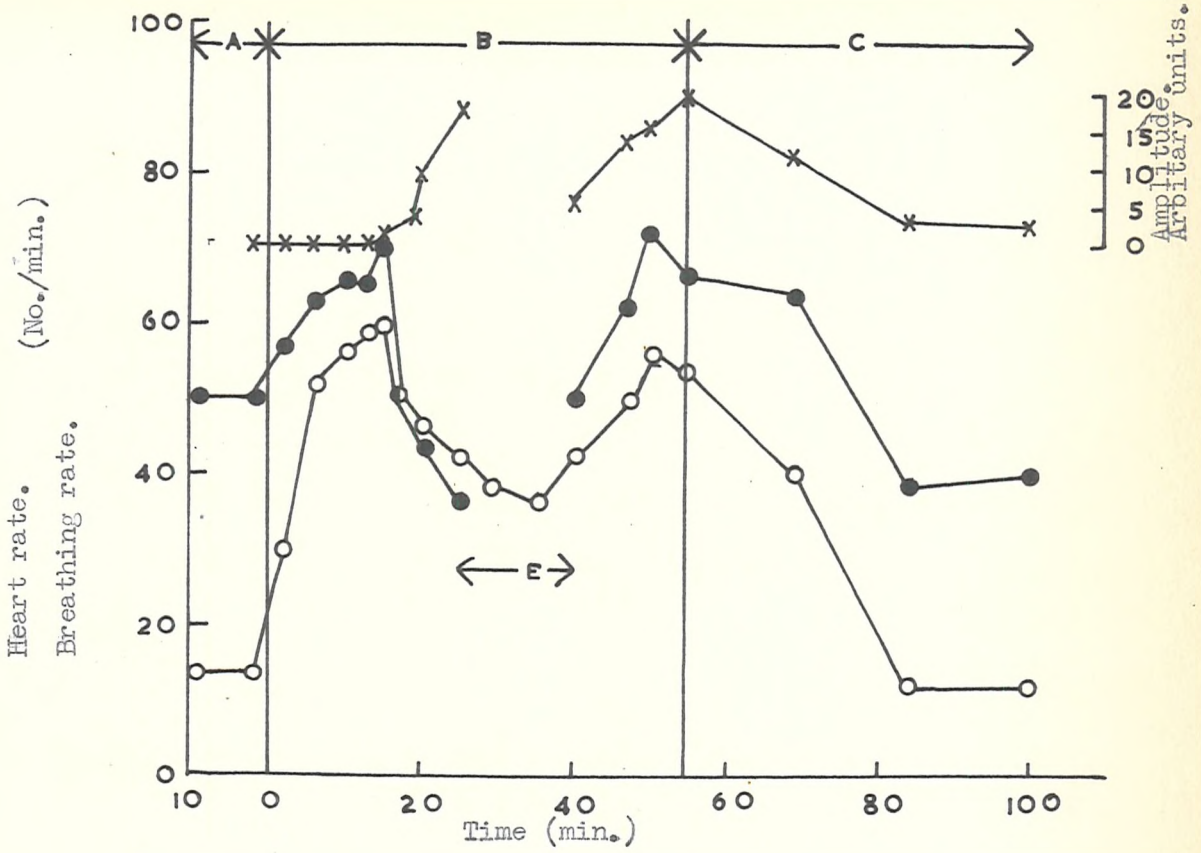
Figure 5. The effect of sudden changes in the flow rate of water over the gills on the heart rate of the tench. Gills perfused with water through a tube inserted into the mouth.

X, mouth forced open by a sudden and large increase in the water flow rate.

If the experimental conditions remained constant, and the fish was left undisturbed, it would remain inactive in the tank for long periods of time. Under these conditions the heart and breathing rates and the breathing amplitude were constant. The term "resting" fish will be used for an animal which is restrained in the experimental tank, but is inactive and has a low and constant heart rate.

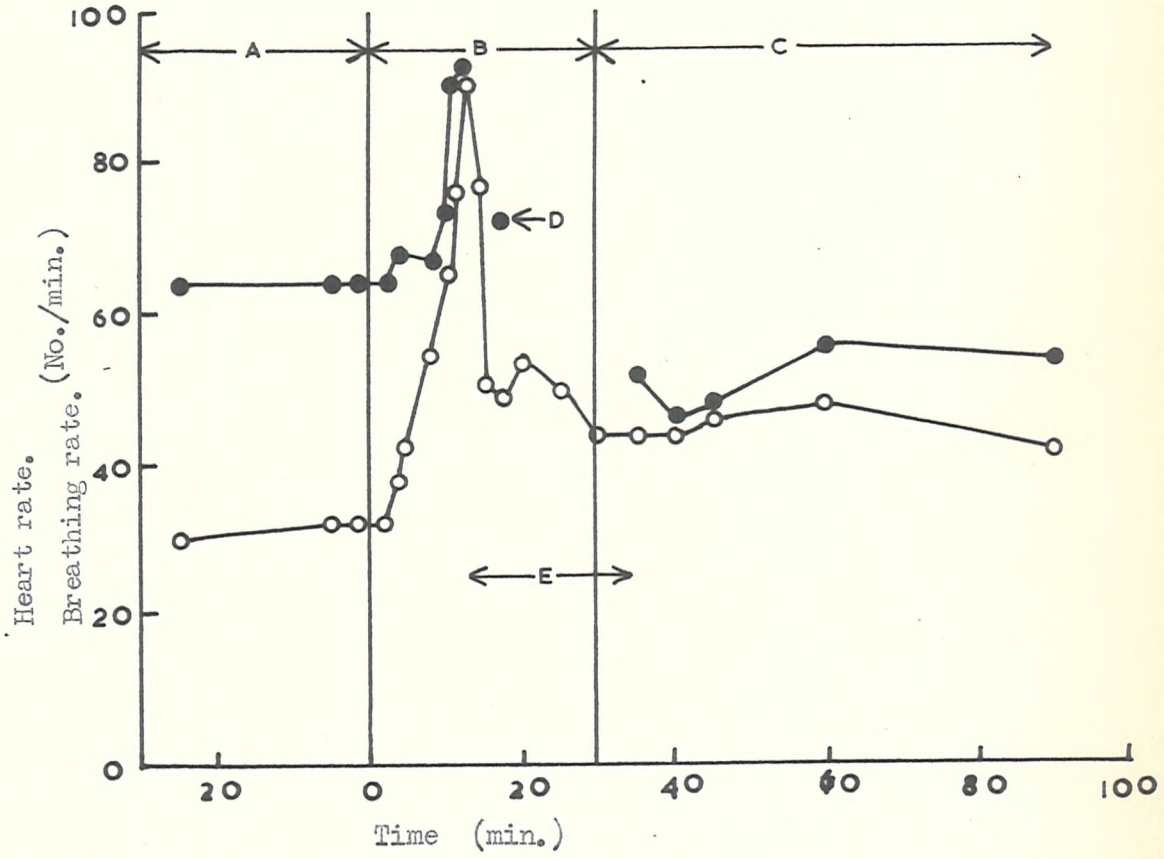
Variations in the flow rate of water in a tail to head direction through the experimental tank of between 75 and 500 ml per minute had no effect on the heart rate of a resting tench. (Fig. 4)

Forced ventilation rates of between 100 and 400 ml per minute, produced by perfusing the gills with oxygenated water through a rubber tube inserted into the mouth of the fish, had no effect on heart rate. Sudden changes in the rate of flow of water over the gills, or keeping the mouth open for long periods of time, however, caused a considerable decrease in heart rate. (Fig.5) Therefore, when forcibly ventilating the gills with water during the MS 222 Sandoz experiments and all subsequent experiments, the flow of water over the gills was maintained at a constant rate, within the limits mentioned above, and passed into the buccal cavity through a thin rubber tube which fitted easily into a small opening between the upper and lower jaws and caused a minimum of interference to the breathing movements.



Breathing rate .
Heart rate.
Breathing amplitude.

Figure 6a. The effect of MS222 Sandoz on the heart rate and the breathing movements of the tench.
A and C. No Anaesthetic. B. MS222 present in concentration of 50mg/litre. E. Period of respiratory failure.



Heart rate.

Breathing rate.

Figure 6b. The effect of MS 222 Sandoz on the breathing and heart rate of the tench.

Aand C. No anaesthetic. B. MS 222 present in concentration of 200 mg/litre. E. Period of respiratory failure. D. Momentary recovery of breathing.

(c) The effect of MS 222 Sandoz on the heart and breathing rates and the breathing amplitude of the intact fish

Because of the effect of handling on the heart and breathing rates of the fish, records for these and all subsequent experiments were not taken until an hour had elapsed after setting up the fish for an experiment.

In solutions of 20 mg/litre of the anaesthetic the responses of the fish to external stimuli are reduced or absent, equilibrium is usually retained, but is occasionally disturbed. Fish have been kept in this concentration of MS 222 for over 24 hours without adverse effects, except that the animal appears very susceptible to oxygen lack. The heart rate is increased, as is the breathing rate and amplitude.

Figure 6a shows the changes in heart rate and the parameters of the breathing movements of the tench that take place when a concentration of 50 ppm of MS 222 is passed through the experimental tank. There is a rapid increase in the heart and breathing rates, the heart rate being doubled within two minutes, and a more variable increase in breathing amplitude. Increases in breathing rate are paralleled by similar increases in heart rate, and when the breathing rate falls and eventually stops there is a corresponding decrease in heart rate.

In concentrations of 100 mg/litre there is a complete loss of equilibrium within 5 to 10 minutes, a sharp rise in heart and breathing rates and a more variable increase in breathing amplitude.

This is followed by respiratory collapse and an associated fall in heart rate, leading eventually to respiratory and cardiac failure within 30-40 minutes. Solutions of 200 mg/litre are lethal if the fish remains in this concentration for longer than 30 minutes.

In general the effect of the anaesthetic on the heart and breathing rates of the tench was more pronounced in higher concentrations of the drug. High concentrations often produced heart rates of 90 per minute or more (Fig.6b), representing a 200% increase of the resting rate.

Variations in the amplitude of the breathing movements did not usually take place directly on the addition of MS 222 Sandoz to the respired water, but were more generally associated with a decline in respiratory rate.(Fig. 6a) It therefore seems probable that the changes in the amplitude of the breathing movements were not directly related to the presence of MS 222 Sandoz, but were a side effect associated with the conditions that lead to respiratory collapse.

C. THE EFFECT OF MS 222 SANDOZ ON THE ISOLATED HEART.

(i) Introduction

Bathing a fish in a solution of MS 222 accelerates the heart rate of the tench. This could be the result of a direct effect of the drug on the heart tissues, or by reflex stimulation of heart rate. A series of experiments was therefore carried out to determine the direct effect of MS 222 on the fish heart.

(ii) Methods

The experiments were carried out on the perfused isolated hearts of the tench, trout, roach and an eel. The hearts were cannulated via the sinus venosus and isolated from the body. They were suspended from the glass cannula, and a heart clip was attached to the ventricle and the beat recorded on a smoked drum. When the hearts were perfused with Young's teleost saline (Lockwood, 1961), the preparations seldom lasted for longer than an hour. Various changes were made to this saline, and eventually a saline of the following formula was arrived at empirically, which maintained isolated hearts in viable condition for up to 32 hours.

Saline:	6.5 grms NaCl	
	0.14 grms KCl	
	0.12 grms $CaCl_2$	made up in a litre
	1.0 grms glucose	of distilled water
	0.125 grms $MgCl_2$	
	0.2 grms $NaHCO_3$	

The saline was kept at room temperature, and continually aerated before use.

The saline was used not only for these experiments but also as a washing fluid during all operative procedures, as well as a solvent for anaesthetics when injected into the body of the fish (except paraldehyde). Other fish Ringers such as those of Burnstock (1958), and Labat et al (1961), did not come to my notice until after the completion of the experiments. These salines were somewhat similar to that used in these experiments

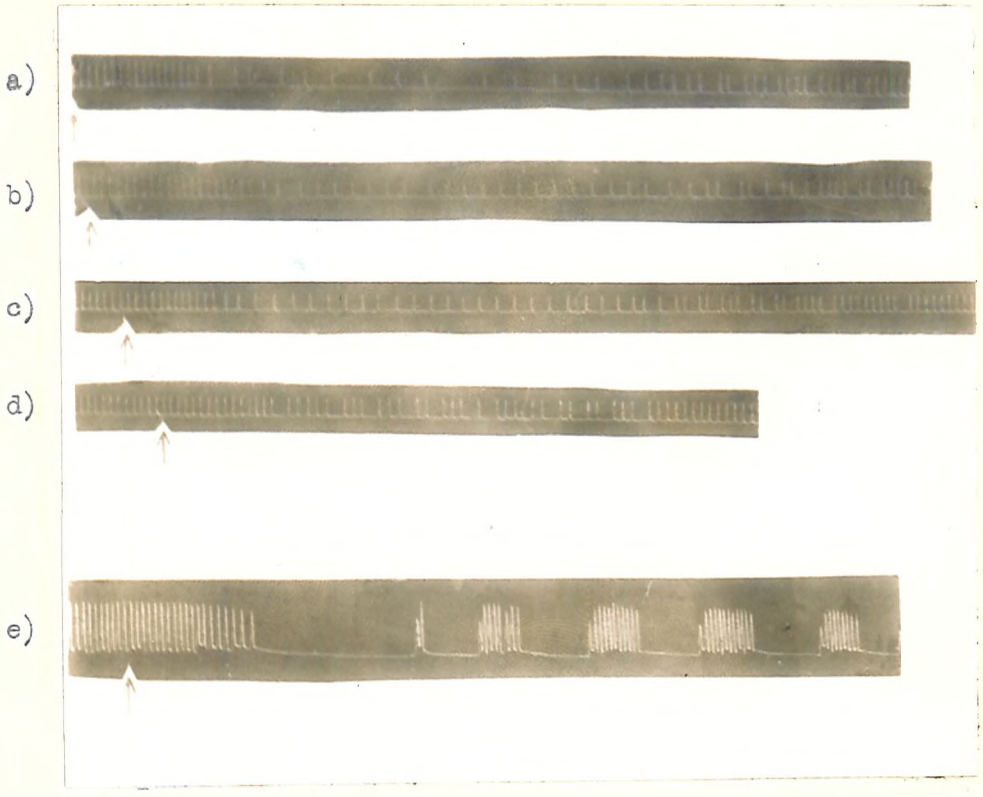


Figure 7a. The effect of MS 222 Sandoz on the isolated heart of the trout. Ventricular beat of heart recorded on a smoked drum. 1ml. of various concentrations of MS 222 added to saline in heart cannula.

a) 250 mg/litre.

b) 125 mg/litre.

c) 62 mg/litre.

d) 30 mg/litre.

7b. Isolated heart of the eel. Ventricular beat recorded.

e) 1ml. of 100mg/litre MS 222 in saline added to cannula.

except for the addition of HPO_4^- to both salines and the absence of Mg^{++} ion from that of Labat et al.

Fish blood clots rapidly, and heparin dissolved in fish saline was often used to prevent clots appearing in the heart. The anticoagulant was either injected into the sinus venosus before dissection, or added to the cannula, as required.

1 ml of solutions of MS 222 Sandoz in saline from concentrations of 15 to 250 mg/litre were then added to the saline in the cannula. The effect of this compound on the rate and amplitude of beat of the isolated heart was recorded on the smoked drum.

(iii) Results

The addition of 1 ml of saline to the cannula had no effect on the rate and amplitude of beat of the isolated heart; if, however, the saline contained MS 222 Sandoz both the amplitude and frequency of the beat decreased. The effect was more marked when higher concentrations of the anaesthetic were added to the cannula. The beat rate of the isolated heart of the trout recorded in figure 7a is 11 per minute; the isolated hearts of the tench and roach had beat rates of between 10 and 17 beats per minute, and that of the isolated eel heart was 14 per minute. The addition of 1 ml of a concentration of 62 mg/litre of MS 222 in saline reduces the beat rate of the trout heart to 4-5 per minute, whereas concentrations of 250 mg/litre reduced the rate to 2 per minute. (Fig.7a) Similar decreases were observed in the beat rate of the isolated hearts of the other species of fish. The

effect of the anaesthetic was most marked 4 to 6 minutes after its addition to the cannula, and the normal beat rate returns within 10 to 15 minutes. MS 222 occasionally caused an auricular-ventricular block, the auricle beating continuously, the ventricle only occasionally in small bursts of 2 or 3 contractions. This was particularly obvious in the single experiment performed on the eel heart. (Fig. 7b)

D. THE EFFECT OF VARIOUS NERVOUS TRANSECTIONS ON THE RESPONSE OF THE HEART TO MS 222 SANDOZ

(i) Introduction

The effect of MS 222 Sandoz on the heart rate in the intact fish is obviously not the result of a direct effect of the drug on the heart tissues, as the effects of MS 222 on the heart rate in the intact fish and the isolated heart are in direct opposition. It is possible, therefore, that in the intact animal the acceleration of the heart could be mediated via a nervous reflex, acting either directly on the heart, or on some part of the blood system to produce changes that result in an increased heart rate. The most relevant nerves in this respect must be the Xth cranial nerve, a branch of which innervates the heart, and the Vth and VIIth cranial nerves which innervate the respiratory musculature .

(ii) Methods

Operative procedures were carried out under urethane anaesthesia, after which the animal was allowed to recover in

oxygenated freshwater. The animal was placed in an open top experimental tank and a small hole was made in the roof of the skull to expose the roots of the IXth and Xth, and the Vth and VIIth cranial nerves. Bilateral sections of the IXth and Xth or Vth and VIIth cranial nerves, or all four nerves together, were carried out, the nerves being cut at their roots as they emerge from the brain. After recovery the gills were perfused with an aqueous solution of MS 222 Sandoz (20 mg/litre), and the effect on the heart rate recorded. The experiments were carried out on ten tench, weighing between 10 and 87 grms. Some of the transections were carried out under MS 222 induced sedation (50 mg/litre).

(iii) Results

Exposing the brain under urethane or MS 222 anaesthesia produced a slight increase in heart rate, which returned to the original resting value within 30 minutes if the animal was left undisturbed. Unfortunately the effect of MS 222 on the heart rate of an animal whose brain is exposed, but whose cranial nerves are still intact was not determined. There was, however, only a small amount of bleeding during the operation, and the fish seemed in a good condition after the operation.

Bilateral vagotomy in the tench resulted in a 15-50% increase in heart rate; cutting the Vth and VIth cranial nerves also produced an increase in heart rate. In both cases the

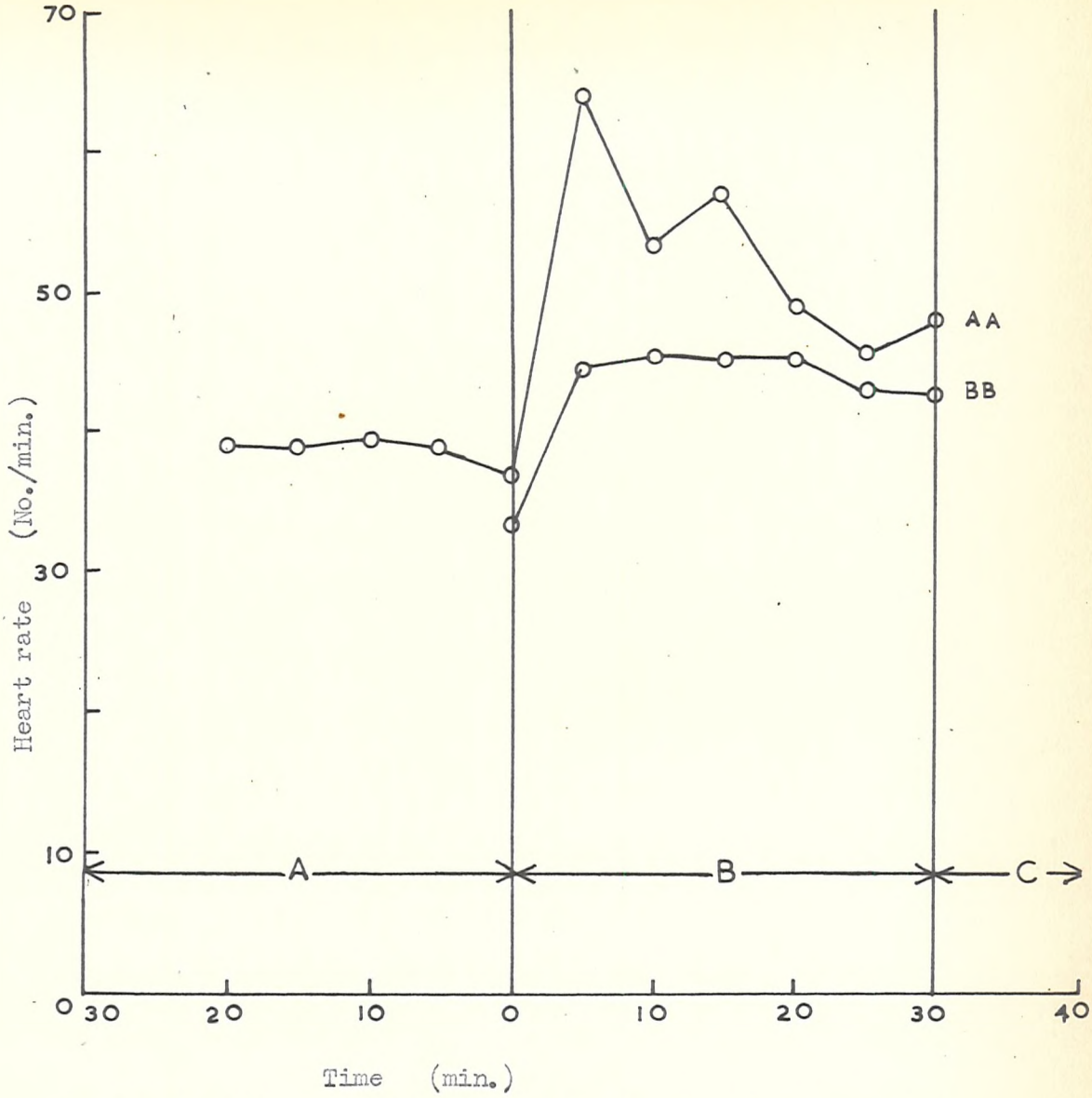


Figure 8. The effect of MS 222 Sandoz on the heart rate of the tench before and after bilateral section of the V, VII, IX and X cranial nerves.

A and C. No anaesthetic. B. MS 222 present in concentration of 20 mg/litre. AA. Intact animal. BB. After bilateral section of the V, VII, IX and X cranial nerves.

resulting increase in heart rate persisted for the duration of the experiment, which often lasted for several hours.

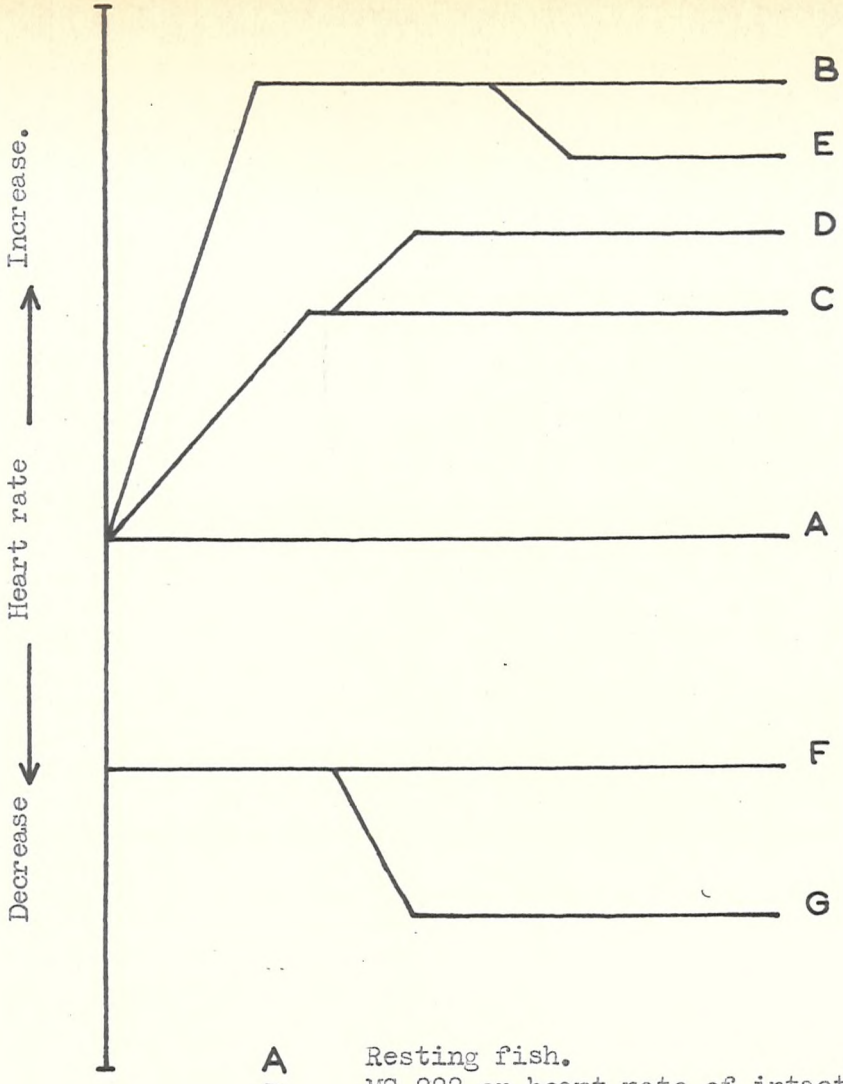
If the IXth and Xth cranial nerves were cut when the fish was anaesthetised with MS 222 Sandoz, and therefore the fish had a high heart rate, then a slight decrease in heart rate ensued.

Forcibly ventilating the gills of the tench with an aqueous solution of MS 222 Sandoz produces a rise in the heart rate of the bilaterally vagotomised animal. This increase, however, is less marked than that seen in the intact animal. Bilateral section of the Vth and VIIth nerves also reduces the response of the heart to MS 222 Sandoz, and sectioning all four pairs of cranial nerves has the same effect on the response of the heart to MS 222 as cutting either V and VII or IX and X alone. (Fig.8)

E. DISCUSSION

The results of the experiments on the effect of MS 222 on the heart rate of the tench are summarised in figure 9. In considering the effect of MS 222 on the heart rate of the intact animal, it can be seen that the response is not caused by a direct effect of the drug on the heart tissues, for this compound decreases the beat rate of the isolated heart.

The bilateral sections, however, indicate that the cranial nerves could be having some effect in the response of the heart to MS 222 Sandoz. The response of the heart to this compound is reduced after bilateral sectioning of the IXth and Xth cranial



A Resting fish.
B MS 222 on heart rate of intact animal.
C IX and X cranial nerves cut.
D MS 222 on heart rate of fish with IX and X cut.
E IX and X cut during MS 222 anaesthesia.
F Beat rate of isolated heart.
G MS 222 on isolated heart.

Figure 9. The effect of MS 222 on the heart rate of the tench.

nerves, and sectioning of these nerves, which normally results in an increase in heart rate, produces the opposite effect if the animal is anaesthetised with MS 222 Sandoz. The most obvious assumption to make under these conditions is that the cardiac branch of the vagus, being the only nerve to innervate the hearts of fishes (Young, 1931; Laurent, 1952-3; Nicol, 1950), contains acceleratory fibres. This is not impossible for the existence of sympathetic fibres in the ramus cardiacus of the vagus of poikilotherms has been recognised for some considerable time (Gaskell, 1887). McWilliam (1885) and Jullien et al (1950)a,b & c) however, have been unable to demonstrate any positive chronotropic effects of vagal stimulation, which is in agreement with the results of section VI, and indicates that there are no acceleratory fibres in the cardiac branch of the vagus of fish. If this is so then the reduced response to MS 222 Sandoz, shown by the denervated heart in situ in the above experiments, is the result of the operative procedure rather than a removal of the nervous innervation of the heart.

If acceleration of the heart is not dependent on direct stimulation of the heart tissues by MS 222 Sandoz, nor on direct stimulation of the heart by acceleratory fibres innervating the heart, then the response of the heart in the intact fish could be due either to the removal of vagal inhibition of the heart or to a release of humoral agents into the blood acting on the heart. Bilateral vagotomy or injections of atropine into the pericardial

cavity (see Section VII), however, only produce a 15-50% increase in heart rate, and it is therefore unlikely that the removal of vagal inhibition alone would result in heart rates of 90 per minute. (Fig.6b) Therefore, although the removal of nervous inhibition may produce part of the acceleration of the heart, there also appears to be some non-nervous mechanism acting on the heart to produce the increases in rate when MS 222 Sandoz is present in the water passing over the gills of the fish. Little is known of the physiological action of the sympathetic system in fish, but it is possible that the liberation of drugs into the blood stream may be producing the increases in heart rate; a further possibility is that vaso-constriction or vaso-dilation of parts of the circulatory system may also be having some effect, but the mechanisms involved, nervous or otherwise, remain obscure. The acceleration of the heart of the tench and other teleosts is discussed more fully in a later section (VII), as is the relationship between the increases in heart rate and breathing rate caused by MS 222 Sandoz. (Section IV)

Section IV

THE RELATIONSHIP BETWEEN HEART BEAT AND RESPIRATION

(i) Introduction

The previous work has shown that the responses of the heart and respiration show some degree of co-ordination. This is perhaps not surprising for the efficiency of oxygen uptake will depend largely on a correlation between blood flow through the gills and the rate of water flow over the gills. In fish one might expect a more pronounced relationship between heart output and ventilation volume than in air breathing forms, for in these animals the amount of blood flowing through the gills will be entirely dependent on cardiac output, and also a much larger effort is involved in ventilating the respiratory surface.

Some aspects of this relationship have already been established in a few teleosts and elasmobranchs. Lyon (1926) recorded synchrony between the heart and breathing rates in the sand shark, the heart beating in a 1:1 relationship with breathing, or at a slower rate than the breathing rate, but still in a simple numerical ratio with respiration. This type of relationship has been demonstrated in other elasmobranchs (Lutz, 1930a, b and c) and Satchell (1960) suggested that the physiological significance of this relationship was that it resulted in coincidence of maximum

flows of blood and water at the gills, and was maintained by inhibition of the heart beat during certain phases of the respiratory cycle.

Occasionally a simple numerical ratio is established between heart and breathing rates in teleosts (Hughes, 1961; Shelton and Randall, 1962), but such a relationship is uncommon (Serfaty and Raynaud, 1957).

Simultaneous inhibition of heart beat and breathing can be induced in a variety of ways (Lutz, 1930a; Otis, Cerf and Thomas, 1957; Labat et al, 1962) in both teleosts and elasmobranchs. This and other results (Campbell and Davis, 1963; Randall, 1962) indicate that there is a close relationship between the medullary centres of heart and breathing in both elasmobranchs and teleosts. This relationship, however, need not be reflected by a numerical ratio between heart and breathing rates. The physiologically significant factors are cardiac output and ventilation volume, and these may be affected by changes in amplitude as well as frequency of movement. Thus the absence of a numerical relationship between heart and breathing rates in teleosts does not necessarily mean that there is no correlation between the flows of blood and water through the respiratory apparatus; indeed, the absence of synchrony may indicate a more precise regulation of the two factors.

In order to obtain a clearer picture of this relationship in the tench a study was made of the position of the heart beat within the breathing cycle, under a variety of conditions.

(ii) Methods

Most of the analyses were carried out on records obtained from the tench, but those from the eel and the goldfish were also used. The animals were placed in sealed experimental tanks and the breathing movements, recorded with the aid of an RCA 5734 transducer, and the E.C.G. were either displayed on a pen recorder or filmed from an oscilloscope. The study included a comparison of the heart rate and the breathing rate recorded under a variety of conditions, and an investigation of the relationship between heart beat interval and the length of each breathing cycle. In order to determine the relative appearance of the E.C.G. in different parts of the breathing cycle, the cycle was divided into ten parts and the position of the ventricular wave of the E.C.G. in each of these parts estimated in over 2000 breathing movements.

(iii) Results

The heart rate of a resting unanaesthetised tench is in the range of 15-30 beats per minute, but occasionally lower rates than these were recorded in mid winter, when the fish were in a torpid state. The range in the eel is from 40-50 beats per minute, and both fish usually have a higher breathing rate than heart rate, being 30-60 respirations per minute in the resting tench, and 40-50 per minute in the eel. The ratio between heart and breathing rates is usually about 1:2 in the resting tench, whereas the ratio in the eel approaches unity, but in either case

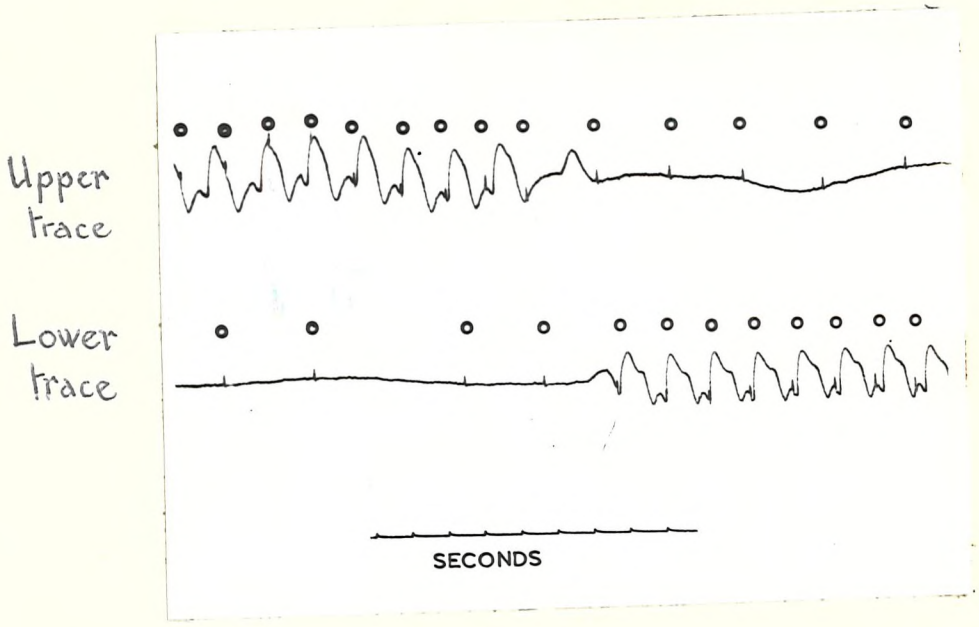


Figure 10. The relationship of the heart rate to breathing in the eel.

E.C.G. superimposed on a record of the breathing movements.

Top trace: Breathing stops; heart rate falls.

Lower trace: Breathing resumes; heart rate increases.

Circles emphasize the position of the E.C.G.

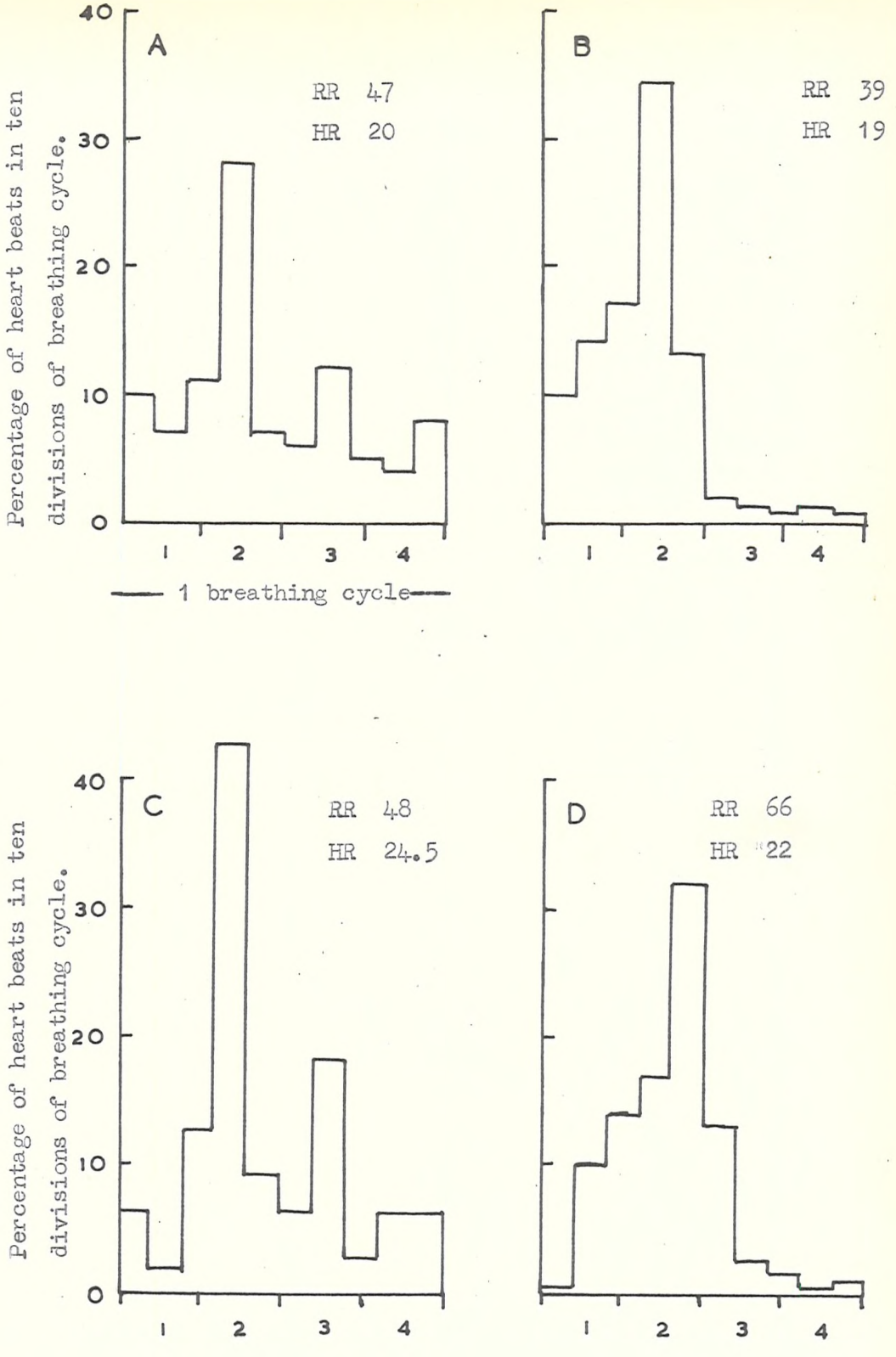


Figure 11.

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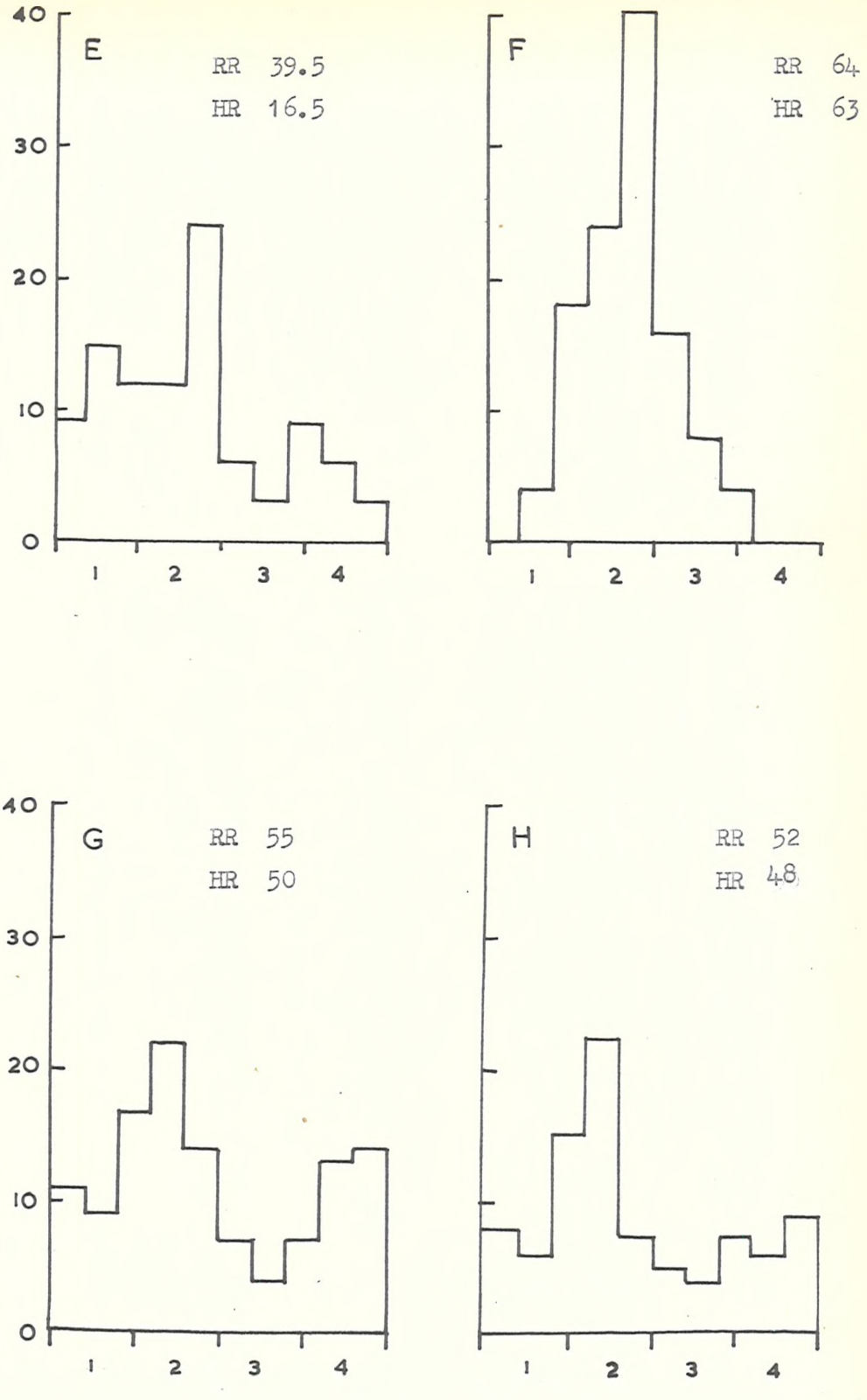

Figure 11

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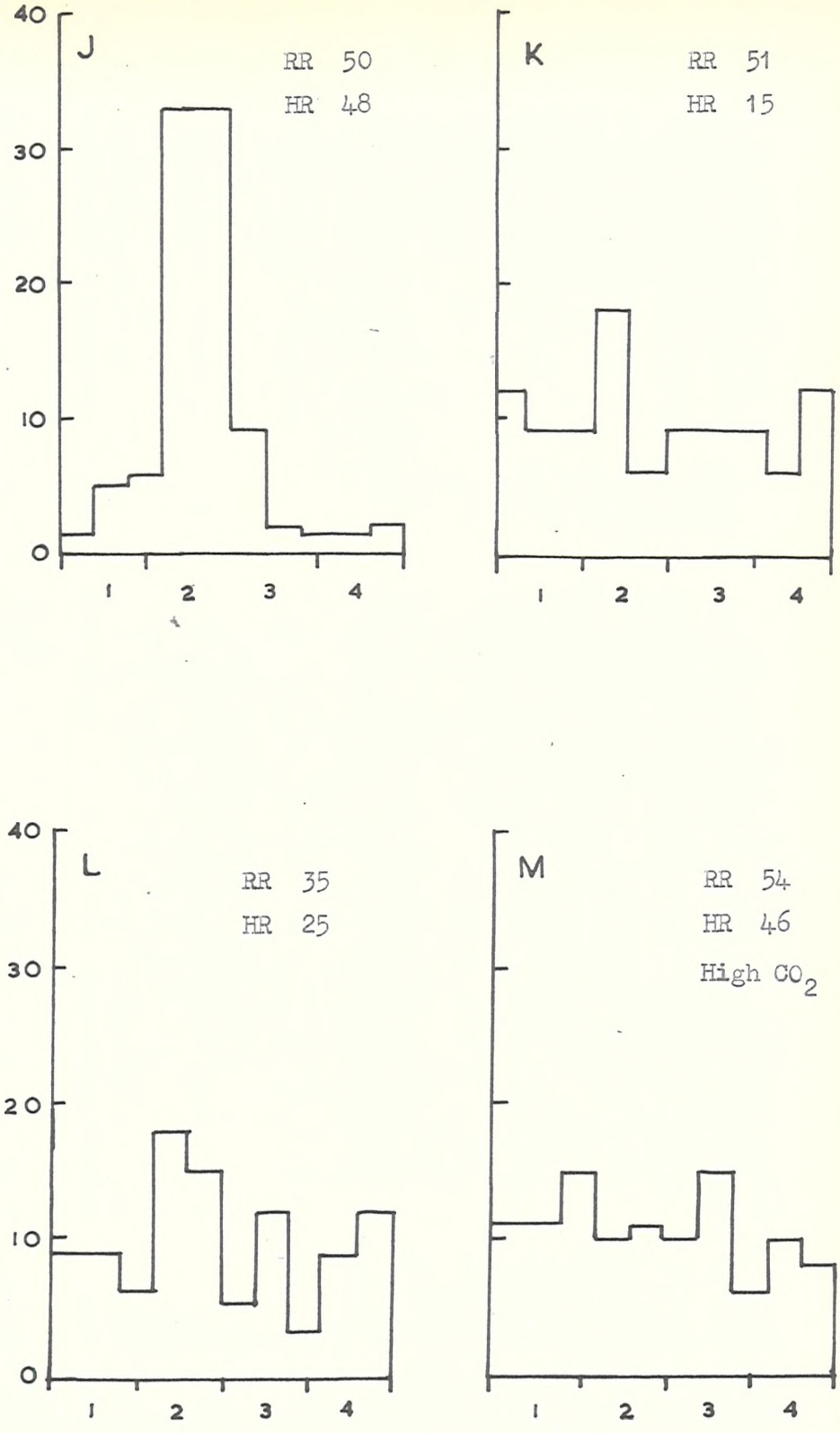

Figure 11.

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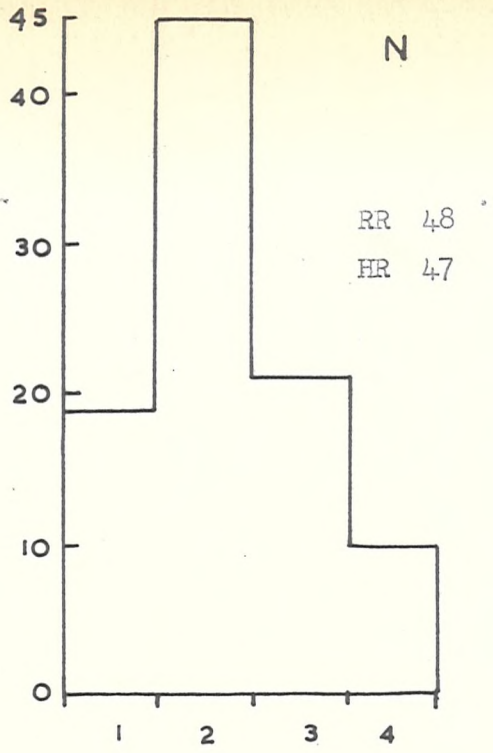


Figure 11. Histograms of the occurrence of the heart beat in relation to the breathing cycle of 12 tench (A-M) and 1 eel (N). Successive breathing cycles are divided into ten equal parts and the occurrence of heart beats in these parts is plotted on a percentage basis in the histograms. For further explanation see text.

Breathing cycle: 1 = mouth open.
2 = mouth closing.
3 = mouth closed.
4 = mouth opening.

RR = Breathing rate.

HR = Heart rate.

a simple numerical relationship between breathing and heart rate is rare. The eel is unusual in that, when in oxygenated conditions, it may ventilate only one side of the branchial chamber (van Dam, 1938) or may cease breathing altogether. During these periods, which may last for 30 seconds or more and be repeated every few minutes, the heart rate falls to approximately half the normal value. (Fig.10)

There appears to be no simple relationship between the changes in heart and breathing rates in response to changes in the external environment. Both may be accelerated simultaneously (MS 222 Sandoz), or the breathing rate may increase while the heart rate remains constant (decreased oxygen, see Section V), or the breathing rate may decrease during periods of tachycardia (carbon dioxide, see Section V). The histograms in figure 11, however, drawn from simultaneous recordings of the E.C.G. and the breathing movements of twelve tench and one eel, illustrate a relationship between the heart beat and each breathing cycle. In figure 11, (A) to (J) it can be seen that a higher proportion of heart beats appear in the mouth closing part of the breathing cycle. Fish (A) to (E) are in a typically resting condition; fish (F) is inactive but has been disturbed and has a high heart rate. Fish (G) to (J) have undergone recent activity. Fish (M) has been subjected to high CO_2 concentrations in the environment and the "mouth closing" relationship of the heart beat with breathing has disappeared. The mouth closing relationship was not apparent

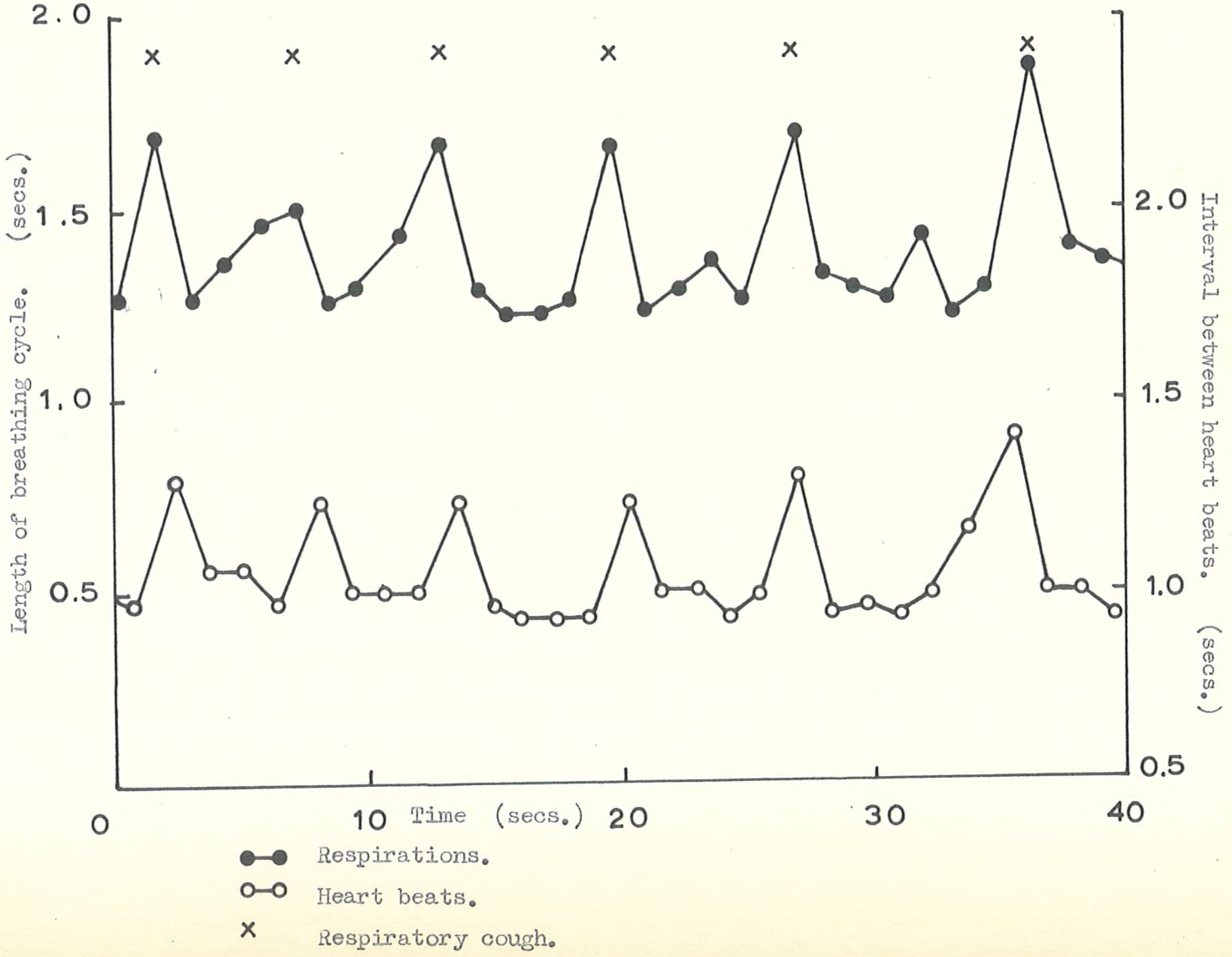


Figure 12. The relationship between heart beat interval and length of breathing cycle in the tench.

in fish (K) or (L) even though they were inactive and undisturbed in the experimental tank. Records from the eel seen in histogram (N) show a similar "mouth closing" tendency for the appearance of the heart beat in this fish. Shelton and Randall (1962) have shown that when synchrony between heart and breathing rates occur in fish anaesthetised with MS 222 Sandoz, the heart beats during the mouth closing phase of the respiratory cycle.

During this study it became obvious that the heart did not beat very often during a cough, and the interval between heart beats was often considerably lengthened by the appearance of a respiratory cough. Figure 12 shows the relationship between the length of each breathing cycle, and the time interval between successive heart beats. The peaks on the breathing trace represent a cough, and it can be seen that the heart beat interval lengthens during these periods.

(iv) Discussion

From the results it would appear that the relationship between heart and breathing rates can be divided into three categories as follows:-

(1) The tendency for the heart to avoid beating during the mouth opening phase of the respiratory cycle and during a respiratory cough.

(2) The synchrony of heart and breathing rates in the resting and anaesthetised fish.

(3) The inhibitory effect on heart rate when breathing ceases.

The synchrony demonstrated by Shelton (in Shelton and Randall, 1962) between respiratory activity in the medulla and the E.C.G. of a tench paralysed with succinyl choline suggests that co-ordination of the heart with the breathing movements is under nervous control of central rather than peripheral origin, and that the relationship is independent of either mechanical or proprioceptive stimulation of the heart by the respiratory musculature. If co-ordination is dependent on nervous control, then in the absence of acceleratory fibres in the cardiac branch of the vagus, the relationship between heart beat and breathing must be explained in terms of purely inhibitory nervous effects on the heart. For this one must assume two forms of inhibition, one cyclic positioning the heart beat within the breathing cycle, perhaps similar to that described by Satchell (1961) in elasmobranchs, and another which depresses heart rate and is itself inhibited by respiration. Combinations of these two types of inhibitory control could then produce the three categories of the heart breathing relationship listed above. First, vagal inhibition during the mouth opening phase of the respiratory cycle and during a respiratory cough would tend to place the E.C.G. in the mouth closing phase of the breathing cycle, and lengthen the interval between successive heart beats during a respiratory cough. Secondly, when respiration stops the inhibitory effect on heart rate would increase and so slow the heart. This inhibition during a respiratory pause may be due to the

cessation of the respiratory movements, the effect of the associated stoppage of water flow over the gills and the subsequent depletion of oxygen, or a central effect in the respiratory centre. Finally, it is noticeable that the mouth closing relationship and synchrony between heart beat and respiration in teleost fish usually occur together and are most evident in the resting or anaesthetised animal. In both cases there is a lack of peripheral stimulation, and during these periods it would seem that cyclic inhibition of the heart is most dominant. If under these conditions the heart rate approximates to the breathing rate, or is half the breathing rate as occasionally happens, then cyclic inhibition of the heart by respiration would tend to produce a rather striking synchrony between heart and breathing rates. This has been recorded occasionally. In figure 11 (M) it can be seen that carbon dioxide destroys the "mouth closing" relationship between heart beat and breathing, as do increases in activity.

The main difficulty in this hypothesis of double inhibitory control of the heart is that it does not sufficiently explain acceleration of the heart. Rates of 90 per minute have been recorded in the tench, and as bilateral vagotomy or injections of atropine (see Section VII) have never resulted in more than a 50% increase in heart rate in the resting animal, it seems unlikely that such rates can be achieved by the removal of vagal inhibition alone, and it is therefore probable that other factors are important in the regulation of the heart rate in these fish.

Section V

THE EFFECT OF VARYING GAS TENSIONS IN THE ENVIRONMENT ON THE HEART RATE AND BREATHING

(i) Introduction

The amount of oxygen taken up from the environment at the respiratory surface will be proportional to the activity of the animal. The exact relationship between oxygen uptake and metabolic rate during any period of time will depend on the proportions of protein, fat and carbohydrate utilised in metabolism, and the extent of any oxygen debt that may have been incurred (Brett, 1962). The respiratory apparatus must be able, especially in freshwater fish, to extract oxygen from an environment in which the oxygen and carbon dioxide tension may vary considerably, and supply oxygen at rates that may be 15 times the quantity required for basal metabolism.

The amount of oxygen crossing the respiratory surface from the water into the blood in fish will be governed by the tensions of oxygen and carbon dioxide in the blood and water, and the flow rates of blood and water through the respiratory surface. Many freshwater fish live in water in which there are large variations in carbon dioxide and oxygen tension. Under these conditions one might expect changes in the relationship between

respiratory volume and cardiac output to counteract any changes in gas tension in the respired water, and so maintain the level of oxygen consumption.

In studies on the effect of oxygen lack and carbon dioxide excess in the respired water on the branchial pump alone, it has been shown that both lead to an increase in the amount of water pumped over the gills, although there is some variation in the exact way in which the parameters of the breathing movements interact to produce these changes (Hughes and Shelton, 1962; van Dam, 1938; Saunders, 1962). The increased ventilation as the oxygen concentration in the water falls will maintain the amount of oxygen available at the respiratory surface, and providing the oxygen tension in the water is adequate to saturate the blood, equilibrium will be maintained. The volume of water ventilated by the gills, however, will not be directly related to the oxygen concentration in the respired water. This is because the amount of oxygen extracted from the water is not directly proportional to the ventilation volume, and the oxygen requirements of the animal do not remain constant, but are increased by the extra work performed by the branchial pump. The increased ventilation in response to carbon dioxide is not so obviously adaptive (Randall and Shelton, 1963), for although there is an increase in the oxygen requirements of the resting animal in high concentrations of carbon dioxide (Basu, 1959; Saunders, 1962), the large increases in ventilation volume are hardly justifiable in this respect.

It also seems unlikely that the increase in water flow will result in the removal of more carbon dioxide from the body, for carbon dioxide is very soluble in water, and therefore any increases in ventilation volume will have a negligible effect.

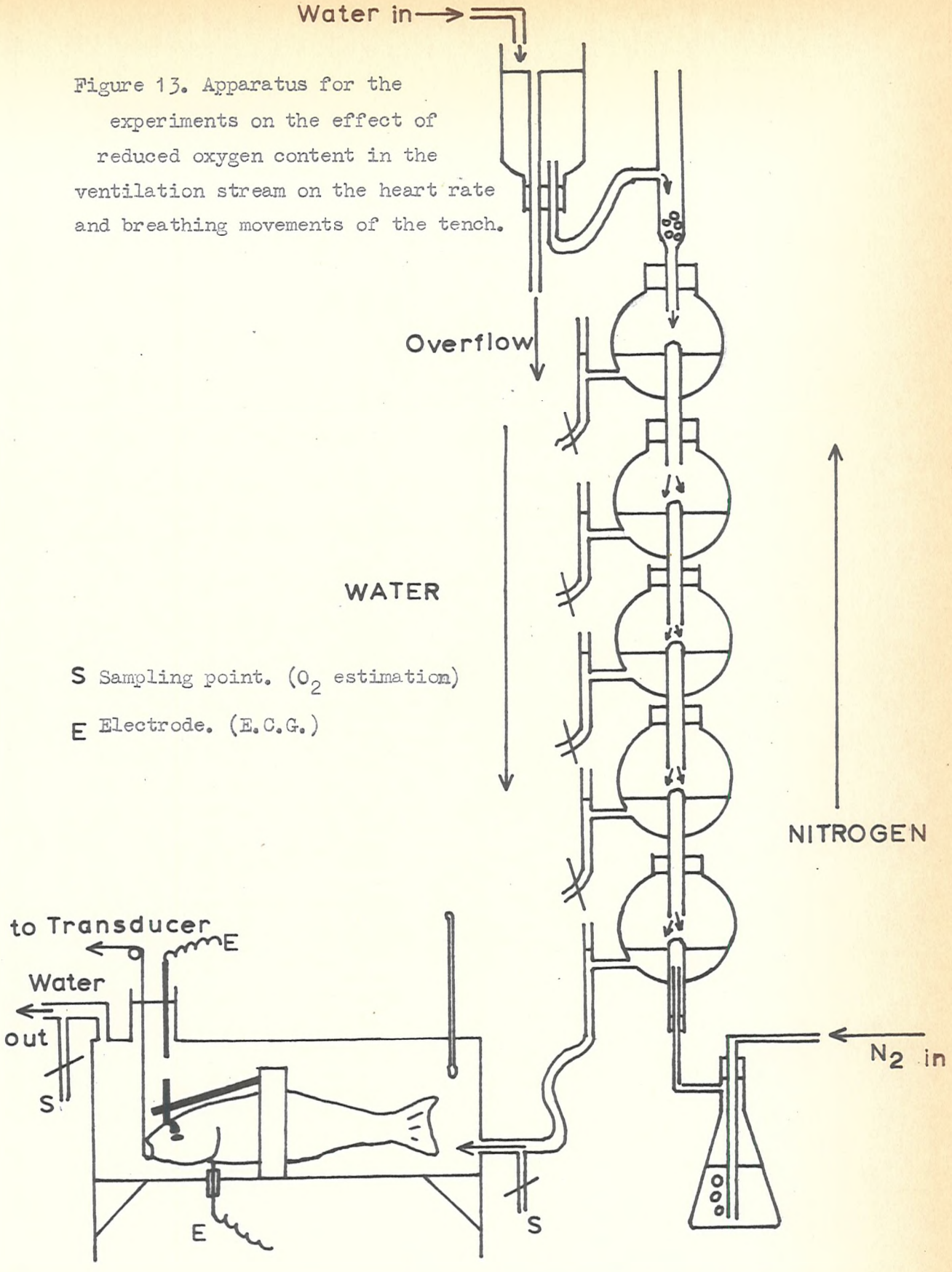
The relationship between gas exchange and the relative flows of blood and water are complicated. Hughes and Shelton (1962) have pointed out the importance of the ratio between the capacity rates (volume flow per unit time multiplied by the solubility of the gas in the fluid) of water passing over the gills and blood pumped through the gills in the effectiveness of gas exchange, and have discussed the relationship between ventilation volume, utilisation of oxygen and the efficiency of respiration. The effectiveness of gas exchange has been shown to be greater if the capacity rate of water is much smaller than that of blood (Hughes and Shelton, 1962). This is achieved in part by the high oxygen carrying capacity of blood compared with that of water, and would be further facilitated by a much higher rate of flow of blood than water through the gills. The function of respiration, however, is to supply the oxygen requirements of the animal as economically as possible, and therefore the relationship between the flow of blood through the gills and the flow of water over the gills must ultimately be a compromise between effectiveness of gas exchange and the efficiency of respiration in terms of economy of effort. Ideally measurement of blood flow through the gills and water flow over the gills, associated with the measurement of the oxygen and

carbon dioxide tensions of the blood and water on entering and leaving the gills, would greatly increase the understanding of these problems. Unfortunately some of these factors are not only difficult to measure but were also beyond the limits of the available apparatus. However, some idea of the changes in the cardiac output-ventilation volume relationship under varying oxygen and carbon dioxide tensions in the respired water can be gained by passing known concentrations of carbon dioxide and oxygen in water over the gills and measuring changes in the heart rate and breathing movements. The limitations of such an experiment lie in accepting changes in the rate and amplitude of the breathing movements as representative of changes in water flow over the gills, and heart rate as a measure of cardiac output. Obviously stroke volume could be as important as heart rate in increasing cardiac output, and the relationship between changes in the parameters of the breathing movements and changes in water flow rate are not known. The measurements are sufficient to allow a preliminary examination of the relationship between the flow of water and blood in the gills during variations in the gas tensions in the environment.

(ii) Methods

The effects of changes in the gas concentrations in the environment on the breathing rate, amplitude and heart rate were studied by placing the animal in a sealed tank of 1800 ml . capacity (Fig.13), identical with that used for the original

Figure 13. Apparatus for the experiments on the effect of reduced oxygen content in the ventilation stream on the heart rate and breathing movements of the tench.



experiments on the effect of MS 222. The heart rate, breathing amplitude and breathing rate were recorded as described in Section III B, except that in a few of the experiments the parameters of the breathing movements were recorded on a smoked drum. Water of varying O_2 and CO_2 tensions was passed through the tank at a rate of 1 litre per minute, completely replenishing the tank every 3-4 minutes. The O_2 and CO_2 concentrations in the water in the tank were measured by sampling at the inlet and outlet.

The O_2 concentration was varied between air saturation (approx. 10 mg/litre) and 0.5 mg/litre. This was achieved by cascading tap water down a glass column against an ascending stream of nitrogen, (Fry,1951). The nitrogen progressively displaces the oxygen in the water and by drawing off the water at various levels down the column a range of oxygen concentrations was obtained which could then be passed through the experimental tank. By maintaining a constant flow of water and nitrogen it was found that the O_2 content of the water tapped off at any level would remain constant for the duration of the experiment. The water from the tap was aerated and its flow through the column stabilised by using a constant head apparatus. The nitrogen flow was controlled by a reducer valve on the cylinder of compressed nitrogen. The glass column consisted of five vertically mounted 1 litre flasks, each flask being able to supply water of a low and constant O_2 content at a rate of up to 1 litre/ minute. The oxygen concentration in the first flask was also controlled

by the number of marbles in the glass tubing mounted above the flasks. The more marbles present the lower the range of O_2 concentrations available from the five flasks. (Fig.13)
My thanks are due to Mr. D.E.Horley who constructed this apparatus.

The O_2 content of the water was determined by Winkler's method as described by Ohle (1953). The samples were collected in 250 ml reagent bottles which were filled via a rubber tube leading to the bottom of the flask, and allowed to overflow 2 or 3 times. 0.5 ml of a 50% solution of $MnSO_4$, prepared by adding 100 grms of $MnSO_4$ to 200 ml of boiled and filtered distilled water, and 0.5 ml of a potassium iodide sodium hydroxide solution, again prepared by adding 100 grms NaOH to 200 ml of boiled distilled water and a subsequent addition of 50 grms KI, were added to the water sample to be analysed by the introduction of a 1 ml pipette below the surface of the water. The flask was sealed and shaken and after 10 minutes the precipitate was fixed by the addition of 3 ml of ortho-phosphoric acid. Two 100 ml portions of this sample were then titrated against N/40 (approx.) sodium thiosulphate, standardised against N/10 potassium dichromate. The oxygen concentration of the sample was then estimated in mg/litre, 1 ml of normal thiosulphate solution being equivalent to 8 mg of oxygen. A correction was made for the addition of the reagents (Ohle, 1953).

An analysis of the tap water, used in all experiments, was obtained from the Southampton Water Works Department, which

indicated that the water contained no oxidising agents in sufficient quantity to affect the chemistry of the Winkler reaction.

The carbon dioxide concentration remained at the normal tap water level of 2 mg/litre during all the experiments on the effect of reduced oxygen content in the respired water on the breathing and heart rate.

Varying carbon dioxide concentrations were obtained by mixing water from two reservoirs, one containing aerated tap water, the other tap water through which carbon dioxide was bubbled continuously. The concentration of carbon dioxide in the experimental tank was varied by altering the relative amounts of water flowing from each reservoir. Samples of water were collected from the inlet and outlet in 250 ml reagent bottles, using methods similar to those employed in the oxygen estimations. The free carbon dioxide content of the water was estimated using two methods, one direct, the other indirect. The direct method was a titration of the sample against N/100 sodium carbonate with phenolpthalein as indicator. Indirect estimations were made from calculations based on the graphs of Moore (1939), which in turn were based on pH and carbonate equilibria in the water and were constructed from determinations using the equations of De Martini (1938). The alkalinity of the tap water was estimated by titrating 100 ml samples against N/50 sulphuric acid, with methyl orange and phenolpthalein as indicators (Welch, 1948). Only

methyl orange alkalinity was present, and to improve the accuracy of the titration this indicator was dispensed with, and instead the samples were titrated to pH4 using a Direct Reading Electronic Instrument pH meter. The addition of carbon dioxide to the water did not affect the alkalinity, and because such large volumes of water were used the effects of micturition were not measurable. The pH of the water was measured after the addition of carbon dioxide, on the pH meter, and as at a given alkalinity, the carbon dioxide concentration is proportional to the pH, the carbon dioxide content of the water passing through the tank could be calculated.

Moore's method of determining free carbon dioxide has been criticized by Milburn and Beadle (1960), the main sources of error lying in the degree of accuracy required in the measurement of pH, and the diffusion of carbon dioxide from the sample to the atmosphere. These drawbacks to the method were reduced by keeping the surface area of the sample to a minimum, and measuring the pH as quickly as possible on a meter accurate to 0.01 pH. Two samples were taken for each pH measurement.

Generally the fish were subjected to carbon dioxide concentrations between 2 and 100 mg/litre, but occasionally concentrations as high as 200 mg/litre were used. Enriching the water with carbon dioxide had very little effect on the oxygen content, reducing it at the highest levels by a calculated 5%.

The following routine was adopted in each experiment except for a few of the earlier experiments on the effects of carbon

dioxide in the respired water. At least an hour was allowed to elapse after setting up the fish in the apparatus before commencing the experiment proper. Water of lowered oxygen content was then passed through the tank for fifteen minutes before the heart rate, breathing rate and breathing amplitude were recorded, so that the water in the tank and the fish could reach equilibrium at the new concentration. It was found that the introduction of carbon dioxide into the tank had an excitatory effect on the animal, but fortunately the animal had usually returned to the resting state within 30 minutes, when the responses of the fish were recorded. Between oxygen and carbon dioxide concentrations the tank was flushed with normally aerated water for 30 minutes. The fish were never exposed to low oxygen concentrations for longer than 30 minutes and to high carbon dioxide for longer than an hour, so the long term effects of these variations in environmental gas tensions were not measured.

The experiments were carried out on 51 tench, weighing between 34 and 135 grms, the majority being close to 70 grms weight, and at temperatures between 12 and 17°C, with variations of $\pm$ 0.5°C during a single experiment. The fish were anaesthetised before being placed in the tank, but were then allowed to recover in oxygenated freshwater. Records were taken from the unanaesthetised animal, breathing normally but restrained in the experimental tank.

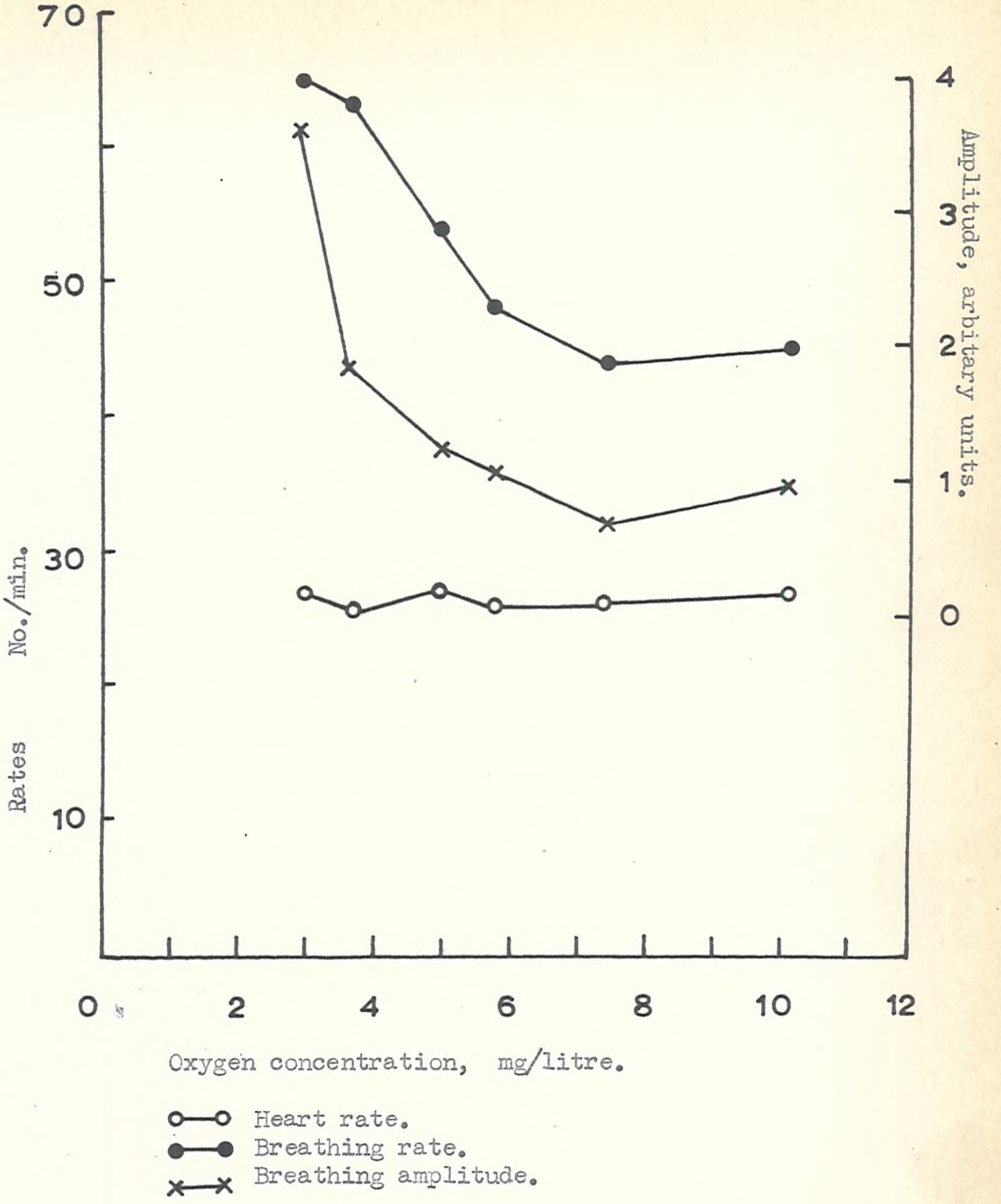


Figure 14. The effects of changes in the oxygen concentration of the water on the breathing movements and heart rate of the tench. Temp. 12°C.

(iii) Results

(a) Oxygen

The effects of lowering the oxygen content of the respired water on the breathing movements and heart rate in the tench are presented in graphical form in figure 14. The curves were plotted from the results obtained from a single fish, and although individual variations exist, they are typical of the responses shown by all the tench exposed to decreased oxygen concentrations in the environment.

Both amplitude and frequency of the breathing movements are increased when the oxygen concentration is lowered, the increase being more marked when the concentration falls below 5 mg/litre. The branchial pump appears fully extended in concentrations of 1-2 mg/litre, and below this level the movements become irregular and eventually stop. In this case survival of the fish is obviously impossible, but in concentrations of 1 to 2 mg/litre the responses of the fish were well co-ordinated and the impression was that the animal was in equilibrium with the environment. The responses of the branchial pump and heart of the tench to very low oxygen concentrations can be seen in figure 15, again plotted from the results taken from a single fish but typical of all the tench examined.

The heart rate is not affected as long as the oxygen concentration in the water remains above 3 mg/litre, but below

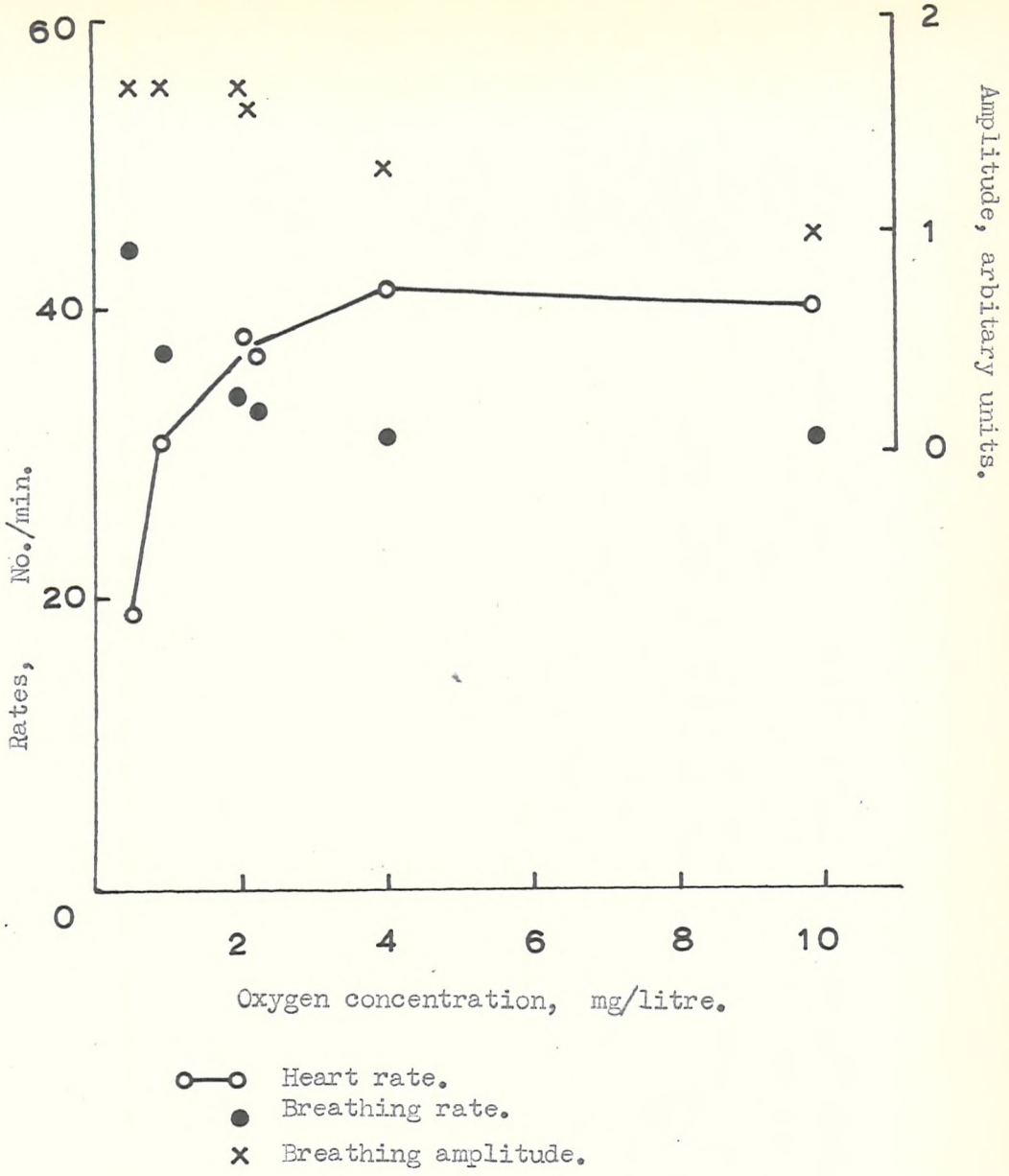


Figure 15. The effect of very low oxygen concentrations on the heart rate of the tench. The depth and frequency of breathing are also shown. Temp. 14°C.

this level the beat frequency falls progressively with the oxygen concentration. The slowing of the heart is at first regular, but in concentrations of 1 mg or less it becomes irregular and successive heart beats may be separated by 10 seconds or more. This inhibition of the heart is very probably part of the same response described by Shelton (in Shelton and Randall, 1962) where perfusion of the gills with deoxygenated water resulted in reflex inhibition of the heart, via the vagus nerve. It is therefore interesting to note that respiration is not inhibited by the low levels of oxygen that affect the heart.

The reactions to changes in oxygen concentration occurred quickly but, because several minutes were needed to establish a new oxygen concentration in the tank, the exact time delay could not be measured.

(b) Carbon dioxide

The effects of carbon dioxide on the tench are complex, for not only are there changes in heart rate and breathing, but also more general effects on the activity of the animal. The bursts of activity associated with the introduction of carbon dioxide into the water of the experimental tank usually persisted for only 15 to 20 minutes, and it can be seen in figure 16 that the changes in heart rate and breathing that occur after the introduction of water of high carbon dioxide content, although irregular at first, have settled at new and fairly

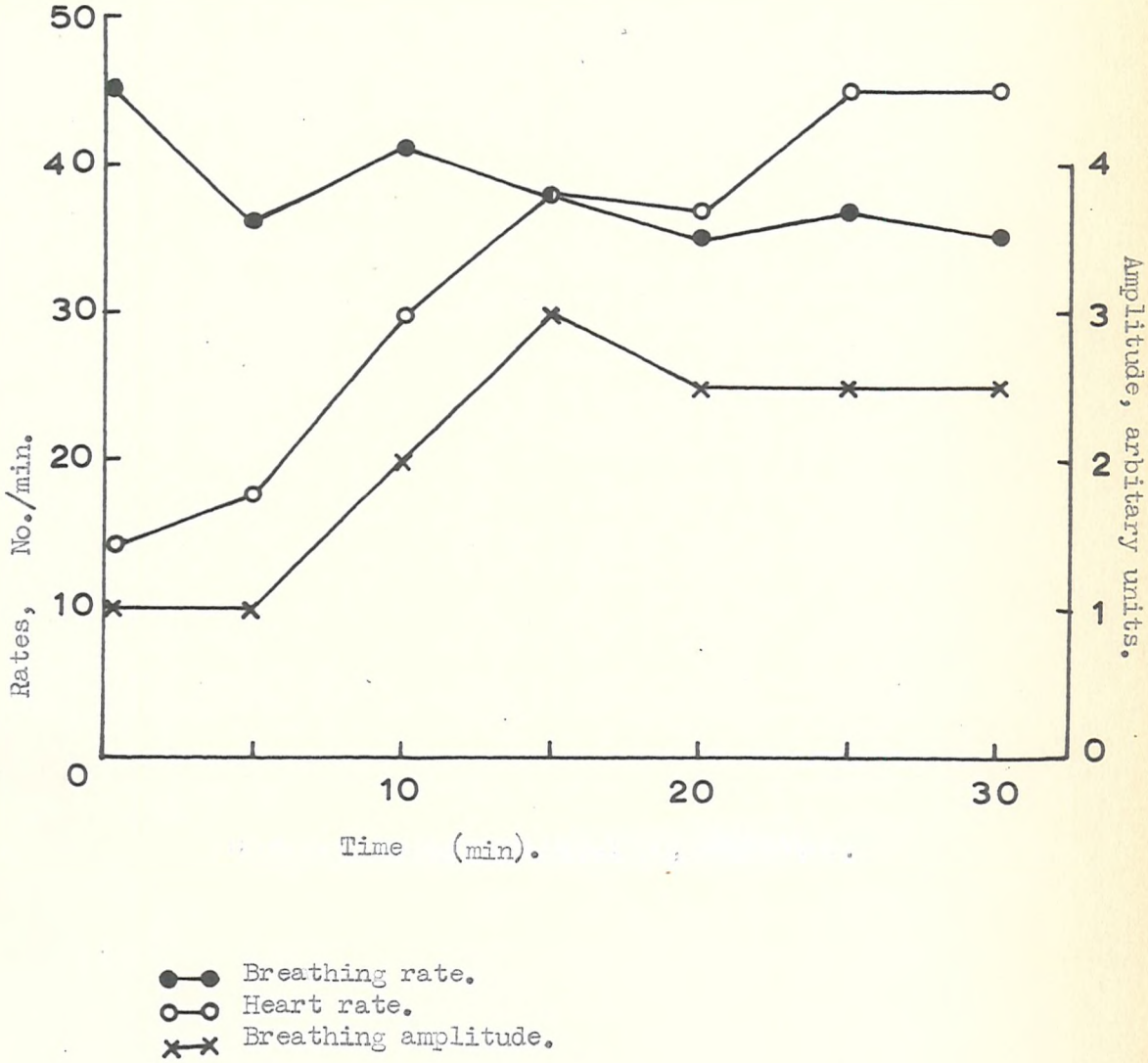


Figure 16. The responses of the branchial pump and heart of the tench to an increase in the carbon dioxide content of the water. The carbon dioxide concentration of the water passing through the tank was increased from 2.5 mg/litre to 80 mg/litre at zero time, and the effect of maintaining the animal in this concentration recorded at 5 minute intervals. Temp. 17°C.

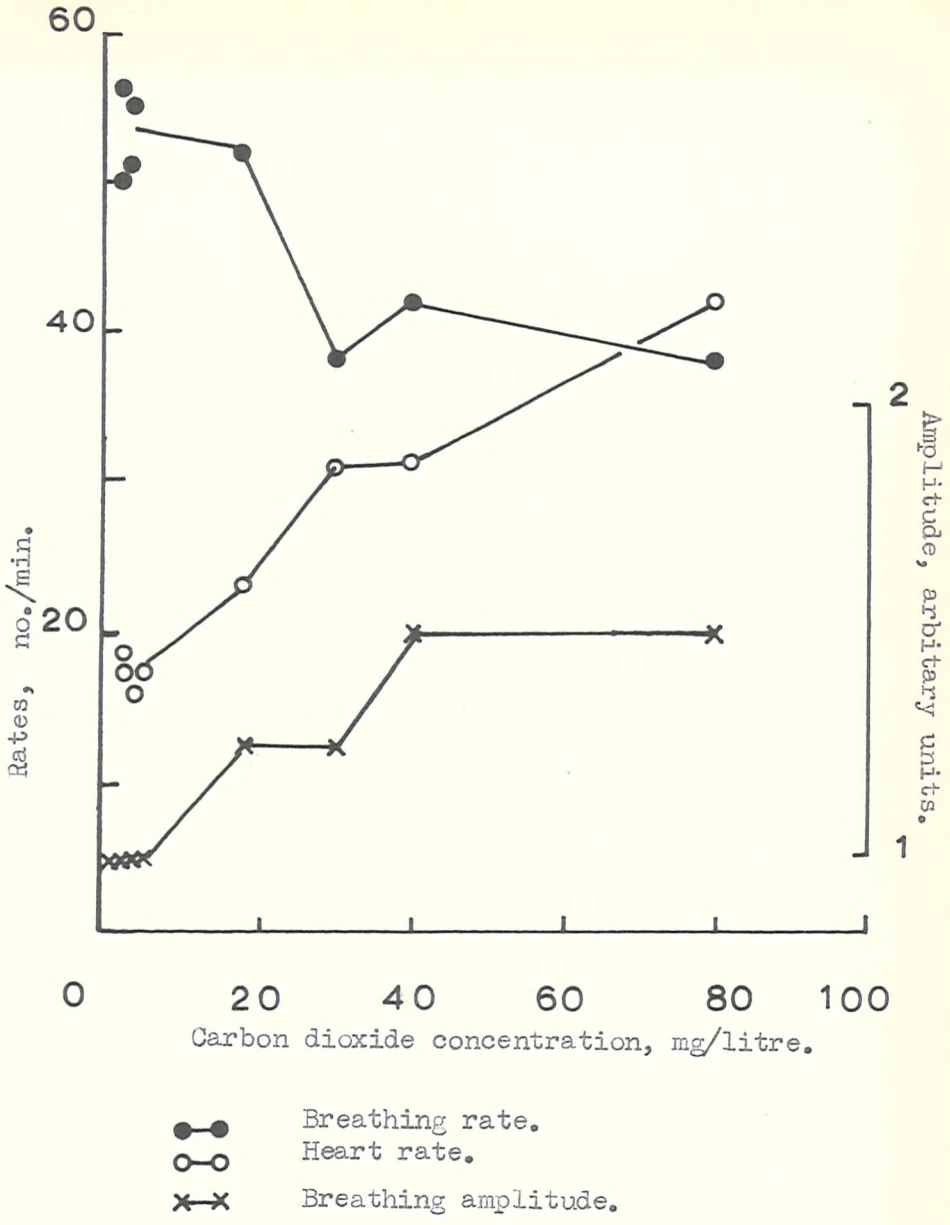
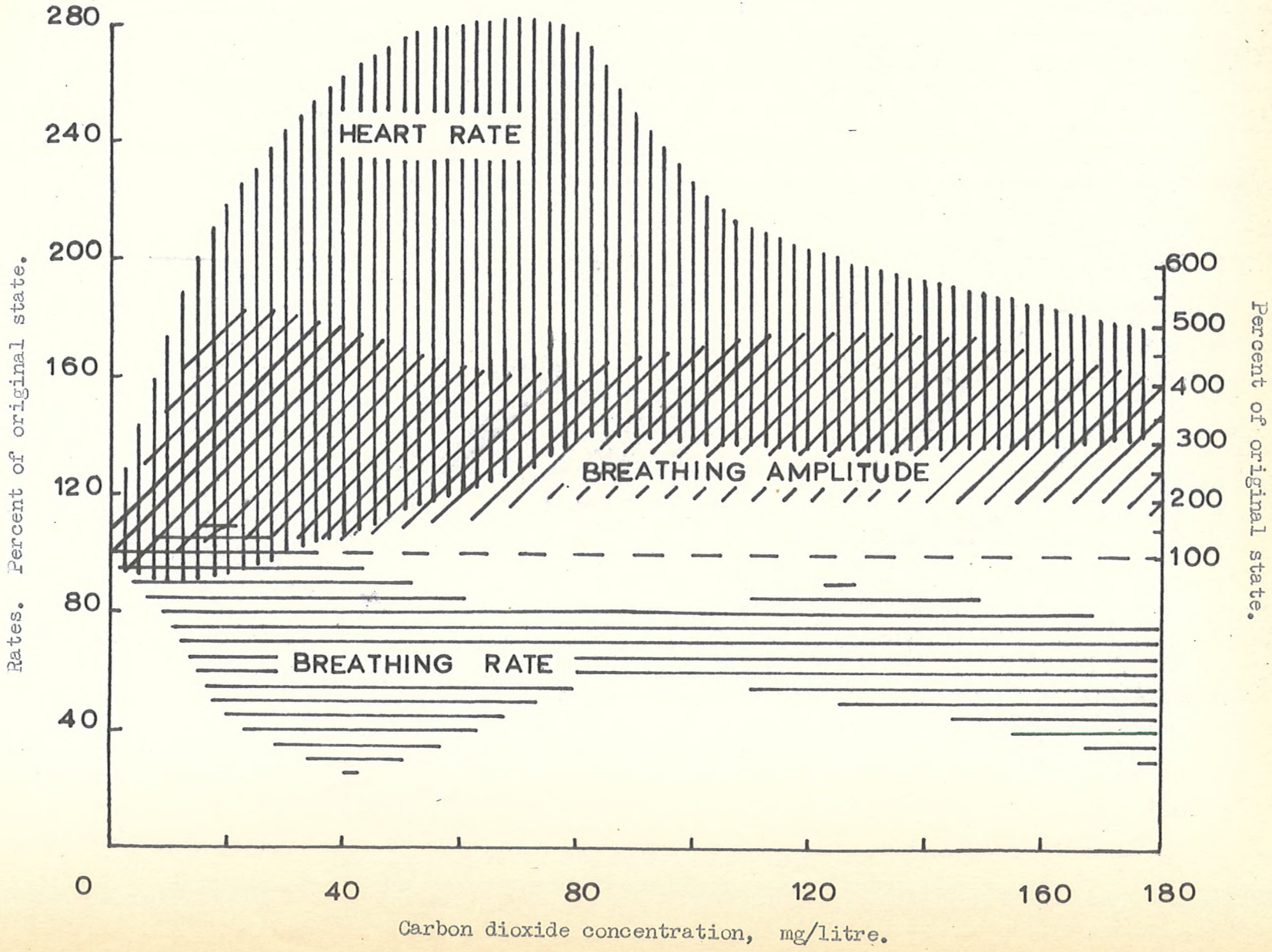


Figure 17. The responses of the branchial pump and heart of the tench to changes in the carbon dioxide content of the water. Temp. 17°C.

Figure 18. The responses of the branchial pump and heart of the tench to changes in the carbon dioxide content of the water. Combined results from 15 tench. All values are expressed as a % of the corresponding value in carbon dioxide free water just before the introduction of water of a high carbon dioxide content. Upper and lower borders of the hatched areas connect maximum and minimum values respectively.


280
240
200
160
120
80
40
HEART RATE
BREATHING AMPLITUDE
BREATHING RATE
600
500
400
300
200
100
0
40
80
120
160
180
Rates. Percent of original state.
Amplitude. Percent of original state.
Carbon dioxide concentration, mg/litre.

constant values within 30 minutes. Consequently steady values for heart rate, breathing rate and breathing amplitude could not be obtained as soon as the carbon dioxide changes were made, and records were only taken after the activity had ceased and the heart rate and breathing had become regular, which was usually 30 minutes after a change in the carbon dioxide content of the water flowing through the tank.

Increasing the carbon dioxide concentration causes a rise in heart rate and breathing amplitude, but a decrease in breathing rate. Figure 17 shows the effect of carbon dioxide on these factors in the tench. The combined results of all experiments presented in graphical form in figure 18 show that the amplitude of the breathing movements become maximal in concentrations of 20-30 mg/litre, and the heart rate in concentrations of 60-100 mg/litre. In higher concentrations both these factors slowly decline, but, even in concentrations of 200 mg/litre, remain at a rate that is higher than that recorded in normally aerated water. The breathing rate declines fairly rapidly at first (0-30 mg/litre) but with further increases in carbon dioxide concentration the fall is less marked.

The slow decline of all factors in high carbon dioxide concentrations is probably a reflection of an anaesthetic effect of carbon dioxide on the fish. The response of the branchial pump is strange in that increases in amplitude are opposed by decreases in frequency. This response is obviously quite

separate from the reaction to low oxygen concentrations, which produce increases in both frequency and amplitude of the breathing movements. The increased amplitude, however, more than offsets the decrease in frequency in the response of the branchial pump to carbon dioxide, and in the tench, as in other teleosts, there is an increase in the volume of water ventilated by the respiratory apparatus (Shelton in Randall and Shelton, 1963).

The above experiments were carried out on the unanaesthetised fish, and because of the effect of carbon dioxide on the activity of the fish, it was thought necessary to repeat the work using an anaesthetic in an attempt to eliminate the burst of activity in the responses of the tench to carbon dioxide. Urethane and nembutal anaesthesia was used, and because a continuous flow of water of varying carbon dioxide concentrations was to be maintained through the experimental tank, injection of the anaesthetic was preferable to bathing the fish in a solution of the drug. In long experiments re-injection was necessary (in the case of urethane) every 2 to 3 hours, for recovery from the light level of anaesthesia became apparent after this period. High carbon dioxide concentrations in the respired water produced a decrease in heart and breathing rates and breathing amplitude in fish anaesthetised with nembutal, a response which can be associated with the effects of this compound on the respiratory centre. Urethane anaesthesia,

however, did remove the effect of carbon dioxide on the activity of the animal, and the changes in heart rate and breathing movement seen in the unanaesthetised animal persisted. It can therefore be assumed that the responses shown in figure 17 are related to the carbon dioxide concentration, and are not a side effect of increased activity in the unanaesthetised animal.

(iv) Discussion

(a) Oxygen

The significance of the responses of the fish to lowered oxygen concentrations in the environment can be explained if one assumes that the regulating factor is the maintenance of an adequate oxygen supply to meet the metabolic requirements of the fish. The results indicate that in the tench, as in many other teleosts, the volume of water ventilated by the branchial pump is increased when the oxygen concentration in the water falls. This increase in water flow offsets the decrease in oxygen concentration, and maintains the quantity of oxygen available at the respiratory surface.

It appears that the respiratory apparatus is able to supply the oxygen requirements of the resting animal even in relatively low concentrations of oxygen, for by comparing Fry and Hart's (1948) value of the standard rate of oxygen consumption for the goldfish at 20^{o}C with Basu's (1959) values of the active oxygen consumption for the goldfish at the same temperature but under varying partial pressures of oxygen, it would appear that

respiration can supply oxygen at the standard rate of 90 ml/kg/hr in environmental oxygen tensions of 22 mm Hg pressure, which in freshwater represents an oxygen concentration of just over 1 mg/litre. This comparison, however, is open to the same criticism as Fry's (1947) concept of the level of no excess activity, for neither take into account the rise in oxygen consumption of the resting fish in low oxygen concentrations produced by the extra work performed by the branchial pump.

The independence of fish in low oxygen concentrations must be largely due to the high affinity of the blood for oxygen (Root, 1931). The blood characteristics of the tench have not yet been determined, but in the closely related carp (Black, 1940) the blood is 95% saturated in oxygen tensions of 17 mm Hg and 50% saturated at tensions of 5 mm Hg. A tension of 17 mm Hg is the same as a concentration of 1 mg/litre of oxygen in the water used in my experiments. The amount of oxygen entering the blood across the gills, however, will depend on the oxygen gradient between water and blood, and it is possible to calculate the pressure gradient necessary to supply the oxygen needs of a hypothetical animal whose respiratory characteristics have been compiled from the results of investigations of a number of fish. If this fish, of 100 grms weight, has a respiratory area of 200 sq cms (100 grm Carp, Saunders, 1962), an oxygen consumption of 100 ml/kg/hr, and the water and blood are separated by connective

tissue of 5μ thickness (pike, Schaperclaus, 1954), which has a diffusion constant for oxygen of 0.115 ml (Krogh, 1919) then a pressure difference of 27 mm Hg is necessary to enable sufficient oxygen to enter the blood to meet the requirements of the fish. If the blood is 95% saturated in tensions of 17 mm Hg (carp, Black, 1940) and is leaving the gills with its full load of oxygen, then tensions of 44 mm Hg are necessary in the respired water, or concentrations of 2.75 mg of oxygen/litre in the water used in these experiments. Although this value is derived from a hypothetical animal, it is nevertheless interesting to note the correlation between the above value, and the concentration at which the heart is first inhibited. The heart rate of the tench remains constant in concentrations of between 3 and 10 mg/litre because the tension of oxygen is sufficient to saturate the blood and changes in ventilation volume compensate for any decreases in oxygen concentration. If the oxygen tension of the blood falls, the heart can respond in either of two ways; it can speed up blood flow through the gills and so increase the water:blood gradient, or it can make no attempt to maintain the normal supply of oxygen to the body and simply cut down the heart output so that blood leaving the gills is carrying close to its maximum load of oxygen. The latter appears to be the case in the tench, but this is based on the assumption that heart rate is proportional to heart output. Johansen (1962)

has shown that in the cod the heart output can be doubled by changes in stroke volume augmented by increased venous return, without affecting the heart rate. It seems unlikely, however, that the decrease in heart rate evident in very low oxygen concentrations in the respired water would be cancelled out by increases in stroke volume to produce an increase in cardiac output.

If the heart output falls, as seems the case in the tench, then one must assume that either there is a decrease in the oxygen requirements of the fish, or that an oxygen debt is gradually incurred. If a debt is developed, then a build up of lactic acid in the blood could cause the bradycardia (Drummond and Black, 1960). This seems unlikely, firstly due to the rapidity of the response (within minutes of reducing the concentration from air saturation values), secondly on account of the similarity of this response to that described by Shelton (in Shelton and Randall, 1962) where nervous inhibition of the heart was produced by a flow of de-oxygenated water over the gills, and finally because Leivestad et al (1957) have shown that during periods of bradycardia in the cod, produced by removing the fish from water, the lactic acid in the muscles rises, but the level in the blood remains constant, and when the animal is put back into the water the lactic acid floods back into the blood stream. It is interesting to note that there is a concomitant rise in heart rate with the increased level of

lactic acid in the blood.

Bradycardia under asphyxial conditions is not only found in fish, but appears typical of many other groups of vertebrates (Scholander et al, 1962). This response has been more widely studied in diving mammals, and it has been found that circulatory changes take place to counteract the effects of bradycardia on the blood pressure, which remains fairly constant as the heart slows. These animals do develop an oxygen debt, but it appears that the reduced blood supply to the muscles prevents the lactic acid formed from entering the blood until the asphyxial conditions have passed.

(b) Carbon dioxide

If one assumes full saturation of the blood leaving the gills in the resting fish in oxygenated water, then during activity the only way in which the blood can deliver more oxygen to the tissues is by increasing blood flow rate; similarly, if the oxygen carrying capacity of the blood is altered, then changes in flow rate of the blood must take place if the oxygen consumption of the fish is to remain at a constant level.

The addition of carbon dioxide to the respired water increases the oxygen consumption of the resting fish (Basu, 1959) which must be a reflection of the increased energy expended in respiration. Carbon dioxide also affects the affinity of the blood for oxygen, reducing the oxygen carrying capacity of the blood. This can be counteracted in the fish by an increase in

blood flow rate, and it is therefore not surprising that a rise in the carbon dioxide content of the water accelerates the heart rate, thus, it is assumed, producing an increase in blood flow rate. A small increase in the quantity of oxygen being brought to the gills by the branchial pump is necessary to deal with the increased oxygen consumption. It seems unlikely, however, that the large increase in ventilation volume (Saunders, 1962) produced by the addition of carbon dioxide to the water is required to deal with the rise in oxygen consumption, which in the resting animal, must be related to the respiratory and circulatory changes. The percentage utilization of oxygen from the water is far less than that of an active fish ventilating at the same rate but in the absence of carbon dioxide. Saunders (1962) and van Dam (1938) attribute this difference to a reduced affinity of the blood for oxygen caused by increased carbon dioxide levels. Although this is an important factor, it is significant that the oxygen consumption has risen, and therefore unless the fish is building up an oxygen debt, the effects of carbon dioxide must have been largely moderated by increases in blood flow. The decreased utilization is more probably a reflection of a superfluous quantity of oxygen in the water passing over the gills. The 'carbon dioxide' resting fish is ventilating its gills at a rate comparable with that of a very active fish, but only requires oxygen at the rate of a moderately active fish.

The only explanation possible at the moment of the response of the branchial pump to carbon dioxide is that the increases in ventilation volume are related to carbon dioxide tensions in the tissues, normally associated with increased metabolism and oxygen lack, rather than to carbon dioxide levels in the water. If this is so then there can be no receptors to carbon dioxide exposed to the medium, for the animal seems unable to determine the amount of carbon dioxide in the environment.

The responses of the branchial pump and heart could be the result of a change in pH produced by increased carbon dioxide content, rather than a response to carbon dioxide. Hall (1931), however, showed that the responses of the puffer fish to acid pH levels produced by the addition of strong acid (HCl) to the water were quite different from those produced by changes in pH as a result of the addition of carbon dioxide to the water. Carbon dioxide produced a large increase in ventilation volume and a decrease in respiration rate, whereas hydrochloric acid produced a decrease in the volume of water pumped over the gills, but a slight increase in the respiratory rate. Thus it appears that the responses of the tench are to changes in the carbon dioxide concentration rather than to changes in pH produced by the increased concentrations of carbon dioxide.

Finally the responses of the branchial pump to high carbon dioxide and low oxygen concentrations in the environment are quite separate, and therefore different mechanisms must be involved in

the responses of the branchial pump and heart to changes in carbon dioxide and oxygen tensions in the environment. Local injections of cyanide and carbonate into the medulla would produce conditions of de-oxygenation and carbon dioxide excess within the central nervous system respectively. It would therefore be of interest of see if these injections showed a similar diversity of response in the fish, as oxygen lack and carbon dioxide excess in the environment.

Section VI

THE ROLE OF THE CARDIAC BRANCH OF THE VAGUS IN THE REGULATION OF THE HEART OF FISH

(i) Introduction

Because of the considerable attention focused on the nervous control of the heart by the results of the previous sections, a series of experiments was designed to determine the importance of the cardiac branch of the vagus in the regulation of the heart.

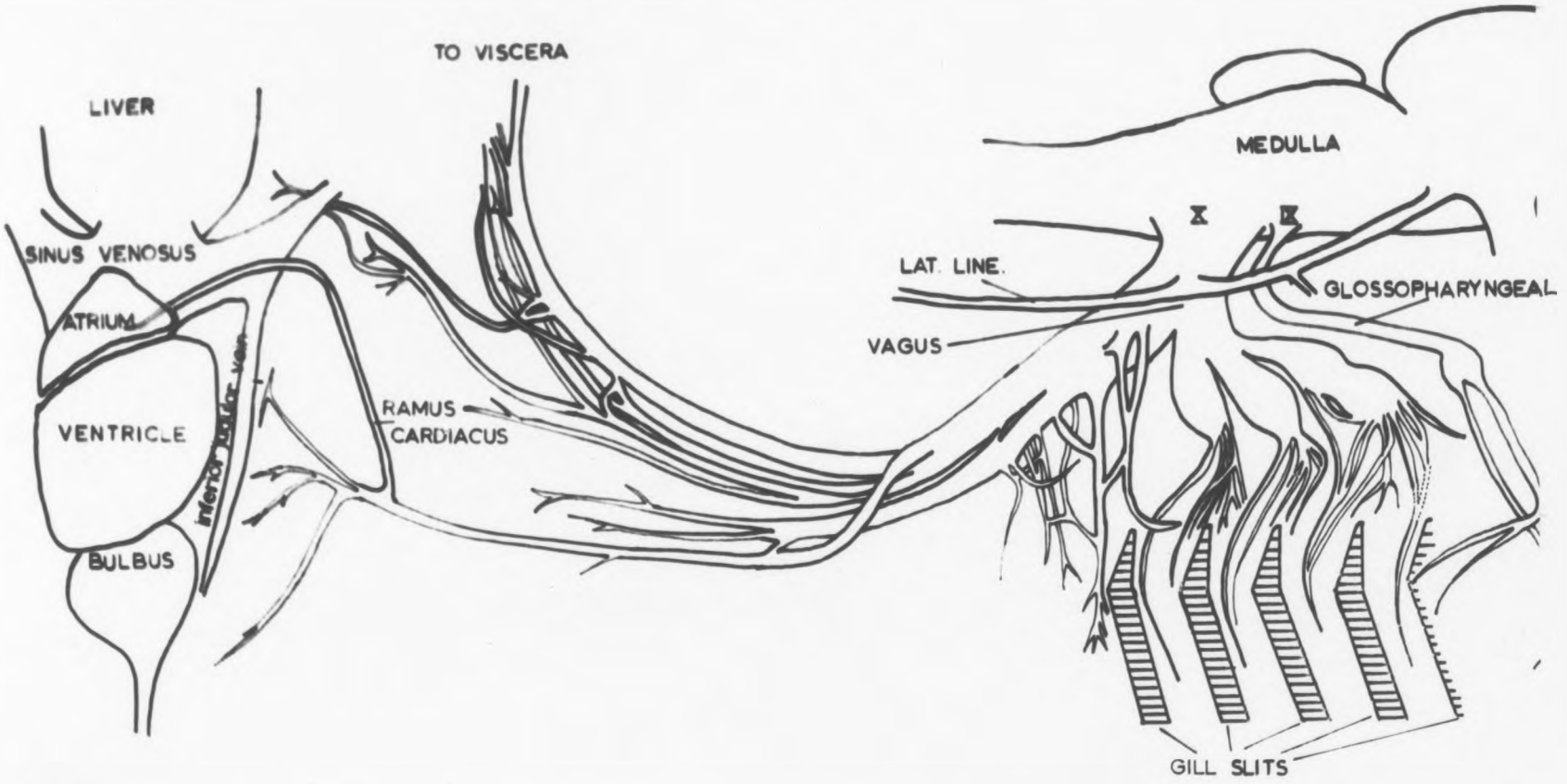
Some functions of this nerve have already been established in fish (Mott, 1957). Sectioning the vagi in fish causes an increase in heart rate, and stimulation of the cut ends of the vagus inhibits the heart (McWilliam, 1885; Lutz, 1930a,b,c) This effect of vagal stimulation on the heart is abolished by atropine. Jullien and Ripplinger have made extensive studies of the role of the cardiac vagus in the regulation of the heart rate in both freshwater and marine teleosts (Jullien and Ripplinger, 1950, a, b and c; Ripplinger, 1950, a and b). They report five effects of vagal stimulation on the heart, the most pronounced being a negative chronotropic effect mediated via cholinergic fibres.

Ripplinger (1950a) suggested that the pacemaker in the hearts of freshwater fish was situated at the base of the sino-

auricular junction and was richly innervated by the vagus. McWilliam (1885) found that the sinus initiated the heart beat in the eel, and that parts of the auricle and ventricle were also capable of beating when isolated from the heart, but usually at a much slower rate than the isolated sinus venosus. He reported that during a strong vagal inhibition the sinus and auricle were inexcitable, whereas the excitability of the ventricle was unaltered. Kisch (1948) found that there were several automatic centres in the fish heart, including one situated in the bulbus arteriosus, and that the normal and retrograde speeds of conduction were fairly equal.

Information on the effects of drugs on the teleost heart in situ is scanty (Mott, 1957). Acetylcholine slows the hearts of fishes (Kisch, 1948) and large doses of darstine, an atropine-like drug, bring about a prolongation of the conduction time of the heart beat (Wilber, 1961). The effects of adrenaline on the heart in situ are unknown, but Keys and Bateman (1932), Lutz and Wyman (1932), Mott (1951) and Burger and Bradley (1951) have studied the effects of adrenaline on the circulation of both teleosts and elasmobranchs. Injections of adrenaline cause a rise in blood pressure in elasmobranchs and teleosts. Keys and Bateman (1932) found that adrenaline constricts perfused vessels of the eel's tail, but dilates the branchial vessels of this species.

Figure 19. The right vagus of the tench, drawn to show the nervous innervation of the heart.


TO VISCERA
LIVER
MEDULLA
X
IX
SINUS VENOSUS
LAT. LINE.
GLOSSOPHARYNGEAL
ATRIUM
VAGUS
RAMUS
CARDIACUS
VENTRICLE
BULBUS
GILL SLITS

In order to clarify the role of the vagus in cardiac control it was decided to repeat some of the vagal stimulation experiments on the tench, and also record activity from this nerve during normal functioning of the heart and respiration.

In order to obtain a suitable preparation for the vagal recording and stimulating experiments, the first stage of this work was a study of the gross anatomy of the vagus in the tench.

(ii) Methods

The vagal innervation of the heart of the tench has already been described by Laurent (1952-3) and by Ripplinger (1950b) who also described the heart and associated blood vessels.

The vagi of 8 tench were traced by dissection, particular attention being paid to the branches leading to the gills and heart. As might be expected there were no major differences in the nervous anatomy of the tench used, but minor variations in the positions of the branches were observed. Figure 19 is a composite diagram constructed from the drawings of the dissection of the right vagus in several fish.

The root of the Xth cranial nerve emerges from the lateral surface of the medulla, and after passing through the vagus foramen in the cranium, separates into two main branches. One component lies in a ventro-lateral position and innervates the viscera and heart, the other passes ventrally to innervate the branchial arches. The branch to the gills immediately

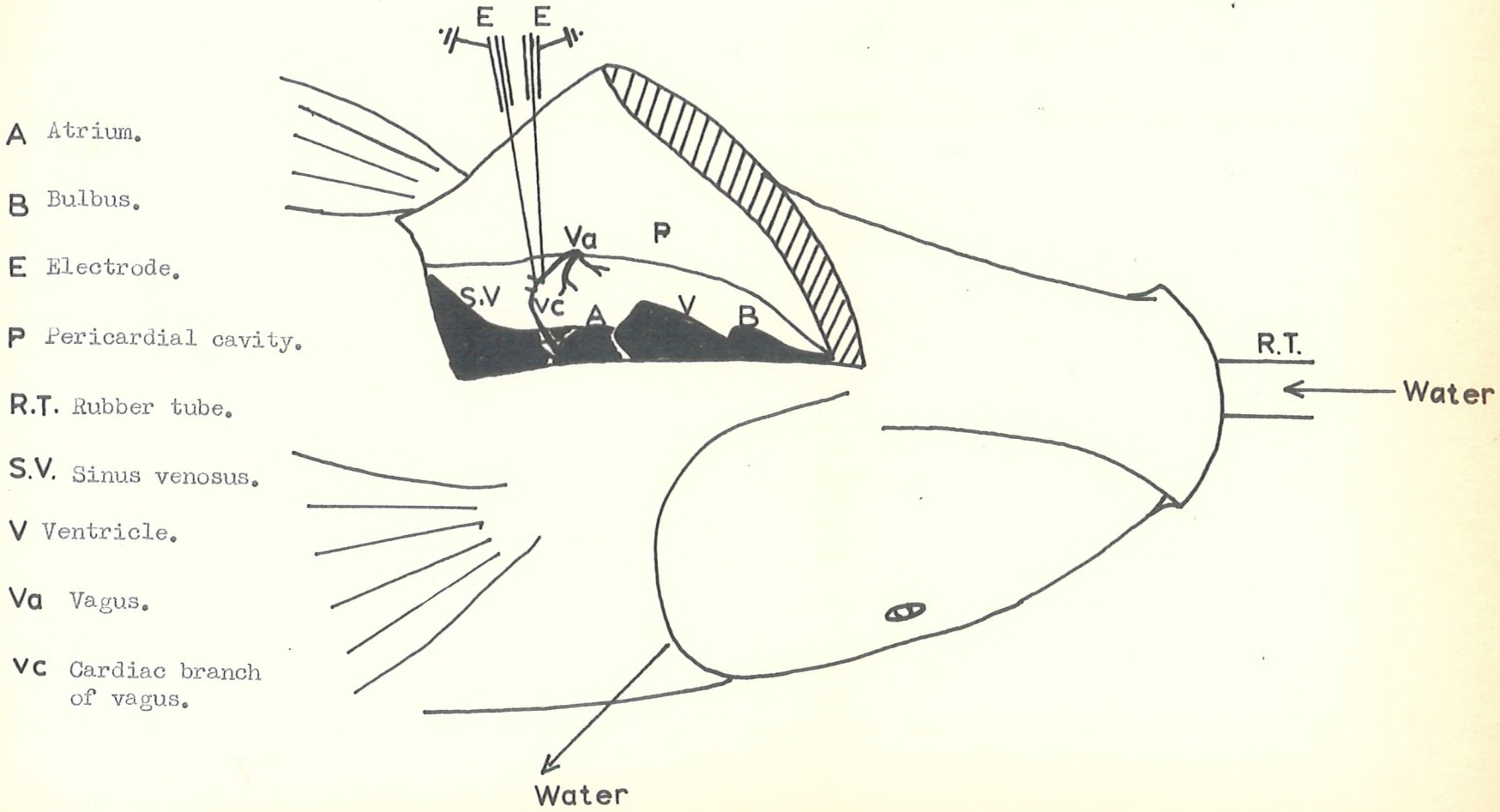


Figure 20. The position of the cardiac branch of the vagus within the pericardial cavity of the tench.

separates into three ganglionic masses, the most anterior of which is oblong and shaped rather like a solid figure 8. Branches from these ganglia innervate the four arches with rami of the glossopharyngeal nerves which pass to the anterior border of the first gill arch. The main vagal trunk passes down the side of the body behind the cleithrum, branching to pharyngeal muscles en route. At the level of the pectoral fins the nerve turns posteriorly and passes back to the viscera, but before doing so branches separate from the main trunk and pass to the oesophageal musculature. Several of these oesophageal rami innervate musculature that forms the dorsal surface of the pericardial cavity. A cardiac branch of the vagus separates from one of these rami and crosses the dorsal surface of the pericardium, passing ventrally over the junction between the inferior jugular vein and the Cuverian duct and along the anterior border of the sinus venosus to the heart; here it divides into two branches at the sino-auricular junction, one branch passing forwards to the auricular-ventricular junction, the other passing dorsad to innervate the auricle and sinus.

When recording from or stimulating the vagus, the electrodes were placed on the ramus cardiacus of the right vagus before it crossed the junction between the inferior jugular vein and the Cuverian duct. (Fig.20)

The experiments were carried out in an open top perspex tank of 2 litres capacity. The fish was held in a clamp that

allowed rotation of the fish around the long axis of the body, and was placed in the tank, ventral surface uppermost. The water level was adjusted to leave the ventral surface of the animal exposed. The gills were perfused with aerated tap water by inserting a rubber tube into the mouth of the fish. Urethane and paraldehyde anaesthesia were used, and when the fish was deeply anaesthetised the skin covering the sternohyoideus muscles was removed. These two muscles were then separated by a longitudinal cut through the connective tissue binding the muscles together. The cut was continued anteriorly to divide the cleithra mid-ventrally at their point of fusion, and the sternohyoideus muscles could then be eased apart to expose the heart and pericardium. After removing the pericardium the right sternohyoideus muscle was cut transversely at a point level with the posterior margin of the pericardial cavity. The cut was extended dorsally up the side of the body, behind the pectoral fin, to a point level with the entrance of the Cuverian duct into the pericardial cavity. The portion of body wall forming the lateral wall of the pericardial cavity could then be held back to expose the dorsal surface of the right half of the pericardial cavity. This section of body wall was held in position by a series of hooks. These were tied to lengths of cotton attached to screws fixed into the walls of the perspex tank. The tension on the cotton could be adjusted by turning the screws. Connective tissue, covering

the oesophageal muscles which form the dorsal surface of the pericardial cavity, was removed to expose the cardiac branch of the vagus. The operation was carried out under a binocular microscope, and the field was illuminated with a 6v lamp with two O N 222 glass heat filters to absorb the heat generated by the lamp. The pericardial cavity was often filled with liquid paraffin to prevent dessication of the nerve.

Two three inch lengths of pointed .01" diameter silver wire, shielded for two-thirds of their length, were used as electrical stimulating and recording electrodes. When recording from the vagus the signals were amplified by a Tektronix type 122 low level preamplifier and displayed on the upper beam of a Tektronix 502 oscilloscope. Permanent records were either photographed directly from the oscilloscope using a Cossor Oscillograph Camera, or the whole experiment was taped on a Ferrograph tape recorder, and then played back into the oscilloscope and photographed. Simultaneous records of either the E.C.G. or the opercular movements were taken while recording from the vagus and displayed on the lower beam of the oscillograph. The glycerine trough transducer was used to record the movements of the operculum, a cotton being tied to the edge of the operculum and looped over the lever arm of the transducer.

The experiments were carried out on 18 tench weighing between 225 and 315 grms. Attempts at recording from the ramus cardiacus of three goldfish were unsuccessful.

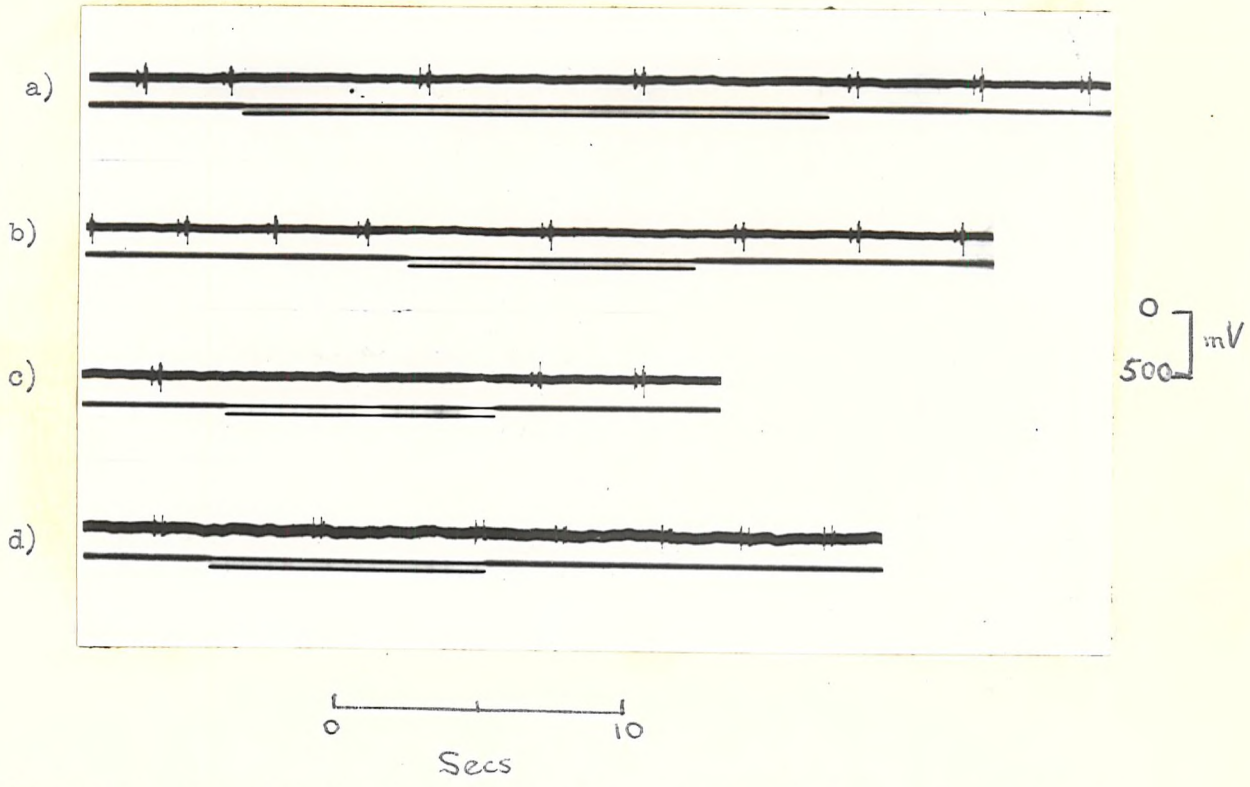


Figure 21. The effect of vagal stimulation on heart rate in the tench.

Upper trace: E.C.G.

Lower trace: Double line marks period of nervous stimulation.

Stimulate; a) Intensity 0.5v, Frequency 10/sec. for 20 secs.

b) 0.5v, 10/sec. 10 secs.

c) 1.0v, 10/sec. 10 secs.

d) 1.0v, 3/sec. 10 secs

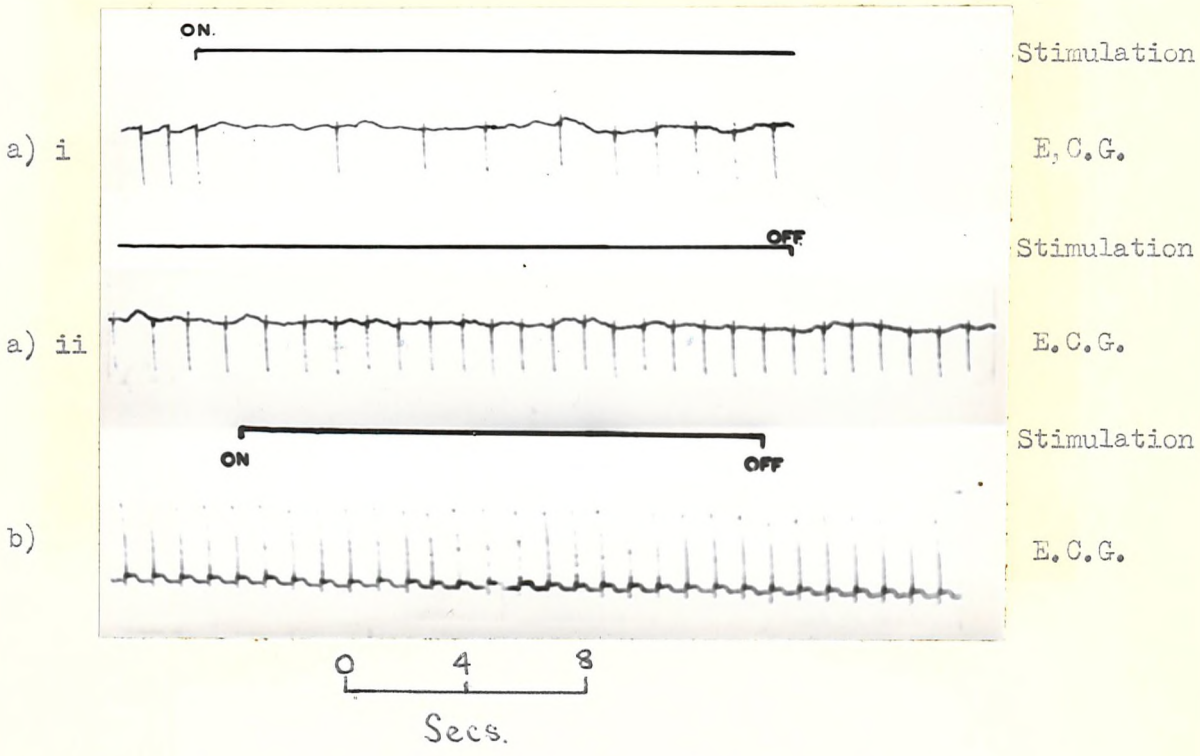


Figure 22. The effect of vagal stimulation on the heart rate of the tench.

a) i & ii : Normal heart, continuous record.

b) Effect on heart after an injection of 0.3 ml. of 10^{-3} atropine into the Cuverian vein.

During experiments in which the activity in the vagus was recorded, the flow of water over the gills was stopped, or the gills were perfused with either de-oxygenated water or with water of high carbon dioxide content, to determine the effects of these on the activity in the vagus.

In the vagal stimulation experiments, the cardiac branch of the vagus was stimulated electrically with square wave discharges produced by a physiological stimulator capable of giving shocks of up to 25 volts intensity, either singly or at rates of 3 to 100 per second. The nerve was stimulated before and after injections of atropine in saline into the Cuverian duct, and the results compared.

(iii) Results

(a) The effects of vagal stimulation on the E.C.G. of the tench

Electrical stimulation of the cardiac branch of the vagus results in a decrease in rate or a total inhibition of the heart beat of the tench. (Fig.21 (a)) During and after stimulation the configuration of the E.C.G. was unaltered except that a decrease in the size of the ventricular wave was sometimes recorded. The spacial relationships of the P, QRS and T waves remained constant before, during and after vagal stimulation in all cases. (Fig.21) No evidence was found to support the negative dromotropic effect recorded by Jullien and Ripplinger (1950c). Repetitive stimulation of the vagus at intensities just

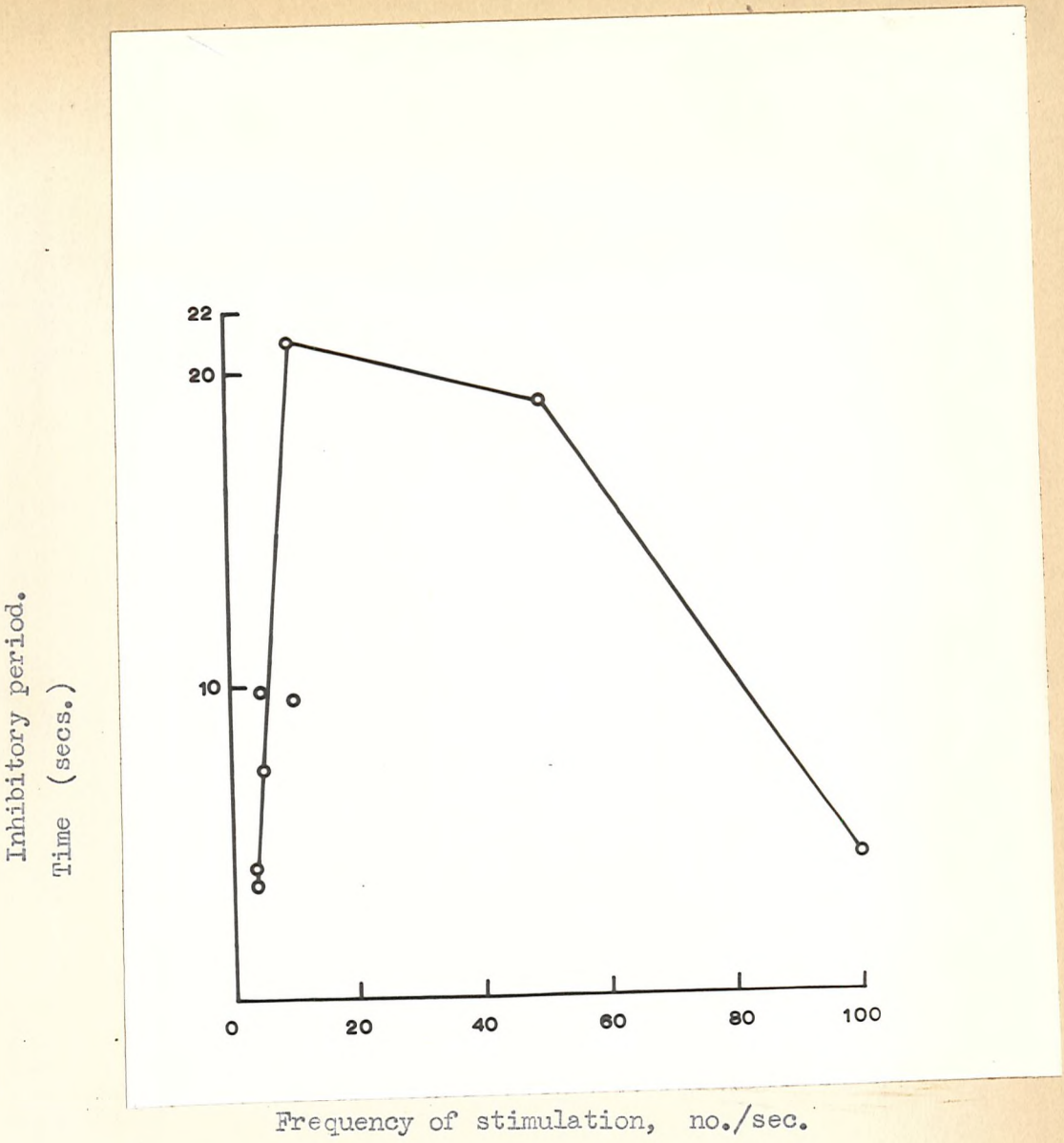


Figure 23. The effect of vagal stimulation on the heart of the tench. Time between start of nervous stimulation and the appearance of the first E.C.G. during stimulation plotted against frequency of stimulation.

above the threshold level seldom inhibited the heart for longer than 20 seconds, after which the heart would beat at irregular intervals of between 3 and 8 seconds. The degree of inhibition was augmented at higher intensities and at certain frequencies. Rates of stimulation of between 10 and 50 per second appeared optimal for inhibition of the heart. (Fig.23)

When vagal stimulation ceased the E.C.G. returned and within three or four beats the pre-stimulation heart rate was re-established; the heart rate did not accelerate beyond the pre-stimulation level on cessation of stimulation.

Stimulation of the vagus between the P and QRS waves did not inhibit the ventricular contraction; vagal inhibition had to precede the auricular beat before the E.C.G. was inhibited. This is in agreement with the results of McWilliam (1885) who found that the excitability of the ventricle of the eel was not affected by vagal stimulation.

An injection of 0.2 ml of 10^{-3} atropine in fish saline into the Cuverian duct causes an increase of up to 15% in heart rate in the tench, but similar injections into the pericardial cavity of three goldfish had no effect on heart rate. It would appear, therefore, that there are interspecific variations in the resting level of vagal activity in the fish.

Vagal stimulation at frequencies of between 3 and 100 stimulations per second, and at intensities of up to 50 times the threshold level for the inhibition of the heart before the atropine

injection, had no effect on the rate and appearance of the E.C.G. of the atropinised heart of the tench. (Fig.22 (b))

(b) Electrical Recordings from the cardiac branch of the vagus of the tench.

Records of the activity in the cardiac branch of the vagus in the tench were taken from a very short length of nerve, of the order of a few millimetres, one end of which was attached to the heart, the other running between the muscles closely associated with respiration. (Fig.20) In the earlier experiments the recordings were taken from the intact nerve, and under these conditions activity could be recorded which was synchronous with either the breathing or heart movements. Visual observations suggested that this was caused by movements of the nerve across the recording electrodes, produced by the heart and breathing movements. In the case of the heart beat there were marked peaks of activity relating to successive heart beats, each peak consisting of a double burst of activity, the first synchronous with the contraction of the auricle, and the second with the contraction of the ventricle, the result being that one could hear the beat of the heart on a loud-speaker connected to the oscilloscope beam on which vagal activity was being recorded. This type of activity was not seen if the nerve was cut at its entry into the pericardial cavity, and a slack length of nerve left between the heart and recording electrodes. Indeed, no activity of any sort was observed from the nerve under these

Figure 24. Activity recorded from the cardiac branch of the vagus of the tench.

a) The relationship between activity in the vagus and phases of the breathing cycle.

Upper trace: Vagal activity.

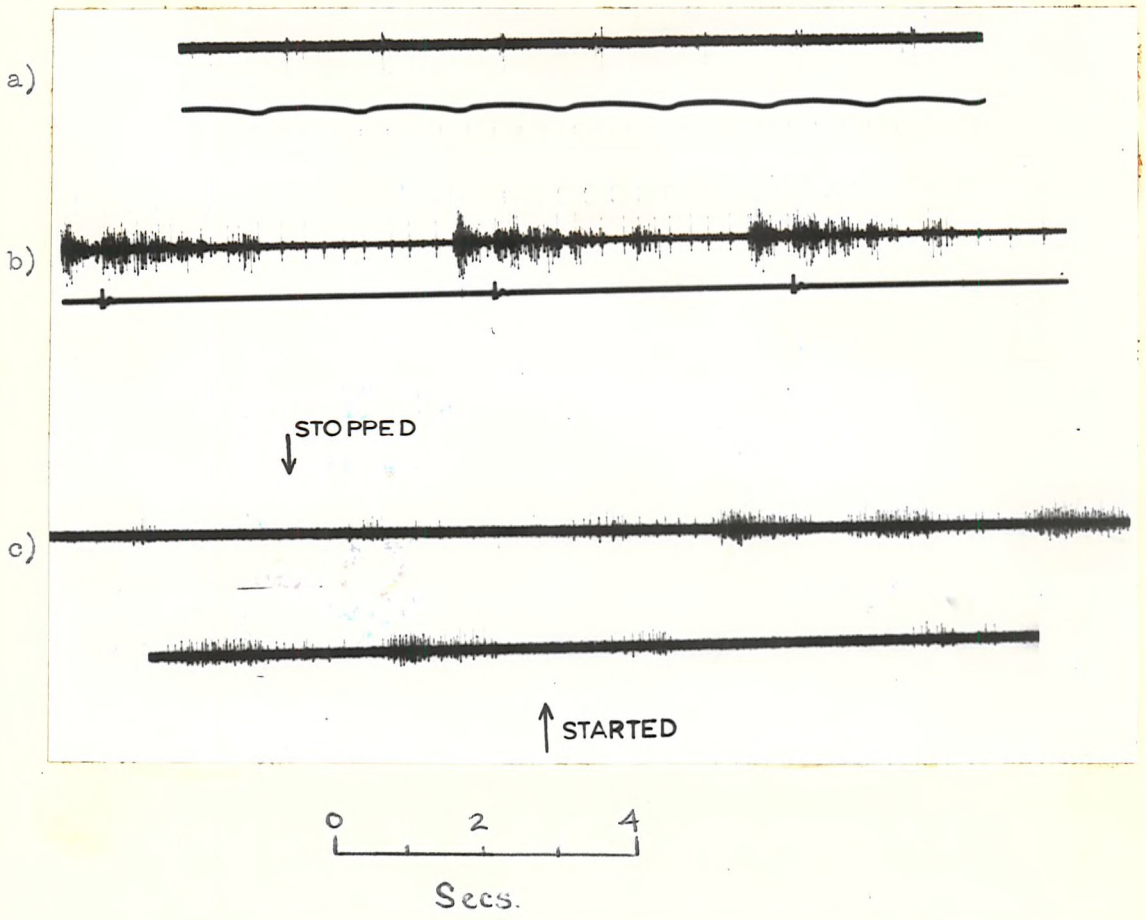
Lower trace: Opercular movements; down on trace = opercular closing.

b) Upper trace: Vagal activity, nerve still attached to heart. Note continuous discharge between activity relating to the E.C.G.

Lower trace: E.C.G.

c) The effect of stopping and starting the flow of water over the gills on activity recorded from the vagus.

Continous record.


a)
b)
STOPPED
c)
STARTED
0
2
4
Secs.

conditions. Similarly, the activity related to the movements of the nerve over the immobile recording electrodes produced by the breathing movements were removed if the vagus was cut close to the heart and a slack length of nerve was left between the electrodes and the main vagal trunk. It would therefore appear that these particular recordings are artifacts relating to the movements of the nerve over the immobile recording electrode.

As stated above, no activity was recorded from the portion of the ramus cardiacus of the vagus still attached to the heart after transection; efferent bursts of action potentials, however, could be recorded from the cardiac branch of the vagus attached to the main vagal trunk, which were associated with the operculum abducted or mouth opening phase of the respiratory cycle. (Fig.24 a) This activity did not occur regularly with each breathing cycle, for sometimes, in oxygenated conditions, there was no activity at all in this nerve. In a single experiment a continuous discharge was recorded under oxygenated conditions from the intact nerve, consisting of a single action potential passing down the nerve at intervals of 0.1 to 0.3 of a second. Unfortunately, because these records were taken while the nerve was still attached to the heart, the record is marred by the superimposed activity related to the movements of the heart. (Fig.24 b)

It was noticeable that the heart did not beat during these periods of cyclic activity in the vagus related to the mouth opening phase of the breathing cycle, suggesting that similar

activity was present in the left cardiac vagus still attached to the heart, and that it was of an inhibitory nature. This relationship between the mouth opening phase of the breathing cycle, vagal activity and the position of the heart beat in relation to the breathing cycle, is interesting when a comparison is made between the results of this section and the histograms in figure 11, showing a tendency for the heart to beat during the mouth closing phase of the breathing cycle. Similarly, during these experiments large bursts of activity were recorded from the vagus during a respiratory cough, and again it can be seen in figure 12 that the heart tends to be inhibited during a cough, which indicates the inhibitory nature of the vagal activity recorded.

Passing de-oxygenated water over the gills, or stopping the flow of water over the gills increases the amount of activity recorded from the vagus; the bursts of activity although still centred around the mouth opening phase of the breathing cycle, contain more action potentials and are of longer duration than those recorded under oxygenated conditions. (Fig.24 c) In some cases de-oxygenation of the water produced a slightly rhythmical but almost continuous discharge of efferent action potentials in the ramus cardiacus of the right vagus.

The effects of high carbon dioxide concentrations in the water perfusing the gills on the vagal activity in the fish have not yet been thoroughly investigated, and only a single permanent record has been obtained from a total of three experiments on this

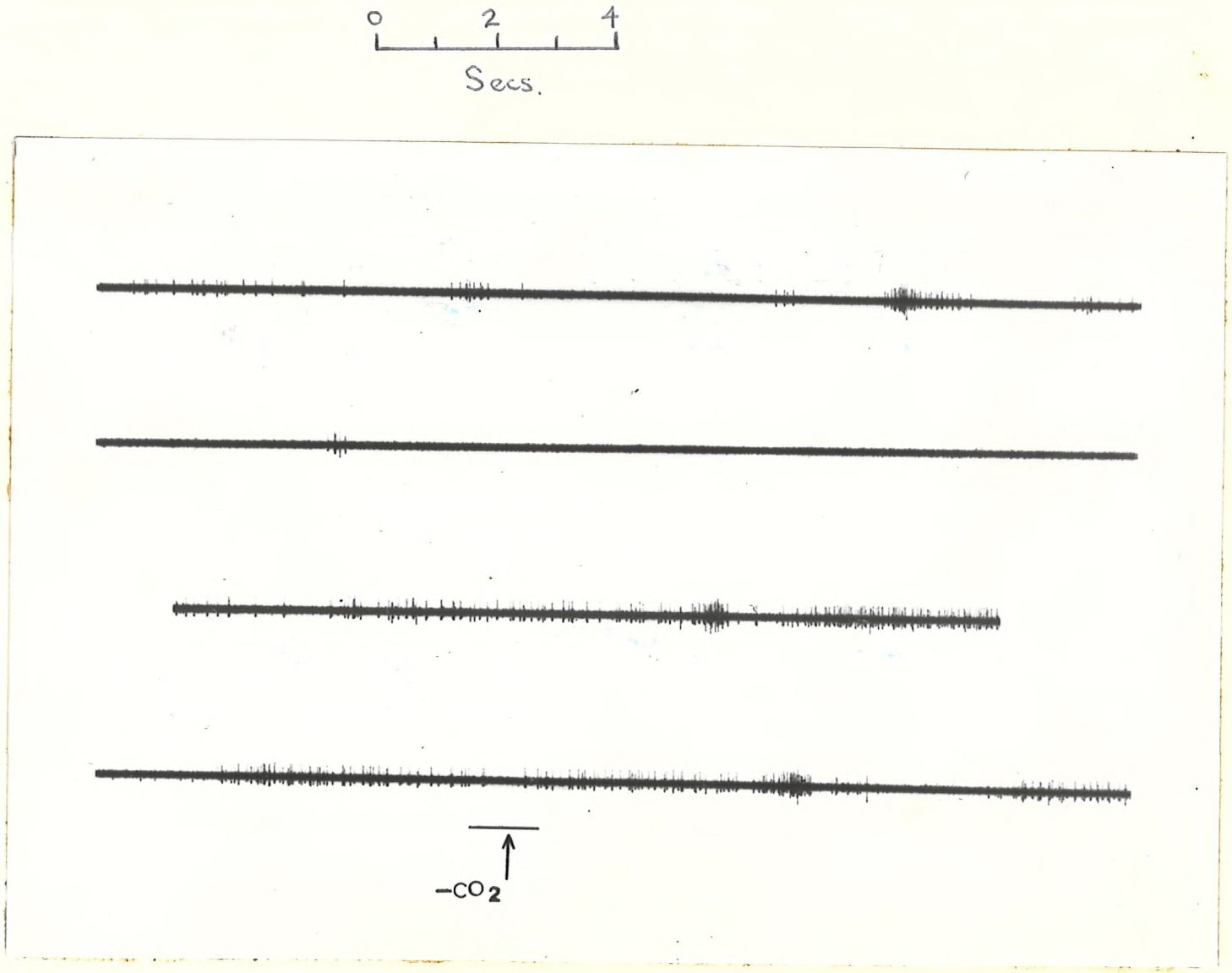
Figure 25. The effect of increased carbon dioxide content in the respired water on the activity recorded from the cardiac branch of the vagus in the tench.

a) Normal oxygenated water passing over the gills.

b) High carbon dioxide content in the water passing over the gills

c) As in b) but 20 minutes later.

d) Water of high carbon dioxide content replaced by a flow of oxygenated water across the gills.


0
2
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a)
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d)
$-CO_2$

problem. (Fig.25) However, from this single record, and from visual observations in the other two experiments, it would appear that perfusing the gills with water of high carbon dioxide content has a very different immediate effect on vagal activity than de-oxygenated water, for all signs of activity in the nerve disappear. This can be correlated with the effect of carbon dioxide in the ventilation stream on the heart rate of the intact animal (Section V), and the breakdown of the "mouth closing" relationship of heart beat and breathing in high carbon dioxide concentrations. (Fig.11) If the gills are perfused with carbon dioxide for a long period of time, however, the activity returns, usually within 20 minutes of the flow of water of high carbon dioxide content over the gills, but not in the original rhythmical form. The activity recorded is a continuous discharge of efferent action potentials, which when the flow of carbon dioxide water is terminated and is replaced with a flow of oxygenated water over the gills, becomes rhythmical. These records closely resemble those seen when perfusing the gills with de-oxygenated water, and indeed, this long term effect of carbon dioxide under these conditions may be the effect of anoxia. In these experiments the gills were perfused at a constant rate; the fish was therefore quite unable to regulate the amount of water ventilating the gills. As high carbon dioxide content in the respired water normally increases the oxygen consumption of the fish (Saunders, 1962), it is possible that under these conditions, the constant

volume of water ventilating the gills is insufficient to meet the oxygen requirements of the animal. Thus the resulting anoxia causes the increased activity in the vagus, rather than it being a direct effect of the high carbon dioxide content of the respired water.

(iv) Discussion

The cardiac branch of the vagus probably contains cholinergic fibres. This is indicated by the fact that vagal stimulation has a negative chronotropic effect on heart rate, which is abolished by injections of atropine. The inhibitory effect on the heart rate gradually disappears on continual stimulation, and total inhibition of the heart in the tench seldom lasts for longer than 20 seconds. The E.C.G. reappears more quickly when the nerve is stimulated at frequencies outside the range of 10 to 50 shocks per second. This effect can be explained if one assumes that successive vagal impulses release diminishing amounts of acetylcholine, which is then being broken down within the heart muscle, and that the heart beats directly the acetylcholine falls below a certain level. The optimal frequency of stimulation with regard to cardiac inhibition, therefore, would depend on the rate of decrease in the amount of acetylcholine released by successive impulses, and the rates of breakdown of this compound within the heart. These factors seem to vary in different species for McWilliam (1885) was able to inhibit the heart of the eel for periods of 15 minutes

or more, whereas in the tench, inhibitory periods of 20 seconds were all that could be produced by continual vagal stimulation. Although one must expect variations in physiological as well as anatomical characteristics between species, the eel is probably atypical of fish in general. This is, in fact, indicated by morphological, ecological and physiological characteristics. The known physiological 'specialities' of this species include intermittant respiration when in well oxygenated water, the ability to respire through only one side of the branchial pump (van Dam, 1938) and the associated variations in heart rate, and, most probably, circulation. These respiratory peculiarities may all be related to the ability of the eel to respire through the general body surface (Krogh, 1904).

The level of tonic activity in the vagi of different species of fish also appears to vary. The heart rate of the goldfish is unaffected by injections of atropine into the pericardial cavity, whereas in the tench, bilateral vagotomy or atropine injections result in an increased heart rate, and in the eel there are large increases in heart rate when the vagi are cut (Mc William, 1885).

Vagal stimulation does not affect the rate of conduction of the wave of contraction over the heart, for the spatial relationships between the P and QRS waves remained constant before, during and after vagal stimulation. The time relationships between these factors, however, does alter normally, but

the mechanisms involved, although apparently independent of vagal stimulation, are not known.

These experiments did not permit an investigation of the effects of vagal stimulation on the tone of the heart muscle, nor was it possible to determine changes in the amplitude and strength of each contraction of the heart from the records of the E.C.G. From visual observations, however, it is thought that the negative tonotropic and +ve inotropic effects recorded by Jullien and Ripplinger (1950 b) from the heart of marine teleosts, could equally be the effect of blood collecting in the sinus and auricle, than a direct effect of vagal stimulation. McWilliam (1885) found that when the heart of the eel was arrested by vagal stimulation the heart remained in diastole, and the jugular veins, sinus and auricle became filled with blood. Similar effects were observed in the tench in these experiments, and the result was that as the sinus and auricle became distended the ventricle was forced towards the ventral surface of the body. Therefore it is thought that such movements may have produced the effects recorded by Jullien and Ripplinger (1950 b)

The records of the activity in the cardiac branch of the vagus suggest that this nerve has an important role in establishing the relationship between heart beat and respiration (see Section IV). Inhibitory impulses in the vagus prevent the heart from beating during a respiratory cough, and during the mouth opening phase of each breathing cycle. The physio-

logical significance of this relationship, however, cannot be determined without some knowledge of the blood flow characteristics through the gills, which are at present unknown. Even so it would seem probable that the basis of such a relationship is to enable a high degree of efficiency in the rate of gas exchange at the respiratory surface. The origin of the vagal activity associated with the inhibition of the heart is also unknown. If it is peripheral origin then one would imagine that afferent fibres connected with this response would originate either from receptors in the gills, or from proprioceptors in the respiratory muscles. If, however, the activity is of central origin then one would expect a nervous connection between the respiratory and cardiac centres in the medulla. This is in fact indicated by other work in which simultaneous inhibition of the heart and respiration has been produced by strong visual stimulation (Otis, Cerf and Thomas, 1957). If the cyclic activity in the vagus originates from a connection between vagal and respiratory centres in the medulla, then the connection could be either one of two types. The activity in the vagus during the mouth opening phase of the respiratory cycle could result from either an overspill of activity into the vagus from the respiratory neurones related to this part of the cycle, or from the inhibition of a continually active vagal centre during the mouth closing phase of the breathing cycle. Van Holst (1934 a and b) has suggested that the spread of activity from automatic cells in the CNS is responsible for

the synchrony of breathing, trunk and pectoral fin movements seen, in the absence of peripheral stimulation, in the goldfish. It is therefore of interest to note that, by comparison with the histograms in figure 11, the cyclic activity in the vagus appears most pronounced in the resting or anaesthetised fish, and that such a system is responsible for the normal regulation of the heart. The rhythmic neurones in this case would be the respiratory neurones in the medulla of the tench connected with the mouth opening phase of the breathing cycle.

In conclusion the results show that the cardiac branch of the vagus contains cholinergic fibres which, when stimulated, inhibit the heart. No tonotropic, inotropic or dromotropic effects could be produced by vagal stimulation, nor could the heart be accelerated. It would therefore appear that the nervous innervation of the heart consists of purely inhibitory fibres affecting heart rate. Activity normally present in this nerve is of cyclic nature, and tends to inhibit the heart beat during the mouth opening phase of the respiratory cycle. This is particularly evident in the resting animal. High carbon dioxide concentrations in the respired water possibly inhibits this vagal activity, which may cause the increased heart rate recorded when the fish is breathing water of high carbon dioxide content. De-oxygenation of the water passing over the gills results in an increase in the amount of activity recorded from the cardiac branch of the vagus, which can be related to the concomitant decrease in heart rate. Reflex inhibition of the

heart when de-oxygenated water is passed over the gills has already been described by Shelton (in Shelton and Randall, 1962). The results of this section add further proof of the existence of this vagal reflex, but add nothing to the other pathways and receptors involved in the mediation of this response.

Section VII

THE REGULATION OF HEART RATE IN TELEOST FISH

(i) Introduction

It has been shown that tachycardia is a common occurrence in the fish used in these experiments. Handling and perfusing the gills with an aqueous solution of MS 222 Sandoz has been shown to accelerate the heart rate. This can result in heart rates of 90 per minute as opposed to a normal resting value of about 25 per minute in the resting tench. If the nervous innervation of the heart of the tench consists of purely inhibitory fibres affecting heart rate, as is suggested in the previous section, and also if the heart rate is determined by nervous regulation alone, then heart rates of 90 per minute must be produced by the removal of vagal restraint on the heart. If these fibres are cholinergic, then similar increases in rate ought to result from injections of atropine into the pericardial cavity.

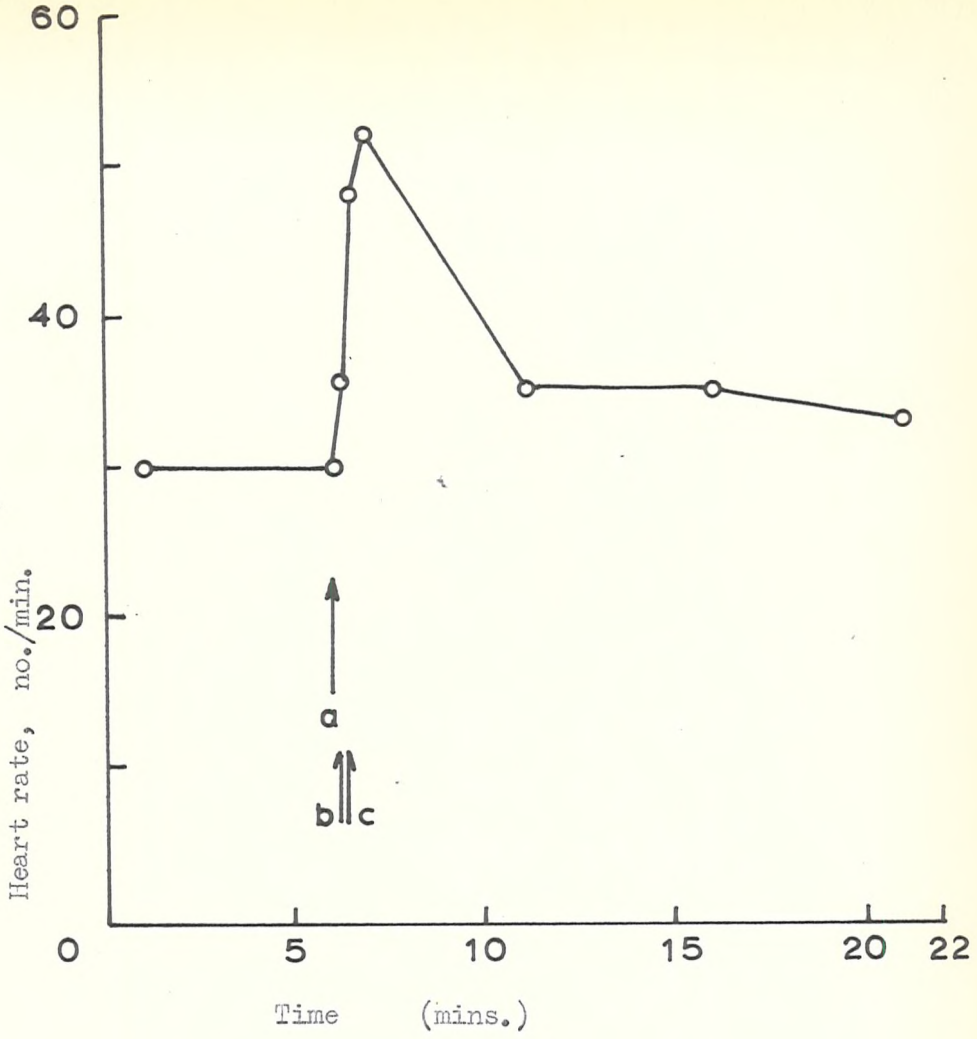
(ii) Methods

A fish was restrained in the tank, ventral surface uppermost, and the E.C.G. was recorded. The effects of

(1) atropine injections into the pericardial cavity of the fish,

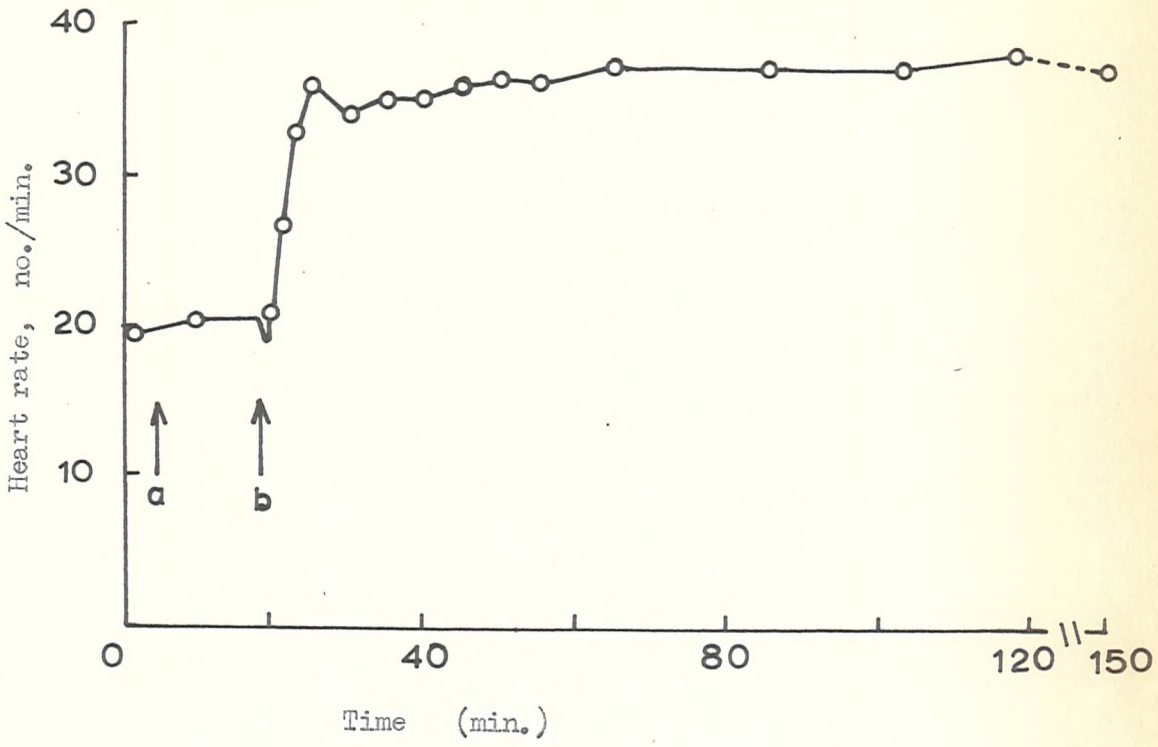
(2) increased activity of the animal, and

(3) injections of adrenaline into the ventral aorta of a single tench,



a Tail pinched with a pair of forceps.
b One lateral movement of tail.
c Two lateral movements of tail.

Figure 26a. The effect of activity on the heart rate of the goldfish. Temp. 16°C.



a Hypodermic inserted into ventral aorta.
b 0.2 ml. of adrenaline in saline injected into ventral aorta.

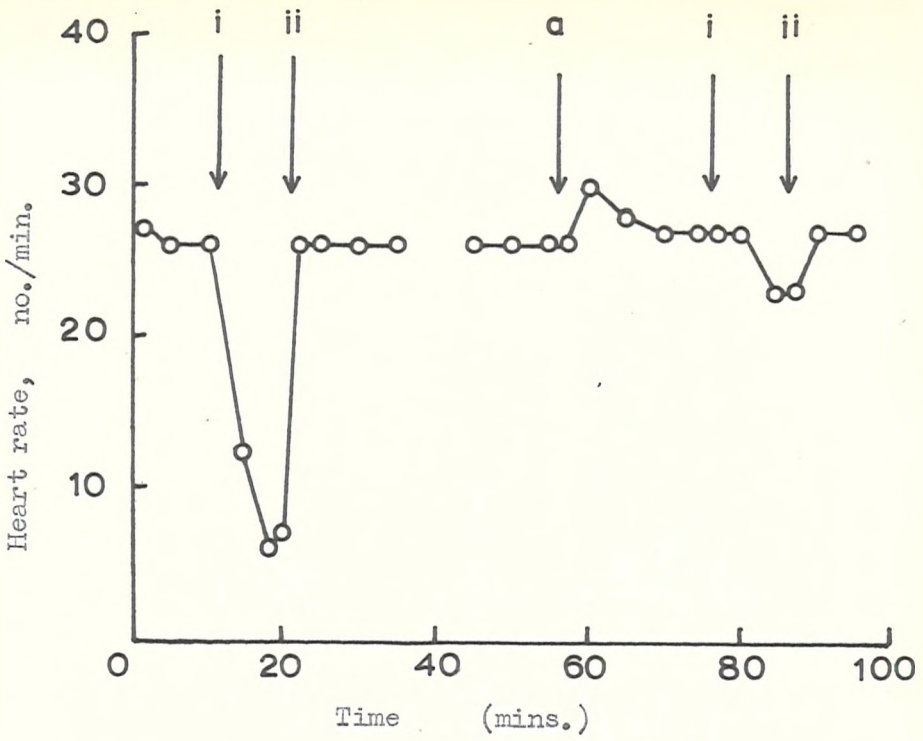
Figure 26b. The effect of injecting 10µg of adrenaline into the exposed ventral aorta on the heart rate of the tench.

on heart rate were then recorded. A 1 ml syringe and a No.20 hypodermic needle were used for the injections. In the single experiment on the effects of injections of adrenaline into the ventral aorta, the heart and ventral aorta were exposed by dissection as described in Section VI. The experiments were carried out on 11 tench (10-232 grms weight, average 73 grms), and 5 goldfish (68-74 grms weight). During the experiments the gills of the fish were forcibly perfused with oxygenated tap water.

(iii) Results

Activity results in a sharp rise in heart rate. In the particular experiment recorded in figure 26a the tail of the goldfish was pinched with a pair of forceps, which resulted in three lateral movements of the tail and a 73% increase in heart rate. The response of the heart was extremely rapid, the highest rate being recorded 2 minutes after disturbing the animal.

Injections of 0.5 ml of atropine into the pericardial cavity produces a slight increase in heart rate in the goldfish (Fig.27), and a 15-20% increase in heart rate in the tench. The flow of water passing over the gills was stopped 10 minutes before and after the atropine injections. (Fig.27) This was done to ensure that the injections were blocking the effects of vagal inhibition, and it can be seen that the injections of atropine remove the inhibitory effect of the vagus on the heart (Fig.27) produced by stopping the flow of water over the gills.



a 0.5 ml. of atropine/saline (10^{-3}) injected into pericardial cavity.
i Water flow over gills stopped.
ii Water flow over gills started.

Figure 27. The effect of injections of atropine into the pericardial cavity of the goldfish, on the heart rate and on the response of the heart to a stoppage of water flow over the gills.

Injections of saline into the exposed but intact ventral aorta of the tench produces a decrease in heart rate, probably due to the rise in blood pressure that must be produced by such an injection. If, however, the saline contains adrenaline, after a primary slowing there is a rapid increase in heart rate, an effect that lasts for several hours. (Fig.26b)

(iv) Discussion

From the results it seems improbable that acceleration of the heart is achieved by the removal of vagal inhibition alone. Bilateral vagotomy (see Section III) and injections of atropine into the pericardial cavity, although removing the effects of vagal inhibition and causing some increase in the heart rate, do not produce such large increases in heart rate as recorded in the intact animal when the gills are perfused with MS 222 Sandoz, or when the activity level of the animal is raised.

If the heart rate is only partially regulated by the nerves that innervate the heart, then it must also be responding to either mechanical or humoral changes in the blood or tissues in or near the heart.

The effects of increased venous return on cardiac output (Johansen, 1962) and heart rate (Labat et al, 1961) have produced variable results. Johansen found that increased stroke volume in the cod was augmented by increased venous return, but that this had very little effect on heart rate. Labat et al produced a marked tachycardia in the catfish by injecting 1 ml of

saline into the hepatic vein. This effect, however, was removed by prior injections of atropine, or by bilateral vagotomy. Johansen has suggested that increased venous return is facilitated by muscular movements during activity, but found that in free swimming teleosts exercise was not accompanied by cardiac acceleration. In the goldfish, however, there is an increase in heart rate associated with muscular movements of the tail, and it is possible that under these conditions, increased venous return is important in raising the heart rate, as demonstrated by Labat et al in the catfish. It is, however, difficult to envisage the significance of increased venous return when the heart is accelerated by MS 222 Sandoz. Indeed, it is difficult to understand how passing a solution of this compound over the gills could produce an increase in venous return, unless it was preceded by an increase in cardiac output. Mott (1951) has demonstrated that a rise in blood pressure in the branchial vessels of the eel results in reflex slowing of the heart. Keys and Bateman (1932) have shown that perfusion of the branchial vessels of this fish with a saline containing adrenaline results in a decrease in the resistance of the gill vessels to the flow of saline. The increased heart rate that followed the injection of adrenaline into the ventral aorta of the tench therefore is probably a reflection of the branchial vasodilatory effects of this drug implicit in the work of Keys and Bateman. The vasodilation would reduce the blood pressure and therefore the level

of vagal inhibition on the heart rate, so producing cardiac acceleration. The injection, however, resulted in an 85% increase in heart rate, which was much larger than any increase resulting from bilateral vagotomy or injections of atropine. It is therefore possible that a reduction in ventral aortic pressure may produce an increase in heart rate directly, and that a similar mechanism is in operation when the heart rate is increased in response to MS 222 Sandoz in the ventilation stream. Another possibility is that humoral agents are being released into the blood, or that adrenaline is being carried back to the heart via the coronary circulation, but little is known of the effects of sympathetic drugs on the heart of fish (Mott, 1957).

The hypothesis that reduced ventral aortic blood pressure, or that the release of humoral agents into the blood may produce an increase in heart rate is based on circumstantial evidence, recorded from experiments, many of which have no relationship to physiological conditions in the intact animal. The conclusions drawn from such experiments therefore, must be of doubtful significance, but the results may give some indication as to possible mechanisms involved in the regulation of the heart in the intact animal.

Section VIII

THE MEASUREMENT OF INTRACELLULAR POTENTIALS FROM THE FISH HEART IN SITU

(i) Introduction

The E.C.G. is a record of the potential changes that take place in the electrical field generated by the heart during a contraction. These changes are directly related to the changes in membrane potential that occur in fibres of the heart muscle when they contract during a heart beat.

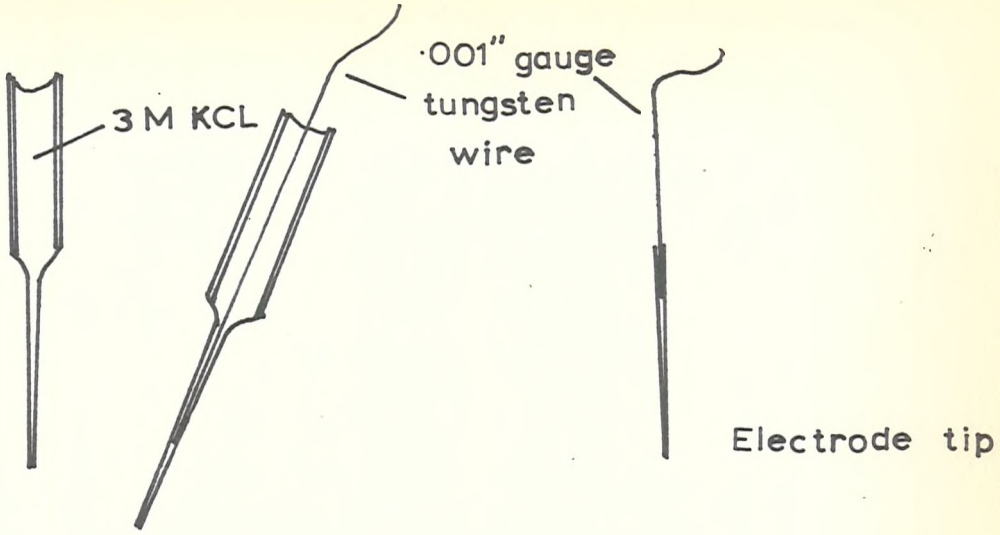
Intracellular potentials have been recorded from the hearts of various vertebrates, both *in situ* (Woodbury *et al*, 1951; Woodbury and Brady, 1956; Hoffman and Suckling, 1952, 1953), and isolated from the body (Weidmann, 1951; Draper and Weidmann, 1951; Hutter and Trautwein, 1955, 1956; Burgen and Terroux, 1953; Cranfield and Hoffman, 1958) using glass micro-electrodes (Graham and Gerard, 1946; Ling and Gerard, 1949).

The membrane potential of the heart muscle fibres during diastole appears to be between 50mV and 100mV, the higher potentials generally being recorded from mammalian hearts. It is not known whether the variations in membrane potential represent species variation, or are the result of differences in micro-electrode characteristics, but Woodbury *et al* (1951) have related small tip diameter of the micro-electrode to high membrane potentials.

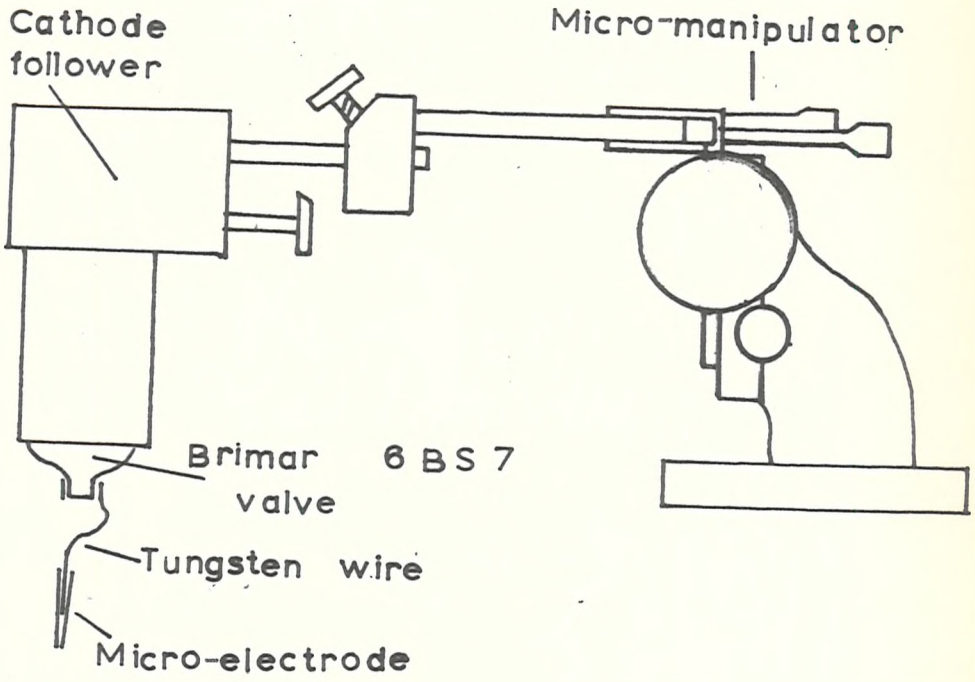
The highest membrane potentials during diastole have generally been recorded from fibres outside the region of the pacemaker fibres.

Intracellular potentials recorded from pacemaker fibres differ from those recorded in other fibres of the heart in that the membrane potential during diastole is not constant, but on reaching a maximum directly after systole, slowly depolarises before changing into a much more rapid action potential associated with the next systole. The effects of vagal stimulation on the pacemaker potential have been investigated in the frog and tortoise (del Castillo and Katz, 1955; Hutter and Trautwein, 1955, 1956). During vagal stimulation the diastolic membrane potential of the pacemaker cells is raised to a level above the maximum recorded between previous beats of the heart. This inhibits the formation of the action potential associated with systole, and so prevents the heart beating. The repolarisation of the action potential, however, is greatly facilitated. Potentials recorded from the auricular fibres are unaffected by vagal stimulation. Sympathetic stimulation of the frog heart accelerates the rate of rise of the pacemaker potential, increasing the heart rate, and augments the size of the action potential.

The main problems associated with intracellular recording from muscle tissues arise from the mechanical movements associated with the action potentials in the fibres. These can be overcome either by immobilising the tissue with reference to



a) The micro-electrode.

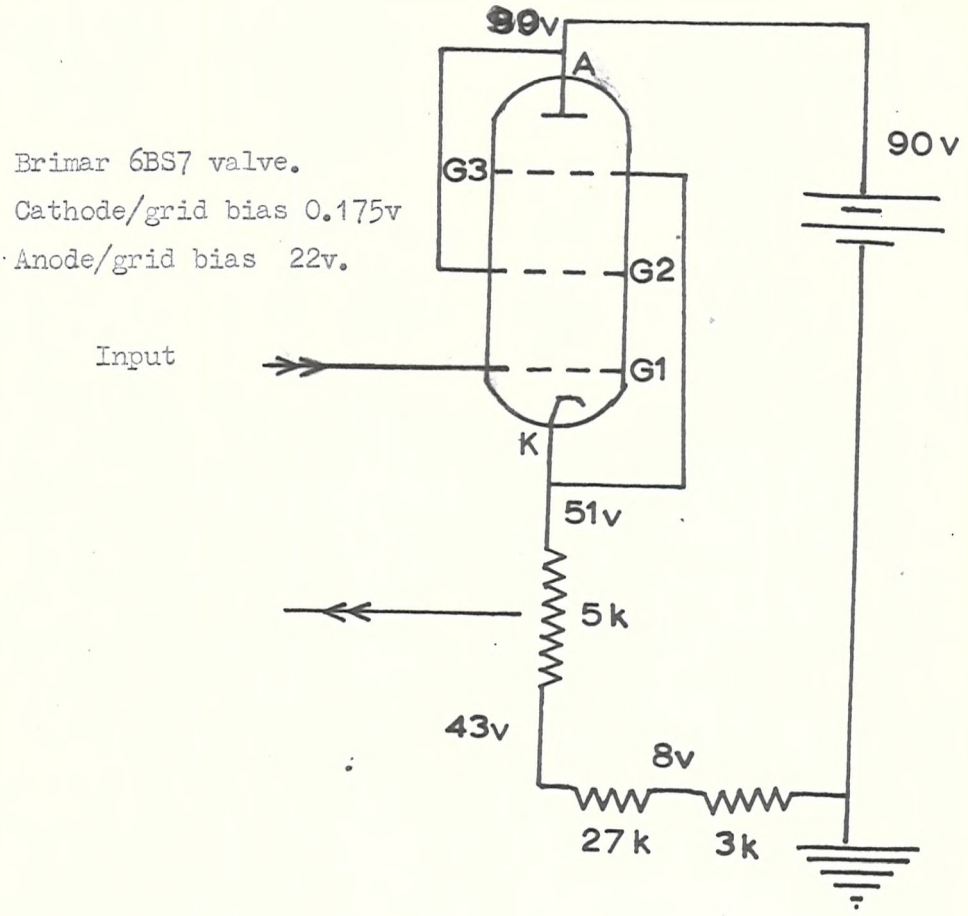


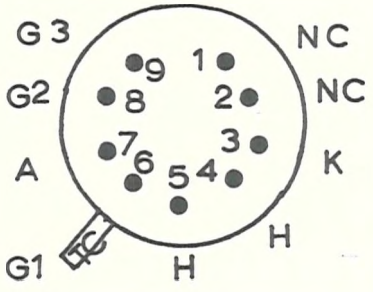
b) Electrode-cathode follower assembly.

Figure 28. Intracellular recordings from the hearts of fish.: Apparatus.

Figure 28.

c) Cathode follower: circuit.





a rigid recording system or by using a floating electrode which parallels the movements produced by muscular contractions. It is obviously impossible to immobilise the heart *in situ* and record intracellular potentials during a normal heart beat; therefore in these experiments a floating electrode similar to that described by Woodbury and Brady (1956) was used to record intracellular potentials from fish hearts *in situ*.

(ii) Methods

Glass microelectrodes were drawn on a Palmer Micro-Electrode Puller (H 101) supplied with a Power Control Unit (H 104) from glass tubing of 1 to 1.25 mm bore and 2 mm outside diameter. The electrodes were filled with distilled water by boiling under reduced pressure, and were then transferred to a 3M KCl solution for a period of 24 hours. A 1" length of 0.001" gauge tungsten wire was then inserted into the tip of the microelectrode until it jammed. The distal centimetre of the electrode was then broken off, and the shank of the electrode was removed, leaving the tip of the microelectrode, filled with 3M KCl, fixed to the end of a 1" length of tungsten wire. (Fig.28a). This was then soldered directly to the grid of a Brimar 6 BS 7 valve, the basis of the cathode follower.

The cathode follower with microelectrode attached, was mounted above the heart on a micromanipulator, an arrangement that enabled the cathode follower and electrode to be moved as a single unit. By lowering the cathode follower the electrode could be pushed into the heart, the tungsten wire acting as a buffer between the mobile heart and electrode and the rigid but moveable cathode follower. (Fig.28b)

The microelectrodes were stored in 3M KCl before use, but were never kept for longer than five days. The surface of the electrode was washed in distilled water before use. The output of the cathode follower was connected to the input of a Tektronix 502 oscilloscope and permanent records were photographed with a Cossor Oscilloscope Camera.

The resistance of the microelectrode was determined by passing a square wave calibration signal through the microelectrode which was connected to the input of the oscilloscope. The voltage deflection was measured, and then various resistances were placed in series with the microelectrode until the voltage deflection on the oscilloscope was halved. The resistance of the microelectrode was then equal to the known resistance introduced into the circuit.

The cathode follower, built to a design originating in the Electronics Department of this University (Fig. 28c), had a grid current of 2.5×10^{-12} Amps when the input impedance was 20 meg Ω.

The procedure for restraining the fish and exposing the heart was similar to that described in Section VI.

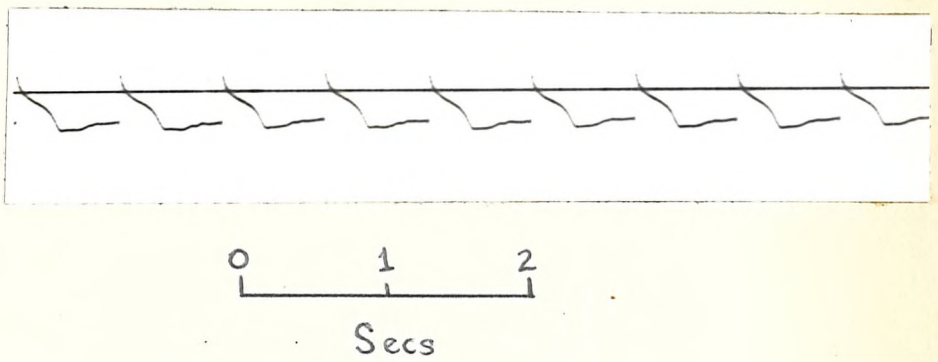
The experiments were carried out on six dace (144-170 grms weight), two tench (149-297 grms weight), three chub (107-131 grms weight) and one roach of 82 grms weight, attemperatures between 11 and 14°C.

These experiments are still incomplete, but the results obtained so far are of interest.

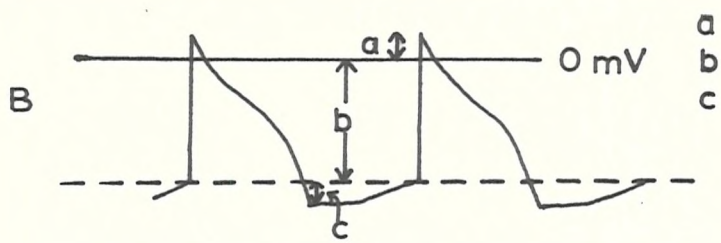
Overshoot potential= +20mV

Diastolic membrane potential= -50mV

Maximum pacemaker potential= 13mV



A



a Overshoot potential.
b Diastolic membrane potential.
c Maximum pacemaker potential.

Figure 29.

A. Intracellular potentials recorded from a ventricular fibre of the roach heart.
B Diagrammatic representation of intracellular potentials, illustrating nomenclature used in text.

(iii) Results

The main problem associated with the methods used to measure intracellular potentials in these experiments was in determining the most suitable length of tungsten wire on which to mount the microelectrodes. If too long a length of wire was used the microelectrode did not penetrate the heart tissue; if the length of wire was too short there was a loss in the mobility of the electrode, and movements of the heart tended to pull the electrode out of the cell. Eventually it was found that lengths of about 1" were most suitable; penetration was relatively easy, and the electrode would remain fixed in the cell. So far intracellular potentials have only been recorded from cells in the ventral wall of the ventricle. The magnitude of the potential changes relating to heart systole and diastole, recorded from these fibres remained constant for the first 5 to 10 minutes after the electrode had penetrated the cell, but after this period the size of the potential changes gradually diminished. This is almost undoubtedly associated with the presence of the microelectrode within the cell.

Figure 29a is a typical record of the potential changes measured from a ventricular fibre during normal ventricular systole. The very rapid depolarisations of the cell membrane are associated with the contractions of the ventricle. Intracellular potentials were recorded from cells in the ventral wall of the ventricle of 12 fish, and up-to 10 fibres in each ventricle,

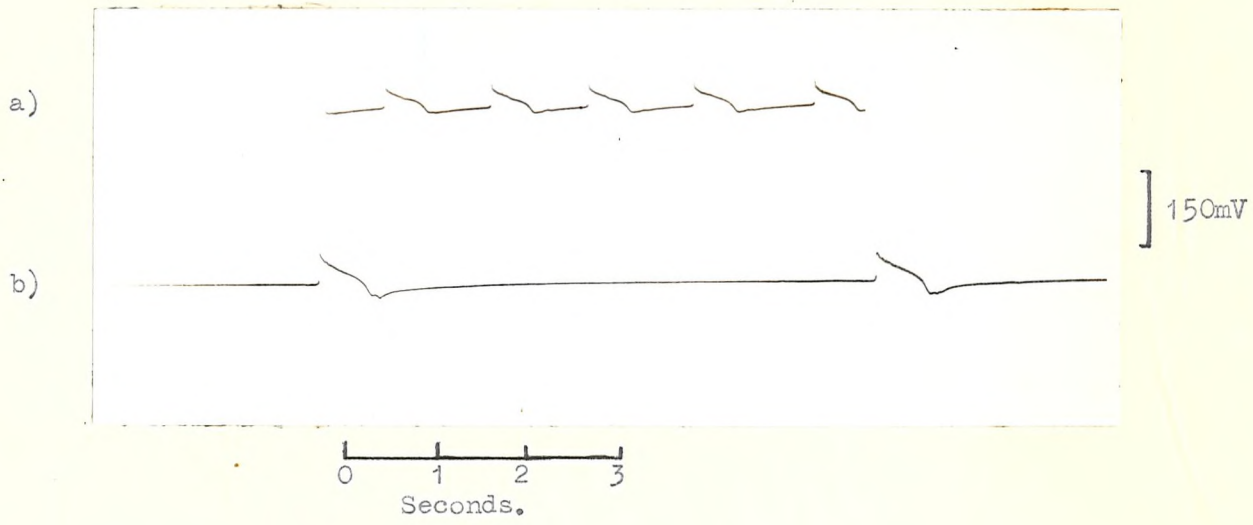
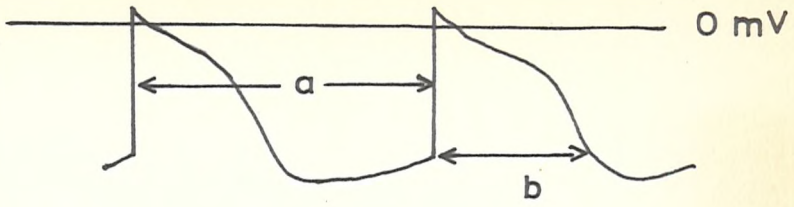


Figure 30a. Intracellular recordings from a ventricular fibre of the heart of a tench.

a) Normal heart beat.

b) Flow of water over the gills stopped; heart rate inhibited.

Diagrammatic intracellular potential from heart muscle fibre.



a Heart beat interval.

b Duration of systolic action potential.

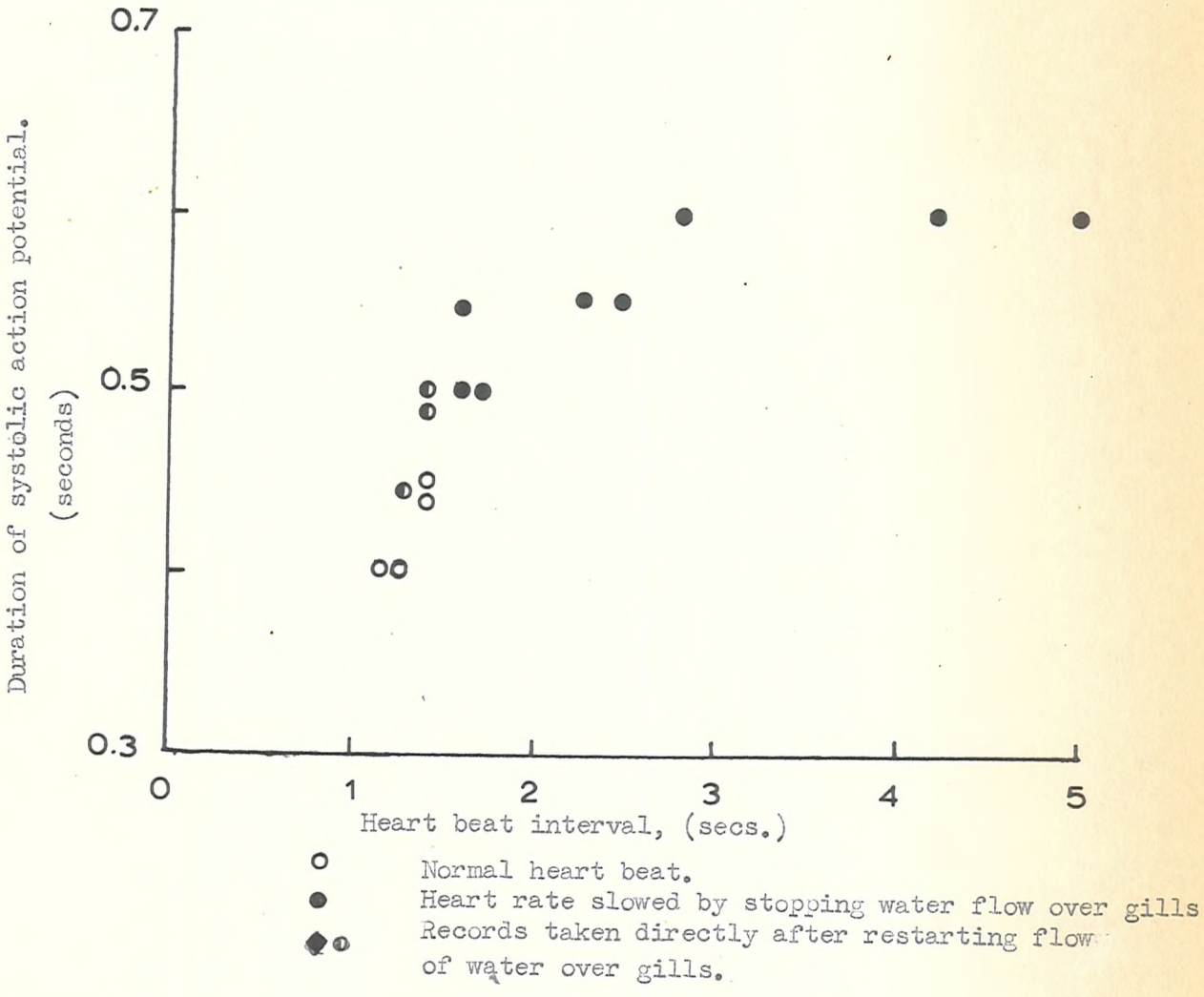


Figure 30b. The relationship between heart beat interval and the duration of the systolic action potential recorded from ventricular muscle fibres of the roach.

and in all cases they resembled those recorded from the pacemaker region of the hearts of other animals (cf. Hutter and Trautwein, 1955). The diastolic membrane potential (see Fig. 29b) was in the range of 40 to 60mV, and the overshoot potential was usually between 5 to 20mV. The values for the diastolic membrane potential appear to be lower than those recorded from other vertebrates. It is not known if this is a reflection of imperfections in the recording technique, or whether these measurements approximate to the actual membrane potential of these cells. It is hoped that further recordings will clarify this matter. The maximum pacemaker potential was normally in the range of 5 to 15mV, but during inhibition of the heart beat this was sometimes raised up to 30mV. Reflex inhibition of the heart was produced by stopping the flow of water over the gills. This inhibition, as well as lengthening the heart beat interval, raised the level of the maximum pacemaker potential (Fig.30) and lengthened the duration of the systolic action potential.

(iv) Discussion

Kisch (1948) has shown that there are automatic centres in the Cuverian veins, sinus, auricle, auricular-ventricular connective tissue, ventricle and bulbus cordis of the fish heart; also McWilliam (1885) found that many parts of the heart of the eel would beat when isolated from the body. This, and the fact that all the potential changes recorded from the ventricu-

lar fibres of the fish heart resembled potential changes recorded from the pacemaker region of the hearts of other vertebrates, suggests that automatically contracting fibres have a much more widespread distribution in the hearts of fish than in some other vertebrate groups. It would seem that in warm blooded animals pacemaker activity is normally confined to the S-A and the A-V nodes. In fish, however, potentials have only been recorded from cardiac ventricular cells. Although there is obviously a fairly wide distribution of pacemaker cells in this region of the heart, these results do not exclude the possibility of other types of cells in the heart, or even in the ventricle. In most vertebrates it appears that the group of autogenic fibres which has the highest intrinsic rhythm dominates the heart rate. McWilliam (1885) suggested that the heart rate of the eel was generally controlled by the sinus tissue close to the auricular-ventricular junction, because this tissue had the highest beat rate when isolated from the body.

The ventricular fibres of the fish heart appear similar to the pacemaker cells of the frog and tortoise hearts (Hutter and Trautwein, 1955) in that during periods of vagal inhibition, the pacemaker potential is raised above the normal diastolic level. The cardiac fibres of the two groups differ, however, in that vagal stimulation of the pacemaker cells of the tortoise heart results in a more rapid repolarisation of the membrane after a systolic action potential, whereas in the fish ventricular

fibres, the duration of the action potential is prolonged during inhibition of the heart. In this respect the ventricular fibres of the fish resemble tetrapod ventricular cells, for Johansen (1959) has shown that during cardiac inhibition in the snake the T wave of the E.C.G., which is associated with the period of repolarisation of the cardiac ventricular cells, is lengthened. The significance of these differences is not known, but it is possible that the fish ventricular fibres represent an intermediate stage between the tetrapod pacemaker cell and those tetrapod ventricular fibres which do not have the power of contracting rhythmically and automatically.

Section IX

FINAL DISCUSSION

The results of this thesis show that there is a relationship between the heart and breathing rates in teleost fish. The significance of such a relationship is probably to be found in terms of the efficiency of gas exchange at the respiratory surface. The important factors in this respect however are not heart and breathing rates, but blood flow through, and water flow over, the gills. The results only indicate a qualitative relationship between blood and water flows, and quantitative measurements of these factors in the intact animal are necessary if the understanding of the relationship between blood and water flows and the efficiency of gas exchange at the respiratory surface is to be increased.

Changes in the blood-water flow relationship at the gills might be expected if the oxygen consumption of the fish varies or if the environmental gas tensions alter. This again is indicated in qualitative terms in the results.

A comparison between air breathing vertebrates and fish shows that in both cases increased carbon dioxide or decreased oxygen content in the respired medium causes an increase in ventilation volume. In both cases carbon dioxide receptors,

closely associated with the respiratory centre, have been located in the medulla. Decreased oxygen content in the ventilation stream increases the nervous activity recorded from the branch of the vagus innervating the carotid glomus of the cat (von Euler, 1940, in Young, 1960). The homology of the carotid arch of mammals with the vessels of the first gill arch of fish, indicates the possibility of oxygen receptors in the gill vessels of fish. The work of Shelton (see Hughes and Shelton, 1962) however, has demonstrated the independence of the response of the branchial pump of fish to decreased oxygen concentrations in the respired water from receptors innervated by the IXth and Xth cranial nerves. It appears, therefore, that there are some differences in the response of respiration in fish and mammals to decreased oxygen content in the ventilation stream.

The fish heart differs from the tetrapod heart in both anatomical and physiological characteristics. The heart of fishes consists of four chambers in series, and because the respiratory and systemic circulations are also in series rather than in parallel as in tetrapods, none of the blood contained in the heart is fully oxygenated, and all of the blood pumped by the heart passes through the respiratory circulation before continuing round the body. Added to these differences there is no sympathetic innervation of the fish heart, and the distribution of cardiac muscle fibres which are capable of automatic rhythmical contractions appears more widespread.

Even in the absence of a direct sympathetic innervation, acceleration of the heart rate is a common occurrence in many teleost fish. This is produced partly by the removal of vagal inhibition and partly by some other non nervous mechanism. In this respect it is of interest to note that in the dog and man much of the increased heart rate due to exercise may be ascribed to the central inhibition of vagal tone (Hoff, 1955). Indeed, although the level of tonic activity appears to be much lower in fish than in mammals, there seem to be many similarities in the parasympathetic innervation of the hearts of fish and tetrapods. Cyclic vagal inhibition of the heart associated with a certain phase of the respiratory cycle has been demonstrated in elasmobranchs (Satchell, 1960) as well as in teleosts, and both are possibly comparable with sinus arrhythmia recorded in many mammals, in which there is a diminution of vagal tone during inspiration, due to central inhibition of the cardiac centre. This results in an increase in heart rate during inspiration and a decrease during expiration. In all cases the response is most obvious in the resting animal. It would seem, therefore, that increased parasympathetic inhibition of the heart rate during a certain phase of the breathing cycle is a reflex common to many vertebrate groups. Reflex inhibition of the heart produced by rises in blood pressure (Mott, 1951) and asphyxial conditions (Scholander et al, 1962) are other vagal reflexes that are apparently widespread throughout many vertebrate classes.

The sympathetic regulation of heart rate in mammals is less clear cut than parasympathetic control, the heart, in general, being only one of several target organs in a generalised sympathetic discharge. This, and the absence of any sympathetic innervation in the heart of fishes, suggests that sympathetic control of the heart is an addition to the more distinct and ancient parasympathetic regulation of the vertebrate heart.

The fish heart with its diffuse distribution of rhythmically active muscle fibres is somewhat similar to the developing heart in a chick embryo. At first the chick heart is a simple straight tube, but gradually, as the myocardium grows back over this tube, the ventricle, atria and then sinus develop. Before the atria form the ventricle beats alone at a very slow rate, but when the atria develop they assume the role of pacemaker because the rate of contraction of the auricle is higher than that of the ventricle, and the beat rate of the whole heart is increased. The intrinsic rhythmicity of the sinus venosus when formed is in turn greater than that of the auricle, and so it usurps the position of the pacemaker and once again the rate of the whole heart is increased. Similarly, in the fish heart, the pacemaker region is not a discrete group of myocardial cells which alone have the power of contracting automatically and rhythmically, but is determined by which group of active fibres has the highest intrinsic rate.

The relationship between cardiac output, stroke volume and heart rate is complex. It was thought that in mammals, increased venous return, producing an increase in stroke volume, was an important factor in augmenting cardiac output. This concept was largely based on results obtained from the heart-lung preparation. More recent work, however, has demonstrated that in the intact animal the significant factor in increased stroke volume is not increased diastolic distension of the heart, but a more complete emptying of the heart during systole (Rushmer, 1960). It has also been shown that during exercise increased heart rate is the significant factor in producing a larger cardiac output in the intact dog, and that this is normally associated with a constant stroke volume (Rushmer, 1959) and an increase in the difference between oxygen tensions in the venous and arterial blood.

The relationship between these factors in the intact fish, although of considerable importance in the interpretation of many of the results of this thesis, is not known. Activity in some fish does result in an increased heart rate, but stroke volume augmented by either an increase in venous return or a decrease in ventral aortic pressure, although not a common control mechanism in tetrapods, may be of importance in fish.

The regulation of respiration and heart rate in teleosts is in certain respects similar to that of other vertebrate groups,

which to some extent is a reflection of the common ancestry of all these groups. Fish, however, are a very specialised group in their own right, and have very successfully colonised aquatic environments. It is therefore not surprising that specialisation to these particular environments is reflected in some of the physiological mechanisms of the regulation of breathing and heart rate in fish.

Section X

SUMMARY

The experiments were carried out on tench, roach, dace, chub, eels, trout and goldfish.

MS 222 Sandoz, handling and activity produce an increase in the heart and breathing rates of the intact fish. The response of the heart to MS 222 is not due to a direct effect of the anaesthetic on the heart tissues, nor is it entirely dependent on the nervous innervation of the heart.

In the resting fish the heart tends to beat during the mouth closing phase of the breathing cycle, which in the tench is due to cyclic inhibitory activity in the vagus associated with the mouth opening phase. The level of tonic activity in the vagi is generally lower in fish than in mammals, and is increased during a respiratory cough, when breathing stops, and when the water flow over the gills is stopped, or contains very little oxygen. The tench heart is innervated by a cardiac branch of the vagus; this contains fibres which, when stimulated, slow the heart. Increases in heart rate are only partially produced by the removal of vagal inhibition.

Decreased oxygen content in the water increases the frequency and amplitude of the breathing movements in the tench. The resulting increase in ventilation volume maintains a sufficient quantity of oxygen at the respiratory surface for the metabolic requirements of the animal. The heart rate is unaffected until the oxygen concentration falls below 3 mg/litre, when it is inhibited.

High carbon dioxide concentrations in the environment produce an increase in heart rate and breathing amplitude, but a decrease in breathing rate. The increased heart rate offsets the Bohr effect. The responses of the breathing apparatus, in the absence of carbon dioxide receptors exposed to the medium, are thought to be related to high carbon dioxide tensions in the tissues.

The distribution of rhythmically active autogenic fibres are widespread in the heart tissues. Ventricular fibres have a typical pacemaker potential which is increased during vagal inhibition; the repolarisation from the systolic action potential, however, is not facilitated but lengthened during this period.

BIBLIOGRAPHY

BALL, J.N. and COWAN, P.N. (1959) Urethane as a carcinogen and as an anaesthetic for fishes. Nature, London, 184, p. 370.

BASU, Satyendra Prasanna (1959) Active respiration in fish in relation to ambient concentrations of oxygen and carbon dioxide. J. Fish. Res. Bd. Canad., 16, pp. 175-212.

BIJTEL, J.H. (1949) The structure and mechanism of movement of the gill filaments in teleostei. Arch. Neerl. Zool. 8, pp. 1-22 and (iii)

BLACK, E.C. (1940) The transport of oxygen by the blood of freshwater fish. Biol. Bull. Woods Hole, 79, 215-229.

BLACK, E.C. (1951) Respiration in Fishes. In Some aspects of the physiology of fish, part III, by Hoar, W.S., Black, V.S. and Black, E.C. Univ. Toronto Studies Biol. Ser. No. 59, Publ. Ontario Fish Res. Lab. 71, pp. 91-111.

BRETT, J.R. (1962) Some considerations in the study of respiratory metabolism in fish, particularly salmon. J. Fish. Res. Bd. Canad. 19. pp. 1025-1038.

BURGEN, A.S.V. and TERROUX, K.G. (1953) On the negative inotropic effect in the cat's auricle. J. Physiol., 120, pp. 449-464.

BURGER, J.W. and BRADLEY, S.E. (1951) The general form of circulation in the dogfish, Squalus acanthias. J. Cell. Comp. Physiol., 37, pp. 389-402.

BURNSTOCK, G. (1958) Reversible inactivation of nervous activity in a fish gut. J. Physiol. 141, pp. 35-45.

CAMPBELL, G.D., and DAVIES, D.H. (1963) Effect of Ethyl m-Amino benzoate (MS 222) on the elasmobranch electrocardiograph. Nature, London, 198, p. 302.

CRANEFIELD, P.F. and HOFFMAN, B.F. (1958) Propagated repolarisation in heart muscle. J. Gen. Physiol., 41, pp. 633-649.

DE MARTINI, F.E. (1938) Corrosion and the Langelier calcium carbonate saturation index. J. Amer. Wat. Wks. Ass., 30, p. 85.

del CASTILLO, J. and KATZ, B. (1955) Production of membrane potential changes in the frog's heart by inhibitory nerve impulses. Nature, London, 175, p. 1035.

DONALDSON, P.E.K. (1958) Electronic Apparatus for Biological Research. Butterworth Scientific Publications, London.

DRAPER, M.H. and WEIDMANN, S. (1951) Cardiac resting and action potentials recorded with an intracellular electrode. J. Physiol., 115, pp. 74-94.

FLEMISTER, L.J. (1958) p. 281. In Handbook of Respiration. (Edited by D. S. Dittmer and R. M. Grebe. W. B. Saunders Company. Philadelphia and London.

FRY, F.E.J. (1947) Effects of the environment on animal activity. Univ. Toronto Studies, Biol. Ser., No. 55, Publ. Ontario Fish Res. Lab., No. 68, pp. 1-62.

FRY, F.E.J. (1951) A fractionating column to provide water of various dissolved oxygen content. Canad. J. Technol., 29, pp. 144-146.

FRY, F.E.J. (1957) The aquatic respiration of fish. In The physiology of fishes. (Edited by M. E. Brown). Vol. I, Academic Press, New York.

FRY, F.E.J. and HART, J.S. (1948) The relation of temperature to oxygen consumption in the goldfish. Biol. Bull., Woods Hole, 94, pp. 66-77.

GASKELL, W.H. (1887) On the action of muscarin upon the heart and on the electrical changes in the non-beating cardiac muscle brought about by stimulation of the inhibitory and augmentor nerves. J. Physiol., 8, pp. 404-414.

GILBERT, P.W. and WOOD, F.G. (1957) Methods of anaesthetising large rays and sharks safely and rapidly. Science, 126, p. 212.

GRAHAM, J. and GERARD, R.W. (1946) Membrane potentials and excitation of impaled single muscle fibres. J. Cell. Comp. Physiol. 28, pp. 99-117.

GRAY, I.E. (1947) The relation between gill surface and activity in marine fishes. J. Elisha. Mitchell Sci. Soc. 63, p. 106.

GRAY, I.E. and HALL, F.G. (1930) Blood sugar and activity in fishes with notes on the action of insulin. Biol. Bull., Woods Hole, 58, pp. 217-223.

HALL, F.G. (1931) The respiration of the puffer fish. Biol. Bull, Woods Hole, 61, pp. 457-467.

HALL, F.G. and GRAY, I.E. (1929) The hemoglobin concentration of the blood of marine fishes. J. Biol. Chem., 81, pp. 589-594.

HOFF, H.E. (1955) Cardiac output: Regulation and Estimation. In A textbook of Physiology (17th edition, Edited by J. F. Fulton). W. B. Saunders and Company, London and Philadelphia.

HOFFMAN, B.F. and SUCKLING, E.E. (1952) Cellular potentials of intact mammalian hearts. Amer. J. Physiol., 170, pp. 357-362.

HOFFMAN, B.F. and SUCKLING, E.E. (1953) Cardiac cellular potentials: Effect of vagal stimulation and acetylcholine. Amer. J. Physiol., 173, pp. 312-320.

HUGHES, G.M. (1960) The mechanism of gill ventilation in the dogfish and skate. J. Exp. Biol., 37, pp. 11-27.

HUGHES, G.M. (1960) A comparative study of gill ventilation in marine teleosts. J. Exp. Biol., 37, pp. 28-45.

HUGHES, G.M. (1961) Gill ventilation in fish. Rep. Challenger Soc., 13 no. 3., pp. 23-24.

HUGHES, G.M. and SHELTON, G. (1957) Pressure changes during the respiratory movements of teleostean fish. Nature, London, 179, p. 255.

HUGHES, G.M. and SHELTON, G. (1958) The mechanism of gill ventilation in three freshwater teleosts. J. Exp. Biol., 35, pp. 807-823.

HUGHES, G.M. and SHELTON, G. (1962) Respiratory mechanisms and their nervous control in fish. In Advances in Comparative Physiology and Biochemistry, Vol. I. (edited by O.E. Lowenstein.) Academic Press, New York.

HUTTER, O.F. and TRAUTWEIN, W. (1955) The effect of vagal stimulation on the sinus venosus of the frog heart. Nature, London, 176, pp. 512-513.

HUTTER, O.F. and TRAUTWEIN, W. (1956) Vagal and sympathetic effects on the pacemaker fibres in the sinus venosus of the heart. J. Gen. Physiol. 39, pp. 715-733.

JOHANSEN, K. (1959) Heart activity during experimental diving of snakes. Amer. J. Physiol., 197, pp. 604-606.

JOHANSEN, K. (1962) Cardiac output and pulsatile flow in the teleost Gadus morhua. Comp. Biochem. Physiol. 7, pp. 169-174.

JULLIEN, A. and RIPPLINGER, J. (1950) a, Le rameau cardiaque du pneumogastrique des poissons est formé d'au moins deux nerfs; un nerf chronotrope cholinergique, et un nerf tomotrope, non cholinergique. C. R. Acad. Sci. Paris, 230, pp. 867-868

JULLIEN, A. and RIPPLINGER, J. (1950) b, Actions comparées du vague sur le coeur de quelques poissons marins. Ann. Sci. Univ. Besançon, 5, pp. 3-23.

JULLIEN, A. and RIPPLINGER, J. (1950) c, Le "temps perdu" dans la réponse du coeur à l'éxcitation du nerf vague chez les poissons d'eau douce. Bull. Soc. Hist. Nat. Doubs., 54, pp. 1-7.

KEYS, A. and BATEMAN, J.B. (1932) Branchial responses to adrenaline and pitressin in the eel.
Biol. Bull., Woods Hole, 63, pp. 327-336.

KISCH, B. (1948) Electrographic investigations of the hearts of fish.
Exp. Med. Surg., 6, pp. 31-62.

KROGH, A. (1904) On the cutaneous and pulmonary respiration of the frog.
Scand. Arch. Physiol., 16, pp. 348-358.

KROGH, A. (1919) The rate of diffusion of gases through animal tissues, with some remarks on the coefficient of invasion.
J. Physiol., 52, pp. 391-408.

KROGH, A. (1941) The Comparative Physiology of Respiratory Mechanisms. 172 pp.
University of Pennsylvania Press, Philadelphia.

LABAT, R; RAYNAUD, P and SERFATY, A. (1961) Réactions cardiaques et variations de masse sanguine chez les téléostéens.
Comp. Biochem. Physiol., 4, pp. 75-80.

LABAT, R; PAYRAUD, C and SERFATY, A. (1962) Réactions électrocardiographique et operculaires provoquées par stimulation lumineuse ou par effet d'approche chez les téléostéen marins.
J. Physiol. Path. Gén., 54, pp. 591-598.

LAURENT, P. (1952-53) L'innervation cardiaque de la tanche. (T. vulgaris Cuv.)
Arch. Zool. Exp. Gén., 84.

LEIVESTAD, H; ANDERSEN, H. and SCHOLANDER, P.F. (1957) Physiological response to air exposure in codfish.
Science, 126, p. 505.

LING, G. and GERARD, R.W. (1949) The normal membrane potential of frog satorius fibres.
J. Cell. Comp. Physiol., 34, pp. 383-396.

LOCKWOOD, A.P.M. (1961) "Ringer" solutions and some notes on the physiological basis of their ionic composition.
Comp. Biochem. Physiol. 2, pp. 241-289.

LUTZ, B.R. (1930) Reflex cardiac and respiratory inhibition in the elasmobranch, Scyllium canicula. Biol. Bull., Woods Hole, 59, pp. 170-178.

LUTZ, B.R. (1930) Respiratory rhythms in the elasmobranch, Scyllium canicula. Biol. Bull., Woods Hole, 59, pp. 179-186.

LUTZ, B.R. (1930) The innervation of the heart of the elasmobranch, Scyllium canicula. Biol. Bull., Woods Hole, 59, pp. 211-216.

LUTZ, B.R. and WYMAN, L.C. (1932) Reflex cardiac and respiratory inhibition of branchio-vascular origin in the elasmobranch Squalus acanthias. Biol. Bull., Woods Hole, 62, pp. 10-16.

LYON, E.P. (1926) A study of the circulation, blood pressure and respiration of sharks. J. Gen. Physiol., 8, pp. 279-290.

McFARLAND, W.N. (1959) A study of the effects of anaesthetics on the behaviour and physiology of fishes. Publ. Inst. Mar. Sci. Univ. Tex., 6, pp. 23-55.

McFARLAND, W.N. (1960) The use of anaesthetics for the handling and the transport of fishes. California fish and game, 46, pp. 407-431.

McWILLIAM, J.A. (1885) On the structure and rhythm of the heart in fishes, with especial reference to the heart of the eel. J. Physiol. 6, pp. 192-245.

MILBURN, T.R. and BEADLE, L.C. (1960) The determination of total carbon dioxide in water. J. Exp. Biol., 37, pp. 444-460.

MOORE, E.W. (1939) Graphic determination of carbon dioxide and the three forms of alkalinity. J. Amer. Wat. Wks. Ass., 31, pp. 51-66.

MOTT, J.C. (1951) Some factors affecting the blood circulation in the common eel. (Anguilla anguilla) J. Physiol., 114, pp. 387-398.

MOTT, J.C. (1957) The cardiovascular system. In The physiology of fishes. (Edited by M.E.Brown). Vol. 1 Academic Press, New York.

NICOL, J.A.C. (1952) Autonomic nervous system in lower chordates.
Biol. Rev., 27, pp. 1-49.

OETS, J. (1950-1952) Electrocardiograms of fishes.
Physiol. Comp., 2, pp. 181-186.

OHLE, W. (1953) Die Chemische und die Elektrochemische Besstimmung des Molekular gelosten Sauerstoffs der Binnengewässer.
Commun. Int. Ass. Theoret. Appl. Limnol., 3, pp. 1-44.

OTIS, L.S; CERF, J.A. and THOMAS, G.J. (1957) Conditioned inhibition of respiration and heart rate in the goldfish.
Science, 126, pp. 263-264.

PEISS, C.N. and FIELD, J. (1950) The respiratory metabolism of excised tissues of warm and cold-adapted fishes.
Biol. Bull., Woods Hole, 99, pp. 213-224.

POWER, E.B. and CLARK, R.J. (Jr.) (1942) Control of normal breathing in fishes by receptors located in the regions of the gills and innervated by the IXth and Xth cranial nerves.
Amer. J. Physiol., 138, pp. 104-107.

RANDALL, D.J. (1962) Effect of an anaesthetic on the heart and respiration of teleost fish.
Nature, London, 195, p. 506.

RANDALL, D.J. and SHELTON, G. (1963) The effects of changes in environmental gas concentrations on the breathing and heart rate of a teleost fish.
Comp. Biochem. Physiol. 9, pp. 229-239.

RICHARDSON, T; TAPPEL, A.L.; SMITH, L.M. and HOULE, C.R. (1962) Polyunsaturated fatty acids in mitochondria.
J. Lipid Res., 3, pp. 344-350.

RIPPLINGER, J. (1950)a, Le coeur des poissons son innervation extrinsèque ses centres automatiques.
Ann. Sci. Univ. Besançon., 5, pp. 45-57.

RIPPLINGER, J. (1950)b, Remarques sur l'automatisme du coeur de tanche.
Bull. Soc. Hist. Nat. Doubs. 54, pp. 1-4.

ROOT, R.W. (1931) The respiratory function of the blood of marine fishes.
Biol. Bull., Woods Hole, 61, pp. 427-456.

RUSHMER, R.F. (1960) Control of cardiac output. In Medical Physiology and Biophysics. (Edited by T.F. Ruch and J.F.Fulton). Saunders, Philadelphia.

RUSHMER, R.F. and SMITH, O.A. (1959) Cardiac Control. Physiol. Rev., 39, pp. 41-68.

SATCHELL, G.H. (1960) The reflex co-ordination of the heart-beat with respiration in the dogfish. J. Exp. Biol., 37, pp. 719-731.

SATCHELL, G.H. (1961) The response of the dogfish to anoxia. J. Exp. Biol., 38, pp. 531-543.

SAUNDERS, R.L. (1961) The irrigation of the gills of fishes. I. Studies of the mechanism of branchial irrigation. Canad. J. Zool., 39, pp. 637-653.

SAUNDERS, R.L. (1962) The irrigation of the gills of fishes. II. Efficiency of oxygen uptake in relation to respiratory flow, activity and concentrations of oxygen and carbon dioxide. Canad. J. Zool. 40, pp. 817-862.

SCHÄPERCLAUS, W. (1954) Fischkrankenheiten. Berlin Akademie Verlag.

SCHOLANDER, P.F.; HAMMEL, H.T.; Le MESSURIER, H.; HEMMINGSEN, E and GAREY, W. (1962) Circulatory adjustment in pearl divers. J. Appl. Physiol. 17, pp. 184-190.

SERFATY, A. and RAYNAUD, P. (1956) Electrocardiographe du poisson dans son milieu naturel. Bull. Soc. Zool. Fr., 81, pp. 121-126.

SERFATY, A. and RAYNAUD, P. (1957) Reflexe aéro-cardiaque chez la truite de rivière (Salmo trutto L.) J. Physiol. Path. Gén., 49, pp. 378-381.

SERFATY, A; LABAT, R and QUILLIER, R. (1959) Les réactions cardiaques chez la carpe (Cyprimus carpio) au cours d'une anesthésie prolongée. Hydrobiologia 13, pp. 144-151.

SHELTON, G. (1959) The respiratory centre of the tench (Tinca tinca L.) I. The effects of brain transection on respiration. J. Exp. Biol., 36, pp. 191-202.

SHELTON, G. (1961) The respiratory centre of the tench (Tinca tinca L.). II. Respiratory neuronal activity in the medulla oblongata. J. Exp. Biol. 38, pp. 79-92.

SHELTON, G. and RANDALL, D.J. (1962) The relationship between heart beat and respiration in teleost fish. Comp. Biochem. Physiol., 7, pp. 237-250.

SOLLMAN, T. (1957) A manual of pharmacology and its application to therapeutics and toxicology. Saunders, Philadelphia and London.

VAN DAM, L. (1938) On the utilization and regulation of breathing in some aquatic animals. Dissertation, Gröningen.

VERNBERG, J. and GRAY, I.E. (1953) Comparative study of respiratory metabolism in excised brain tissue of marine teleosts. Biol. Bull., Woods Hole, 104, pp. 445-449.

VON HOLST, E. (1934) Reflex und Rhythmus im Goldfischrückenmark. Zool. Anz. (Suppl. 7) pp. 93-95.

VON HOLST, E. (1934) Studien uber Reflexe und Rhythmen biem Goldfisch (Carassius auratus). Z. Vergl. Physiol. 20, pp. 582-599.

WEIDMANN, S. (1951) Effect of current flow on the membrane potential of cardiac muscle. J. Physiol. 115, pp. 227-236.

WELCH, P.S. (1948) Limnological methods. The Blakiston Company. Philadelphia and Toronto.

WILBER, G.C. (1961) Some physiological characteristics of the heart of the toadfish, Opsanus tau. Chesapeake Science, 2. pp. 72-75.

WILBER, G.C. (1962) The biology of water toxicants in sub-lethal concentrations. 28p. Report. Kent State University.

WOODBURY, L.A; HECHT, H.H. and CHRISTOPHERSON, A.R. (1951) Membrane resting and action potentials of single cardiac muscle fibres of the frog ventricle. Amer. J. Physiol. 164, pp. 307-318.

WOODBURY, J.W. and BRADY, A.J. (1956) Intracellular recording from moving tissues with a flexibly mounted ultramicroelectrode. Science. 123, pp. 100-101.

YOUNG, A.C. (1960) Regulation of respiration. In Medical Physiology and Biophysics (Edited by T.F.Ruch and J.F.Fulton). Saunders, Philadelphia.

YOUNG, J.Z. (1931) On the autonomic nervous system of the teleostean fish Uranoscopus scaber. Quart. J. Micr. Sci., 74, pp. 491-535.

DRUMMOND, G.I & C.C.BLACK. (1960). Comparative Physiology: Fuel of muscle metabolism. Ann. Rev. Physiol., 22, pp 169-190

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