

NON-RELEASE OF LACTIC ACID FROM ANAEROBIC SWIMMING MUSCLE OF PLAICE *PLEURONECTES PLATESSA* L.: A STRESS REACTION

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SUMMARY

1. Plaice caught by trawl net and plaice exercised in laboratory tanks all show high levels of lactic acid (33-44 mmol/kg) in the anaerobic swimming muscle. During exhausting exercise 2 moles of lactate are formed from 1 mole of glycogen glucose. After an 8 h rest 50-80% of the muscle glycogen is restored.

2. Blood lactate levels remain low (0.5-2 mmol/l) in the majority of plaice caught by trawl. In a small number of plaice, peak levels over 5 mmol/l are reached 2-4 h after capture. Low blood lactate levels could be guaranteed in all fish exercised 24 h after the stress of capture and in tank-adapted fish exercised and injected with the β -adrenergic stimulating drug, isoxsuprine hydrochloride. The blood lactate in plaice, tank-adapted for more than 8 days and then exercised, may reach peak levels up to 5 mmol/l 2-4 h later.

3. High blood lactate levels were obtained by injecting the β -adrenergic block propranolol to stressed exercised fish. The α -adrenergic block did not have this effect. All plaice with blood lactate levels reaching 5-12 mmol/l died.

4. The results indicate that the muscle cells regulate the release or non-release of their lactate load to the blood stream and increases in the blood circulating to the muscle do not influence this release. The non-release mechanism may be activated by a catecholamine circulated in the blood stream following a stress.

INTRODUCTION

The anaerobic white muscle tissue forms the bulk of the swimming muscles on either side of the backbone in a teleost fish. New measurements have shown that the maximum speed of a fish is limited by the contraction time of the swimming muscle; and the shortening time gets longer as the fish gets larger (Wardle, 1975, 1977). Plaice have been observed and filmed swimming at the high maximum speeds predicted by their muscle contraction time in trawls and seine nets (Hemmings, 1973; C. S. Wardle, unpublished). The endurance of fast swimming is limited by the anaerobic energy released when the stored glycogen is transformed by the Embden Meyerhof cycle to form lactic acid within the muscle cells (Black, Robertson & Parker, 1961). The short endurance of the fish swimming at these higher speeds is a major reason for the successful capture of fish by these types of fishing gears. The

observed short swimming endurance of fish in the mouth of seine or trawl gear can be explained by the depletion of a finite glycogen store. Measurements of power output by swimming fish agree reasonably with the energy available in the white muscle glycogen stores (Wardle & Reid, 1977). Once the glycogen store is depleted the fish's scope of behaviour is limited and typically fish maintain/shelter and rest for a period of up to 24 h, while the muscle regains its full performance potential (Black, 1958a; Black *et al.* 1961). During the capture of plaice by seine net or trawl the fish are made to exhaust their muscle glycogen store in the region of the mouth of the gear and eventually they drop back into the funnel of the net and are guided by the netting into the codend (C. S. Wardle, unpublished). During a series of experiments examining the levels of lactic acid in the blood of plaice following capture by trawl it became clear that although the lactic acid in the muscle had reached maximum levels and glycogen was depleted, the level of lactate in the blood could be very low throughout the period after capture (Wardle, 1967).

Experiments examining blood glucose levels in plaice (Wardle, 1972) had demonstrated that capture and transfer to sea-water tanks initiated a stress reaction in the plaice even though the sea water properties were kept identical. The process of capture not only exhausts the muscles of the fish but also imposes a stress stimulus on the 'wild' fish, detected by the sensory system. This stimulus interpreted as stressing by the brain initiates a series of physiological changes as the fish adapts to the aquarium. The present study examines in more detail the factors controlling release and non-release of lactic acid from muscle to blood in plaice and relates these findings to the stress of capture. A related study (Batty & Wardle, 1978) examines the recovery cycle leading to restoration of the glycogen in plaice anaerobic muscle.

MATERIALS AND METHODS

Adult plaice, *Pleuronectes platessa* L. were caught either by short trawl hauls (30 min) or by seine net (30–50 min) and selected fish between 400 and 500 g weight were immediately transferred to aquaria. The experiments investigating the changes following capture were carried out entirely on research boats. Other experiments made us of fish caught from local fishing grounds and brought back to the Aberdeen Laboratory the same day. Time of capture could not be determined precisely but where stated refers to the time when landed on the deck. No fish included in these experiments took food subsequent to capture. Aquaria on research ships had circulating sea water at sea temperature and shore tanks contained recirculated sea water kept at 9 °C.

To ensure high levels of lactic acid in the muscle of plaice in some experiments a rubber-tipped rod was used to chase plaice for a maximum period of 15 min.

Injections were made to the renal portal vein as shown in Wardle (1971, 1972).

Phenoxybenzamine hydrochloride (Dibenylin) was dissolved (10 mg) in ethanol (0.2 ml) and was made up with saline solution (1 ml, 0.18 M) just before injecting as a suspension.

Isoxuprine hydrochloride (Duvadilan, 2 ml ampoules 5 mg/ml, Crookes) was made isotonic (0.18 M).

Propranolol (Inderal) was made up in saline (10 mg/ml, 0.18 M).

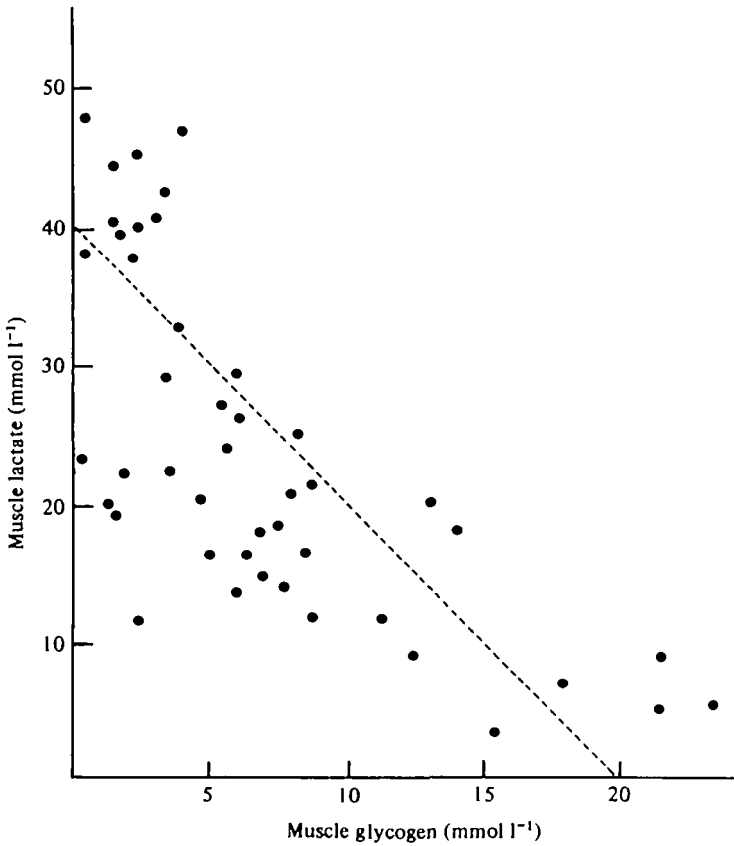


Fig. 1. Plaice in different states of rest (bottom right) and exercise (top left). The values of muscle lactate are plotted against muscle glycogen for each individual. The dashed line represents equimolar conversion of glycogen to lactic acid. Two moles of lactate are formed from 1 mole of glycogen glucose.

The muscle circulation was examined using ^{51}Cr -labelled erythrocytes and iodinated human serum albumin (^{131}I HSA or ^{125}I HSA) as described in Wardle (1971). ^{51}Cr -labelled erythrocytes when injected to the blood stream pass freely into the muscle capillaries and as soon as the labelled cells have mixed thoroughly with the blood (from 5 min post-injection) the blood volumes in the muscle reach constant levels between 0.07 and 0.2 ml per 100 g (Wardle, 1971). In the course of experiments attempting to measure the volume of blood in plaice muscle it was discovered that ^{131}I HSA was not limited to the vascular space but passed into a lymph circulation in the muscle (Wardle, 1971). The rate of formation of the lymph in experiments with plaice could be assessed if the level of the isotope label in the muscle was measured at a constant time interval (3 h) following injection to the blood (Wardle, 1971).

Blood samples for lactic acid analysis were taken either from the severed tail of plaice killed at different times following capture or periodically by syringe from the renal portal vein of an identified living plaice. The sample (0.20 ml) of whole blood was transferred immediately to aqueous perchloric acid solution (1.0 ml, 0.6 M)

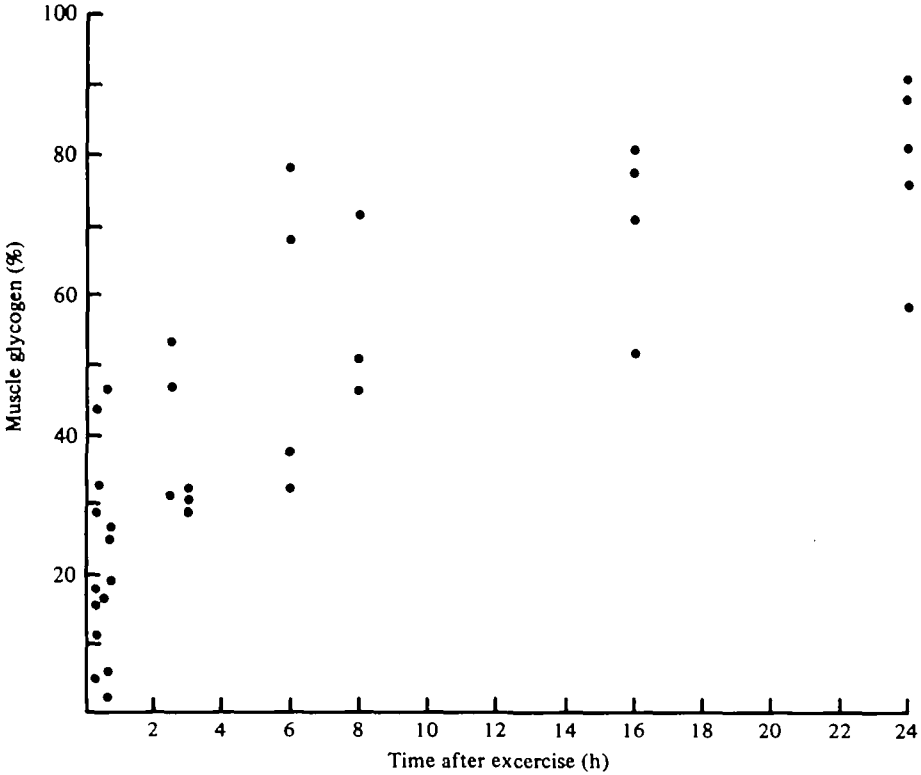


Fig. 2. Muscle glycogen levels in plaice rested in aerated sea water and killed at various times after an initial exhausting exercise period. Glycogen level is expressed as percentage of total anaerobic fuel level (lactate + glycogen as glucose).

contained in polystyrene disposable test tubes (4 ml) and sealed with Parafilm. The contents were shaken mechanically, centrifuged and the supernatant liquor decanted.

Muscle samples ($4 \times 1 \times 1$ cm) for both lactate and glycogen analysis were cut from the dorsal right lateral muscle block of fresh-killed fish. The pieces were placed in small polythene bags and frozen immediately in liquid nitrogen (-173°C). The samples were trimmed in a cryostat (at -15°C) to remove skin and blood-stained surfaces, chopped to small pieces and weighed. For lactate analysis the frozen pieces were homogenized in perchloric acid (pca) solution (0.5 g muscle to 5 ml 0.6 M pca) for 60 s then diluted to 15 ml (0.6 M pca) and centrifuged.

For glycogen analysis the frozen pieces (1 g) were added to 5 ml of a digestion mixture (30 g potassium hydroxide: 100 ml water and 5 ml saturated sodium sulphate) in a Pyrex test tube heated in a boiling water bath until clear then cooled and made up to 10 ml with more digestion mixture.

Aliquots of pca supernatant for lactic acid analysis were neutralized using the indicator methyl orange and potassium carbonate (69 g/100 ml water). Lactate in neutralized pca extracts of muscle or blood was analysed according to the method of Hohorst (1963) using L-lactate dehydrogenase and nicotinamide adenine dinucleotide (NAD).

For glycogen analysis duplicate aliquots of the muscle digests were twice pre-

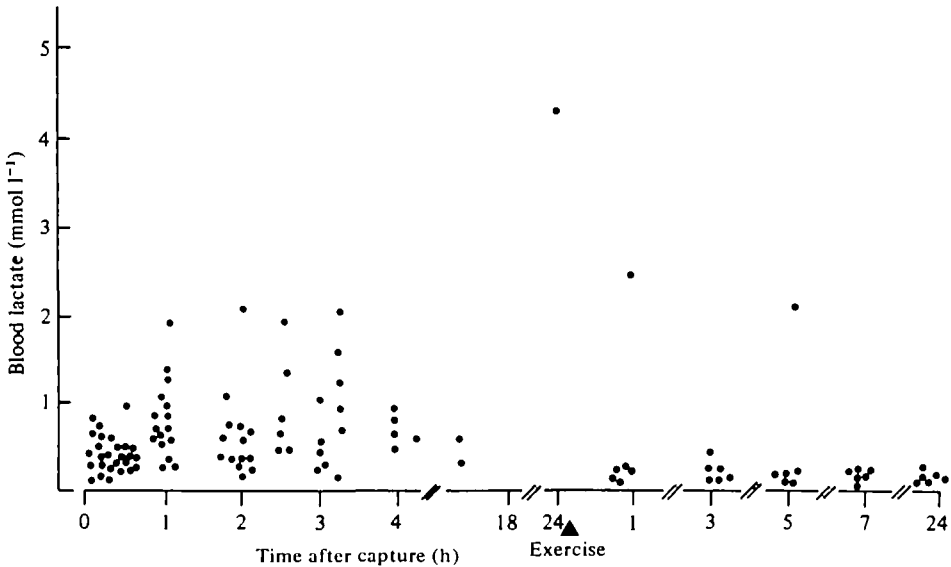


Fig. 3. The changes in blood lactic acid level in plaice held in sea-water aquaria after capture by trawl for the times indicated. Each point represents the blood lactate in a single fish. Twenty-four h after capture the swimming muscles of the rested plaice were exhausted by chasing and low blood levels of lactate were characteristic during the next 24 h.

precipitated with ethanol and analysed according to Handel (1965) using anthrone reagent. All glycogen measurements are expressed as hydrolysed glycogen in terms of mmol of glucose per kg wet muscle.

RESULTS

1. *Lactic acid from glycogen*

The values of the glycogen and lactic acid levels in muscle samples from 69 plaice in various states of exercise are shown in Fig. 1. Because the lactic acid remained in the muscle after exercise, the values for muscle lactic acid and glycogen glucose for each individual were added to give the total anaerobic fuel capacity of the muscle. It was found that immediately after severe exercise muscle glycogen could be as low as 5% of the total anaerobic fuel store and in plaice rested for more than 24 h could be as high as 90% of the store. In order to examine the speed of recovery of the muscle glycogen store, muscle samples were taken from plaice which had been exercised and killed after various periods of recovery. The glycogen levels, shown in Fig. 2, are expressed as a percentage of the total anaerobic fuel level (lactic acid + glycogen) and plotted against time of recovery. The slope of this graph indicates that 50–80% of the glycogen might be restored in plaice muscle within 8 h.

2. *Release of lactic acid from muscle to blood*

Plaice caught at sea by trawl or seine net (30–50 min fishing time) were allowed to rest for various periods in sea-water aquaria. They were then killed and blood samples

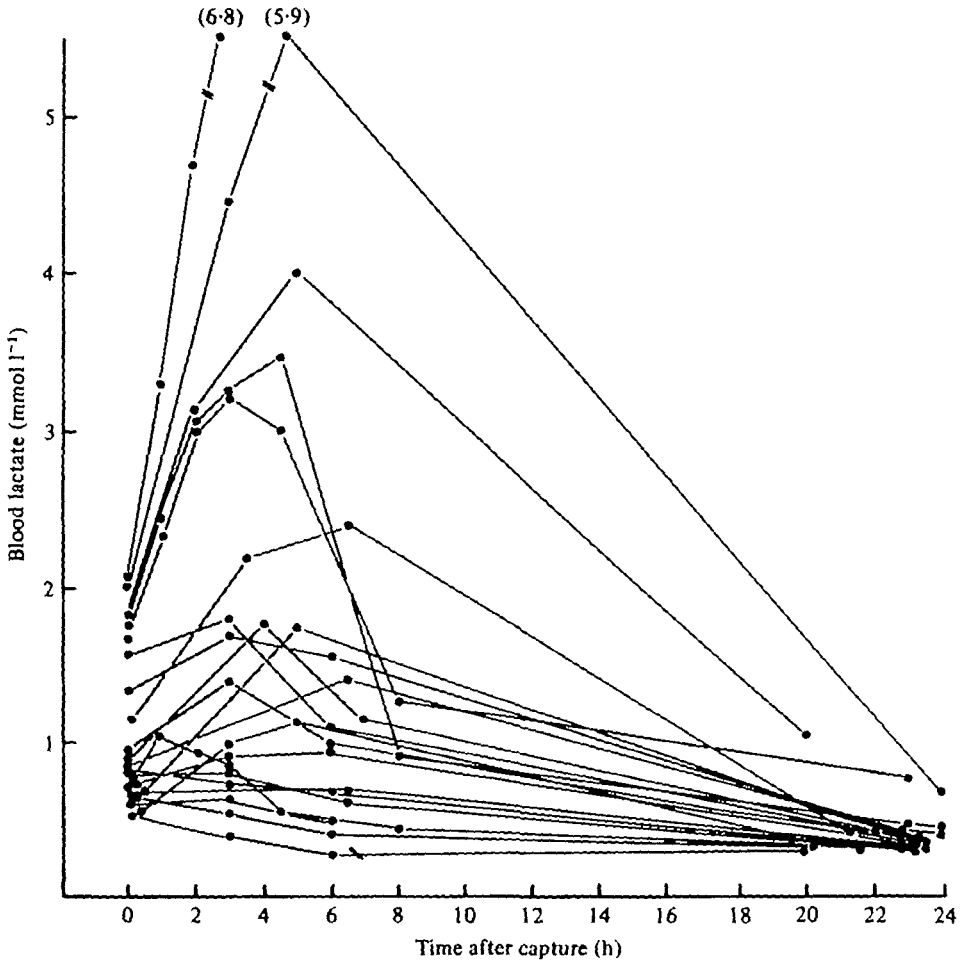


Fig. 4. The changes in lactic acid level of blood collected serially from individual fish after capture and transfer to sea-water aquaria.

taken for lactic acid analysis; the results are shown in Fig. 3. As we have seen, when plaice are landed exhausted on the deck of the fishing boat their muscle lactic acid is at maximum levels (between 30 and 50 mmol/kg muscle). The blood lactate does not reflect the high muscle lactate levels and the values rarely rise above 2 mmol/l (Fig. 3). In a further experiment, analysis of serial blood samples taken from 21 plaice confirmed that a similar large proportion of plaice caught by trawl showed little or no elevation of blood lactate during the recovery period (Fig. 4). When newly caught plaice were rested for 24 h in aquaria and then exercised to complete exhaustion by 10 to 20 min of constant chivvyng, the muscle lactate was found to be high (between 33 and 44 mmol/kg; Fig. 1, left-hand side), and the blood lactate remains characteristically low (between 0 and 0.5 mmol/l) in the majority of these fish (Fig. 3).

When 3 fish were carefully tank-adapted and then exercised without previous disturbance, their blood lactate (Fig. 5) measured in serial samples rose to levels between 2.7 and 4.7 mmol/l. When the same fish were again exercised 24 h later the

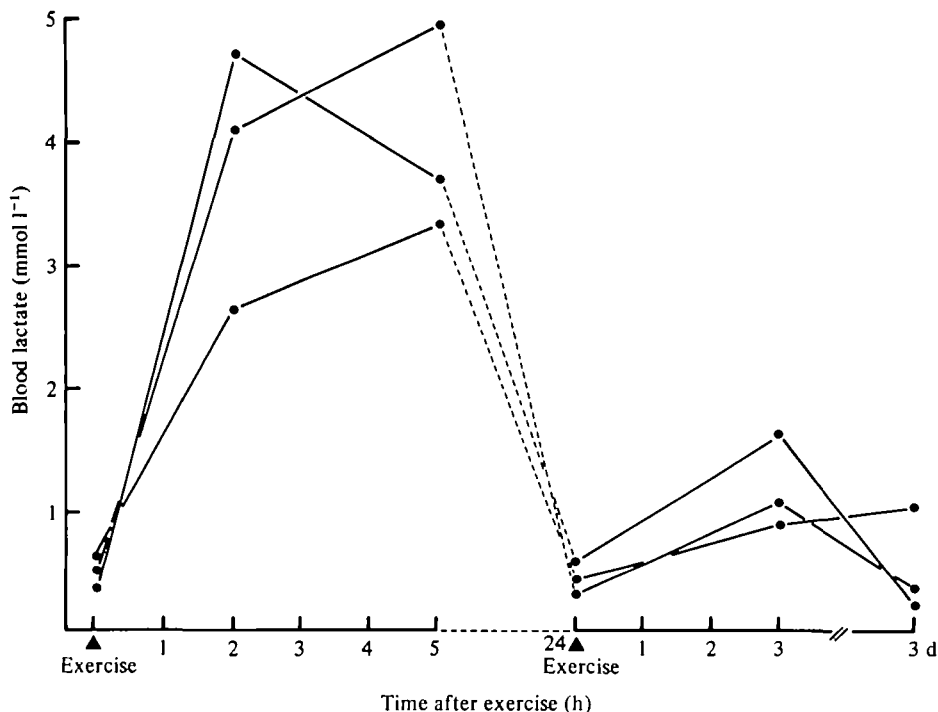


Fig. 5. The effect of exercise repeated on day 1 and day 2 on the blood lactate level of previously undisturbed tank-adapted plaice.

release of lactic acid to the blood was inhibited (Fig. 5). A similar inhibition of lactate release after 1 day and an increased release after 8 days of tank adaptation was obtained in 22 plaice where both muscle and blood levels were measured (see Table 3).

An experiment with eight haddock, *Melanogrammus aeglefinus* (C. S. Wardle, unpublished), showed that, 24 h after a first exercise period, a second exercise period raised lactic acid levels in the muscle tissue to a range 42–84 mmol/kg tissue $2\frac{1}{2}$ h after the exercise, whereas the blood lactic acid levels sampled at the same time remained at low levels, between 0.5 and 2.0 mmol/l blood.

These experiments have shown that soon after capture from the wild the elevation of muscle lactic acid was not necessarily followed by a rise in blood lactate. Blood lactate levels did rise if plaice were exercised when they had been kept in aquaria for more than five days while all forms of stressful stimuli were avoided. These experiments have indicated that any form of handling or disturbance inhibits the release of lactic acid from the exercised muscle to the blood. It is well known that diving birds and mammals can bring about similar control of metabolic exchange by modifying the blood circulation pathways (Scholander, 1940), and some further experiments were designed to examine the circulation and its relation to lactic acid release.

3. Muscle circulation during release and non-release

In an experiment to investigate the effect of tank-adaptation time and exercise on the state of the circulation, plaice 1–10 (Table 1) were tank-adapted for one day and

Table 1. *The effect of tank adaptation time and exercise on muscle circulation and lactate release and non-release*

	1-day-adapted				8-day-adapted			
	Fish no.	Blood lactate (mmol/l)	Muscle blood volume (ml/kg)	Muscle lymph volume* (ml/kg)	Fish no.	Blood lactate (mmol/l)	Muscle blood volume (ml/kg)	Muscle lymph volume* (ml/kg)
Unexercised	1	0.18	1.0	5.8	11	0.86	1.2	5.6
	2	0.47	0.8	3.0	12	0.32	1.0	4.5
	3	0.16	1.3	5.8	13	0.22	1.2	5.5
	4	0.50	1.2	4.4	14	0.50	0.7	5.3
	5	0.22	1.0	2.9	15	0.28	0.8	5.6
Exercised	6	3.50	1.3	3.3	16	0.10	0.9	3.4
	7	0.50	1.4	4.1	17	0.14	1.2	4.3
	8	1.81	1.6	4.7	18	0.087	1.4	6.3
	9	0.50	1.2	4.1	19	7.50	1.7	6.8
	10	0.66	1.5	4.2	20	0.078	4.0	5.3

* Volume labelled in 3 h by ¹²⁵IHSA.

then injected with a mixture of ⁵¹Cr-labelled erythrocytes and ¹²⁵IHSA. Fish 1–5 were unexercised and fish 6–10 were exercised for 15 min. Plaice 11–20 were tank-adapted for 8 days and injected in the same way. Fish 11–15 were unexercised and fish 16–20 were exercised. This treatment guaranteed muscle lactate levels between 20 and 50 mmol/kg in the exercised fish. Three hours after the appropriate treatments all the fish were killed, blood was collected from each fish for lactic acid analysis and the volume of blood and lymph in the muscle was estimated. After exercise fish 6 and 8 (Table 1) showed release of lactic acid to the blood, fish 7, 9 and 10 did not. Fish 19 of the eight-day-adapted group showed considerable lactic acid release. However, the blood and lymph volume measurements which indicate the amount of lymph and blood circulating through the muscle showed no difference between those individual fish that released and those that did not release lactic acid from the muscle to the blood. In this preliminary experiment no consistent evidence was obtained that an opening or closing of the circulation was associated either with the release or non-release of lactate in the exhausted fish or with the state of rest or exercise.

4. *Pharmacological modification of the blood circulation*

Further experiments were made using well-known drugs to modify the flow of blood and lymph in the plaice muscle. Isoxsuprine hydrochloride is known to stimulate adrenergic β -receptors in vertebrates (Goodman & Gillman, 1965). Plaice carefully tank-adapted and injected with isoxsuprine HCl (1 mg) to the blood stream showed at least double the muscle blood volume of the saline-injected controls 2 h after the injection (Table 2a). When measured 6 h after the injection the effect on the capillaries was still present but had decreased (Table 2b). However it was also found that this β -adrenergic stimulating drug not only increased the tissue blood volume but also decreased the level of lactic acid in the blood of exercised fish if treated more than 2 h after sampling (cf. Table 2a and 2b). In serial samples taken from a further 8 plaice, 4 treated with isoxsuprine HCl (Fig. 6), the lowered release of lactate from the exercised muscle to the blood was confirmed. Following the β -adrenergic stimula-

Table 2. *Effect of Isoxsuprine HCl on muscle blood volume and lactate release in exercised plaice*

(a)

Treatment	No. of fish	30 min after injection: no exercise		120 min after injection, 90 min after exercise			
		Blood lactate (mmol/l)		Blood lactate (mmol/l)		Muscle blood volume (ml/kg)	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Saline	4	0.58	0.16	3.39	1.33	1.1	0.2
Isox HCl β -Stim	4	1.00	0.64	2.41	0.74	3.1	1.5

(The plaice were injected 30 min before the first blood sample. They were then exercised and 90 min later they were killed and blood and muscle samples taken.)

(b)

Treatment	No. of fish	2½ h after injection and exercise		6 h after injection and exercise			
		Blood lactate (mmol/l)		Blood lactate (mmol/l)		Muscle blood volume (ml/kg)	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Saline	3	3.29	0.61	5.06	1.51	0.6	0.3
Isox HCl β -Stim	3	0.77	0.07	0.468	0.03	1.0	0.2

(The plaice were exercised and injected: 2½ h later blood samples were collected: after 6 h the fish were killed and blood and muscle samples taken.)

tion there was an immediate increase in the volume of blood in the muscle, but the inhibition of lactate release from the lactate-loaded muscle took 2 h or more to develop.

5. Pharmacological increase of muscle lymph flow

Twelve 1-day-adapted and ten 8-day-adapted plaice were injected with ^{131}I HSA in order to measure lymph flow. Half of each group were injected with saline (0.2 M) and the rest were injected with the α -adrenergic blocking agent phenoxybenzamine hydrochloride (1 mg) to increase lymph flow (Kutner, Schwartz & Adams, 1967; Goodman & Gillman, 1965). All the fish were then exercised for 15 min and rested for 3 h before they were killed and muscle and blood samples taken.

A greater lymph flow value was associated with the injection of α -adrenergic block in most of the 1-day- and 8-day-adapted fish (Table 3). The 1-day-adapted fish showed no release of lactic acid to the blood stream. The 8-day-adapted fish showed high levels of lactic acid in the blood. The highest blood lactate levels were seen in the 8-day-adapted fish treated with α -adrenergic block (Table 3).

6. β -adrenergic block and lactate release

It appeared likely that the lactate non-release mechanism in muscle was activated by β -adrenergic stimulation which at the same time also increased the flow of blood through the muscle tissue.

In order to test further the role of β -adrenergic stimulation in inhibiting lactic acid

Table 3. *The effect of tank adaptation time and modification of lymph circulation on release of lactate to blood from exercised plaice muscle*

Treatment	Fish no.	One-day-adapted		
		Muscle lactate (mmol/kg)	Blood lactate (mmol/l)	Muscle* lymph (ml/kg)
Saline controls	1	45.47	0.46	9.0
	2	31.62	0.22	5.1
	3	35.73	0.3	6.7
	4	39.54	0.83	8.1
	5	28.02	0.2	9.9
	6	51.97	0.58	8.7
Phenoxybenzamine HCl	7	73.9	1.42	11.9
	8	53.29	0.8	13.6
	9	52.77	0.91	10.4
	10	36.98	0.33	5.8
α -adrenergic block	11	54.24	0.47	12.4
	12	47.68	0.67	14.8
		Eight-day-adapted		
Saline controls	13	43.67	2.29	4.8
	14	47.67	1.94	4.9
	15	41.40	4.87	4.1
	16	47.63	3.89	6.9
	17	32.62	2.01	6.3
Phenoxybenzamine HCl	18	45.6	2.81	6.0
	19	31.27	4.72	5.1
	20	39.48	5.14	9.0
α -adrenergic block	21	37.52	6.81	3.6
	22	43.62	6.94	9.3

* Volume labelled in 3 h by ^{125}I HSA.

release from muscle to blood, experiments were made using the drug propranolol which is used in higher vertebrates and man to block the stimulation of β -adrenergic receptors (Goodman & Gillman, 1965). When 11 plaice, caught 24 h previously and expected to be non-releasers, were exercised and injected with propranolol (1 mg) it was found that the blood lactate of 6 of these plaice rose during 5 h to high levels, well above any previously measured, and these fish died. The remaining fish maintained low blood levels (Fig. 7). When propranolol was injected to plaice adapted for longer periods in the aquaria there was no effect on the release of lactate to the blood. It appeared that plaice were sensitive to this drug at the time when β -adrenergic stimulation was probably naturally high due to the stresses of capture.

Injection of lactic acid to the blood stream was shown to cause death in plaice. Serial samples of blood taken from 4 plaice following a single injection of lactic acid to their blood stream (40 mg lactic acid DL in 0.2 ml) were analysed and are shown in Fig. 7 (open circles). The two fish which did not regulate their blood levels died.

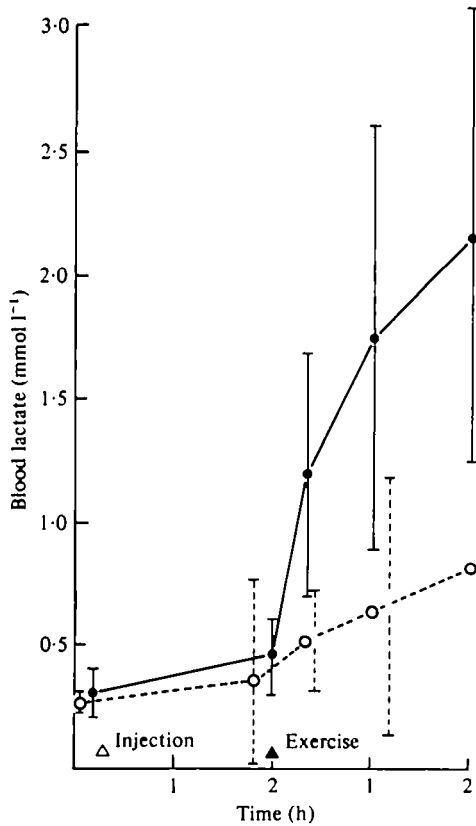


Fig. 6. The modifying effect of β -adrenergic stimulation on blood lactate levels in exercised plaice. The tank-adapted plaice were injected with saline (continuous line) or isoxsuprine hydrochloride (dashed line). Two h after the first blood sample, a second blood sample was taken and the fish exercised. Four fish in each group, vertical bars indicate standard deviation.

DISCUSSION

1. Muscle lactic acid

The present experiments and others, with both marine and freshwater teleosts, have shown that during vigorous swimming movements there is a fall in the glycogen levels in the anaerobic lateral muscle tissue and an associated rise in the lactic acid levels (Black *et al.* 1962; Wittenburg & Diaciuc, 1965; Burt & Stroud, 1966; Stevens & Black, 1966; Beamish, 1968; Dando, 1969; Burt, 1969). Immediately following exercise in plaice the yield of lactic acid is similar to the amount of glycogen lost. A similar stoichiometry was found in trout (Stevens & Black, 1966).

Maximum fuel levels expressed as lactate or glucose were found between 3 and 5 g/kg of muscle tissue in plaice when sampled from the wild but can be lower in tank-adapted non-feeding fish. This finding agrees with those of Love (1958) and Fraser, Punjamapirou & Dyer (1961).

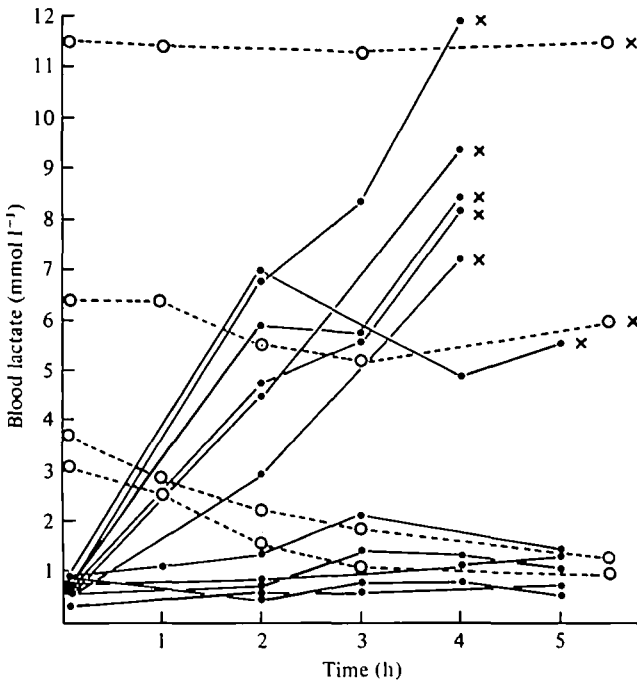


Fig. 7. Eleven plaice (continuous lines) caught 24 h earlier were exercised and injected with β -adrenergic blocking agent. Six fish developed high subsequent blood lactate levels and died. The remaining five fish survived with low levels. For comparison, four fish were injected with lactic acid (40 mg DL in 0.2 ml, dashed lines), two fish regulated their blood lactate to low levels and survived and two fish kept high levels and died. x, All these fish died.

2. Blood lactic acid

There have been numerous measurements of levels of lactic acid in blood following exercise in plaice (Bagge, 1970; Dando, 1969) and other teleosts (Beamish, 1968; Stevens & Black, 1966; Driedzic & Hochachka, 1976; Wood, McMahon & McDonald, 1977). A general observation is that the release of lactic acid to the blood is small in relation to the amount of lactic acid in the muscle and the blood peak level occurs 2–3 h after the exercise. In explanation, suggestions have been made that there is a sluggish or limited circulation, a closing of the circulation, or shunting of blood.

It has been shown here that high levels of lactic acid in the muscle tissue are not necessarily reflected as high blood levels. Non-release of lactic acid from muscle to blood was also observed in plaice by Bagge (1970) who suggested that release was inhibited by low temperature. However, if Bagge's experimental methods are examined carefully and related to the present finding, it seems likely that the low levels of lactic acid in the blood of exhausted plaice are due to the handling and the psychological stresses associated with lowering the aquarium temperature at an appropriate time interval before the blood lactate was measured. Caillouet (1964) exercised carp in various conditions and again the non-release of lactic acid which he attributes to a seasonal cycle appears to correspond to handling the fish 24 h before the particular experiment. Wood *et al.* (1977) measured low peaks (2 mmol/l) of lactate in blood of

starry flounder. The experiment took place 3 days after a chronic cannula had been fitted.

The non-release of lactic acid from muscle to blood can be guaranteed in the plaice by treatment with the β -adrenergic stimulator, which was also found to increase the volume of blood in the muscle capillaries. It is concluded from the present results that the lactic acid is held in the muscle cells when stimulated by catecholamines and that the slow release observed is not due to inhibition of either the blood or the lymph circulation. The β -block propranolol caused release of lactic acid from the exhausted muscle to the blood stream of plaice. Evidence for a similar catecholamine control in humans is seen, but not noted, in fig. 2 of Harris, Bergstrom & Hultman (1971), where propranolol caused double the rate of loss of lactic acid from the exhausted thigh muscle. Retention of lactic acid in human muscle has been suggested by experiments of Hermansen *et al.* (1975).

3. Death and high blood lactate levels

Death of fish, particularly following capture, has very often been correlated with high levels of lactic acid in the blood (Black, 1958*b*; Parker & Black, 1959; Parker, Black & Larkin, 1959; Beamish, 1964). It seems unreasonable that exercise of the highly developed anaerobic muscle of the teleost should itself cause death and much more likely, in the cases cited, that the animal's homeostasis is upset by aspects of the experiments other than the exercise so that the lactate-retaining mechanisms are weakened. In the present study high blood lactic acid was associated with death in plaice treated with the β -adrenergic block propranolol and in plaice injected with lactic acid. The fish that showed no control of blood lactic acid levels died, and survivors showed an ability to regulate lactic acid at low blood levels. Jonas, Sehdev & Tomlinson (1962) injected lactic acid into *Salmo gairdneri* and demonstrated that all survivors maintained high blood pH and death occurred only if the blood pH was not regulated. The variation observed between individuals may be caused by a variation in the available space into which the injected lactic acid can diffuse. Batty & Wardle (1968) have demonstrated considerable uptake of lactate by muscle tissue in recovering exhausted fish, and the state of release or non-release of the muscle cell may influence the ability of the fish to cope with an introduced lactate load.

If the non-release of lactic acid from the muscle cell is an active process of the living cell the lowering of the metabolism of that cell due to any cause may allow lactic acid to be released by the muscle cells and flood into the circulation. Jonas *et al.* (1962) indicated that lactic acid might lower the pH of the already weak animals resulting in destruction of other regulatory systems. Wood *et al.* (1977) found that the major blood pH change from pH 7.9 in resting fish to pH 7.5 immediately after exhaustion of starry flounders (*Platichthys stellatus*) was due to blood P_{CO_2} , rising from 2 to 8 mmHg. After 4 h of recovery the contribution of lactate to pH increased to nearly 50% as the P_{CO_2} decreased. The pH was already back to pH 7.7, 2-3 h after exhaustion at the time when the blood lactate reached peak levels (2 mmol/l).

4. *The significance of retaining lactate in the muscle cell*

It is worth remembering that the teleost is able to carry the relatively large volume of powerful anaerobic muscle with its limited endurance for use on rare occasions because the muscle tissue acts as neutrally buoyant packing to form the streamlined shape of the body. The superior burst swimming speed achieved for very short periods allows survival of the animal by increasing the hunting and escape performance while the general overall metabolic level remains low and is unimpaired. Wardle (1971) measured the relative volumes of blood and muscle, and the amount of blood and lymph circulating through the lateral muscle of plaice. Plaice lateral muscles make up 35% of the body weight, and after exhaustion the whole of this volume will contain lactic acid at levels between 33 and 44 mmol/kg. The total blood volume of a plaice is only 3% of the body weight, and if all the muscle lactate is transferred to this small blood volume levels higher than 500 mmol/l could result. Plaice with a maximum aerobic metabolic rate never greater than 300 mg O₂/kg/h does not have the same capacity as a warm-blooded mammal to make sensible use of large amounts of lactate as substrate for other reactions. It is reasonable that there should be alternative mechanisms to prevent this overload.

5. *Muscle lactate retention and stress*

The present experiments with plaice have shown that the slow release or the non-release of lactic acid from the exhausted anaerobic muscle may be under the control of the catecholamine hormones involved in the fright and fight reaction. The results have also indicated that the active uptake and retention of lactic acid by the anaerobic muscle is a metabolic function of the muscle cells and that this mechanism is easily weakened by damaging metabolic processes.

These results suggest that following an appropriate stimulus catecholamine released to the blood will stimulate the lactate-loaded muscle cells to retain and take up lactic acid against a gradient, and at the same time the blood flow through the white muscle will be increased allowing greater dissolved gas and metabolite exchange. This is a satisfactory mechanism for a fish in the wild that has exhausted its anaerobic escape apparatus and must seek and maintain shelter (Black, 1958*a*; Black *et al.* 1961) while its anaerobic energy store is rebuilt. The elevation of blood glucose by β -adrenergic stimulation (Wardle, 1971) may form part of a restorative process in which the glucose passes from the liver glycogen stores to the blood, to the muscle cells, supplying both raw material and energy for the rebuilding of muscle glycogen. However, Batty & Wardle (1978) demonstrate that insufficient glucose is mobilized to restore completely the muscle glycogen level.

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