RESPIRATORY AND CIRCULATORY RESPONSES OF RAINBOW TROUT LARVAE TO CARBON MONOXIDE AND TO HYPOXIA

By G. F. HOLETON[*]

Department of Zoology, University of Bristol

INTRODUCTION

The purpose of this investigation was to explore the ontogeny of certain respiratory responses of rainbow trout. Adult trout respond to hypoxia with an increase in the flow of water over the respiratory surfaces, and with a reflex bradycardia (Holeton & Randall, 1967). When adult rainbow trout are exposed to 5 % carbon monoxide (CO) and thus rendered anoxaemic while at the same time their arterial blood and the environmental water are at a high P_{O_2}, they do not show the reflex bradycardia. In fact their heart rate increases considerably (Holeton, 1971). One possible interpretation of the differences in the response of the hypoxic adult compared with the CO-poisoned fish is that the response to CO is the basic response to anoxaemia, and that the response to hypoxia is the same but with the bradycardia reflex superimposed upon this.

By examining the responses to hypoxia and to CO by larval trout, it was hoped that a temporal or ontogenetic separation in the development of responses would indicate whether more than one set of responses was involved, and if so, which was the more 'primitive' or basic.

The use of CO on newly hatched trout presupposes that haemoglobin blockage will have some effect, and therefore that the haemoglobin in the larval fish is serving an oxygen transport function. The hazard of tissue metabolism interference by 5 % CO was investigated by Holeton (1971) and discounted. Thus if the respiration of the larval fish is affected by CO, then it is reasonable to assume that their haemoglobin is serving a respiratory function.

An investigation such as the present one is greatly facilitated by the transparency of the newly hatched trout. The breathing movements and circulatory dynamics can be observed visually without disturbance to the fish.

MATERIALS AND METHODS

Rainbow trout eggs were obtained from the Ubley Trout Hatchery, Somerset, England, and kept in near darkness under aerated running water at 10 °C. The eggs were fertilized on 5 January 1968 and hatching occurred between 5 and 7 February. The experiments commenced on 8 February. Experiments were conducted on groups

[*] Present address: Department of Zoology, University of Toronto, Toronto 5, Ontario.

of four fish of normal appearance which were selected from the rearing trays. Fish which were deformed or abnormal-appearing were not used.

A gas mixture of 5 % CO, 21 % O_2 and 74 % N_2 (± 5 % accuracy) was obtained from Messrs Hilger I.R.D. Ltd., Loughton, Essex.

The apparatus consisted of a recirculating system which included a fish chamber, a 2 l glass reservoir and a float-type flow indicator. The water in the system was circulated with a small centrifugal pump. All of the system except the pump was immersed in a water bath with transparent sides, and in which the temperature was controlled to 10 ± 0.1 °C. Gas tensions in the system were controlled by bubbling suitable gas mixtures into the reservoir through an aquarium air-diffusing stone. The reservoir was provided with a vent to allow the gases to escape. Flow rate of the recirculating water was adjusted to produce a water velocity in the experimental chamber of approximately $0.5-1.0$ cm/sec.
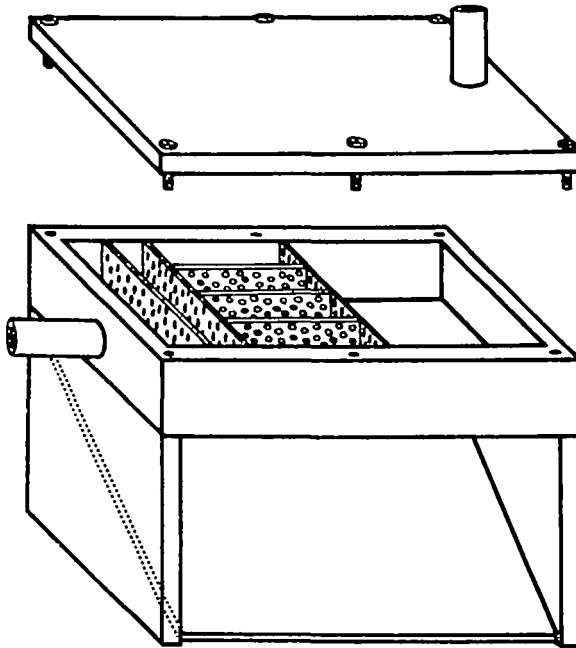

Fig. 1. The chamber used to observe heart movements and breathing movements of larval fish. Mirror underneath is angled at 45° to permit observation of the underside of fish through the transport floor of the chamber. Note compartment for filtering material immediately in front of the four stalls which hold the fish.

The fish chamber was constructed to permit observation of the underside of the fish by means of a mirror angled at 45° (Fig. 1). The sides of the chamber were painted black but the top and bottom were left transparent. The inside of the chamber was partitioned into four stalls which kept the fish separated and restricted the movements of the fish to a space 0.5 by 3.5 cm. The partitions were perforated to ensure even distribution of water flow to each fish. Water passing into the chamber travelled through a layer of cellulose fibre to filter detritus from the water before

◗ reached the fish. By placing the chamber near the side of the water bath it was possible to observe the fish with the aid of a dissecting microscope.

Fish were selected and gently transferred to the experimental stalls in a spoon. The lid was placed on the chamber and secured by means of brass screws. A thin smear of Vaseline assured an air-tight fit. The chamber was lowered into the water bath and a microscope lamp set to a very low reddish intensity was placed to shine down on the chamber. The complete apparatus was shaded as much as possible with sheets of black polyethylene. The fish were left for several hours to recover from the handling. Observations of the fish were made periodically and the heart and breathing rates were determined with the aid of a stopwatch. The microscope lamp was left on at all times and observations at night were made with this source of light only. Particular care was taken to avoid any sudden movements or jarring of the apparatus which might frighten the fish.

The effects of hypoxia were examined on the fish, 1 2 and 8 days after hatching. Hypoxia was created by bubbling nitrogen into the reservoir. The P_{O_2} of water entering the fish chamber was monitored with a Beckman Macro oxygen electrode coupled to a Beckman Model 160 Physiological Gas Analyser.

Fish aged 1, 6, and 18 days post-hatching were exposed to 5 % CO for 3 h and their respiratory and circulatory responses were observed. The CO was administered by bubbling the mixture of 5 % CO, 21 % O_2, and 74 % N_2 into the reservoir.

RESULTS

After hatching, contractions of the heart and movement of red blood cells in the blood vessels of the larvae were easily observed. Ventricular end-systolic volume was nearly zero under most conditions for at least a week after hatching. Venous blood flow was pulsatile in the major veins, with blood coming completely to rest between heart beats.

Changes in the heart and breathing rates during the first 3 weeks after hatching are summarized in Fig. 2. Heart rate increased from an average of 57 to an average of 66 beats/min during the first 3 days and then stayed relatively constant until the 9th day. After 18 days had elapsed the resting heart rate had dropped to an average of 49 beats/min.

The buccal and opercular respiratory pumps of the newly hatched trout were very small and not well developed. Breathing movements immediately after hatching were irregular, poorly co-ordinated, and weak. It is doubtful whether much water is moved over the gills of newly hatched trout. At this stage in development the gill filaments are small and the large well-vascularized yolk sac appears to offer a considerably larger gas-exchange area. The pectoral fins of the young larvae were often moving rhythmically and motion of the occasional particle of detritus in the water around the fish indicated that fin movements created a considerable current of water over the fish. With more vigorous movements of the pectoral fins it could be seen that the leading edges of the fins were brushing the longer trailing tips of the gills which protruded slightly from the opercula. As the fish grew older the movements of the buccal and opercular respiratory mechanisms became stronger and well co-ordinated. Breathing rate of the newly hatched alevins increased considerably between the 6th and 18th days after hatching (Fig. 2).

The scope for changes in the resting heart rate and breathing pattern increase with age. Struggling resulted in increased heart and breathing rate at all times, but by the third week the changes were more pronounced. The magnitude of the increase in heart and breathing rate resulting from struggling can be seen in Fig. 2.
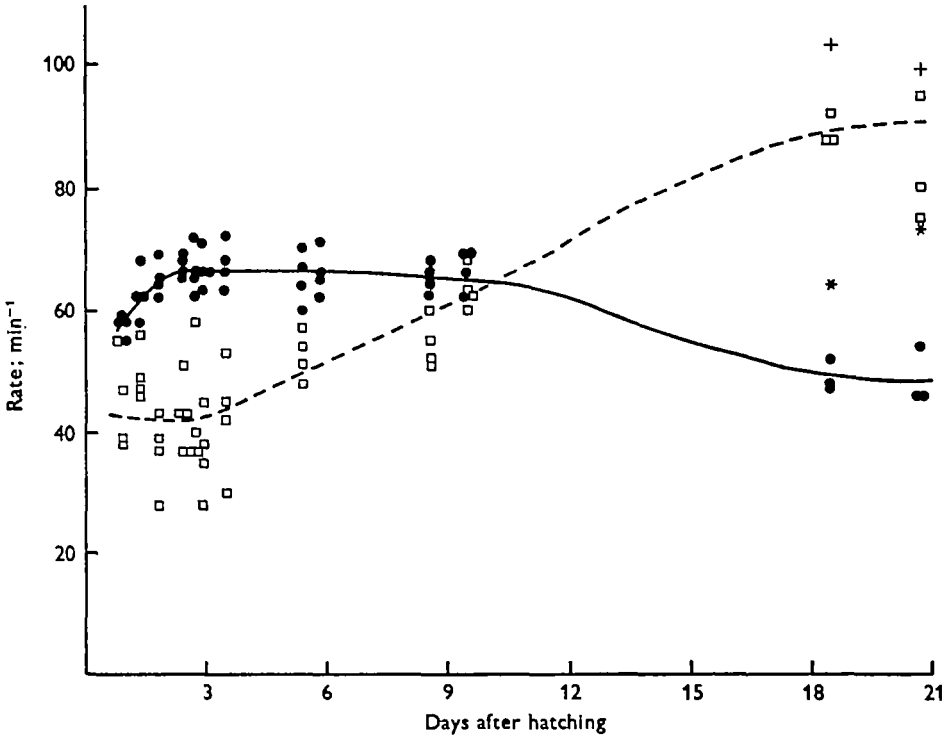

Fig. 2. Changes in heart rate and breathing rate of resting rainbow trout after hatching. □, Breathing rate; ●, heart rate; +, *, active fish.

THE RESPONSE TO HYPOXIA

Three experiments were conducted with groups aged 1, 2 and 8 days post-hatching. In all cases the initial lowering of the environmental P_{O_2} resulted in an increase in body movements. Motion of the pectoral fins increased with the 1-day and 2-day fish, but this was not observed in the 8-day fish. The effects of hypoxia on heart and breathing rates of the 1-day and 2-day fish were virtually the same and are summarized in Fig. 3, which shows the responses of 1-day fish. The responses of the 8-day fish are shown in Fig. 4.

The breathing movements of 1-day and 2-day fish increased in rate and became more regular as the oxygen level in the water was lowered. However, when the environmental P_{O_2} had dropped to 45–50 mmHg the breathing rate began to decline, and by the time the environmental P_{O_2} was down to 16 mmHg the breathing rate had dropped back to resting levels, but the movements were jerky and of greater amplitude than normal. Restoring oxygen levels to normal did not result in an elevation of breathing rate such as shown by adult trout, and the pattern of breathing returned to normal within 20 min.

The heart rate of 1-day and 2-day fish increased slightly until falling environmental P_{O_2} had reached 45–50 mmHg (Fig. 3). Further decreases in the environmental P_{O_2} to 16 mmHg caused a moderate drop in the amount of blood effectively pumped by the heart. In some fish, blood in the major veins was reversing the direction of flow momentarily between heart beats. Heart rate returned to normal within a few minutes when the oxygen levels in the water were restored to normal.
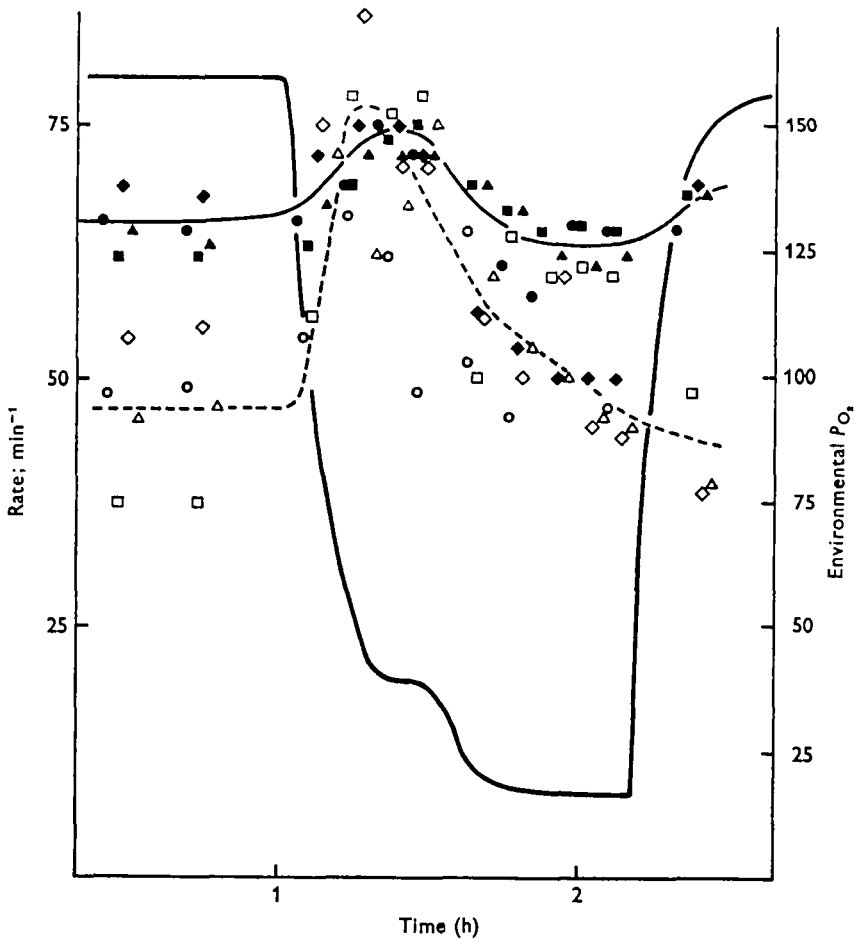

Fig. 3. The effect of hypoxia at 10 °C upon heart rate (solid line, solid figures) and breathing rate (dotted line, open figures) of rainbow trout 1 day after hatching. Heavy line indicates environmental water P_{O_2}. Individual fish are represented by a particular shape of symbol.

The breathing responses of the 8-day fish to hypoxia were more pronounced than those of the 1- and 2-day fish. Breathing amplitude increased and breathing rate nearly doubled as the water P_{O_2} was gradually lowered to 60 mmHg and held there for about 1 h (Fig. 4). Further lowering of the environmental P_{O_2} to 5 mmHg provoked a sharp drop in the rate of breathing movements. Breathing movements of three of the fish stopped completely (Fig. 4). Restoration of the oxygen level to 65–75 and then to 160 mmHg P_{O_2} was followed by recovery of breathing movements, with rates reaching levels higher than resting. The recovery of one fish was very slow

and its breathing rate was only 8/min 1 h after the oxygen levels had been restored (Fig. 4).

Heart rate of the 8-day fish increased slightly when the P_{O_2} was dropped to 60 mmHg and held there. Further lowering of the P_{O_2} to 5 mmHg resulted in a decrease in heart rate to 30–35 beats/min (Fig. 4). Restoring the oxygen levels to 65–75 mmHg and then to normal resulted in recovery of heart rate, but this took several minutes and was not sudden like the reflex recovery of heart rate shown by adult trout under similar circumstances (Holeton & Randall, 1967). The recovery of one fish lagged behind the others and this was the same individual which showed a poor recovery of breathing.
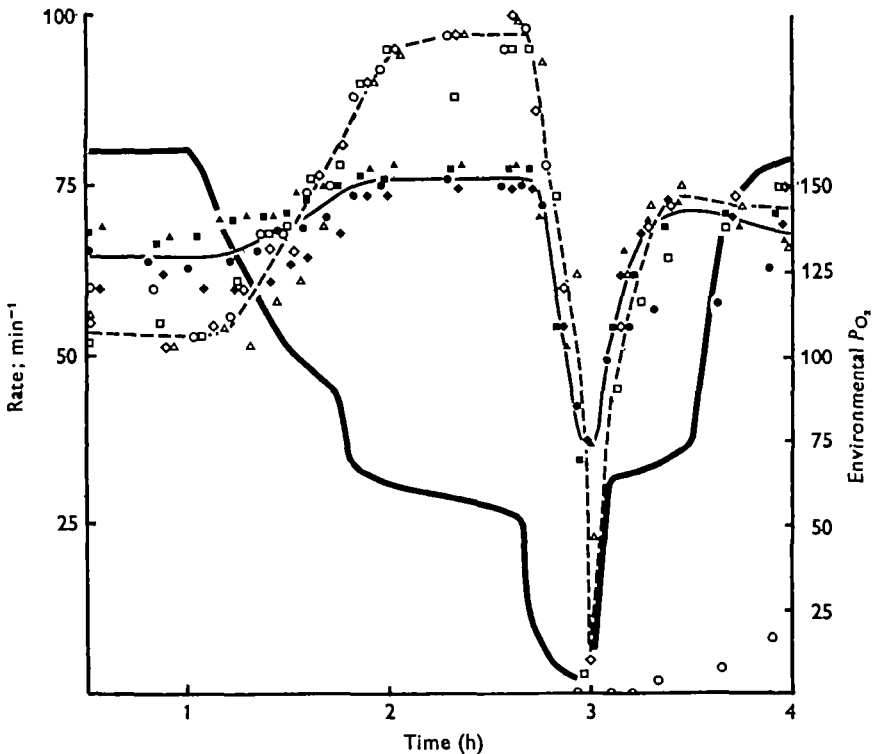

Fig. 4. The effect of hypoxia at 10 °C upon heart rate (solid line, solid figures) and breathing rate (dotted line, open figures) of rainbow trout 8 days after hatching. Heavy line indicates P_{O_2} of water. Individual fish are represented by a particular shape of symbol.

THE EFFECT OF CARBON MONOXIDE

The larvae were exposed to 5 % CO for 3 h at 1, 6 and 18 days after hatching. None of the fish died or showed ill effects as a result of the exposure to CO.

Changes in breathing rate of the three groups of fish are summarized in Fig. 5. Breathing rates of 1-day trout exposed to CO did not change markedly and there was only a slight increase in amplitude of breathing movements. These fish became restless and moved about more than usual during the first ½ h of exposure to CO. The 6-day trout increased their breathing rate and to a lesser extent their breathing

Amplitude. The breathing pattern of the 6-day fish returned towards normal within $\frac{1}{2}$ h of the removal of the CO. The 18-day fish showed only a slight increase in the breathing rate, but the amplitude of the movements increased a great deal.

The circulatory responses to CO-exposure made by the three age-groups are summarized in Fig. 6. In all cases, but particularly with the 1-day fish, there was a noticeable change in the colour of the blood to a lighter and brighter shade of red. The 1-day and 6-day fish showed moderate increases in heart rate which returned
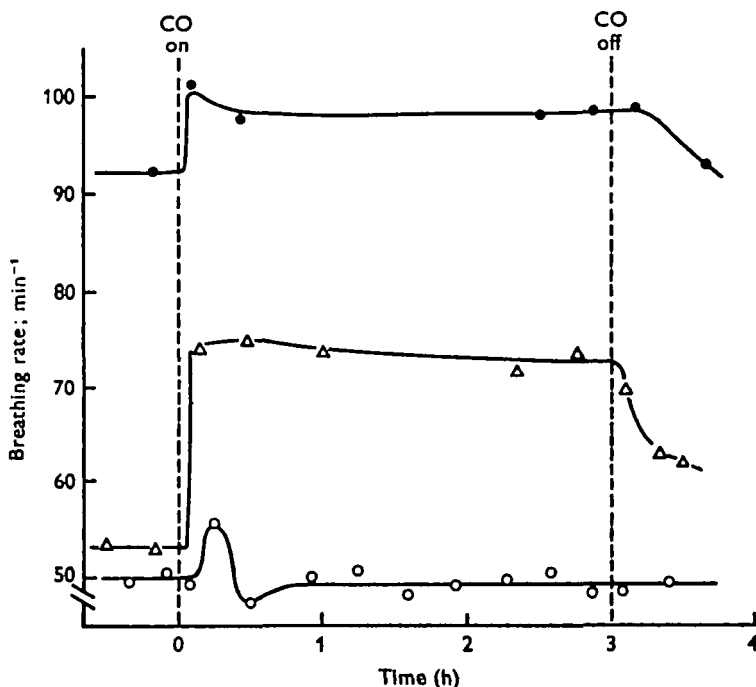

Fig. 5. The effect of exposure to 5 % CO at 10 °C on the breathing rate of rainbow trout 1 day (O), 6 days (△) and 18 days (●) after hatching. The points plotted are means derived from experiments on four fish.

to normal shortly after the CO had been removed (Fig. 6). The heart rate of the 6-day fish remained slightly higher than that of the 1-day fish throughout the experiments, but the response to CO was very similar. Although the 18-day fish had the lowest initial heart rate, exposure to CO resulted in a large increase in heart rate such that these fish had the highest heart rates of the three groups tested (Fig. 6). Removal of the CO was followed by a gradual decrease in heart rate of the 18-day fish. Their heart rate took several hours to return to normal.

DISCUSSION

The eggs of trout contain a large amount of yolk which is metabolically inert (Hayes, Wilmot & Livingston, 1951). It is probable that the vascularized surface of the yolk sac is the principle site of gas exchange for trout embryos just before and shortly after the time of hatching.

Newly hatched trout must undergo a transition from cutaneous respiration to gill respiration. It is between the time of hatching and the time that the yolk sac is absorbed and the skin thickened that the fish must develop the gills structurally and functionally to the extent that they can assume the role of primary gas-exchange organs.

The pattern of respiration of newly hatched trout was characterized by movements of water over the body by the pectoral fins rather than by irrigation of the gills by the respiratory pumps. The mouth and gills of newly hatched trout are very small.
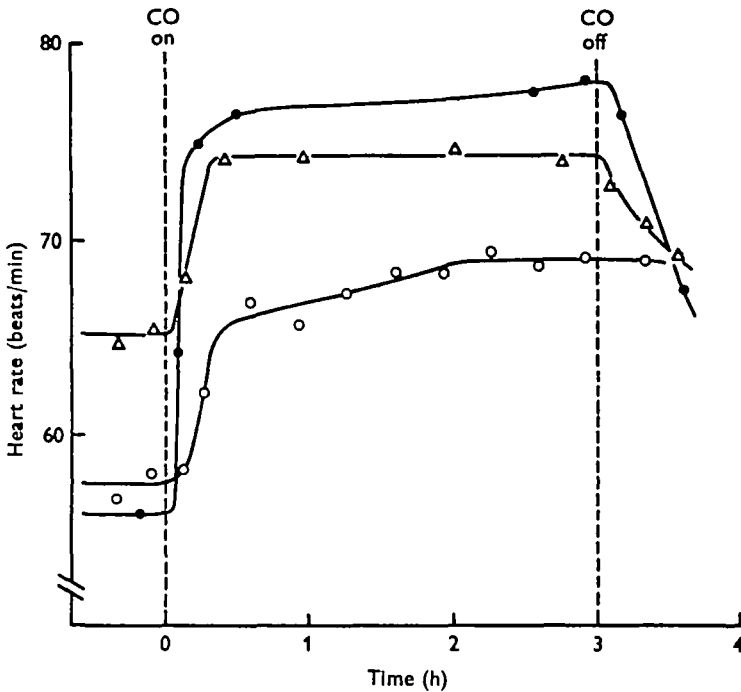

Fig. 6. The effect of exposure to 5 % CO at 10 °C on the heart rate of rainbow trout 1 day (O), 6 days (△) and 18 days (●) after hatching. The points plotted are means derived from experiments on four fish.

The rapid growth and development of the respiratory pumps is reflected in the progressively stronger and faster respiratory movements which occur during the first 18 days after hatching.

Well before the time of hatching, the circulatory system of the trout was developed and a positive circulation of red blood established. After hatching the heart rate increased slightly over the first few days and then stabilized at approximately 66 beats/min. Some time between the 9th and 18th days after hatching there was a drop in resting heart rate down to about 49 beats/min. It was not determined what caused this drop, nor, unfortunately, were the fish observed closely enough during the critical times to determine whether the drop in heart rate occurred suddenly, or gradually as depicted in Fig. 2. Data obtained by Grodzinsky (1950) on the heart rates of developing *Salmo trutta* L. show an increase in heart rate during the early stages of development followed by a decrease a few weeks after hatching, much like

the changes found for rainbow trout in this study. Grodzinski's data shows that these heart-rate changes are consistent over a wide range of temperatures.

One possible explanation for the drop in heart rate between the 9th and 18th days is that inhibitory nervous control of the heart via the vagus nerve is established during this period. 'Vagal tone' of the heart has been reported for the tench *Tinca tinca* and the goldfish *Carassius auratus* (Randall, 1966), and for the resting sucker *Catostomus macrocheilus* (Stevens & Randall, 1967). The bradycardia of hypoxic tench and goldfish is due to increased inhibitory vagal activity (Randall, 1966). However, Stevens & Randall (1967) were not able to find evidence of vagal tone in the heart of the trout.

The heart rate of the 18-day fish showed greater increases under stress than the heart rate of fish younger than 9 days. This was supported by observations of the young trout when they were active, and the heart-rate increases of the 18-day fish were considerably greater than those of the 9-day or younger fish.

The resting heart rate and breathing rate of the 18-day and 21-day fish are very similar to those of juvenile rainbow trout of approximately 10 cm length at 10 °C (Holeton, unpublished observations).

HYPOXIA

The responses shown by the 1-, 2- and 8-day fish to hypoxia are essentially similar. The breathing responses of the 8-day fish were more pronounced and reflect greater development of the respiratory pumps at that age.

Unlike the adult trout which show a marked bradycardia at reduced oxygen levels (Randall & Smith, 1967; Holeton & Randall, 1967), the newly hatched trout show a tachycardia. However, when oxygen was dropped to a very low level there was a decrease in both heart rate and breathing rate. This is most clearly demonstrated by the experiment on the 8-day fish (Fig. 4), where the water P_{O_2} was held at approximately 60 mmHg for over 45 min. During this period both heart rate and breathing rate remained elevated, demonstrating that the responses were not transient. When the environmental P_{O_2} was dropped still further to a very low level of 5 mmHg P_{O_2}, there appeared to be considerable weakening of the fishes' movements, heart rate slowed, and breathing movements slowed and ceased. At the lowest heart rates it was obvious that very little blood was being pumped by the heart. This last finding is in agreement with the observations of Gottwald & Kaniewski (1967). They examined the effects of low oxygen upon lake-trout larvae at 7 °C and observed a slowing of the heart rate at lower oxygen levels (0·9 mg/l). They found that when the oxygen was lowered to very low levels the heart reached a critical stage at which it still pulsated, but was unable to pump blood.

With hypoxic adult trout the return of oxygen-rich water is characterized by an abolition of the bradycardia within a few beats of the heart (Holeton & Randall, 1967). The heart rate of the hypoxic larval trout did not increase rapidly when oxygen was restored to the water, but increased gradually over a period of 10 min.

The bradycardia reflex of adult fish to low environmental oxygen levels is mediated by the inhibitory action of the cardiac branch of the vagus nerve (Randall, 1966, 1968). The present experiments with rainbow trout larvae 1, 2 and 8 days after hatching at 10 °C show that the vagus-mediated bradycardia reflex to low oxygen is not

functional up to at least the 8th day after hatching. The low heart rates these fish have at the very low oxygen tensions are more likely to be associated with oxygen starvation of the heart. The levels of oxygen used to test for the bradycardia reflex should have been low enough to detect the reflex if it existed. It is not likely that the 8-day fish could have survived at an environmental P_{O_2} of 5 mmHg for very long as evidenced by the cessation of breathing movements and the slow recovery of one of the fish.

CARBON MONOXIDE

None of the 1-, 6- and 18-day trout larvae died when exposed to 5 % CO for 3 h, nor did they show any ill effects as a result of the exposure. At the same temperature adult rainbow trout and brown trout die if exposed to 5 % CO for much longer than 30 min (Holeton, 1971).

No attempt was made to determine how much of the haemoglobin of the larvae was combined with CO during these experiments. The blood of adult rainbow trout has approximately 94 % of the haemoglobin in the carboxy form when equilibrated with 5 % CO (Holeton, 1971). However, the CO affinity of the blood of larval trout may be different from that of adult trout blood. Changes in haemoglobin dissociation characteristics during early development occur in a wide variety of vertebrates (Manwell, 1960; Prosser & Brown, 1961). It was assumed that the blood of the larvae would be similar enough to that of adults that a large portion of the haemoglobin would combine with CO. This assumption is supported by the evidence that the fish were affected sufficiently by the CO to show respiratory and circulatory changes and by the slight colour change in their blood.

All the larval trout responded to CO in a similar manner and the response intensified with age. These responses, particularly those of the 18-day larvae, are similar to the changes in adult trout (Holeton, 1971).

The larval trout showed very similar responses to both hypoxia and to CO, whereas in adults the responses are quite different (Randall & Smith, 1967; Holeton & Randall, 1967; Holeton, 1971). The adults show a reflex bradycardia when hypoxic whereas larval trout up to 8 days after hatching do not. The 8-day larvae in the present study showed a bradycardia only after the oxygen was lowered to a level below that reported lethal for a number of species of larval salmonids (Bishai, 1960; Hamdorf, 1961; Silver, 1963). The recovery of heart rate in this instance was slow and not characteristic of a reflex response.

The results of these experiments may be relevant to an understanding of the functional integration of the respiratory and circulatory systems of fishes. Fish differ from terrestrial animals in that not only must they adjust their respiratory and circulatory systems to supply oxygen to their tissues economically but they must also contend periodically with very low oxygen levels in the environment. When oxygen levels in the water are high a general increase in the supply of oxygen to the tissues can be effected by increasing the quantity of oxygen presented to the gills (increased breathing) and increasing the capacity to move oxygen from the gills to the tissues (increased circulation). However, if the oxygen level in the water falls considerably, then the normal response of increased breathing may be offset, with the result that there is little net increase in the quantity of oxygen presented to the

gills. Under such circumstances there would be little benefit in increasing circulation. It is in this context of hypoxic conditions that an overriding control which slows circulation would be of benefit.

The experiments with larval trout indicate that the bradycardia reflex in response to hypoxia is not functional for at least 8 days after hatching. This lends support to the suggestion that the response of the newly hatched trout to CO and to hypoxia, and the adult response to CO is the basic response to anoxaemia. The adult brady-cardia response to hypoxia would thus appear to be a superimposed response which Holeton (1971) concluded must be initiated by changes in P_{O_2} of the environment or arterial blood.

The absence of the bradycardia reflex in the larval trout probably does not represent a handicap since their respiration just after hatching is primarily cutaneous and the gill region is poorly developed. A co-ordination of circulatory efforts with the supply of oxygen reaching the gills would be of little use under these circumstances.

SUMMARY

1. Larval trout undergo a transition from cutaneous respiration to gill respiration which at 10 °C is well advanced by the 18th day after hatching.

2. Resting heart rate of newly hatched trout increases during the first few days, stabilizes for a while, and then drops between the 9th and 18th day after hatching. This drop may be the result of establishment of 'vagal tone'.

3. The basic breathing response of larval trout when hypoxic is an increase in fin movements and an increase in rate and amplitude of breathing, but breathing movements slow and weaken if environmental P_{O_2} falls much below 40 mmHg.

4. With trout up to 8 days after hatching hypoxia causes a tachycardia. At very low P_{O_2} levels there is a drop in heart rate and in the amount of blood pumped by the heart. Upon restoring oxygen to very hypoxic larvae, the heart rate recovers slowly, unlike the quick abolition of bradycardia of adult fish. It appears that up to the age of 8 days from hatching, trout do not have a functional bradycardia reflex associated with hypoxia.

5. The responses of larval trout to CO and to hypoxia, and of adult trout to CO, are similar in nature. It is proposed that the basic response to anoxaemia is an increase in breathing and circulation and that the adult bradycardia response to hypoxia is a superimposed mechanism which relates circulation to the quantity of oxygen presented to the gills.

REFERENCES

BISHAI, H. M. (1960). The effect of gas content on larval and young fish. *Z. wiss. Zool.* **163**, 37–64.

GOTTWALD, S. & KANIEWSKI, A. (1967). Blood system disorders in lake-trout larvae (*Salmo trutta morpha lacustris* L.) due to low oxygen level in water. *Roczniki Nauk Polniczych.* Tom 90, Ser. H, Zeszyt **3**, 407–22.

GRODZINSKI, Z. (1950). Susceptibility of the heart in the sea trout embryo *Salmo trutta* L. to small changes in temperature. *Bull. acad. Polon. Sci.* Ser. B II, 173–82.

HAMDORF, K. (1961). Die Beeinflussung der Embryonal- und Larvaltwicklung der Regenbogenforelle *Salmo irrideus* Gibb. durch die Umweltfaktoren O_2-partialdruck und Temperatur. *Z. vergl. Physiol.* **44**, 523–49.

HAYES, F. R., WILMOT, I. R. & LIVINGSTON, D. A. (1951). Oxygen consumption of salmon eggs and embryos. *J. exp. Zool.* **116**, 377–95.

HOLETON, G. F. (1971). Oxygen uptake and transport by the rainbow trout during exposure to carbon monoxide. *J. exp. Biol.* **54**, 239–54.

HOLETON, G. F. & RANDALL, D. J. (1967). Changes in blood pressure in the rainbow trout during hypoxia. *J. exp. Biol.* **46**, 297–305.

MANWELL, C. (1960). Respiratory pigments. *Ann. Rev. Physiol.* **22**, 191–244.

PROSSER, C. L. & BROWN, F. A. (1961). *Comparative Animal Physiology*, 2nd ed. Philadelphia: W. B. Saunders.

RANDALL, D. J. (1966). The nervous control of cardiac activity in the tench (*Tinca tinca*) and the goldfish (*Carassius auratus*). *Physiol. Zool.* **39**, 185–92.

RANDALL, D. J. (1968). Functional morphology of the heart in fishes. *Am. Zool.* **8**, 179–89.

RANDALL, D. J. & SMITH, J. C. (1967). The regulation of cardiac activity on fish in an hypoxic environment. *Physiol. Zool.* **40**, 104–13.

SILVER, S. J., WARREN, C. E. & DOUDOROFF, P. (1963). Dissolved oxygen requirements of developing steelhead, trout, and chinook salmon embryos at different water velocities. *Trans. Am. Fish. Soc.* **92**, 327–43.

STEVENS, E. D. & RANDALL, D. J. (1967). Changes in blood pressure, heart rate, and breathing rate during moderate swimming activity in rainbow trout. *J. exp. Biol.* **46**, 307–15.