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THE EFFECTS OF HYPOXIA AND REOXYGENATION ON FORCE DEVELOPMENT IN MYOCARDIA OF CARP AND RAINBOW TROUT: PROTECTIVE EFFECTS OF CO_2/HCO_3^-.

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SUMMARY

Isometrically mounted and electrically paced myocardial ventricular strips of carp have a much higher capacity to develop force during severe hypoxia and to redevelop force after it than those of rainbow trout.

When the concentrations of CO_2 and HCO_3^- in the solutions surrounding the strips were increased together, such that pH remained constant, the force developed during hypoxia increased. The concentration of CO_2 was raised from 0–4%; that of HCO_3^- from 0–25 mM. The effect was much more pronounced in the carp strips than in the trout strips.

With the carp strips, the force recovery upon reoxygenation was unaffected by the variations in CO_2 and HCO_3^-. The trout strips, however, recovered better when CO_2 and HCO_3^- had been raised during either hypoxia or reoxygenation.

INTRODUCTION

The concentrations of H^+, CO_2 and HCO_3^- are interrelated variables in the cellular environment. Of these concentrations only that of H^+ proved to be of functional importance in having a negative inotropic effect in a study of an isolated heart preparation supplied with oxygen (Vaughan Williams & Whyte, 1967). During hypoxia the concentrations of CO_2 and HCO_3^- may be important in determining myocardial contractility. In this situation the cellular production of lactic acid and thus of H^+ ions increases, and recently, CO_2 and HCO_3^- have been suggested to be constituents of mechanisms which increase the outflow of H^+ ions from the cell (Boron & de Weer, 1976; Mainwood & Worsley-Brown, 1975; Strome, Clancy & Gonzalez, 1976). An intracellular acidosis is associated with a depressed anaerobic energy liberation (Ui, 1966) and probably also with a decreased functional capacity of the contractile elements themselves (Katz & Hecht, 1969; Williamson *et al.* 1976).

The present study concerns the influence of extracellular CO_2 and HCO_3^- on the contractility of isolated heart strips during severe hypoxia. The concentrations of CO_2 and HCO_3^- were increased together, such that pH remained constant. Myocardia of carp and rainbow trout were selected for two reasons. Firstly, the myo-

cardium of carp, in contrast to that of trout, was found in preliminary experiments have a high capacity to develop force during hypoxia. This capacity is correlated with the capacity for anaerobic energy liberation as shown in a study of hearts from marine fishes (Gesser & Poupa, 1974) and from turtles (Reeves, 1963). Secondly, the carp, a fish lives in ponds and swamps, probably experiences hypoxia together with an increase in the environmental total concentrations of CO_2 (Carter, 1934), and so may be expected to show metabolic adaptations to these conditions.

MATERIAL AND METHODS

Adult carp (*Cyprinus carpio*) and rainbow trout (*Salmo gairdneri*) of both sexes were used. They were kept in aquaria with filtered recirculating water at 15–20 °C. The Ringer solution used for the heart muscle preparations consisted of: 152 mM-Na^+, 2·93 mM-Ca^{2+}, 5·14 mM-K^+, 0·94 mM-Mg^{2+}, 2·00 mM-PO_4^{3-}, 0·94 mM-SO_4^{2-}, 5 mM Tris-HCl, 5 mM glucose, and Cl^- and HCO_3^- in varying concentrations. The sum of the Cl^- and HCO_3^- concentrations was always 161 mM, and the maximum HCO_3^- concentration was 25 mM. The Ringer solution was equilibrated with different gas mixtures delivered from a gas mixing pump (Wösthoff type 1 M 301/a–F). When CO_2 and HCO_3^- were used, the ratio of their concentrations was kept constant at 1% CO_2: 6·25 mM-HCO_3^- or at 4% CO_2: 25 mM-HCO_3^-. The Tris-HCl buffer stabilized the pH between 7·48–7·52 at 20 °C. The pH was continuously measured in the muscle bath with a glass electrode connected to a pH meter (Radiometer type PHM 71) allowing readings of 1/100 of a pH unit.

Each experimental fish was decapitated. The heart was rapidly excised and placed in Ringer solution containing 25 mM-HCO_3^- equilibrated with 96% O_2 and 4% CO_2. A myocardial strip preparation, taken from a similar site on each ventricle, was mounted for isometric force recording in a bath containing 100 ml Ringer solution and equilibrated with oxygen and CO_2. The temperature of the bath was thermostatically controlled (Lauda type K2) at 20 °C. One end of the strip was firmly fixed with a plastic clip. The other end was tied with surgical silk to a force displacement transducer (Grass FT 03), connected to a recorder (Brush Mark 220). The strip was electrically paced at 12 beats/min with square pulses of 5 ms duration by a stimulator (Grass SD9) through two parallel platinum electrodes, one on each side of the strip. The voltage was about 20% above the minimum necessary to give a maximal mechanical response. Spontaneously beating strips were discarded.

After 15 min at a low applied stretch the strip was stretched to the apex of the curve of contractile force to length. After an additional period of 15–30 min of stabilization, hypoxia was induced by replacing O_2 with N_2 in the gas mixture. During the stabilization period 4% and 25 mM, or 1% and 6·25 mM, of CO_2 and HCO_3^-, respectively, was present in the Ringer solution. In some of the experiments these concentrations were left unchanged. In others, about 30 s before onset of hypoxia CO_2 was removed from the gas mixture while simultaneously the Ringer solution was exchanged for one without HCO_3^-. After 30 min of hypoxia the prehypoxic conditions of O_2, CO_2 and HCO_3^- were restored. All solutions were pre-equilibrated with the appropriate gas mixtures.

The force developed during hypoxia and reoxygenation was calculated as the

percentage of that developed after stabilization just before the onset of hypoxia. Some strips developed force irregularly with successive contractions during hypoxia. For these strips the force value given represents a mean of the value obtained upon four stimulations. Before concluding an experiment the length of the strip at the apex of the length–contractile force curve was measured. The strip was then blotted and weighed. The cross sectional area was calculated assuming cylindrical form and a specific gravity of 1·00. Absolute force values in mN/mm^2 could therefore be calculated. Data are presented as mean ± S.E. Differences were analyzed with Student's *t* test. The level of significance was set at 5%.

RESULTS

After stabilization in well oxygenated conditions the forces developed by the strip preparations were 6·87 ± 0·78 mN/mm^2 (n = 21) for carp and 7·16 ± 0·69 mN/mm^2 (n = 25) for trout. The total concentration of CO_2 and HCO_3^- in the Ringer during the stabilization period had no effect on the force developed. The cross sectional area of the strips was 1·02 ± 0·43 mm^2 for carp and 1·10 ± 0·40 mm^2 for trout.

Fig. 1 shows that the force development of the myocardium of both carp and trout decreased during 30 min hypoxia. In the carp, this decrease was strongly affected by the muscle bath concentrations of CO_2 and HCO_3^-. In the presence of 4% CO_2 and 25 mM-HCO_3^- the force levelled off at the end of the hypoxic period at about 80% of the pre-hypoxic value. When 1% CO_2 and 6·25 mM-HCO_3^- were used the force was still declining at the end of hypoxia, at which time it had reached a value of 34% of the original. In the absence of CO_2 and HCO_3^- the force decrease was even more accentuated, and the final value reached was 10% of the pre-hypoxic force. In the trout the decrease was less affected by CO_2 and HCO_3^-. In their absence, or in the presence of 1% CO_2 and 6·25 mM-HCO_3^-, there was a decrease to about 10% after 30 min hypoxia. After hypoxia in the presence of 4% CO_2 and 25 mM-HCO_3^-, there was a decrease to about 30% of the prehypoxic value. For both species there was a tendency for the myocardium to develop force irregularity with successive contractions when CO_2 and HCO_3^- were absent.

Fig. 2 shows recovery of force development during re-oxygenation for 30 min in the presence of 1% CO_2 and 6·25 mM-HCO_3^-; the 30 min of hypoxia having either been in the absence of CO_2 and HCO_3^- or in the presence of 1% CO_2 and 6·25 mM-HCO_3^-. The trout myocardium recovered more rapidly and to a greater extent after CO_2 and HCO_3^- had been present in the bath than after the bath had been CO_2 and HCO_3^- free. The carp myocardium recovered without showing a dependence on the CO_2 and HCO_3^- concentration of the bath during hypoxia.

Fig. 3 illustrates the recovery of force after 30 min of hypoxia during which CO_2 and HCO_3^- had been absent from the Ringer solution. The trout myocardium recovered more force if the reoxygenation occurred in the presence of 4% CO_2 and 25 mM-HCO_3^- than in the presence of 1% CO_2 and 6·25 mM-HCO_3^-. The force recovery of the carp heart was similar in the two conditions. It can be seen from Figs. 2 and 3 that the carp myocardium recovers force more rapidly and to a greater extent than the trout myocardium.

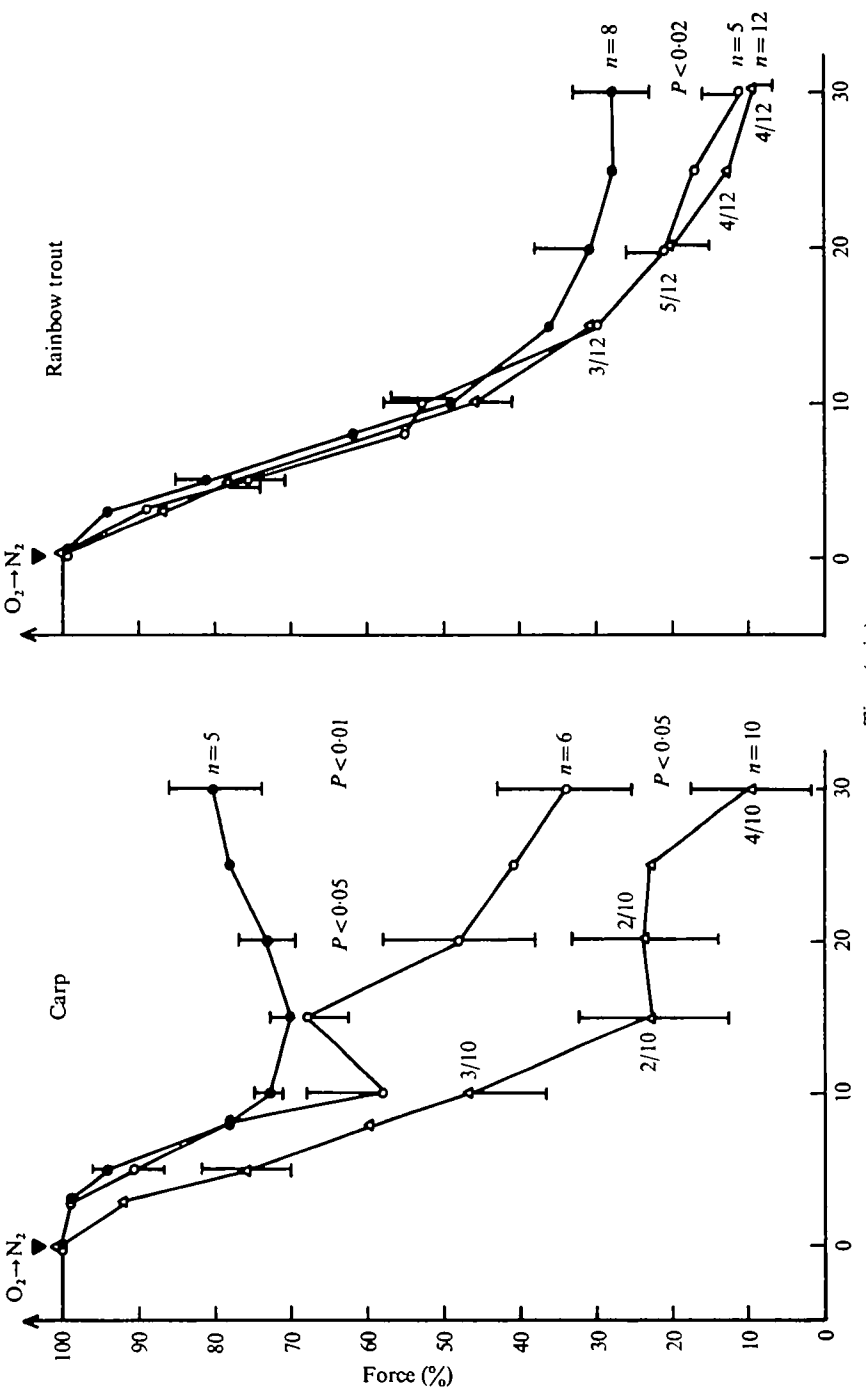

Fig. 1. Force development (mean ± S.E.) during hypoxia in different concentrations of carbon dioxide and bicarbonate: ●—●, 4 % CO_2, 25 mM-HCO_3^-; ○—○ 1 % CO_2, 6·25 mM-HCO_3^-; △—△, 0 % CO_2, 0 mM-HCO_3^-. The fractions indicate the proportion of preparations in which force was developed irregularly with successive contractions.

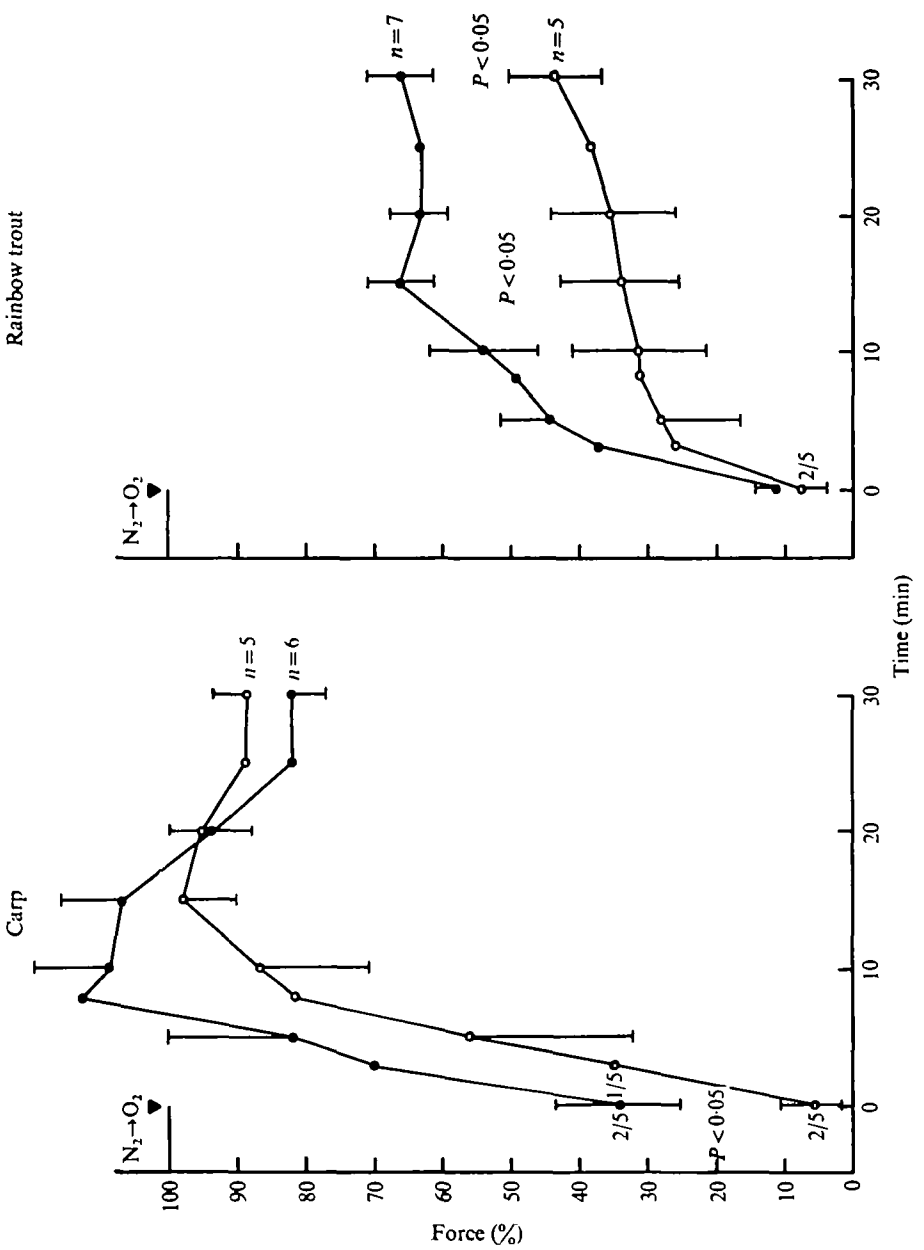

Fig. 2. Force recovery (mean ± S.E.) in the presence of 1 % CO_2 and 6·25 mM-HCO_3^-, after 30 min of hypoxia in either 1 % CO_2, 6·25 mM-HCO_3^- (●—●) or in absence of these substances (○—○). Fractions indicate as for Fig. 1.

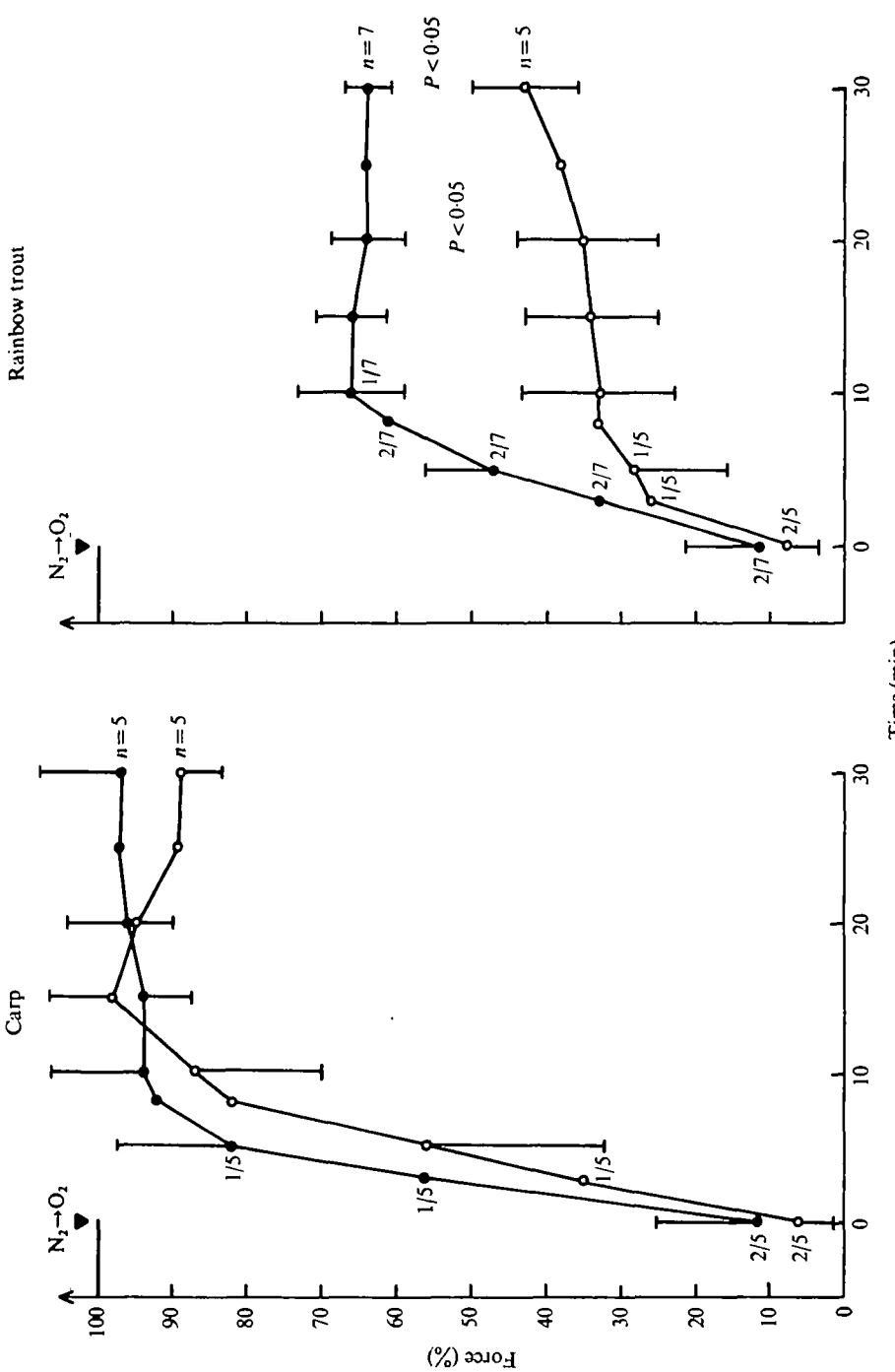

Fig. 3. Force recovery (mean ± S.E.) in either 4% CO_2, 25 mM-HCO_3^- (●—●) or 1% CO_2, 6·25 mM-HCO_3^- (○—○) after 30 min of hypoxia in the absence of CO_2 and HCO_3^-. Fractions indicate as for Fig. 1.

In some experiments the period of recovery was extended to 90 min. The trout heart, however, never reached the initial force level of 100%. It was probably irreversibly damaged during hypoxia.

DISCUSSION

Myocardial force development during hypoxia was kept at a much higher level in the carp than in the trout. There was also a more rapid and complete recovery in the carp. This presumably reflects the adaptation of the carp to waters in which oxygen availability is frequently very low, and the lack of such adaptation in the trout. The cause of the overshoot in contractile force seen initially during recovery for carp heart (Fig. 2) is not known, but is being studied in this laboratory.

During hypoxia the carp myocardium would presumably profit more than the trout myocardium from factors counteracting an intracellular acidosis, because a high hypoxic force development is thought to be associated with a high rate of formation of lactic acid and hence of hydrogen ions (Gesser & Poupa, 1974; Reeves, 1963). An excesses of these ions inside the myocardial cell has been claimed to depress the glycolytic capacity (Ui, 1966), and the uptake of Ca^{2+} by the cell (Williamson *et al.* 1976). Furthermore, an intracellular acidosis is alleged to inhibit in a competitive way the binding of Ca^{2+} to troponin (Katz & Hecht, 1969).

Counteraction of intracellular acidosis may be expected to be produced by an increase in extracellular HCO_3^- concentration. Such an increase is likely to raise the outflow of undissociated lactic acid from muscle cells (Mainwood & Worsley-Brown, 1975), and, furthermore, CO_2 and HCO_3^- seem to take part in a mechanism equivalent in effect to an active transport of H^+ ions out from the cell (Boron & de Weer, 1976; Strome *et al.* 1976). It may be, therefore, that the effect of a raised CO_2 and HCO_3^- concentration upon the carp myocardium during hypoxia, to reduce the depression of force development, may be explained by a counteraction of intracellular acidosis.

During the introductory stabilization period, CO_2 and HCO_3^- levels in the bath were either 4% and 25 mM or 1% and 6·25 mM. This difference did not affect the force values obtained before the onset of hypoxia. Neither did it affect the force developed during hypoxia, when CO_2 and HCO_3^- had been removed. Thus, the contractility during hypoxia seems to be a function of the absolute levels of CO_2 and HCO_3^- and not of the extent of change in these parameters.

The cause of the greater effect of hypoxia upon the trout than the carp myocardium, and the means by which CO_2 and HCO_3^- aid recovery in the trout is a subject inviting further studies.

In interpreting the results it must be remembered that a complete absence of CO_2 and HCO_3^- in the extracellular fluid is alien to an *in vivo* situation. 1% CO_2 and 6·25 mM HCO_3^- are within the range found in fish arterial blood while 4% and 25 mM align to values typical for terrestrial vertebrates (Howell, 1970). The values in the immediate vicinity of the fish myocardium are hard to estimate. The heart muscle cells in fish are to a great extent nourished by blood returning from central systemic veins, likely to be higher in CO_2, HCO_3^- and H^+-ions than the arterial blood leaving the gills.

It is of interest that for the carp a decrease in ambient oxygen availability might be concomitant with a sharp increase in CO_2 and HCO_3^- in the surrounding water (Carter, 1934). Such an increase will cause elevated concentrations of the same substances in the blood (Janssen & Randall, 1975) and hence may counteract the effects of hypoxia. Also, in habitual divers, oxygen lack is combined with a raise in CO_2 and HCO_3^- in the blood due to the interrupted gas exchange with the environment (Scholander, 1940).

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REFERENCES

BORON, W. F. & DE WEER, P. (1976). Active proton transport stimulated by CO_2/HCO_3^-, blocked by cyanide. *Nature, Lond.* **259**, 240–41.

CARTER, G. S. (1934). The fresh waters of the rain-forest of British Guiana. *J. Linn. Soc. London (Zool.)* **39**, 147–93.

GESSER, H. & POUPA, O. (1974). Relations between heart muscle enzyme pattern and directly measured tolerance to anoxia. *Comp. Biochem. Physiol.* **48**, 97–104.

HOWELL, B. J. (1970). Acid-base balance in transition from water breathing to air breathing. *Fedn Proc.* **29**, 1130–4.

JANSSEN, R. G. & RANDALL, D. J. (1975). The effects of changes in pH and pCO_2 in blood and water on breathing in rainbow trout, *Salmo gairdneri*. *Respir. Physiol.* **25**, 235–45.

KATZ, A. M. & HECHT, H. H. (1969). Editorial: The early 'pump' failure of the ischemic heart. *Am. J. Med.* **47**, 497–502.

MAINWOOD, G. W. & WORSLEY-BROWN, P. (1975). The effects of extra-cellular pH and buffer concentration on the efflux of lactate from frog sartorius muscle. *J. Physiol.* **250**, 1–22.

REEVES, R. B. (1963). Energy cost of work in aerobic and anaerobic turtle heart muscle. *Am. J. Physiol.* **205**, 17–29.

SCHOLANDER, P. F. (1940). Experimental investigations on the respiratory function in diving mammals and birds. *Hvalrdd. Skr.* **22**, 5–121.

STROME, D. R., CLANCY, R. L. & GONZALEZ, N. (1976). Myocardial CO_2 buffering: Role of transmembrane transport of H^+ or HCO_3^- ions. *Am. J. Physiol.* **230**, 1037–41.

UI, M. (1966). A role of phosphofructokinase in pH-dependent regulation of glycolysis. *Biochim. Biophys. Acta* **124**, 310–22.

VAUGHAN WILLIAMS, E. M. & WHYTE, J. M. (1967). Chemosensitivity of cardiac muscle. *J. Physiol., Lond.* **189**, 119–37.

WILLIAMSON, J. R., SAFER, B., RICH, T., SCHAFFER, S. & KOBAYASHI, K. (1976). Effects of acidosis on myocardial contractility and metabolism. *Acta Med. Scand. Suppl.* **587**, 95–112.