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Effects of Moderate and Severe Exercise in Rainbow Trout on Some Properties of Arterial Blood, Including Red Blood Cell Deformability

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Abstract Effects of exercise on arterial blood gas and haematological properties including red blood cell deformability were studied using chronically cannulated rainbow trout. No significant change in P_{a,O_2} was observed after severe ($2.0\ L\cdot s^{-1}$) exercise but there was some indication of an increase after moderate ($1.6\ L\cdot s^{-1}$) exercise. A significant increase in pH_a following moderate exercise was observed and the same specimens showed a decrease in pH_a after severe exercise. Although there was a tendency for red blood cell deformability to be increased after moderate and especially severe exercise, the changes were statistically not significant.

Many studies have been made on haematological features of blood of fish under various conditions including hypoxia and during exercise. Little consideration has been given, however, to the possible importance of changes in the physical properties of the red blood cells (RBC). One of the most characteristic features of these cells is their remarkable deformability which enables them to squeeze through the fine vessels of the microcirculation both in gas exchange organs and in the tissues. The importance of the microcirculation has been much appreciated in mammalian studies but so far has attracted little attention of the comparative physiologist. Recent studies on the deformability of fish red blood cells have used the Nuclepore filtration method which incorporated a considerable improvement in reliability (Kikuchi et al., 1980, 1983) to give an index of RBC deformability. In this method the time is taken for a given volume of blood to pass through a Nuclepore filter containing straight cylindrical pores of a constant defined diameter under a pressure difference of 10 cm H_2O. Marked effects of temperature on RBC deformability was shown in yellowtail (Hughes et al., 1982) and carp (Kikuchi et al., 1982). The effects of temperature have also been studied in rainbow trout and *Raia* (Hughes and Kikuchi, in preparation) and Pacific salmon (Kikuchi et al., in preparation). These studies indicate differences in the effect of temperature which are related to the normal temperature of the environment in which these species live. There is also evidence to suggest that RBC deformability is affected by hypoxia (Hughes and Kikuchi, 1984) and some transient effects have been observed in salmon red blood cells during transfer from seawater to rivers (Kikuchi et al., in preparation).

The present studies were undertaken to test whether exercise has any marked effect on RBC deformability of rainbow trout. An increase in deformability might be expected to improve gas transfer from gills to mitochondria. However, under the present conditions no conclusive results were obtained; an increase in deformability seemed clear in some specimens but an opposite effect, i.e., a slight decrease in deformability was also observed in some others. Nevertheless, it is discussed that an increase in RBC deformability is probable especially during severe exercise in which tissue hypoxia and similar changes in some plasma factors may be involved as in the case of environmental hypoxia (Hughes and Kikuchi, 1984).

Materials and methods

Rainbow trout (body length 32.8±0.4 cm [Mean and S.E.], body weight 312±7.5 g) were anaesthetized (MS222, $0.1\ g\cdot l^{-1}$) and chronically cannulated in the dorsal aorta using modifications of methods of Soivio et al. (1975) similar to those described in Hughes et al. (1983). Subsequent to cannulation, a fish was transferred to a Brett (1964) tunnel respirometer and allowed to recover overnight in a water flow of 15 $cm \cdot s^{-1}$ before the swimming trial was conducted. For each fish, two levels of exercise were employed, 'moderate' and 'severe'. Preliminary experiments indicated that the critical speed for local trout is 2.0 body lengths (L) per sec ($L \cdot s^{-1}$). For 'moderate' exercise, after withdrawing 0.9 ml blood onto 0.1 ml heparinized saline (Cortland saline containing heparin at 5,000 I.U. per ml), water velocity was increased by 0.2 $L \cdot s^{-1}$ (approximately 6 $cm \cdot s^{-1}$) every 10 min until a swimming speed of 1.6 $L \cdot s^{-1}$ was attained. After 30 min at this speed, a blood sample was taken and water flow reduced to 15 $cm \cdot s^{-1}$. Following a 3 h recovery period, another blood sample was obtained and water flow was then increased to 2.0 $L \cdot s^{-1}$ (approximately 65 $cm \cdot s^{-1}$) using the same increments as in 'moderate' exercise. At this speed, most fish fatigued within 20 min as was apparent by considerable burst swimming and struggling and an inability to swim off the retaining grid. When this occurred, a blood sample was taken. However, some fish continued to swim for the prescribed 30 min. All swimming trials were conducted at 15°C.

P_{O_2} of the blood samples, i.e., P_{a,O_2}, was measured with a Radiometer P_{O_2} electrode (E5046) in a thermostatted cuvette and displayed on a Strathkelvin 381 oxygen meter. pH_a was measured with an Ingold pH microelectrode assembly and Pye Model 290 pH meter. Haematocrit value (Ht) was obtained using heparinized microhaematocrit tubes and a Hawksley microcentrifuge. Red blood cells (RBC) were counted using an improved Neubauer counting chamber. Plasma osmolality was measured using the plasma portion from the microhaematocrit tube and a 5100B vapour pressure osmometer (Wescor, Inc.).

Usually 0.35 ml of blood was used for each measurement of the deformability of the red blood cells. The heparinized blood is placed in a 0.5 ml syringe and flows through a Nuclepore filter containing pores of 8 μm diameter under a pressure difference of 10 cmH_2O. The time for the blood to pass through the filter which was determined for every 0.05 ml blood volume was converted to the mean pore passage time of single red blood cells by inserting the blood passage time, haematocrit value (Ht), and mean corpuscular volume (MCV) into a previously derived equation (Kikuchi et al., 1980, 1983). This equation was obtained by analyzing factors influencing blood flow through the filter, to which the major resistance is obviously provided by red blood cell deformability.

Results

Table 1 summarizes the average values obtained together with results of comparisons between resting and exercised fish using Student's paired t-test. The general impression gained from these experiments was as follows:

(i) No significant change in arterial P_{O_2} was observed after severe exercise and probably not after moderate exercise although a change in the latter was significant at 10% level.

(ii) There was a significant increase in pH following moderate exercise. After a period of 3 hours for recovery from the moderate exercise, the same fish when subjected to severe exercise showed a decrease (significant at 10% level) in arterial pH. The statistical significance of these effects was greater ($P<0.01$) when additional unpaired measurements were taken into account (see Table 1).

(iii) The haematocrit value generally showed a slight increase following exercise, only significant at 10% after moderate exercise, but no significant change in mean corpuscular volume. Taken together this gives some indication that the change in haematocrit value was due either to the release of new red blood cells into the circulation or the water shift from plasma to tissues.

(iv) The passage time for whole blood through 8 μm filters decreased in spite of the increase in haematocrit value, indicating a larger decrease in RBC pore passage time and hence an improved RBC deformability. However the changes were not significant after moderate and severe exercise.

Discussion

Although in some specimens an increase in RBC deformability following exercise seemed to occur, the present results show an absence of any significant change in deformability following either moderate or severe exercise. Nevertheless, as a change in deformability was found in half of the experiments, it remains possible that under different conditions of exercise (e.g., swimming to fatigue) and/or blood sampling (e.g., reduced sample volume and number of times) some significant effects might be found. Under the conditions of the present experiment, however, significant changes were observed both in arterial pH and P_{O_2}. The increase in pH after moderate exercise and decrease after severe exercise are of especial interest and have been confirmed in preliminary experiments using an extracorporeal circulation (Hughes, Thomas and Duthie, unpublished observations, 1982).

The changes in P_{a,O_2} during exercise have also been confirmed by a more extensive series of experiments over a wide range of swimming speeds (Duthie, in preparation). This rise in P_{a,O_2} should not greatly affect arterial O_2 content because all oxygen tensions measured in the present study are sufficient to produce at least 90% saturation (Eddy, 1971). For the same species, Kiceniuk and Jones (1977) found a slight fall in P_{a,O_2} during exercise. Holeton et al. (1982) could find no significant difference in P_{a,O_2} between stationary and swimming mackerel.

A possible interpretation of these results seems to be that moderate exercise is accompanied by hyperventilation partly due to increased ventilatory movements but also the inevitable ramjet component as swimming increases. The increased flow of water across the gills would encourage outflow of CO_2 and a consequent transitory increase in blood pH. During severe exercise an increased production of muscle lactate would lead to an increased concentration in the blood and lowered blood pH. No measurements of lactate were made in the present experiments, but the osmolality measurements suggest an increase in some plasma constituent which could have been lactate. The stimulus for increased ramjet ventilation is probably concerned with mechanoreceptors either on the outside of the fish or within the buccal cavity (Roberts, 1975; Randall, 1982) and there seems

Table 1. Comparison of values for blood parameters before and after moderate and severe exercise (see Methods for definition). Number of observations in each group is given in parentheses.

	Moderate			
			Paired t-test	
	Pre-exercise	Post-exercise	t-value	level of significance
P_{a,O_2} (mmHg)	94.73±5.96 (6)	104.82±2.36 (6)	−2.0970	10%
*pH_a	7.931±0.025 (6)	8.050±0.018 (6)	3.2946	5%
Blood passage time (sec)	4.90±1.59 (6)	4.52±0.57 (6)	−0.5254	ns
MCV (μm^3)	212.80±7.76 (6)	208.52±3.68 (6)	−0.7429	ns
Ht (%)	17.1±2.6 (6)	18.4±2.9 (6)	2.2523	10%
RBC pore passage time (msec)	1.18±0.36 (6)	1.03±0.17 (6)	−0.7636	ns
Osmolality (mOsm/kg)	296.1±7.4 (5)	303.0±4.5 (5)	1.5727	ns

	Severe			
			Paired t-test	
	Pre-exercise	Post-exercise	t-value	level of significance
P_{a,O_2} (mmHg)	102.92±6.92 (4)	98.15±3.60 (4)	−0.7388	ns
*pH_a	8.005±0.035 (4)	7.942±0.014 (4)	−2.2771	10%
Blood passage time (sec)	4.92±0.77 (4)	3.52±0.56 (4)	−1.2302	ns
MCV (μm^3)	205.85±6.40 (4)	217.07±18.05 (4)	0.7150	ns
Ht (%)	16.7±3.3 (4)	17.0±2.9 (4)	0.3948	ns
RBC pore passage time (msec)	1.43±0.58 (4)	0.95±0.12 (4)	−0.9038	ns
Osmolality (mOsm/kg)	296.5 (2)	318.7 (2)	—	—

* When additional unpaired observations are added (N=12) and compared using the t-statistics for 2 groups of means, there is a significant increase in pH_a after moderate exercise at the 1% level and a significant decrease after severe exercise at the 5% level.

to be little evidence that changes in blood pH are involved.

An increase in RBC deformability has been observed in rainbow trout subjected to aquatic hypoxia (Hughes and Kikuchi, 1984). In that case the blood oxygen level itself appeared to be irrelevant since little change was observed when blood samples were subjected in vitro to comparable changes in P_{O_2}. This finding probably supports the possibility that an increase in RBC deformability may take place during exercise. Especially during severe exercise tissue hypoxia is produced and hence plasma lactate and adenosine will be increased. Increases in circulating cathecolamines are also known to occur both during hypoxia and exercise (Randall, 1982; Hughes, Le Bras and Duthie, unpublished observations, 1983). The red blood cell membrane might be affected by such substances and consequent changes in its physical properties might further result in an altered cellular deformability. Thus a change in RBC deformability is expected to occur during exercise because of the operation of mechanisms similar to those during hypoxia. Such an increase in RBC deformability will play a role in cardiovascular responses to hypoxia and exercise. A decrease in the peripheral resistance due to an increase in RBC deformability will contribute to increases in blood flow through the gills, heart and working muscles. Especially, the muscle circulation may be more dependent on RBC deformability since the bore of their blood vessels becomes much smaller when they are compressed by the contracted muscles.

Any changes in RBC properties may be thus related to the altered blood chemistry but are complicated by possible changes in the RBC population. The repeated blood sampling in the present experiments may have induced the release of new RBC into the circulation. If they had a lower deformability than those acclimatized to the circulation, any increase in RBC deformability should have been obscured. Modifications in haematocrit value could also involve increases or decreases in plasma volume and there is evidence for an increased liberation of RBC during exercise of some fish species (Yamamoto et al., 1980; Randall and Daxboeck, 1982).

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Literature cited

Brett, J. R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Bd. Can., 21: 1183–1226.

Eddy, F. B. 1971. Blood gas relationships in the rainbow trout, *Salmo gairdneri*. J. Exp. Biol., 55: 695–711.

Holeton, G. F., M. G. Pawsen, and G. Shelton. 1982. Gill ventilation, gas exchange and survival in the Atlantic mackerel (*Scomber scombrus* L.). Can. J. Zool., 60: 1141–1147.

Hughes, G. M. and Y. Kikuchi. 1984. Effects of in vivo and in vitro changes in P_{O_2} on the deformability of red blood cells of rainbow trout (*Salmo gairdneri* R). J. Exp. Biol., 111: 253–257.

Hughes, G. M., Y. Kikuchi and H. Watari. 1982. A study of the deformability of red blood cells of a teleost fish, the yellowtail (*Seriola quinqueradiata*) and a comparison with human erythrocytes. J. Exp. Biol., 96: 209–220.

Hughes, G. M., C. Albers, D. Muster and K. H. Gotz. 1983. Respiration of the carp (*Cyprinus carpio*) at 10° and 20°C and the effects of hypoxia. J. Fish Biol., 22: 613–628.

Kiceniuk, J. W. and D. R. Jones. 1977. The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. J. Exp. Biol., 69: 247–260.

Kikuchi, Y., T. Arai and T. Koyama. 1983. Improved filtration method for red cell deformability measurement. Med. Biol. Eng. Comput., 21: 270–276.

Kikuchi, Y., G. M. Hughes and C. Albers. 1982. Temperature dependence of the deformability of carp (*Cyprinus carpio*) red blood cells. Experientia, 38: 822–823.

Kikuchi, Y., M. Horimoto, T. Koyama, Y. Koyama and S. Tozawa. 1980. Estimation of pore passage time of red blood cells in normal subjects and patients with renal failure. Experientia, 36: 325–326.

Randall, D. J. 1982. The control of respiration and circulation in fish during exercise and hypoxia. J. Exp. Biol., 100: 275–288.

Randall, D. J. and C. Daxboeck. 1982. Cardiovascular changes in the rainbow trout (*Salmo gairdneri*, Richardson) during exercise. Can. J. Zool., 60: 1135–1140.

Roberts, J. L. 1975. Active branchial and ram gill ventilation in fishes. Biol. Bull., 148: 85–105.

Soivio, A., K. Nyholm and K. Westman. 1975. A technique for repeated sampling of the blood of individual resting fish. J. Exp. Biol., 62: 207–217.

Yamamoto, K., Y. Itazawa and H. Kobayashi. 1980. Supply of erythrocytes into the circulating blood from the spleen of the exercised fish. Comp. Biochem. Physiol., 65A: 5–11.

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ニジマス動脈血 P_{O_2}, pH, Ht および赤血球変形能に及ぼす中度および強度運動負荷の影響

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運動負荷による血液酸素運搬能の変化の有無を検討した．背動脈にカニュレーションしたニジマスを 1.6 体長/秒の速度で 30 分間（中度）泳がせ，3 時間の休止後，再び 2.0 体長/秒の速度で 30 分間（強度）泳がせ，それぞれの運動負荷の前後でヘパリン採血した血液について，P_{O_2}, pH, Ht, 赤血球数，血漿浸透圧および赤血球変形能を測定した．赤血球変形能は 10 cm H_2O の圧力差の下での赤血球の 8 μm ニュークリポアフィルター通過時間の平均値を指標とした．pH は中度運動負荷後増加し，強度運動負荷後減少した．前者は過呼吸を，後者は乳酸産生増加を反映しているものと思われた．P_{O_2}, Ht, 赤血球数は中度運動負荷後増加したが，強度運動負荷後の変化は明らかでなかった．赤血球変形能は中度，殊に強度運動負荷後高まる傾向があったが，有意ではなかった．運動中の血液酸素運搬能の増加について考察した．

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