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CHANGES OF GAS CONCENTRATIONS IN BLOOD AND WATER DURING MODERATE SWIMMING ACTIVITY IN RAINBOW TROUT

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INTRODUCTION

In a previous paper (Stevens & Randall, 1967) changes in blood pressure, heart rate and breathing rate during activity were reported. Interpretation of these results as to the physiological significance of such changes can be expanded with a knowledge of any concurrent changes in gas concentrations in blood and water afferent and efferent to the gills. Recent technical developments permitting gas analysis on small quantities of blood, associated with the development of suitable handling and cannulation techniques (Holeton & Randall, 1967*a*, *b*), allow measurement of blood–gas concentrations in unanaesthetized, unrestrained, intact fish.

The object of this study was to measure changes in gas concentrations in blood and water afferent and efferent to the gills of rainbow trout before, during, and after moderate swimming activity.

METHODS

The experiments were carried out on fifty hatchery-raised rainbow trout (*Salmo gairdneri*) weighing between 200 and 400 g. The fish were maintained and cannulated as previously described by Stevens & Randall (1967).

When all cannulae were in place, a fish was placed in a respirometer tube similar to that described by Brett (1964). After recording pre-exercise levels, the water velocity was increased stepwise once every minute for 5 min., maintained at a maximum level for 5 min. (51·8 cm./sec.), and then decreased stepwise once every minute for 5 min.

Blood P_{O_2} and blood P_{CO_2} were determined separately on small blood samples with electrodes in a Beckman Modular Cuvette and a Beckman Physiological Gas Analyser (model 160). P_{O_2} was measured with a Beckman oxygen macro-electrode and P_{CO_2} was measured with a Severinghaus-type P_{CO_2} electrode. The blood sample was maintained at the same temperature as the fish during the determination, and was returned to the fish after each determination. In any single experiment blood was sampled from only one blood vessel and only P_{O_2} or P_{CO_2} was determined. Blood–gas concentrations could not be determined every minute because of the slow response time (1–5 min.) of the electrodes as low temperatures.

Oxygen consumption was measured on eleven fish in the respirometer by periodically permitting the fish to utilize about 10% of the oxygen available (Brett, 1964). Oxygen was determined using the unmodified Winkler method.

The temperature of the dechlorinated fresh water during the experiments was the same as that in the holding tanks. The temperature was 4–8° C.; however, it never varied more than ±0·5° C. during any one experiment. Oxygen consumptions were determined at 5° C. on fish acclimated to that temperature.

RESULTS

In general, the fish swam continuously throughout the period of maximum water velocity. The tail-beat frequency during this period was about 160 beats/min.

The P_{O_2} of ventral aortic blood before exercise was 19 mm. Hg. During exercise it decreased 3 mm. Hg, 30 min. after exercise it had returned to pre-exercise levels, and 60 min. after exercise it was 3 mm. higher than pre-exercise levels (Fig. 1 and Table 1).

Table 1. *Blood P_{O_2}, P_{CO_2} afferent and efferent to the gills, and opercular water P_{O_2} during and after exercise in rainbow trout. Values given are means ± standard errors*

Condition	Blood P_{O_2} (mm. Hg)		Blood P_{CO_2} (mm. Hg)		Opercular water P_{O_2} (mm. Hg)
	Ventral aorta $n = 9$	Dorsal aorta $n = 13$	Ventral aorta $n = 6$	Dorsal aorta $n = 7$	$n = 12$
Pre-exercise	19±1·4	85±4·7	5·7±·15	2·3±·11	121±3·0
Exercise					
Beginning of maximum level	17±1·9	90±7·0	—	—	121±2·7
End of maximum level	16±1·8	78±6·3	8·0±1·2	2·2±·13	121±3·0
Post exercise (min.)					
0	18±1·8	71±5·4	—	1·7±·08	121±3·1
5	—	81±7·6	—	—	120±3·1
10	17±1·8	79±6·0	—	—	118±3·1
30	19±2·4	73±5·8	8·8±1·4	2·1±1·0	117±3·3
60	22±2·3	72±6·1	7·9±2·0	2·2±·12	121±1·9
90	19±1·2	80±8·9	9·6±3·3	1·9±·28	121±1·8

The P_{O_2} of dorsal aortic blood before exercise was 85 mm. Hg. The changes in P_{O_2} during and after the exercise were not statistically significant (Table 1), nor are these changes significant physiologically since the blood is fully saturated at a P_{O_2} of about 70 mm. Hg (Randall, Beaumont & Holeton, unpublished).

The P_{O_2} of opercular water (i.e. afferent to the gills) was 121 mm. Hg and did not change during or after exercise. The P_{O_2} of buccal water (i.e. efferent to the gills) was 134 mm. Hg (Fig. 1).

The P_{CO_2} of ventral aortic blood before exercise was 5·7 mm. Hg. It increased during exercise and remained elevated throughout the recovery period (Fig. 2 and Table 1).

The P_{CO_2} of dorsal aortic blood before exercise determined on thirty-two samples from eleven fish, was 2·3 mm. Hg. Changes during or after exercise did not exceed 1 mm. Hg (Fig. 2 and Table 1).

Resting oxygen consumption was 36 mm./kg./hr. During exercise it increased 5-fold and returned to pre-exercise levels within 30 min. (Fig. 3).

Cardiac output was calculated by the Fick principle using two oxygen capacities: 9·00 vol. % (Holeton & Randall, 1967) and 13·8 vol. % (Irving, Black & Safford, 1941).

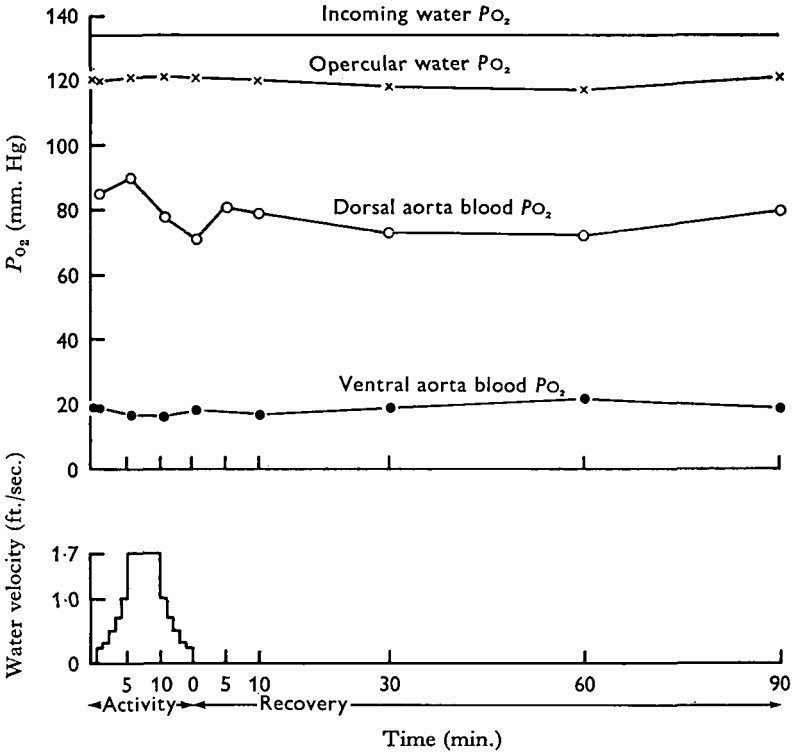

Fig. 1. Changes in P_{O_2} of venous blood, arterial blood, and exhaled water during and after moderate swimming activity.

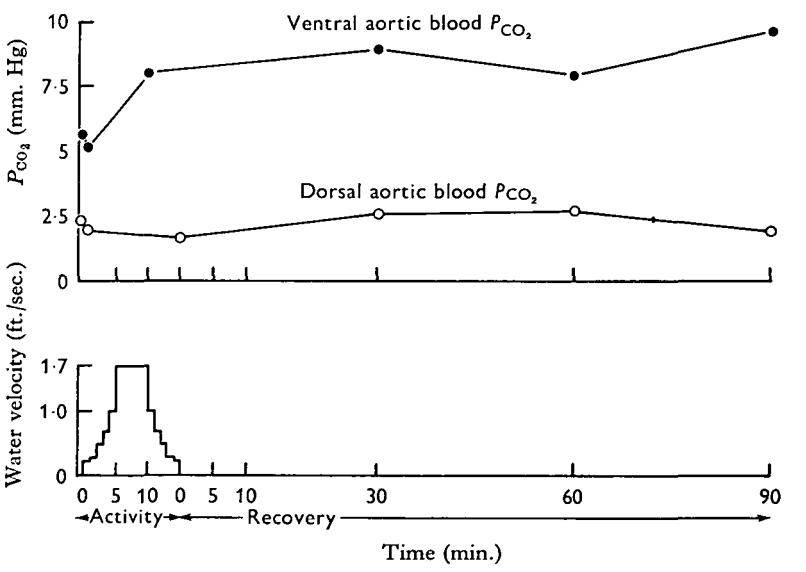

Fig. 2. Changes in P_{CO_2} of venous and arterial blood during and after moderate swimming activity.

Cardiac output was also calculated from the P_{CO_2} data assuming a respiratory quotient of 1·0 and using the CO_2 dissociation curves of Black, Kirkpatrick & Tucker (1966). Pre-exercise cardiac output was 6 ml./min. and increased about 4·5-fold during exercise. It remained elevated for about 10 min. after exercise and then gradually returned to pre-exercise levels (Fig. 4).

Stroke volume was calculated from cardiac output and heart rate (Stevens & Randall, 1967). Stroke volume increased almost 5-fold during exercise, whereas heart rate increased by only 15%. Stroke volume remained elevated for about 10 min. after exercise, whereas heart rate returned to pre-exercise levels rapidly within 10 min. (Fig. 4).

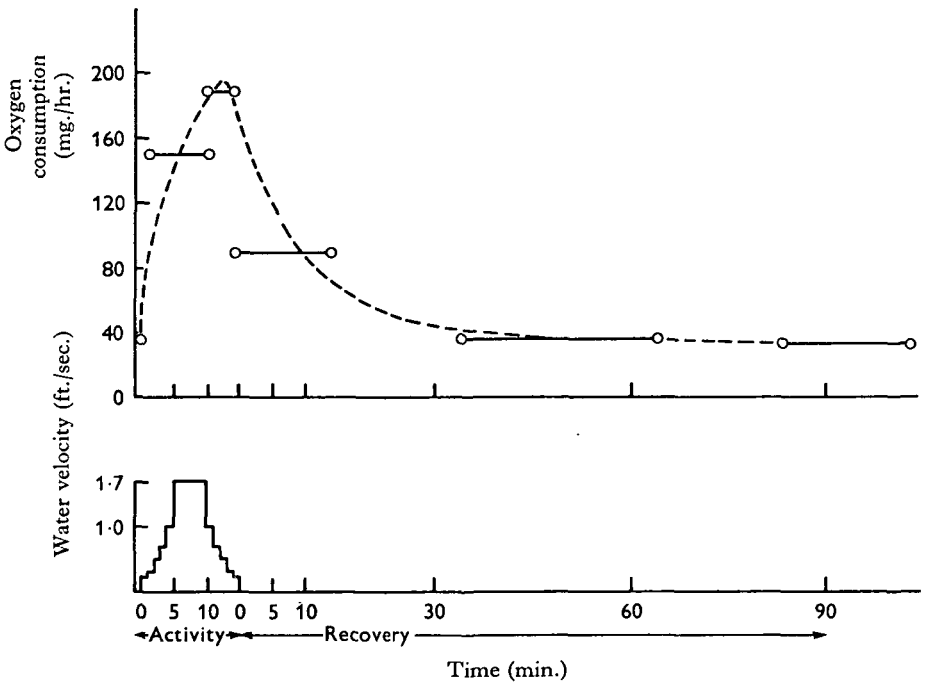

Fig. 3. Changes in oxygen uptake during and after moderate swimming activity.

Ventilation volume was calculated by the Fick principle using a solubility coefficient for oxygen of 0·005718 ml./l./mm. Hg. Resting ventilation volume was 571 ml./min., and increased 5-fold during exercise. At 10 min. after exercise it was 2·5-fold above resting level but returned to pre-exercise levels within 30 min. after exercise (Fig. 5).

The volume of water moved by each breath was calculated from the ventilation volume and the rate of respiration (Stevens & Randall, 1967). Breath output increased about 4·4-fold during exercise, whereas rate increased by only 30%. The breath output remained elevated for about 10 min. after exercise, whereas respiratory rate returned to pre-exercise levels within 5 min. (Fig. 5).

Of the total amount of oxygen delivered to the tissues only 8·3% was carried in physical solution. This value decreased to 6·7% of the total during exercise since the increase in venous P_{CO_2} caused relatively more oxygen to be discharged from the haemoglobin to the tissues (Table 2).

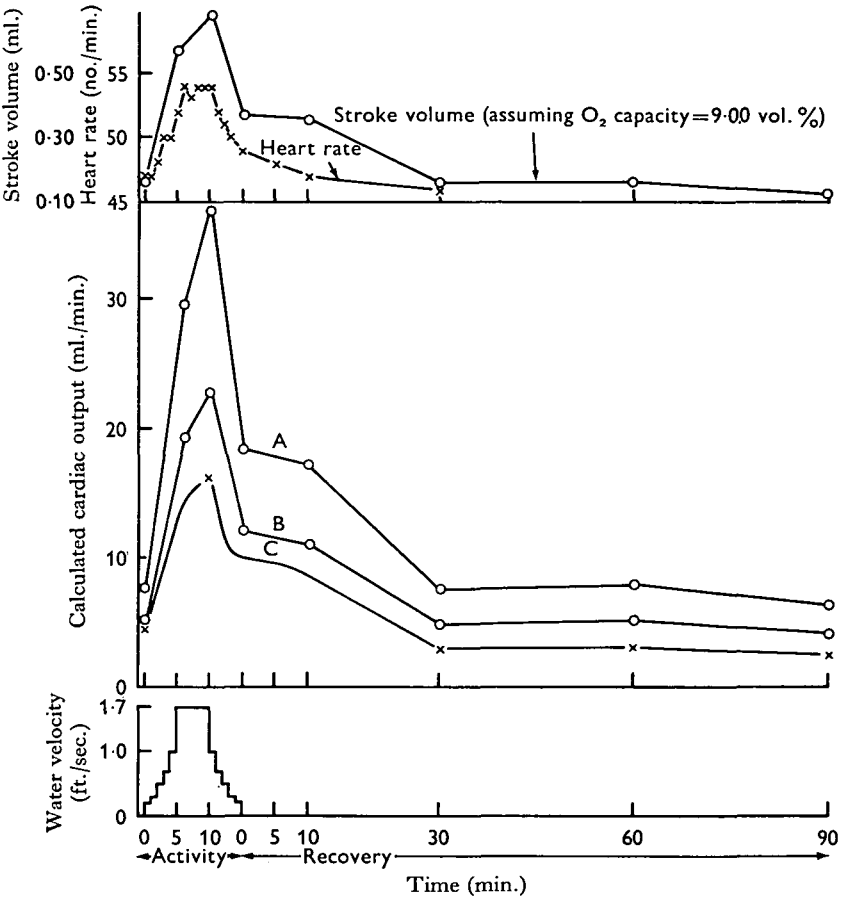

Fig. 4. Changes in cardiac output, stroke volume, and heart rate during and after moderate swimming activity. Cardiac output was calculated by the Fick principle assuming an oxygen output capacity of 9·00 vol. % and of 13·8 vol. %. A. From oxygen uptake data assuming O_2 capacity = 9·00 vol. %. B. From oxygen uptake data assuming O_2 capacity = 13·8 vol. %. C. From CO_2 output data assuming respiratory quotient = 1·0.

Table 2. *Changes in the percentage saturation and* CO_2 *content of the blood during moderate swimming activity*

Condition	Saturation of blood with oxygen (%)		CO_2 content of blood (vols. %)		% of total O_2 delivered to tissues which is in physical solution
	Dorsal aorta	Ventral aorta	Dorsal aorta	Ventral aorta	
Pre-exercise	99·6	37·8	12·3	22	8·3
Beginning of maximum exercise	100	34·2	—	—	8·8
End of maximum exercise	98·6	28·7	12·1	25·5	7·0
Post-exercise (min.)					
0	96·4	32·4	—	—	8·4
10	98·5	30·7	—	—	7·0
30	96·9	34·0	11·9	26·5	6·9
60	96·7	37·7	12·1	25·4	6·7
90	98·7	34·0	11·6	27·5	7·3

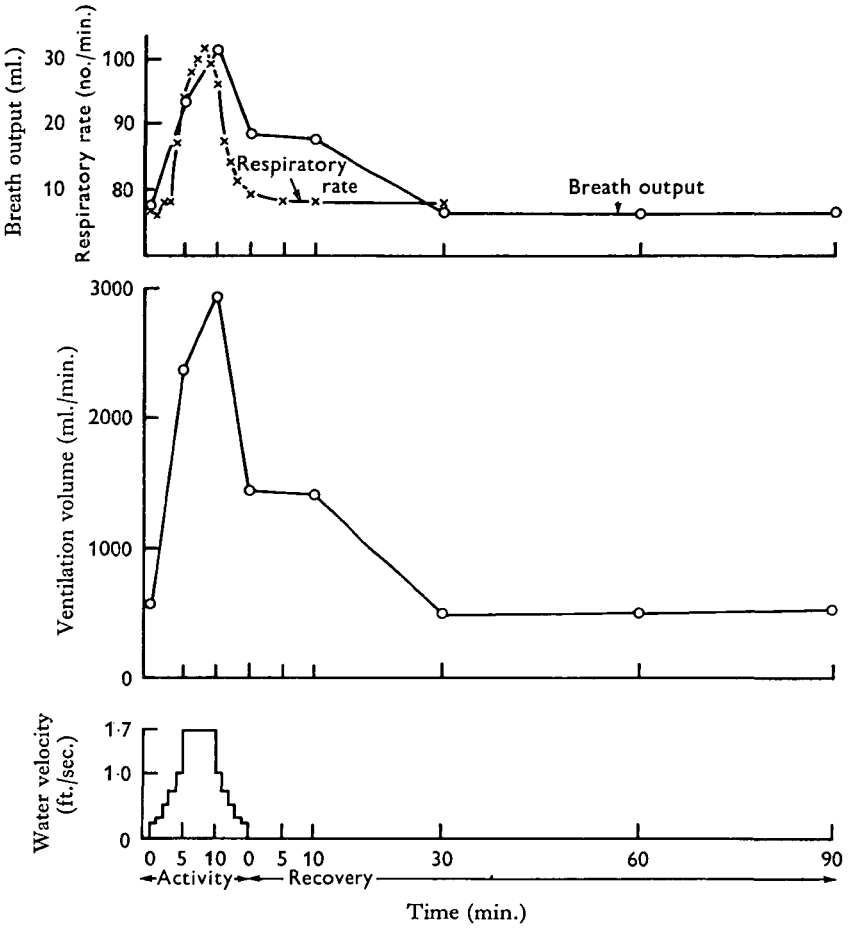

Fig. 5. Changes in ventilation volume, breath output and rate of respiration during and after moderate swimming activity.

DISCUSSION

In the present study which was carried out at 4–8 °C., blood in the ventral aorta of the resting fish was 38 % saturated with oxygen (Table 2). Ferguson & Black (1941) reported a range of 0–3 % saturation of venous blood in the same species of fish but at a higher temperature (15–20° C.). Holeton & Randall (1967) working on the same species of fish at a temperature similar to that of Ferguson & Black (1941) estimated the saturation of venous blood to be 70 %. The P_{CO_2} of venous blood measured by Holeton & Randall (1967) was 2·5 mm. Hg compared with 5 mm. Hg recorded in this study. Because of the large root effect in fish blood, such small changes in P_{CO_2} have a large effect on the percentage saturation of the blood with oxygen, and are sufficient to explain the difference in the calculated percentage saturation of the blood in the two studies. Differences in blood flow and CO_2 production in the tissues determine the P_{CO_2} in venous blood returning to the heart. In both this study and that of Holeton & Randall (1967) blood flow was calculated using the Fick principle. The estimate of blood flow is dependent on the measurement of P_{CO_2}, as blood oxygen content was calculated

rather than measured directly. In this instance, therefore, differences in blood flow cannot be used to explain the differences in venous P_{CO_2}. The accuracy of the estimated values for cardiac output depend largely on estimated values for the percentage saturation of venous blood. Because of the large Bohr and Root effects small errors in the measurement of P_{CO_2} can have a large effect on the estimated values for oxygen content of venous blood, and hence on the estimated values for cardiac output. The P_{CO_2} of fish blood is low, and accurate values at low temperatures are not easily obtained using the methods employed in this study.

The reported values for the percentage saturation of the venous blood of carp, suckers and catfish (Ferguson & Black, 1941) and brook trout (Black *et al.* 1966) are similar to those of the rainbow trout reported here, but higher than the value of 10% saturation reported by Itazawa (1957) for the carp.

The percentage saturation of the venous blood decreased to only 28% during exercise (Table 2). Exercise in this case was only moderate, but there was, however, a 5-fold increase in oxygen uptake by the animal, and it is surprising that this increase in oxygen utilization by the tissues does not result in a larger amount of oxygen being removed from a unit volume of blood (venous blood saturation could theoretically be reduced to zero).

Arterial blood in the dorsal aorta was always more than 95% saturated with oxygen. This is due to the high affinity of the blood for oxygen, and indicates that ventilation of the gills is always adequate to saturate the blood under the conditions of this experiment.

The delivery of oxygen to the tissues during and after exercise is facilitated by an increase in cardiac output, rather than an increase in arteriovenous oxygen difference. The amount of oxygen delivered to the tissues per unit volume of blood increased by only about 15% whereas the oxygen uptake increased by 400–500%.

The increased cardiac output was the result of a 15% increase in heart rate and a 5-fold increase in stroke volume. Thus the increased rate of delivery of oxygen to the tissues was largely the result of large increases in stroke volume of the heart, with adjustments in breathing to maintain saturation of the blood in the face of an increased blood flow through the gills during moderate exercise.

The P_{O_2} in the exhaled water in the opercular cavity was much higher than that reported by Saunders (1962) or van Dam (1938). The respirometer was flushed at a rate of approximately 10 l./min., producing a water velocity of about 1 cm./sec. in the absence of the pump. At rest the water velocity, with the pump on, was 7 cm./sec., and the fish only began to swim when the water velocity was increased to 20 cm./sec. The water velocity must have a considerable effect on the ease with which water is pumped over the gills, and in turn may effect the percentage utilization of oxygen from the water as it passes over the gills. The percentage utilization did not change during exercise in spite of the large increase in oxygen uptake. The increase in oxygen delivery to the gills is therefore the result of a large increase in ventilation volume. This increase in ventilation volume is produced by an increase in both rate and amplitude of breathing. The increase in water velocity must, however, also play a considerable part in this increase in ventilation volume. Thus the general body musculature, in maintaining the position of the fish in face of the increased water velocity, is contributing to the effort required to increase ventilation volume during exercise. The muscles active during swimming are not only propelling the fish through the water, but are also

moving water over the gills. This is possible only in a dense environment like water. During more violent exercise in the sockeye salmon breathing ceases as the animal moves forward through the water (Brett, personal communication) and in this case ventilation is due entirely to the action of the general body musculature.

It is possible that the nature of ventilation of the gills as well as the velocity of water flow may affect the percentage utilization of oxygen in the water. Such factors may contribute to the differences between values for percentage utilization reported here and the highest levels of percentage utilization recorded by van Dam (1938) and Saunders (1962).

The changes in blood P_{CO_2} are small, but physiologically significant because of the large Bohr effect in fish blood (Krogh & Leitch, 1919). For example, Irving, Black & Safford (1941) showed that increasing the P_{CO_2} from 1 to 10 mm. Hg increased the P_{O_2} required for half-saturation from 14 to 31 mm. Hg in the blood of the rainbow trout. Ferguson & Black (1941) reported venous P_{CO_2} of 8–10 mm. Hg in the rainbow trout at 15–22° C. Black *et al.* (1966) reported a venous P_{CO_2} in brook trout at 0° C. of 5–6 mm. Hg. These data are in good agreement with those of the present study. In contrast to these values, Koyama (Hughes, 1964) reported a venous P_{CO_2} of 32 mm. Hg in the carp.

P_{CO_2} in the dorsal aorta remained at a low level before, during, and after exercise. This is to be expected in a direct ventilation system exchanging O_2 and CO_2 between water and blood. The respiratory quotient is of the order of unity, so that approximately the same quantity of CO_2 is removed from the blood as of oxygen taken into the blood. The solubility of CO_2 in water is higher than that of oxygen, and CO_2 diffuses through the tissues more readily than oxygen. Even though the solubility coefficient of CO_2 in blood is normally higher than that of oxygen, the exchange system is such that CO_2 can move more easily between blood and water than oxygen can move from water to blood. Thus if the blood leaving the gills is fully loaded with oxygen the CO_2 level in the blood efferent to the gills cannot be very different from that of the water in the buccal cavity. That is, if the P_{CO_2} in the water afferent to the gills was nearly zero, one would expect P_{CO_2} levels in the blood in the dorsal aorta also to approach zero. An increase in CO_2 content in the water would also produce an increase in the CO_2 content of the blood afferent to the gills. This would markedly decrease the oxygen-carrying capacity of the blood. Values for P_{CO_2} of dorsal aortic blood reported here are similar to those reported by Rahn (1966) for the tautog, carp and mackerel.

During exercise the oxygen uptake of the animal increased about 5-fold. Both the resting and active values reported here are similar to those of young salmon at 5° C. (Brett, 1964).

SUMMARY

1. Changes in partial pressures of O_2 and CO_2 in blood and water afferent and efferent to the gills are reported. These variables were measured before, during and after moderate swimming activity in rainbow trout.

2. Neither blood P_{CO_2} nor water P_{O_2}, afferent or efferent to the gills, changed markedly before, during or after exercise.

3. Arterial blood was always more than 95 % saturated with oxygen. Venous blood was 38 % saturated, falling to a minimum of 28 % during exercise.

4. P_{CO_2} of arterial blood was 2·3 mm. Hg. P_{CO_2} of venous blood increased from 5·7 to 8·0 mm. Hg during exercise and remained elevated throughout the recovery period.

5. Cardiac output (calculated using the Fick principle) stroke volume, ventilation volume and the volume of water pumped per breath all increase by a factor of between 4 and 5 during exercise. All tended to remain elevated for between 10 and 30 min. after exercise and then gradually decrease to pre-exercise levels.

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REFERENCES

BLACK, E. C., KIRKPATRICK, D. & TUCKER, H. H. (1966). Oxygen dissociation curves of the blood of brook trout (*Salvelinus fontinalis*) acclimated to summer and winter conditions. *J. Fish. Res. Bd Can.* **23** (1), 1–13.

BRETT, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Bd Can.* **21** (5), 1183–226.

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

FERGUSON, J. K. W. & BLACK, E. C. (1941). The transport of CO_2 in the blood of certain freshwater fishes. *Biol. Bull. mar. biol. Lab. Woods Hole.* **80** (2), 139–52.

HOLETON, G. F. & RANDALL, D. J. (1966*a*). Changes in blood presure in the rainbow trout during hypoxia. *J. Exp. Biol.* **46**, 297–305.

HOLETON, G. F. & RANDALL, D. J. (1966*b*). The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. *J. Exp. Biol.* **46**, 317–327.

HUGHES, G. M. (1964). Fish respiratory homeostasis. *Symp. Soc. Exp. Biol.* **18**, 81–107.

IRVING, L., BLACK, E. C. & SAFFORD, V. (1941). The influence of temperature upon the combination of oxygen with the blood of trout. *Biol. Bull. mar. biol. Lab. Woods Hole* **80** (1), 1–17.

ITAZAWA, Y. (1957). Gas content of the blood in response to that of medium water in fish. *Bull. Jap. Soc. sci. Fish.* **23**, 71–80.

KROGH, A. & LEITCH, I. (1919). The respiratory function of the blood in fishes. *J. Physiol.* **52**, 288–300.

RAHN, H. (1966). Aquatic gas exchange: theory. *Resp. Physiol.* **1**, 1–12.

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. *Can. J. Zool.* **40**, 817–62.

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**, 307–315.