THE EFFECT OF SWIMMING ACTIVITY AND SECTION OF THE VAGUS NERVES ON HEART RATE IN RAINBOW TROUT

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INTRODUCTION

In fish, periods of activity with intervening rest periods probably cause the most rapid changes in metabolic rate that the animal is likely to undergo. Blood circulation, which is closely associated with respiratory exchange, must be capable of rapid adjustment to these varying metabolic rates. The study of the effect of swimming activity on heart rate is therefore of particular interest in understanding the mechanisms of cardiac regulation in fish.

Stevens & Randall (1967a, b) have demonstrated that when trout swim at moderate speeds large changes in cardiac output occur which are the result of large increases in stroke volume and small increases in heart rate. Sutterlin (1969) also measured increases in heart rate corresponding to different swimming speeds in several species including trout. As this previous work was confined to a limited range of swimming speeds, one of the aims of the present study was to investigate the changes in heart rate over a wide range of swimming speeds up to the maximum sustainable speed when oxygen debt would be incurred. Comparisons are then possible with the metabolic studies of Brett (1964), Webb (1971a, b) and others. The experiments were carried out at two temperatures, 6·5 and 15 °C, which are likely to be encountered in winter and summer respectively.

The vagus nerves which innervate the heart were cut in some of the test fish in order to investigate the function of the nerves in regulation of heart rate. Randall (1970) stated that in trout there is no vagal tone to the heart during rest or exercise as long as the fish is in well aerated water, therefore regulation of heart rate could not be attributed to changes in vagal cardiac inhibitory tonus. However, Gannon & Burnstock (1969) have shown that stimulation of the vagi can cause cardioacceleration in trout as well as inhibition, and suggested that this was mediated by adrenergic fibres in the vagus leading to the heart. As some of Randall's conclusions were based on experiments involving the blocking of vagus function with atropine (Stevens & Randall, 1967a) which would not have affected any adrenergic cardioacceleratory function, the precise function of the vagus in active trout remains obscure.
Tests were carried out at two temperatures, 6.5 and 15 °C, on three groups of fish; intact, bilaterally vagotomized and unilaterally vagotomized. The experiments consisted essentially in the measurement of electrocardiograms of the fish during swimming at various speeds in the test apparatus described below.

Hatchery-reared rainbow trout (*Salmo gairdneri*) between 23.0 and 28.2 cm long were used. They were kept in an 800-litre circular tank at the proposed experimental temperature (±1.5 °C) for at least 4 weeks prior to swimming tests or the vagotomy operation. A slow circulation of water was maintained in the acclimation tank against which the fish would usually swim.

**Test Apparatus**

The apparatus (Fig. 1) consisted of a flume around which water was circulated by a 3/4 horse-power pump in a bypass circuit. Water velocity was varied by a valve controlling the output of the pump. The channel was 23 cm square in cross-section and was constructed of timber and marine-grade plywood painted to give a smooth waterproof finish. The fish was confined to a 56 cm long transparent Perspex section of this channel by a 2.5 cm mesh grid. A longitudinal slot in the test-chamber cover and a special suspended swivel holding the electrode cable attached to the fish allowed complete freedom of movement of the fish in this space. The water velocity was measured using a Pitot-tube system with sloping water manometers. The water temperature was kept constant within ±0.5 °C by a thermostatically controlled immersed refrigeration coil. The water was renewed at intervals and well aerated throughout the experiments.
VAGOTOMY TECHNIQUE

The vagus nerve was cut using a technique modified from Labat (1966). The operation was carried out in a room maintained at the appropriate experimental temperature. The fish was anaesthetized in 1:10 000 MS 222 until breathing movements ceased. It was then held lying on one side on an angled table with the head pointing away from the operator, and the gills were irrigated with a regulated dilution of MS 222 (which just inhibited breathing movements) from a tube inserted into the fish’s mouth. When the fish’s head was angled upwards it was possible to operate under the operculum looking from the posterior aspect with the aid of a binocular dissection microscope.

A small incision was made posterior to the fifth branchial arch along the anterior edge of the cleithrum. A segment of the then easily exposed cardio-visceral branch of the vagus nerve was removed taking care not to cause haemorrhage from the major veins in this region. Two sutures were found to be necessary to close the wound. Approximately 35 min were required to effect a bilateral vagotomy on a fish. Within 1 h after return to a recovery tank the fish regained its normal pale colour.

The vagotomized fish were allowed 12 to 22 days for recovery in a 180-litre tank before implantation of ECG electrodes. Success of vagotomy and absence of vagus regeneration were then tested for in the lightly anaesthetized fish by checking the absence of cardiac inhibitory reflexes when the operculi were held shut. The absence of approach reflexes in the ECG (Labat 1966) and post-mortem anatomical examination provided additional verification of lack of vagus function.

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

RECORDING OF ELECTROCARDIOGRAMS AND SWIMMING TESTS

Feeding was discontinued 24 h before implantation of ECG electrodes. The fish was then anaesthetized in MS 222 and two silver 30 swg wire electrodes were implanted subcutaneously, one at the ventral surface of the pectoral girdle and the other anterior to the anus. These were sutured in place and the attached insulated wires were led around the side of the body and anchored by suture just anterior to the dorsal fin. The fish was then introduced into the apparatus to recover from anaesthesia.

Electrocardiograms were recorded from the implanted electrodes using a Devices M2 heat-pen recorder and an AC7C pre-amplifier. Heart rates were subsequently determined by counting the number of heart beats in each minute. A screen prevented movements in the laboratory being seen by the fish and care was taken to avoid disturbance of the fish throughout the experiments.

The fish was kept in a water current of about 5 cm/sec in the test chamber against which it would orientate but not necessarily swim. It was thus allowed to rest overnight before the first speed trial. A speed trial consisted of exposure for 30 min to an elevated water velocity against which the fish would swim in order to maintain station. Two such trials were carried out per day with at least 3 h rest (5 cm/sec flow) in between. Trials were continued for up to 6 days with each animal at a wide range of velocities up to the maximum which could be sustained for 30 min.
Table 1. Comparison of heart rates in intact and vagotomized fish at 6.5 and 15 °C

<table>
<thead>
<tr>
<th>Condition of fish</th>
<th>Basal heart rate (beats/min)</th>
<th>Maximum heart rate (beats/min)</th>
<th>Regression line for intermediate heart rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Equation</td>
<td>Correlation coefficient</td>
</tr>
<tr>
<td>Intact 6.5 °C</td>
<td>32.54 ± 3.81</td>
<td>log H.R. = 1.3342 + 0.3038 (V/L)</td>
<td>0.8665</td>
</tr>
<tr>
<td>Unilateral vagotomy 6.5 °C</td>
<td>34.56 ± 1.82</td>
<td>log H.R. = 1.3821 + 0.2658 (V/L)</td>
<td>0.8669</td>
</tr>
<tr>
<td>Bilateral vagotomy 6.5 °C</td>
<td>48.25 ± 4.27</td>
<td>log H.R. = 1.6846 + 0.0363 (V/L)</td>
<td>0.3726*</td>
</tr>
<tr>
<td>Intact 15 °C</td>
<td>45.52 ± 6.18</td>
<td>log H.R. = 1.5639 + 0.1937 (V/L)</td>
<td>0.8290</td>
</tr>
<tr>
<td>Unilateral vagotomy 15 °C</td>
<td>37.26 ± 3.88</td>
<td>log H.R. = 1.4894 + 0.2505 (V/L)</td>
<td>0.9617</td>
</tr>
<tr>
<td>Bilateral vagotomy 15 °C</td>
<td>45.13 ± 9.46</td>
<td>log H.R. = 1.5277 + 0.1826 (V/L)</td>
<td>0.8292</td>
</tr>
</tbody>
</table>

H.R. = heart rate. P = level of significance of regression correlation.
* These values were calculated by incorporating all the heart-rate figures above 0.2 (V/L).

RESULTS

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

Specific speed (V/L) = \( \frac{\text{velocity (cm/sec)}}{\text{body-length (cm)}} \).

The analysis may be obscured if the heart rate is different in fish of different sizes but no such effect was observed in the narrow size range used. The results of Nomura et al. (1972) for much larger rainbow trout confirm that any size effect is probably small.

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

Intact fish

Four intact rainbow trout were tested at 6.5 °C. With the fish at rest the heart rate stabilized at 25-35 beats/min (Table 1). Fig. 2a shows a typical ECG at rest, and it can be seen that the beat-to-beat interval varies considerably. During periods of excitement, or if the stomach was full, the resting heart rate was elevated for long periods up to a maximum rate of 45 beats/min. Bursts of spontaneous activity gave rise to maximum heart rates of about 60 beats/min for periods of not more than 1 or 2 min. During a typical burst of this kind lasting about 10 sec the heart often slowed or stopped, and the peak heart rate was reached approximately 30 sec after the peak of activity.

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

At higher test velocities heart rate rapidly increased to a uniform level which was sustained throughout the test period after which the rate rapidly fell to the resting
Heart rate in rainbow trout

Fig. 2. Electrocardiograms of fish at 6·5 °C. (a) Intact fish showing irregularity in the beat-to-beat interval. (b) Bilaterally vagotomized fish with a regular cardiac rhythm.

Fig. 3. Intact fish at 6·5 °C. Heart rates during the course of three speed trials. ●, 1·07 (V/L). ▲, 1·38 (V/L). ■, 1·66 (V/L).

levels. A typical trial of this kind at 1·07 (V/L) is shown in Fig. 3; in this case there was also some spontaneous activity after the trial. This active heart rate increased with swimming velocity up to approximately 1·5 (V/L) when the maximum heart rate of about 56 beats/min was reached. In trials in excess of this critical speed no further increase in heart rate took place and a longer period for return to the resting rate was observed (Fig. 3, compare 1·38 (V/L) and 1·66 (V/L)). As test speed and recovery times increased the maximum speed which could be sustained for 30 min was rapidly approached.
For each speed trial the mean was calculated of all the heart-rate readings, excluding the first five, in order to discount the disturbances associated with the initial acceleration.

The logarithms of these mean values are plotted against swimming speed in Fig. 4A. The mean of the readings at rest for 20–30 min prior to each test are also plotted.

The time taken after a speed trial for the heart rate to fall half-way to the prior resting level was determined, and the inter-relationship between this time for 50% recovery and trial speed is shown in Fig. 5A.

For analysis in Fig. 4A the velocity \( v \)–heart-rate relationship is broken into three segments. The mean resting level \((0.2 \, (V/L))\) is shown as an initial horizontal line. The maximum heart rate was taken as the mean of all the heart rates in trials with 50% recovery times in excess of 5 min (i.e. speeds in excess of the critical). The remaining intermediate points were used to calculate the regression line between the two extremes.

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

The effect of unilateral vagotomy

One fish was vagotomized on the right side only, with a sham operation on the left. The results of swimming tests were in no way significantly different from those in the intact fish as shown in Figs. 4A and 5B, and all the features of the ECG were normal.

The effect of bilateral vagotomy

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

The heart rate at rest varied from 41 beats/min to the maximum 56 beats/min. The
Heart rate in rainbow trout

Fig. 5. The relationships between swimming speed during a trial and the time taken during recovery for the heart rate to fall half-way back to the level prior to exercise (50% recovery time).
lower rates were only recorded after several days acclimation to the apparatus and then there was some evidence that the heart rate tended to decline to close to the resting levels in intact fish. However, the slightest disturbance caused a rapid rise in heart rate which took a very long time to fall again so that extended periods at the lowest rates proved impossible to record. The mean resting heart rate of 48.25 was significantly higher than that in the intact fish. There was no evidence in the ECG of irregularity in the beat-to-beat interval as noted in the intact fish (Fig. 2).

Cardiac response to exercise was variable, and even in cases where the resting heart rate was low the pattern of recovery after a test was erratic (Fig. 6). Training was required before the fish could sustain the higher swimming speeds and the maximum speed attained, 1.47 (V/L), was slightly lower than that in the intact individuals. Heart rates during swimming activity were significantly higher than at rest \( P < 0.001 \) but the correlation between heart rate and swimming speed was not significant and no coherent analysis could be made of recovery times or critical speeds. Thus the only line shown in Fig. 4B is the mean resting heart rate.

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

Intact fish

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

Fig. 7 shows three cardiac responses to different levels of swimming activity. The general pattern is exactly as described at 6.5 °C except that the maximum heart rate is
Heart rate in rainbow trout

over 90 beats/min and the critical speed, at which that is attained, is approximately 2·0 (V/L). In the test at 1·97 (V/L) the fish executed some spontaneous movements just prior to the test period so that the heart rate began to rise prematurely (Fig. 7). The steep increase in recovery times at 2·0 (V/L) is shown in Fig. 5C. The maximum time observed for complete recovery in heart rate from fatigue was 5½ h.

The relationship between heart rate and swimming speed was analysed as before and is shown in Fig. 8A. Despite large variations in resting heart rates the maximum frequencies are remarkably constant. The slope of the regression is not as steep as at 6·5 °C.

The effect of unilateral vagotomy

One individual was vagotomized on the right side only, and the results are shown in Figs. 8A and 5D. There was little deviation from the normal pattern at this temperature except that the resting heart rates tended to be low but were not entirely outside the range of variation in the intact fish. It is of interest to note that although there was this difference at the lower activity levels, there was no significant difference above 0·7 (V/L) (Fig. 8A).
The effect of bilateral vagotomy

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

Fig. 9 shows the changes in heart rates associated with swimming at three different speeds in one of the fish. In contrast to the heart rate of the vagotomized fish at 6·5 °C, the heart rate at 15 °C was well regulated and closely correlated with activity. However, the mean maximum heart rate (83·88 beats/min) was significantly lower than in the normals. The resting heart rate ranged from 31 to over 60 beats/min, two fish having high resting rates and one a low resting rate (Fig. 8 B). The pattern of increase in recovery time at the critical speed of about 2·0 (V/L) was very close to that in normal fish (Fig. 5 E).

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

DISCUSSION

When a fish swims at moderate speeds it is probable that respiratory function and blood flow keep pace with the rate of metabolism and hence cardiac output closely reflects changes in metabolic rate. Stevens & Randall (1967 b) have shown that changes of P CO₂ and P O₂ in arterial and venous blood during and after moderate exercise in
rainbow trout are small. Assuming that there are no large changes in blood oxygen-carrying capacity, cardiac output is proportional to oxygen consumption.

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

\[ \log O_2 = a + b(V/L). \]

Values for \( b \) ranging from 0.2 to 0.47 have been obtained for several species by different authors: Rao (1968), Kutty (1968), Tytler (1968), Webb (1971b) and Morgan (1974).

For the purpose of this discussion the value of \( b \) for rainbow trout will be taken as 0.3 at all temperatures. Thus, if no changes in cardiac stroke volume take place, applying the Fick principle, the slope of the relationship between the logarithm of heart rate and specific speed should be 0.3. Comparison of the actual data with this 'ideal' value enables estimates to be made of the relative importance of changes in stroke volume and heart rate in regulation of cardiac output.

At 6.5 °C there was no change in heart rate until a speed of 0.6 \((V/L)\) was reached,
when the estimated stroke volume was 1.5 times the resting value. At higher speeds the increase in heart rate accounted for much of the increase in oxygen transport up to 1.4 \( (V/L) \) above which there was no further increase in heart rate. At 1.5 \( (V/L) \) the 50% recovery-time curve begins to rise steeply as an oxygen debt is presumably incurred and the equilibrium between respiratory exchange and metabolism breaks down. At this critical point heart-rate increase accounted for 39% of the estimated increase in cardiac output and the estimated stroke volume was 2.1 times the resting volume. It is probable that at 6.5 °C there was a tendency for the heart rate not to increase until the stroke volume was quite large. Sutterlin (1969) found a similar effect in brown trout at 8 °C when there was no increase in heart rate until a speed of 0.8 \( (V/L) \) was reached.

In the experiments at 15 °C the heart rate began to increase at 0.45 \( (V/L) \) at an estimated stroke volume of 1.36 times the resting value. At 2.0 \( (V/L) \), the critical speed, the change in heart rate had accounted for 36% of the overall increase in estimated cardiac output and the corresponding stroke volume was 2.9 times that at rest. In this case there appears to have been a steady rise in stroke volume throughout the range of heart rates.

The estimates of stroke-volume changes are smaller than the fivefold increase reported by Stevens & Randall (1967b) in rainbow trout at 5 °C at similar swimming speeds. However, in view of the possible errors in the assumptions made in the present estimates the difference cannot be regarded as significant.

Brett (1964) showed that increased oxygen consumption due to oxygen-debt repayment after fatigue in sockeye salmon took up to 6 h to return to resting levels, which corresponds to the recovery times recorded in this work.

The fluctuation in beat-to-beat interval noted particularly at the lower temperature may be due to fluctuations in vagal tonus (Labat, 1966) which are linked with the synchronization of cardiac function with breathing movements. However, Randall & Smith (1967) have shown that in well-oxygenated water there is no synchrony of the cardiac rhythm with breathing in rainbow trout.

The cardiac inhibition observed during spontaneous bursts of activity and during the initial stages of acceleration of a speed trial is similar to the bradycardia noted by Stevens et al. (1972) in Ophiodon elongatus during exercise. The absence of this effect in vagotomized fish confirms that this is a vagus effect probably of the type observed in approach reflexes (Labat, 1966).

At both temperatures unilateral vagotomy had no significant effect on heart rates during activity or on any cardiac responses to stimuli. The significantly lower resting heart rates in the unilaterally vagotomized fish at 15 °C may be due to individual variation as heart rates of the same order were recorded in intact fish. Basically it seems that the vagotomy operation and subsequent recovery period have no effects on the fish which could obscure interpretation of the data from vagotomized fish.

Bilateral vagotomy had distinctly different effects at the two temperatures. There was little effect on the general pattern at 15 °C although cardiac inhibitory reflexes and the associated periods of bradycardia were absent. At 6.5 °C the resting heart rates were high, and changes associated with swimming did not appear to be well regulated. This gave rise to the paradox that the intrinsic heart rate, in the absence of vagus function, was higher at 6.5 °C than 15 °C. Bennion (1968) has shown by in vitro
Heart rate in rainbow trout

studies on the trout heart that at 6 °C adrenaline had a tachycardiac effect whereas at 15 °C this effect was minimal although stroke volume was increased. Thus the catecholamines which are likely to be released into the circulation during disturbance in the experimental situation (Nakano & Tomlinson, 1967) could elevate the heart rate at 6.5 °C but have no effect at 15 °C. In the intact fish this effect was presumably masked by the bradycardiac effect of the vagus.

At 15 °C the main significant difference in vagotomized fish was a lower maximum heart rate than in the intact fish; this may be evidence of a cardiac excitatory function of the vagus. It is conceivable that in order to sustain absolute maximum heart rates appropriate vagus stimulation is required. Gannon & Burnstock (1969) have demonstrated an adrenergic cardiac excitatory effect in the vagus of trout which may function in this way. In the present study the vagus nerve was sectioned distal to the ramus communicans from the sympathetic ganglion so that any adrenergic fibres entering the heart along this route would also have been severed.

Cobb & Santer (1972) found that, in plaice, stimulation of the vagus could cause cardiac excitation or inhibition according to the frequency of stimulation. This effect was apparently cholinergically mediated (Santer, 1972). Saito (1973) also found cholinergic excitatory effects during or after vagus stimulation in the carp, although she suggested that this was caused by effects associated with deterioration of the experimental preparation. It is evident that there are great interspecific differences in the autonomic nervous system of fish and this may give rise to much of the controversy regarding sympathetic inervation of the heart (Satchell, 1971; Randall, 1970; Campbell, 1970).

The fact that the vagotomized fish had not fed during the post-operative recovery period may have been the cause of the lowered maximum heart rates (I. G. Priede, unpublished), although the unilaterally vagotomized fish which went through exactly the same procedure had a normal maximum heart rate. It is concluded that before a continuous vagal cardiac excitatory effect during swimming at high speeds can be postulated with certainty, detailed research is required on this aspect, which was outside the scope of the present study.

It is evident from the present study that considerable regulation of cardiac rate can take place in the absence of vagus function. Apart from the possible function of the adrenergic fibres entering the heart along the coronary vessels (Gannon & Burnstock, 1969) the changes in heart rate observed in the bivagotomized fish must be of aneural origin. Randall & Stevens (1967) could explain changes in blood pressure in salmon in terms of interaction of adrenaline with α receptors in the blood vessels. The dorsal aorta ligament (De Kock & Symons, 1959) and valves in the veins of the caudal region (Sutterlin, 1969) could produce a pumping action during swimming movements which would increase venous return. Any increase in venous return or the direct effect of adrenaline on the heart (Bennion, 1968) could account for tachycardia during exercise in bivagotomized fish. The secretion of catecholamines is probably rapid enough to explain the increase in heart rate as exercise begins (Nakano & Tomlinson, 1967; Randall & Stevens, 1967). However, the rapid fall in heart rate after exercise at lower speeds (Fig. 9) may be more difficult to explain in the absence of vagus inhibitory function as it may take some time for catecholamines to be removed from the circulation.
SUMMARY

1. Heart rates associated with swimming activity were measured in intact and vagotomized fish at 6.5 and 15 °C.
2. Low swimming speeds had no effect on heart rate but above a threshold speed it increased logarithmically with swimming speed up to the critical speed and maximum heart rate.
3. Times for recovery after exercise increased rapidly above the critical speed.
4. Bilaterally vagotomized fish at 6.5 °C showed high resting heart rates and erratic cardiac responses to exercise.
5. In bilaterally vagotomized fish at 15 °C heart rates were normal except for a low maximum rate.
6. It is concluded that the vagus nerve can function differently at different temperatures.

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