

Energy Metabolism and Contractility in Ectothermic Vertebrate Hearts: Hypoxia, Acidosis, and Low Temperature

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I. INTRODUCTION

A. Objectives

Survival of vertebrate animals is dependent on uninterrupted heart function in all but extreme cases (e.g., freeze-tolerant frogs, Ref. 330). Cardiac muscle is therefore of particular interest in its ability to maintain pump performance under different physiological conditions. Hearts of ectothermic vertebrates face far greater ranges in extracellular gas content, acid-base status, and temperature than hearts of endothermic animals. This is a function of fluctuating environments, behavior, and organization of cardiovascular systems. In this review the ecophysiological approach is utilized to gain insight into strategies of cardiac adaptation and to identify mechanisms that allow maintenance of appropriate cardiac function.

Heart performance is dependent on the transient changes in cytosolic Ca^{2+} interacting with contractile proteins and energy derived from the hydrolysis of ATP to support contractility. These features are influenced

by numerous external factors that impact on heart performance. The specific objective of the present review is to examine the processes of cardiac energy metabolism and Ca^{2+} -regulated contractility in ectothermic vertebrates under conditions of oxygen limitation, acidosis, and hypothermia. Comparisons are made to mammalian hearts where appropriate.

B. Animals of Study and Their Ecophysiology

Most studies in comparative/environmental cardiology have been with species that are either cultured or may be readily captured and maintained under laboratory conditions. Body sizes typically range from ~150 to 1,500 g. Most of the experimental species are aquatic with body temperatures from ~0 to 25°C under field conditions. Experimental animals include representatives from all the major classes of ectothermic vertebrates. Common and generic names of the most frequently investigated species are listed below and are referred to only by common name hereafter. Within the class Agnatha, the most frequently studied species is

Atlantic hagfish (*Myxine glutinosa*). Of the Elasmobranchii (i.e., cartilaginous fish), the usual species are a variety of dogfish and skates. Within the class Pisces (i.e., bony fish), investigation has been restricted largely to the teleost subclass, the most studied organisms being cod (*Gadus morhua*), eel (*Anguilla anguilla*), goldfish (*Carassius auratus*), trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*), and sea raven (*Hemitripterus americanus*). The common grass frog (*Rana pipiens*), European frog (*Rana temporaria*), and marine toad (*Bufo marinus*), all anurians, are the most frequently utilized representatives from the class Amphibia. Within the class Reptilia, most studies relevant to the topic of this paper have been conducted on painted turtles (hereafter referred to as turtle), which in the original literature are classified to genus as either *Pseudemys* or *Chrysemys*. Unless otherwise stated, this paper deals exclusively with this group of organisms, encompassing a number of subspecies.

By virtue of their aquatic habitat, these animals face wide extremes in oxygen availability due to such factors as ice covering, plant respiration, burrowing into the substrate, and diving by obligate air breathers. Gill-breathing animals also face challenges of high environmental CO₂ and subsequent low pH usually associated with nighttime plant metabolism. Breath holding or intense activity can also lead to large decreases in plasma pH, which may remain depressed for many hours following a return to air-breathing or resting conditions. Tolerance to hypoxia and acidosis is extremely species specific. For most fish, amphibians, and reptiles, body temperature equilibrates with environmental temperature. Therefore, these animals may be subjected to wide fluctuations in body temperature on either a daily or seasonal basis.

C. Heart Anatomy and Vascularization

In fish, a single ventricle receives venous blood that is subsequently pumped to the gills for oxygen uptake. Hagfish lack coronary arteries, so the heart, which is relatively thin walled, is nourished only by the venous blood that it pumps. In teleosts, the ventricle consists of a spongy inner layer that in some species is surrounded by varying degrees of an outer compact layer. The compact layer is enhanced in animals with high sustained swimming speeds and may account for as much as 35% (e.g., trout and eel) to 70% (e.g., tuna) of the total ventricle (299). In teleosts, the spongy layer is supplied primarily by the venous blood from the intertrabecular spaces of the ventricular lumen, whereas the compact layer is nourished by discrete coronary arteries providing oxygenated blood (345). In elasmobranchs, the compact layer represents ~25% of the ventricle in numerous species (299) and subepicardial arteries anastomose with the intertrabecular spaces of the spongy layer to provide this area with oxygenated blood as well (345). Coronary perfusion becomes relatively more important in situations where venous PO₂ decreases and/or myocardial oxygen demand increases (73–75).

The amphibian heart is similar to the teleost heart in that the single ventricle is predominantly of the spongy trabeculate type, lacking a coronary supply. Chelonian (turtles) and squamate (snakes and lizards) reptiles have a single large ventricle that consists of three distinct compartments. The turtle heart has a thin compact layer constituting ~10% of the ventricle. The balance of the ventricle appears to be of the spongy type, and coronary arteries nourish only the thin outer layer (44).

In overview, the ventricle of ectothermic vertebrates consists for the most part of a spongy trabeculated inner section surrounded by a layer of compact musculature. Only in the reptiles is the ventricle divided into functionally discrete chambers. The compact layer is most extensive in highly active teleosts and is vascularized by coronary arteries. The spongy layer potentially faces marked decreases in oxygen level, low pH, and high CO₂ levels. This arrangement is in contrast to mammals and birds in which the heart is well vascularized and receives arterial blood of high PO₂ and nearly constant pH.

D. In Situ Heart Performance

It is valuable to have an appreciation of in situ performance, since this is the physiological context in which the contractile system and energy metabolism must operate. A full review of ectothermic cardiac performance is well beyond the scope of this paper. This discussion focuses on those species or closely related species used most frequently for investigation at the cellular level. Emphasis is placed on aspects most relevant to contractility and energy metabolism, notably heart rate, pressure development, cardiac output, and power output. Typical resting levels of cardiac performance under normoxic conditions are presented in this section. When not given in the original literature, power outputs were calculated from the product of mean aortic pressure, cardiac output, and gravitational acceleration.

1. Fish

At 10°C, Atlantic hagfish have a heart rate of ~22 beats/min, a mean ventral aortic blood pressure of 1 kPa, and a cardiac output of 9 ml · min⁻¹ · g heart⁻¹. This results in a power development of ~0.15 mW/g (13, 121). In *Eptatretus cirrhatus*, ventral aortic pressure is slightly higher (121, 122), and isolated perfused hearts can achieve power outputs of 0.4 mW/g (119).

For teleosts and elasmobranchs, typical heart rates are 10–60 beats/min, cardiac outputs are 6–40 ml · min⁻¹ · kg body wt⁻¹, and mean ventral aortic pressures range between 3 and 6 kPa (11, 108, 110, 122, 215, 216, 337). Power outputs by representative species are as follows: short finned eel (*Anguilla australis*), 0.9 mW/g heart at 18°C (176); sea raven, 1.7 mW/g at 11°C (12); cod, 1.8 mW/g at 10°C (14); trout, 1.5 mW/g at 10°C

(206) and 2.5 mW/g at 15°C (378); *Pagothenia bernacchii*, 0.6 mW/g at 0°C (11).

2. Amphibians

Measurements of cardiac performance in amphibians are restricted almost exclusively to the anurians. Assessments of total cardiac output necessary for the calculation of power output are few due in part to anatomic restrictions in the implantation of flow probes and cutaneous respiration that disallows calculations of cardiac output via the Fick principle. In air-breathing toads at 22°C, heart rate is 26 beats/min with a mean arterial pressure of 3 kPa and a systemic arch blood flow of 36 ml·min⁻¹·kg body wt⁻¹ (376). Our estimate of total cardiac output is ~30 ml·min⁻¹·g heart⁻¹, yielding a power output of ~1.5 mW/g. Similar levels of cardiovascular parameters have been recorded in the bullfrog (*Rana catesbeiana*) (332, 338). In grass frogs at 20°C, heart rates are much higher at ~60 beats/min (305). This is probably due to body size, since grass frogs are only ~10% the size of bullfrogs or marine toads (25 vs. 250 g). Arterial pressure, cardiac output per gram heart, and hence calculated power output per gram is similar to the larger species of anurians (90, 305).

3. Reptiles

Air-breathing turtles at 20–25°C have heart rates of 30–40 beats/min, cardiac outputs of ~50 ml·min⁻¹·g heart⁻¹, and an aortic pressure development of ~3 kPa. This corresponds to a power output of ~2.5 mW/g heart. A limited amount is known about cardiovascular performance in terrestrial lizards. At 35°C both Savannah monitors (*Varanus exanthematicus*) and green iguana (*Iguana iguana*) have resting heart rates of 40 beats/min with cardiac outputs of ~40 (*V. exanthematicus*) and 70 ml·min⁻¹·g heart⁻¹ (*I. iguana*). During maximum sustainable exercise, heart rate increases to ~100 beats/min and cardiac output increases to 115 ml·min⁻¹·g heart⁻¹ (141). Systemic blood pressure, even in inactive *V. exanthematicus*, approaches 9 kPa (50). Cardiac power output during activity may exceed 17 mW/g in this species.

4. Comparison of *in situ* performance with endotherms

Resting heart rate of a typical 500-g ectothermic vertebrate, at 15–20°C and under nonlimiting oxygen conditions, is 20–60 beats/min. Mean arterial blood pressure is usually 3–5 kPa, with cardiac output on the order of 10–50 ml·min⁻¹·kg body wt⁻¹. This converts to power outputs of 1–4 mW/g heart. Hagfish hearts are an exception to the above in the development of lower pressures and power outputs.

Heart performance is enhanced during exercise via increases in heart rate, blood pressure, and cardiac out-

put. Reliable estimates of maximal power output are typically ~7 mW/g heart. This does not preclude higher cardiac power outputs in particularly fast swimmers such as tuna (47), which may have very high cardiac outputs or in varanid lizards that develop higher pressures than other ectotherms (50). These animals, however, are not usual candidates for studies at lower levels of physiological organization.

During periods of oxygen limitation and/or low temperature, heart rate and cardiac output usually decrease (details are presented in sects. II and IV). For instance, in turtles, heart power output when diving at 3°C is only a very small fraction (~1/300th) of that which occurs when air breathing at 20°C.

Typical cardiovascular parameters for mammals may be estimated from body size relationships (182). A 500-g resting mammal operating at 37°C has a heart rate of 280 beats/min, a left ventricular output of 125 ml·min⁻¹·g⁻¹, and an aortic pressure development of 13 kPa. Calculated power output is 25 mW/g left ventricle and would be higher in an active state. Cardiac power output per gram in birds estimated in a similar fashion is somewhat lower because of the much greater heart size (150). Regardless, heart performance by comparably sized birds and mammals at usual physiological temperatures is manyfold higher than ectothermic vertebrates. Scaling has an immense effect on cardiovascular parameters such that a 100-kg mammal has a heart rate of 75 beats/min, a left ventricular output of 23 ml·min⁻¹·g⁻¹, and a calculated power output of 5 mW/g left ventricle. These values are approached by a typical 500-g fish, frog, or turtle.

E. Cellular Features

The basic features of cellular energy liberation, as well as the contractile mechanisms (i.e., the actin-myosin interaction and its regulation by Ca²⁺) appear to be similar in vertebrate heart and skeletal muscle and need not be reviewed here. Relevant species differences are addressed in this section. Myocardial cells are, however, unique with regards to excitation-contraction (E-C) coupling (i.e., the regulation of contractility through the cytoplasmic Ca²⁺ activity) (e.g., Ref. 296). The myocardial E-C coupling is unclear in many aspects and shows species differences of probable ecophysiological significance. Therefore, a brief account of this aspect is provided with emphasis on species variations and with respect to the subsequent discussion of ecophysiological aspects. Several reviews deal with the cardiac E-C coupling, which almost exclusively has been studied in mammalian and amphibian species (e.g., Refs. 28, 60, 61). Except for amphibians, the information is sparse for ectothermic vertebrates as made apparent in a review of cardiac E-C coupling in fish (340).

1. Surface-to-volume ratio

The cellular surface-to-volume ratio is of importance to the exchange of substances such as Ca²⁺ be-

tween the intra- and extracellular spaces. This ratio increases as cell diameter decreases. The diameter of myocardial cells tends to be smaller in ectothermic than endothermic vertebrates. For ventricular cells of the rat, a diameter of 20–25 μm has been recorded (207). This value is larger than those reported for the diving turtle (5 μm ; Ref. 114), frog (2–3 μm ; Ref. 317), and fish, where it ranges from 2 μm (e.g., *Squalus acanthias*) to 12.5 μm (*Ostheoglossum bicirrhosum*) (298). With a diameter of $\sim 4.5 \mu\text{m}$ (298), cardiac cells of plaice (*Pleuronectes platessa*) have a surface-to-volume ratio of about six times that of rat cardiac cells. Such differences may be diminished, though, since transverse tubules apparently occur only in myocardial cells of endothermic vertebrates (see sect. IE4). The importance of transverse tubules to the surface-to-volume ratio is, however, unclear (61).

2. Active calcium extrusion

The cytoplasmic Ca^{2+} activity during diastole is $\sim 10^{-7} \text{ M}$, and during systole it is $\sim 10^{-4}$ times below its equilibrium value. With an extracellular Ca^{2+} concentration of $\sim 10^{-3} \text{ M}$ and a sarcolemma leaky to Ca^{2+} , Ca^{2+} has to be actively extruded from the cell. Two sarcolemmal mechanisms for this have been identified: the $\text{Na}^+-\text{Ca}^{2+}$ exchange and the outwardly directed Ca^{2+} -transport adenosinetriphosphatase (ATPase).

The sarcolemmal $\text{Na}^+-\text{Ca}^{2+}$ exchange has been reviewed several times (37, 60, 61, 96). It has a coupling ratio of three Na^+ to one Ca^{2+} (i.e., it is electrogenic). The cytoplasmic Ca^{2+} activity (Ca_i^{2+}) approached is given as a function of the extracellular Ca^{2+} activity (Ca_o^{2+}), intra- (Na_i^+) and extracellular activities of Na^+ (Na_o^+), and membrane potential (E) by the equation

$$\text{Ca}_i^{2+} = \text{Ca}_o^{2+} (\text{Na}_i^+/\text{Na}_o^+)^3 e^{(EF/RT)}$$

where R , F , and T have their usual meanings. Hence, the electrochemical gradient for Na^+ due to the Na^+-K^+ -ATPase results in an inward downhill gradient for Ca^{2+} . Apart from its effect as substrate, intracellular Ca^{2+} exerts a controlling influence, such that the rate of the $\text{Na}^+-\text{Ca}^{2+}$ exchange increases with the intracellular Ca^{2+} activity (37, 161, 162).

The $\text{Na}^+-\text{Ca}^{2+}$ exchange operates at a rate comparable to that of twitch relaxation (e.g., Ref. 45) and appears to be the major mechanism for relaxation in frog (62) and trout myocardium (341).

The reversal potential for a $3\text{Na}^+-1\text{Ca}^{2+}$ exchange is surpassed during the action potential so that Ca^{2+} might be brought into the cell (9, 61, 221, 306). According to other studies, though, the $\text{Na}^+-\text{Ca}^{2+}$ exchange adds little to the amount of activator Ca^{2+} under normal conditions (29, 69) but may be of significance under conditions when intracellular Na^+ is augmented and the sarcolemmal Na^+ gradient decreases (29). As to this discussion, recently reviewed evidence (56) suggests that the Na^+

concentration near the inner face of the sarcolemmal is higher than in the cytoplasmic bulk solution.

The myocardial $\text{Na}^+-\text{Ca}^{2+}$ exchanger from dog, frog, and trout displays a high degree of similarity. However, there are large differences in temperature dependence, probably due to differences in primary protein structure (31, 342).

In mammalian myocardia, the $\text{Na}^+-\text{Ca}^{2+}$ exchange is thought to represent a high-capacity low-affinity system, whereas the reverse is true for Ca^{2+} -ATPase, which presses cytoplasmic Ca^{2+} down to the final value (55).

3. Excitation and calcium transient

Excitation (i.e., the action potential) is followed by a short-lasting increase in the myoplasmic Ca^{2+} activity, known as the Ca^{2+} transient. The peak value attained by this transient increase depends not only on the size of the amplitude of the Ca^{2+} transient but also on the starting level (i.e., the diastolic Ca^{2+} activity) (27). The amount of total Ca^{2+} that is redistributed during the Ca^{2+} transient depends on the intracellular buffering of Ca^{2+} and may vary among species. Furthermore, the relative contribution of extracellular relative to intracellular derived Ca^{2+} to the Ca^{2+} transient is unclear and appears to vary largely among species.

The cardiac action potential depends on Ca^{2+} entering the cell mainly through the L-channels in the sarcolemma. In addition to the L-channels, mammalian atrial and ventricular cells also possess another Ca^{2+} channel, the T-channel (26). This is true also for frog atrial cells (26, 39), whereas, in contrast, frog ventricular cells have only the L type (10). The physiological consequences of this and the situation in other ectotherms is not known.

According to patch-clamp experiments, the Ca^{2+} current density in frog atrial cells is in the lower end of the range observed for mammalian cardiac cells (242). Similar studies on other ectotherms are lacking, but the binding of Ca^{2+} -channel antagonists suggests that ventricular myocytes of trout have a high-channel density relative to mammalian myocytes (341). The durations of the myocardial action potential in ectotherms as exemplified by frog (262), trout, flounder (178), crucian carp (*Carassius carassius*) (361), and lizard (*Lacerta sicula campestris*) (59) are in the upper range or above the values recorded in mammals. A prolonged action potential most likely indicates a prolonged time with open Ca^{2+} channels and should also favor a possible Ca^{2+} influx via the $\text{Na}^+-\text{Ca}^{2+}$ exchange at positive membrane potentials. The surface-to-volume ratio together with these data suggest that Ca^{2+} influx during the action potential contributes relatively more to the Ca^{2+} transient in ectothermic than in endothermic vertebrates.

4. Sarcoplasmic reticulum

The amount of Ca^{2+} entering during the action potential in the mammalian myocardium is generally be-

lieved to be too small alone to elicit the concomitant contraction. The main bulk of activator Ca^{2+} is released from the sarcoplasmic reticulum (SR) in a way that is strictly related to the action potential-dependent influx of Ca^{2+} across the sarcolemma (e.g., Refs. 66, 103, 104, 252). It is unclear, however, how this model for myocardial activation applies to ectothermic species.

Sarcoplasmic reticulum or SR-like structures appear to be present in the myocardia of all vertebrates examined, including hagfish (170), and are more abundant in atrium than in ventricle (40). Regarding basic ultrastructural properties, myocardial SR in ectotherms, as studied predominantly in frog and toad, resembles that in mammals (e.g., Refs. 243, 273). One distinction, however, is that myocardial cells of ectotherms, as shown for species of fish, amphibians, and reptiles, have no transverse tubules (40, 118, 298), although shallow sarcolemmal invaginations, caveolae, have been described for frog heart (243).

Quantitatively, the myocardial SR development varies largely among species. Relative to mammals, it is sparse in frogs (e.g., Refs. 243, 273) and in many fish, whereas other fish, such as trout and mackerel (*Scomber scombrus*), display a much more abundant SR complement (298). Large differences are encountered among reptilian species, too (118). Measurements of the SR-dependent Ca^{2+} uptake in myocardial homogenates provided some unexpected results. Thus the maximal uptake rate was found to be about as high in trout as in rat myocardium (95, 240), which has an extremely well-developed SR (e.g., Ref. 322). The Ca^{2+} uptake rate was low in frog myocardium in accordance with the ultrastructural development (95, 360).

The release mechanism also shows important differences. With skinned myocardial cells, Ca^{2+} -induced releases of SR bound Ca^{2+} occurred in all the mammalian and avian species examined. In lizard (*Anolis carolinensis*), this response requires the presence of sarcolemmal fragments. This is also true for frog atrial tissue, except that higher Ca^{2+} activities are required to induce Ca^{2+} release. In contrast to this, the SR of frog ventricular cells does not show any Ca^{2+} -induced release (102), but consistent with a function in Ca^{2+} metabolism, it contains calsequestrin like mammalian myocardial SR (243).

The functional significance of myocardial SR in ectothermic species is unclear and may differ between atrium and ventricle. As to the most extensively examined ectotherm, however, the frog, arguments have been advanced for (e.g., Refs. 7, 60, 61) and against (e.g., Ref. 306, 371) the concept that myocardial SR contributes directly to the Ca^{2+} transient. Tentatively, it might do so under specific conditions only, for example, during transitions from one heart rate to another (261) and by mediating α -adrenergic control of twitch force (262).

The significance of the SR in the E-C coupling is unclear in myocardia in which the SR appears to be richly developed from an ultrastructural and biochemical aspect. The relationship between force and frequency is usually interpreted in terms of the Ca^{2+} trans-

sient (e.g., Refs. 123, 374) and has been used together with inhibitors of the SR function such as ryanodine to assess the importance of SR (e.g., Refs. 270, 322). In isolated atrial tissue from skipjack tuna (*Katsuwonus pelamis*), the SR appears to function like that of mammalian myocardial tissue in that twitch force was reduced by ryanodine both at high and low contraction rates (201). Also, trout ventricular muscle showed a depression of force development by ryanodine, but under certain conditions only. Hence, as a finding of possible ecophysiological importance, a strong inhibition occurs at 25°C, which gradually disappears as temperature is lowered to 5°C (183). It is, however, puzzling that the importance of the SR as assessed with ryanodine only appears at frequencies far below the physiological range (99, 183, 184). This point is further illustrated by a study of the twitch force elicited by premature single stimulations interrupting a stimulation rate within the physiological range. The increase in this twitch force with the preceding interval (i.e., mechanical restitution) was not affected by ryanodine in trout ventricular muscle as it is in mammals (249).

Alternatively or additionally to contributing directly to the Ca^{2+} transient, SR, not showing Ca^{2+} -induced Ca^{2+} release, may serve as a long-term buffer of cellular Ca^{2+} (102). As an interesting possibility, SR may modulate the cytoplasmic Ca^{2+} activity and thereby the Ca^{2+} current through the sarcolemmal Ca^{2+} channels (243). Rapid increases in the cytoplasmic Ca^{2+} activity have been shown to stimulate the Ca^{2+} current in frog myocardium (152). Here, the control of the $\text{Na}^+-\text{Ca}^{2+}$ exchange by intracellular Ca^{2+} (161, 162) should also be recalled.

5. Mitochondria

Mitochondria exchange Ca^{2+} with the cytoplasm. Calcium is taken up through an electrophoretic uniporter and is released in exchange for Na^+ and H^+ . The "time averaged" intramitochondrial and cytoplasmic activities of Ca^{2+} covary (241). The exchange mechanisms seem to act too slowly to allow any significant influence of mitochondria on the Ca^{2+} transient at least in the mammalian myocardium (e.g., Refs. 24, 55). The relationship between the covarying activities of cytoplasmic and intramitochondrial Ca^{2+} should tentatively be affected by the cytoplasmic activities of Na^+ and H^+ . Such possible effects are discussed with regard to the influence of acid base state on contractility in section III C.

6. Sarcolemma

The hypothesis has been advanced that the inner side of the sarcolemma functions as a mobilizable store for Ca^{2+} . It has been proposed to be directly implicated in the E-C coupling (231) or intimately coupled to the $\text{Na}^+-\text{Ca}^{2+}$ exchange (219). These proposals are problematic, though, seemingly requiring a diffusion barrier be-

tween the cytoplasm around the sarcolemma and the bulk of cytoplasm (219). However, such a diffusion barrier seems to exist (56). Furthermore, activation seems to involve Ca^{2+} bound at the outside of the sarcolemma (218, 220). The externally bound Ca^{2+} may reach the inside by the Ca^{2+} channels and by the $\text{Na}^+-\text{Ca}^{2+}$ exchange (210).

Existing data do not allow comparisons between endo- and ectothermic species or within the ectothermic group regarding the sarcolemma as a store of activator Ca^{2+} . Interesting differences may exist though. For instance, a study of myocardium of the *M. glutinosa* showed that force decreased only very slowly after removal of extracellular Ca^{2+} (282).

7. Extracellular calcium

Extracellular Ca^{2+} varies with conditions in species having a bony skeleton and more so in ectothermic than in endothermic species (295). Turtles seem to be extreme in this respect, since total plasma Ca^{2+} may increase from 3 to >100 mM during prolonged diving (187). The effect of this on turtle cardiac function is unknown. In other species, total plasma Ca^{2+} is at most doubled upon severe exercise and acidosis (295). These changes should have modest but positive effects on cardiac function under different "stress" conditions (294). In this respect, it should be noted that the ectothermic myocardium relative to the mammalian one tends to display larger changes in contractility upon changes of extracellular Ca^{2+} (263).

8. Conclusions

Ectothermic relative to endothermic vertebrates have myocardial cells with a high surface-to-volume ratio and long action potentials. These features are likely to enhance the importance of the sarcolemmal Ca^{2+} transport in the E-C coupling. Cardiac SR is well developed in several ectothermic species such as trout, but its function remains to be clarified. Other structures implicated in the cellular Ca^{2+} balance such as mitochondria and the inner surface of the sarcolemma also have to be considered, for example, with respect to acidotic challenges, as does the cellular Na^+ balance and its effects on the sarcolemmal $\text{Na}^+-\text{Ca}^{2+}$ exchange.

II. IMPACT OF OXYGEN-LIMITING CONDITIONS

A. In Situ Performance

1. Fish

Atlantic hagfish may burrow into soft mud remaining without exchange of water for at least 1 h (331) and may also feed inside the body cavity of dead animals

(155). Both situations are associated with extended periods of oxygen limitation. In the laboratory, Atlantic hagfish can survive anoxic water at 5°C for at least 20 h (153). Heart rate, ventral aortic blood pressure, and cardiac output are maintained at normoxic levels in hypoxic water ($\text{PO}_2 \approx 2$ kPa; 15–35 min) under conditions where oxygen content of blood perfusing the heart must be extremely low (13).

Teleosts live in water with variable oxygen content and have a wide range of swimming capabilities. Both factors impact on energy demand and oxygen delivery to the heart. Swimming results in increases in heart rate (≈ 20 –60%), ventral aortic pressure (≈ 20 –60%), and cardiac output (≈ 50 –200%) such that cardiac power output increased in trout, cod, and sea raven by factors of 4.7, 1.8, and 2.0, respectively (12, 14, 206). In trout, ventral aortic PO_2 falls from 4.43 kPa at rest to 2.13 kPa during maximum activity (206). Thus blood with a lower PO_2 nourishes the spongy layer at a time when cardiac energy demand is elevated. Intense exercise may be associated with substantial decreases in plasma pH (e.g., Refs. 248, 380) and increases in Ca^{2+} (295), K^+ (180), and catecholamines (52, 380), all of which have bearing on heart performance.

Anoxic tolerance is highly species specific. Salmonids have limited ability to cope with hypoxia (81), whereas eel and goldfish can sustain anoxia for a few hours at elevated temperatures (354, 362). At 4°C, the survival period is extended to about a week for goldfish (362). Hypoxic exposure is often associated with a rapid reflex bradycardia as demonstrated in a variety of species (107, 125, 129, 237, 287), including cod (127). Heart rates are typically decreased to $\sim 50\%$ of control level at the limit of animal viability. In contrast, hypoxic bradycardia is weak or absent in sea raven (300), winter flounder (*Pseudopleuronectes americanus*) (58), and some other species (e.g., Refs. 11, 30, 125). Under hypoxia, blood pressure increases in trout (181, 379), sea raven (300), and cod (127) but decreases in other species (58, 107, 125). In trout and cod, cardiac output is maintained under hypoxia due to an increase in stroke volume (126, 379), but in lingcod (*Ophiodon elongatus*) it decreases (107). Variability in response is probably due to species differences, experimental approaches, and depth of hypoxia. But, it appears that some species attempt to maintain cardiac performance before succumbing to oxygen limitation, whereas in others performance immediately declines under environmental hypoxia and power development may be reduced to $\sim 50\%$ of normoxic levels. It is unlikely that the heart of any teleost enters a prolonged hypometabolic state as occurs in turtles. Finally, it should be noted that hypoxia tends to increase levels of plasma catecholamines in some species (e.g., Ref. 339).

2. Amphibians

Cutaneous respiration is important for gas exchange, especially in water, for all amphibians and can

elevate the level of oxygenation in the systemic venous return to the heart (192). During submergence in the grass frog, there is a marked bradycardia and decreases in blood pressure and total cardiac output (305) such that power output is decreased by ~75% following 30 min underwater at elevated temperatures.

3. Reptiles

Turtles have been the subject of extensive investigation due to their ability to withstand prolonged periods of anoxia. At natural winter temperatures of 3°C, freshwater turtles survive months without oxygen. At temperatures of 20–25°C, survival time is hours to days (352). During forced submergence there is a gradual reduction in heart rate that may reach values as low as 4–8 beats/min in some individuals (172, 277, 373). In extreme cases, cardiac output is reduced to 5% of the predive level, and pressure development is ~2 kPa. As such, even at high temperatures, there may be a 30-fold decrease in the mechanical power output of the heart during extensive dives. Shutdown of cardiac performance is under neural control as atropine administration blocks the bradycardia (373). Diving at low temperature results in heart rates of ~0.5 beats/min and mean arterial pressures of ~0.5 kPa (172).

Diving has a profound impact on acid-base balance in that pH may drop by as much as 1 unit during prolonged apnea (see sect. III).

B. Importance of Myoglobin

One strategy for coping with decreased extracellular oxygen levels is to extend the lower limit at which oxygen extraction from the extracellular space is possible. In some species this is achieved by elevated levels of the intracellular protein myoglobin. It is well recognized that myoglobin facilitates diffusion of oxygen in mammalian skeletal muscle (377). A similar role is clearly demonstrated in hearts of fish which may face low PO_2 levels due to either environmental hypoxia or intense exercise.

Myoglobin content in fish hearts is extremely variable among species. Antarctic icefish, which are devoid of hemoglobin, have undetectable (115, 310) or possibly very low levels of cardiac myoglobin (82). Very low levels of myoglobin are not restricted to icefish, since some species of nonpolar teleosts, such as lumpfish (*Cyclopterus lumpus*), monkfish (*Lophius piscatorius*), and ocean pout (*Macrozoarces americanus*) have either zero or marginally detectable myoglobin contents (91, 310). Isolated perfused hearts from these species appear white after blood washout (85). At the other extreme, some species have hearts that are extremely rich in myoglobin such as tuna (*Thunnus thynnus*; ~580 nmol/g wet wt), mackerel (*S. scombrus*; 332 nmol/g wet wt), and carp (*Cyprinus carpio*; 488 nmol/g wet wt) (139, 310). Comprehensive listings of myoglobin levels in hearts of

various species are provided elsewhere (86, 139). Fish that exhibit high levels of sustainable swimming invariably have high levels of cardiac myoglobin. Elevated levels are also found in species that are tolerant of environmental hypoxia such as carp and eel. Low levels of myoglobin are only found in species that are relatively inactive.

The oxygen binding characteristics of fish myoglobin are well suited for function at physiological temperatures. At 20°C, the half-saturation values (P_{50}) for myoglobin from buffalo sculpin (*Enophryns bison*) and coho salmon (*Oncorhynchus kisutch*) heart are significantly higher (i.e., bind with a lower affinity) than myoglobin from rat heart or sperm whale. Affinity increases with a decrease in analysis temperature. If fish myoglobins bound oxygen with the same affinity as mammalian myoglobin, unloading would be restricted at physiological temperatures (259).

Unlike the situation in skeletal muscle, the presence or absence of myoglobin does not correlate with a predilection toward either enhanced aerobic or anaerobic energy metabolism. In three independent studies, comparisons between myoglobin-rich and myoglobin-poor hearts from ectotypically similar species showed no difference in maximal activities of key enzymes from carbohydrate metabolism, fatty acid metabolism, the citric acid cycle, or the electron transport system (91, 115, 310). In a study encompassing a wide spectrum of species with different behavioral characteristics, there was no correlation between heart myoglobin content and any of eight key enzymes of energy metabolism (310). Regardless of the myoglobin content, fish hearts have an aerobic metabolic profile similar to red-type skeletal muscle and quite distinct from anaerobic white muscle (91, 115, 116).

Indirect evidence for myoglobin function under hypoxia was first obtained from performance studies of isolated hearts. Hearts from ocean pout (~5 nmol myoglobin/g wet wt) and sea raven (~75 nmol myoglobin/g wet wt) were treated with iodoacetic acid (IAA) to block glycolysis and subjected to hypoxia (5 kPa; 0.07 mM O_2). Ocean pout hearts failed more rapidly than sea raven hearts. Conversion of myoglobin to a form incapable of binding oxygen resulted in more rapid failure of sea raven but not ocean pout hearts (92). These studies were subsequently extended to include oxygen consumption measurements. A stepwise change in input PO_2 from 21 kPa (0.3 mM O_2) to 5 kPa resulted in a decrease in oxygen consumption by ocean pout but not sea raven hearts. Oxidation of myoglobin with hydroxylamine led to a decrease in oxygen consumption by sea raven hearts under both normoxic and hypoxic conditions. Hydroxylamine treatment had no effect on ocean pout hearts under normoxia and only a small effect under hypoxia (15). In later experiments, isolated hearts from American eel (*Anguilla rostrata*) (239 nmol myoglobin/g), sea raven, and lumpfish were subjected to stepwise changes in input oxygen content from 21 to 1.3 kPa (20). Hearts were force-filled with perfusate at set flow rates, afterload, and frequency of contraction that resulted in mainte-

nance of stroke volume and stretch on the ventricle. Sea raven and lumpfish hearts were treated with sodium nitrite to block myoglobin function. Sodium nitrite treatment is completely reversible (16) and does not impair respiration of isolated mitochondria even at oxygen levels approaching zero (20). Myoglobin-rich hearts were able to extract a constant amount of oxygen until perfusate P_{O_2} had fallen below 10.6 kPa. At this point oxygen uptake began to decline, but these hearts consumed oxygen until input P_{O_2} was 1.3 kPa. Naturally myoglobin-poor lumpfish hearts and nitrite-treated sea raven hearts were unable to maintain constant levels of oxygen consumption in the face of declining perfusate P_{O_2} and failed at an input P_{O_2} of ≈ 4.6 kPa. Nitrite treatment had no effect on lumpfish hearts. Half-maximal oxygen consumption values were attained by myoglobin-rich hearts at a lower input P_{O_2} than either untreated lumpfish hearts or nitrite-treated sea raven hearts. The impact of myoglobin in the support of oxygen consumption was evident at relatively high extracellular P_{O_2} (5.3–10.6 kPa) in this model system.

An independent study with fish hearts failed to reveal myoglobin-facilitated oxygen consumption. The inactivation of myoglobin with phenylhydroxylamine had no effect on cardiac performance or oxygen consumption by isolated buffalo sculpin (*E. bison*) hearts even under hypoxic conditions (260). Hearts were perfused in a fashion similar to that utilized in the study of sea raven and ocean pout cited above (16), and myoglobin levels are similar in buffalo sculpin and sea raven hearts. The apparent lack of myoglobin function in this study is perplexing. It is possible that for any given species myoglobin plays a critical role in only a narrow band of oxygen supply and energy demand. As such, it may be very difficult to hit on the set of conditions that illustrates the function of this protein.

In summary, the bulk of the evidence supports the concept that the presence of myoglobin allows hearts of some fish to maintain oxygen consumption at lower levels of extracellular P_{O_2} than would otherwise be possible. This is similar to findings with mammalian heart which show that the presence of myoglobin allows better maintenance of relaxation times (43) and defense of high-energy phosphates (336) under hypoxic conditions. The fish studies were the first to show a direct impact of myoglobin on oxygen consumption in intact heart for any species.

C. Metabolic Rates and High-Energy Phosphates

1. Metabolism *in vivo*

I) FISH. Atlantic hagfish subjected to 20 h of anoxia (water $P_{O_2} < 0.3$ kPa) showed a decrease in cardiac glycogen (22 to 0.95 $\mu\text{mol glucose/g}$) in association with an increase in tissue lactate (0.4 to 11 $\mu\text{mol/g}$). Blood glucose doubled from 0.5 to 1 $\mu\text{mol/ml}$. Glycolysis was es-

sential to the maintenance of heart function. Pharmacologically immobilized animals maintained cardiac performance at control levels for up to 3 h under anoxic conditions following treatment with cyanide or azide to impair oxidative phosphorylation; however, glycolytic blockage with IAA severely impaired performance even under normoxic conditions (153).

Cardiac glycogen levels in teleosts typically range between 40 and 150 $\mu\text{mol glucose/g}$ (89, 91, 93, 94, 194, 245); however, a value as low as 5 $\mu\text{mol glucose/g}$ has been reported for European eel (354). During exposure to environmental hypoxia there is usually a decrease in glycogen (89, 93, 94, 245) with a concomitant increase in heart lactate (89, 93, 94, 194, 194, 307). Blood glucose increased under oxygen limitation in bluegill sunfish (*Lepomis macrochirus*) (163), goldfish (307, 362), flounder (*Platichthys flesus*) (194), eel (354), and African lungfish (*Protopterus aethiopicus*) (94), but no increase was noted in trout (93) or cutthroat trout (*Salmo clarki*) (163).

The use of exogenous glucose under anoxic conditions by heart was clearly shown in goldfish, since injection of [¹⁴C]glucose resulted in labeled glucose, glycolytic intermediates, and lactate in the heart. The specific activity of tissue lactate exceeded that of blood lactate (307).

African lungfish (94), eel (354), and flounder (195) maintained ATP, ADP, and AMP levels under conditions of environmental hypoxia which led to either decreases in glycogen and/or increases in lactate concentration. Creatine phosphate (CP) levels were defended in flounder and lungfish heart as well (94, 195). In contrast, trout showed a decrease in CP and ATP levels while ADP and AMP concentrations remained the same under hypoxia (194). With the exception of trout, it appears that high-energy phosphate levels are maintained rather well via anaerobic metabolism within the range of sustainable life.

II) TURTLES. In turtles maintained with free access to air, cardiac glycogen levels are typically 250–400 $\mu\text{mol glucose/g}$ or 4–7% of the heart wet weight (25, 65, 76, 277). Glycogen is mobilized during oxygen limitation, and at elevated temperatures (20–25°C), reserves were reduced to very low levels in ~ 3 h in animals exposed to nitrogen (65, 76). Blood glucose levels gradually increased during this period (65, 76, 277) and approached values as high as 45 mM following 24 h of anoxia (76). Heart lactate levels also increased (65) and reached amounts as high as 85 $\mu\text{mol/g}$ heart following 24 h of submergence in anoxic water (277). Lactate levels continued to increase long after glycogen mobilization ceased (65), indicating the use of exogenous glucose as a metabolic fuel.

At 3°C, there was a depletion of glycogen in association with accumulation of lactate, although the process was very much prolonged in that it took 4 wk for glycogen to approach minimal levels (25). In some individuals, blood glucose levels were elevated and approached 40 mM after 25 wk of anoxia; however, in other individuals blood glucose remained at pre dive concentra-

tions. Blood lactate was ~ 200 mM after such prolonged dives (353).

Similar sequences of events have been reported for tortoise (*Testudo hermanni*) (236) and musk turtle (*Sternotherus odoratus*) (188).

Turtles exposed to N_2 at $22^\circ C$ showed a significant decrease in heart ATP and CP after 3 h. Levels continued to fall until they approached zero after 19 h of anoxia (65). Animals exposed to anoxic water at $18^\circ C$ displayed a decrease in heart ATP, ADP, and AMP levels after 1 h, but after 5 h, ATP increased over the initial content and ADP returned to the initial level (202). The difference between these two studies may relate to N_2 exposure with concomitant alterations in CO_2 and pH balance versus a more natural diving state as described by Wasser et al. (368). At $3^\circ C$ there was a tendency for CP and ATP to be lower following 2 and 4 wk of forced submersion (234).

III) OVERVIEW OF IN VIVO STUDIES. The in vivo studies reveal that glycogen is mobilized and lactate accumulates in heart during periods of oxygen limitation. Glycogen levels are especially elevated in turtles, which can sustain life without oxygen for the longest period of time. The extent of alterations in glycogen and metabolite levels depends on the level and length of time under hypoxia. For the most part, high-energy phosphate levels are held fairly constant under reversible oxygen deprivation challenges; that is, glycolytic potential is adequate to maintain whole tissue ATP at a level sufficient to meet ATP demand. There is evidence that ATP levels may actually increase under some conditions.

2. Metabolism in isolated preparations

Isolated preparations are useful in that they allow simultaneous assessment of performance and the calculation of rates of anaerobic metabolism, since glucose and lactate levels can be quantitated for tissue and the bathing medium.

I) FISH AND AMPHIBIANS. The importance of anaerobic glycolysis to the maintenance of contractility was evident by a much accelerated failure of perfused frog hearts (63) and trout ventricle strips under anoxia, with IAA in the media to block glycolysis, relative to preparations with glycolysis intact (154). Extracellular glucose extends survival time under anoxia for both fish (89) and frog (64) hearts.

Perfused isolated hearts of hagfish (*E. cirrhatus*) challenged with low PO_2 (≈ 2 kPa) sustained low levels of power output (≈ 0.2 mW/g), with 70% of the required energy being derived anaerobically. Short-term peak lactate production rate was $2.5 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ (120). Perfused isolated sea raven, ocean pout (351), and smooth dogfish (*Mustelus canis*) (84) hearts and ventricle strips of trout (264) all entered contractile failure under anoxia in association with an increase in lactate production. In each of these studies the preparations began to fail before a significant decrease in ATP levels (Fig. 1). When trout ventricle strips received an eleva-

tion in external Ca^{2+} , twitch force development increased even under anoxia in association with an increase in the rate of lactate production and no change in ATP levels (264).

Alterations in the CP pool are more difficult to define. There was no decrease in CP content in the case of sea raven, ocean pout, or dogfish; however, levels at the initiation of the perfusion period were low, and it may be that CP had already discharged (84, 351). Creatine phosphate levels decreased in trout ventricle strips under anoxia (264).

The rate of release of lactate by trout ventricle strips, maintained in media of $1.25 \text{ mM } Ca^{2+}$, was linear over the 60-min anoxic challenge. The rate of lactate production taking into consideration both release and accumulation in the tissue was $\approx 0.25 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. With the assumption of linearity of lactate release for the perfusion period, comparable values for sea raven and ocean pout were 0.5 and $0.13 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$, respectively. These values are all well below the maximal potential of glycolysis suggested by in vitro enzyme activity levels (Table 1). It is probable that the isolated preparations were working at submaximal levels for reasons other than a limitation of energy production mechanisms.

II) TURTLES. Turtle heart is often regarded as extreme in tolerance to oxygen lack. A similar tolerance is, however, displayed by myocardia from other reptiles such as the lizard (*Varanus exanthematicus*; Ref. 281) and viper (*Vipera berus*; Ref. 135). Therefore, tolerance to oxygen lack is not restricted to turtles among the reptiles. Our discussion is restricted to turtles, however, since almost all information regarding mechanisms has been obtained with these animals.

Force development by ventricle strips bathed in Ringer solution and subjected to anoxia at $28^\circ C$ decreased by 50% over 1 h. The presence or absence of glucose in the medium did not affect the rate of decay; however, glycolytic blockage with IAA resulted in complete mechanical failure within 5 min (32). Ventricle strips subjected to anoxia plus acidosis at $20^\circ C$ and paced at 12 beats/min developed only $\sim 20\%$ of the initial force after 1 h of treatment. The greater the rate of contraction, the more rapid was the rate of failure. Lactate production was enhanced at elevated contraction frequencies (364). These studies emphasize the dependence of an intact glycolysis for maintenance of contractility and that the lower the rate of energy demand the greater the length of survival. Experiments with ventricle strips are consistent with in vivo performance and metabolic characteristics.

Studies with isolated working perfused preparations are particularly insightful in elucidating the anaerobic potential of turtle hearts (289, 290, 297). Hearts were perfused at $23-25^\circ C$ via the cannulated right atrium, and contents were expelled against an afterload via the left radix artery such that power output was $2-5$ mW/g heart (i.e., a physiological level). The Ringer solution was fortified with plasma from the same animal. A transition from aerobic to anoxic conditions resulted

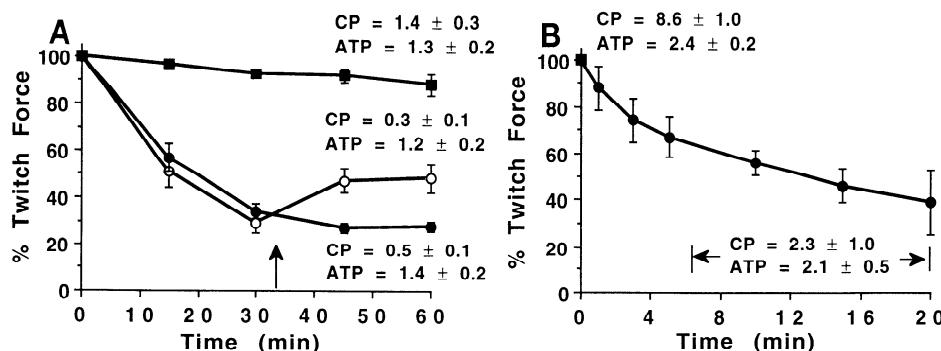


FIG. 1. Performance of trout ventricle strips (*A*) and rabbit papillary muscle (*B*) under anoxia. All preparations were paced at 12 beats/min at maximum length of active force and at a temperature of 20°C. Squares, performance under oxygenated conditions; circles, performance under anoxia. Force of contraction of rabbit muscle had remained stable for 2 h before transition to anoxia. Up arrow in trout study indicates an increase in Ca^{2+} from 1.25 to 5.0 mM. In the case of the trout, creatine phosphate (CP) and ATP concentrations were determined at 60 min. In the case of the rabbit, CP and ATP concentrations were determined at time 0 and between 6 and 20 min. Values are expressed in $\mu\text{mol/g}$ weight wt, assuming 85% wet weight. Rates of lactate production were 0.25 and 0.6 $\mu\text{mol} \cdot \text{g}$ wet wt $\cdot \text{min}^{-1}$ for trout and rabbit heart preparations, respectively. [Redrawn from Nielsen and Gesser (264) and Mast and Elzinga (238).]

in a 25% decrease in rate, but power output was maintained for at least 3–4 h. Lactate production was zero under aerobic conditions but increased under anoxia in direct proportion to power output. Lactate production was sustained during the anoxic period and achieved maximal rates of $8 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. Maximal rate of glycogen utilization was $1 \mu\text{mol glucose} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. Hence maximal rate of utilization of exogenous glucose was $\sim 3 \mu\text{mol glucose} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. Rates of ATP turnover under aerobic conditions (calculated from oxygen consumption) were the same as rates under anoxia (calculated from lactate production), indicating about a 10-fold increase in glycolysis, dependent on the phosphate-to-oxygen ratio and the source of hexose units. At low work loads and/or early in the anoxic challenge, hearts preferentially used endogenous glycogen, but at higher work loads and after glycogen was depleted exogenous glucose was called upon. Maximal power output was higher when glucose was available in the perfusion medium. In an independent study (278), hearts were subjected to retrograde perfusion via the right aortic arch at 22°C. Under anoxia, heart rate gradually decreased by $\sim 25\%$, and lactate was produced at a linear rate of $\approx 2 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ for 1 h.

Perfused isolated working hearts maintained levels of ATP and ADP following 1 h of anoxia. Under these conditions there was a significant increase in P_i (291). In hearts perfused in a retrograde mode, ATP showed a small but significant increase after 15 min of anoxic treatment and remained elevated for up to 60 min of perfusion. Adenosine 5'-diphosphate increased three-fold following 15 min of anaerobic perfusion and remained elevated (278). Wasser et al. (365) extended these findings by tracking intracellular pH (pH_i) and high-energy phosphate intermediates with ^{31}P -nuclear magnetic resonance (NMR). Hearts were perfused at 20°C in the working mode similar to the preparation used by Reeves (289). Upon transition from aerobic to

anaerobic conditions, hearts maintained performance at preanoxic levels for 3 h before beginning to deteriorate over the next hour. The ATP levels were maintained over the 4 h. During the first hour of anoxia, CP decreased by 50%, P_i doubled, and pH_i dropped from 7.4 to 7.2. These parameters remained constant for the next 3 h. The direction of these changes as well as cardiac performance were restored during recovery under oxygenated conditions. Alterations in performance, CP, P_i , and pH_i were in the same direction but markedly accentuated when hearts were perfused with anoxic-acidotic (35 mM lactate, pH 7.0 as opposed to pH 7.8) medium. Again ATP levels remained constant, and original conditions were restored during a recovery period.

III) OVERVIEW OF IN VITRO EXPERIMENTS. Studies with isolated heart preparations corroborate *in vivo* findings in that they emphasize the importance of the activation of anaerobic metabolism under hypoxia with the use of both endogenous glycogen and exogenous glucose as fuels. Heart preparations from teleosts and elasmobranchs invariably fail. Maximal rate of lactate production is only $\sim 0.25 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$, and hearts enter failure before total ATP pools are decreased. Ventricle strip preparations from turtle similarly fail under anoxia even though glycolysis is activated. Perfused turtle hearts can maintain power output provided the medium contains turtle plasma. The critical factors in the plasma are unknown. Hearts can generate lactate at a rate of $8 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$, reflecting about a 10-fold increase in glycolysis. Under these conditions ATP and ADP levels are maintained, but there is an increase in P_i released from the breakdown of CP. Maintenance of performance by isolated turtle hearts is paradoxically opposed to the *in vivo* situation where rate and power output are decreased during diving. These studies, however, illustrate the substantial glycolytic capacity of this tissue which may be utilized only occasionally under natural conditions.

TABLE 1. Metabolic enzyme activities in hearts of ectothermic and endothermic vertebrates

Species	HK	PFK	PK	LDH	CS	Reference No.
Agnathids						
<i>Myxine glutinosa</i>	3.4	5.1	71.9	228	13.8	153, 310
Elasmobranchs						
<i>Potamotrygon magdalena</i>	0.0	0.1		39	14.4	313
<i>Raja erinacea</i>	6.8	10.6	81.8	215	14.6	250, 310
<i>Squalus acanthias</i>	8.7	20.8	133.0	502	42.6	310
Teleosts						
<i>Anguilla rostrata</i>	11.5	21.7		454	23.0	18, 233
<i>Cyprinus carpio</i>	12.8	3.9	83.4	370	9.0	310
<i>Dicentrarchus labrax</i>	16.5	19.1	93.7	416	26.4	310
<i>Esox niger</i>	4.8	1.5		336	15.6	18, 310
<i>Gadus morhua</i>	12.5	19.3	117.0	660	24.3	153, 310
<i>Gadropsar vulgaris</i>	22.0	16.1	136.0	264	17.9	310
<i>Hemitripterus americanus</i>	7.3	3.7	106.0	443	33.6	91, 302, 310
<i>Lophius piscatorius</i>	10.3	7.7	36.5	224	14.1	310
<i>Macrozoarces americanus</i>	7.0	3.3	103.0	365	36.5	91
<i>Makaira nigricans</i>			104.0	708	14.2	334
<i>Micropodus dolomieu</i>	9.9				37.1	303
<i>Morone americanus</i>	7.9	4.8	138.0	504	17.2	303, 310
<i>Morone saxatilis</i>	23.9	19.1	59.7	298	9.6	310
<i>Myoxocephalus octodecimspinosis</i>	14.5	10.4	105.0	891	23.5	70
<i>Onchorhynchus mykiss</i>	17.5	41.9	79.5	331	35.1	5, 48, 112
<i>Perca flavescens</i>	12.4				32.8	303
<i>Protopterus aethiopicus</i>			101.0	594	17.9	94
<i>Salmo salar</i>	27.0	30.8	116.0	430	69.6	101
<i>Salvelinus fontinalis</i>	13.6	31.1	75.4	297	33.4	90, 101, 233
<i>Scomber scombrus</i>	7.6	19.9	69.7	312	38.1	310
<i>Tautoga onitis</i>			66.5	294	28.8	70
<i>Zoarces viviparous</i>		100.0	89.2		36.6	88
Amphibians						
<i>Rana pipiens</i>	8.3	14.5	103.0	231	35.1	90, 233
<i>Necturus maculosus</i>	2.5		28.8	178	7.3	90
Reptiles						
<i>Pseudemys</i> sp.	7.5	14.5	74.6	857	30.7	90, 233
<i>Chrysemys picta</i>		2.8			25.4	267
<i>Iguana iguana</i>	10.7		44.7	257	29.0	90
<i>Scleroporus undulatus</i>	7.3		141.0	332	24.5	90
<i>Mabuya striata</i>			197.0	368	11.8	151
<i>Chameleo jacksonii</i>			97.8	407	48.5	151
Mean ectotherms	10.9 ± 1.2	17.6 ± 4.2	94.7 ± 6.7	360 ± 25	26.2 ± 2.3	
Mammals						
Alpaca			91.9	133	40.3	177
Cow	1.7	22.1			51.6	36
Dog	1.2	33.4			68.6	36
Guinea pig	2.7	26.7			68.5	36, 90
Llama			96.7	125	38.2	177
Mouse	4.7	29.7			137.0	5, 36
Ox			57.7	241	40.4	5, 251
Pig	1.4		158.0	350	38.9	5, 90
Rabbit	1.6	26.8			58.0	5, 36
Rat	4.2	18.5	69.4	381	98.0	5, 36, 90, 233
Sheep			317.0	232	58.0	5
Taruca			94.5	448	94.1	177
Weddell seal	0.9				12.5	251
Birds						
Budgerigar					120.0	90
Chicken	2.3		58.2	343	58.1	5, 90
Finch					146.0	90
Hummingbird	3.8		191.0	135	71.5	333
Sandpiper	1.2				78.7	87
Pigeon	2.8	6.6	74.9	253	114.0	5, 90, 233
Mean endotherms	2.4 ± 0.04*	23.4 ± 3.4	121 ± 26	264 ± 36	73.3 ± 8.3*	

Values are expressed as μmol substrate converted to product · g wet weight $^{-1} \cdot \text{min}^{-1}$. Assay temperatures were between 10 and 25°C for ectotherms and between 25 and 39°C for endotherms. For comparative purposes, activities of all enzymes were converted to 25°C on the basis of a Q_{10} value of 2. When activities for enzymes from the same species were reported more than once, values were averaged. HK, hexokinase; PFK, phosphofructokinase; PK, pyruvate kinase; LDH, lactate dehydrogenase; CS, citrate synthase. * Statistically significant difference in enzyme activity between ectotherms as a group and endotherms as a group.

D. Metabolic Control at the Enzyme Level

1. Relative maximal enzyme activity levels

The maximal in vitro activity of enzymes expressed in tissue homogenates is often used to gain insight into the organization of energy metabolism in vivo. There is a sound theoretical and experimentally supported basis for the use of enzymes that catalyze rate-limiting and nonequilibrium reactions as predictors of metabolic flux rates in vivo (257). It is also generally accepted that the in vitro activity of enzymes that catalyze reactions maintained close to equilibrium in vivo provide at least qualitative information regarding the presence or absence of particular metabolic pathways. This experimental approach has been taken many times in the study of heart metabolism. Table 1 presents the activity of selected enzymes of energy metabolism from ectotherms and endotherms. Maximal enzyme activities are interpreted to reflect amount of protein present. References have been selected that present data for a number of enzymes assayed under similar analytical conditions. Data have been normalized to 25°C assuming a Q₁₀ of 2, which will not be accurate for all enzymes, but this should not negate the comparative value of looking at individual enzymes across the various classes. Antarctic teleosts have not been included in this analysis in light of the particular uncertainty of adjusting enzyme activities from assay temperatures of close to 0°C to the comparative temperature of 25°C. In this analysis no attempt has been made to distinguish enzyme activities in spongy versus compact layers, since differences occurring at this level are small relative to between-species variation (101, 112).

In terms of anaerobic metabolism, the important feature of these data is that hexokinase (HK), phosphofructokinase (PFK), pyruvate kinase (PK), and lactate dehydrogenase (LDH), all requisite enzymes of glycolysis, are routinely detected in vigorous activities. The mean activity has been determined for each enzyme for the collective ectotherms and endotherms. Hexokinase, which catalyzes the first step in the utilization of exogenously supplied glucose, is about five times more active in hearts of ectotherms than endotherms. This is consistent with the use of exogenous glucose as a metabolic fuel to support anaerobic performance. There is no significant difference in activity of PFK, PK, or LDH between the two groups, implying that the capacity for anaerobic metabolism reached its maximum early in the development of vertebrates possibly at the level of the agnathids.

Within the ectothermic group, the maximum in vitro activity levels of enzymes of cardiac energy metabolism are not obviously related to survival capabilities at either the whole animal or isolated tissue level. There are some descriptive relationships though that warrant comment. When ectothermic vertebrates are considered as a single group, there is a significant linear expansion of HK versus LDH and PK versus LDH when enzyme

activities are plotted at physiological temperatures (Fig. 2). As the capacity to channel carbon to the level of pyruvate is enhanced, there is a parallel increase in capacity to convert pyruvate to lactate, the final electron acceptor.

On the basis of three species only, a linear relationship exists between maximal PFK activity/resting work and capacity of in vitro preparations to maintain performance under anoxia (309). In seven species of marine fish, there was a linear correlation between maximal in vitro activities of PK/cytochrome oxidase versus the time under anoxia required to reduce initial level of force to 50% (134). These studies suggest modifications in enzyme activity levels in association with anoxic cardiac performance. However, there is no simple relationship between energy metabolism and functional integrity. As discussed in section II C 2, the full glycolytic potential is not necessarily elicited by oxygen lack.

Although not related to anaerobic metabolism, for comparative purposes Table 1 also includes levels of citrate synthase (CS), which is an indicator of aerobic metabolism. Citrate synthase catalyzes the first reaction of the citric acid cycle in which acetyl CoA is condensed with oxaloacetate to form citrate. Acetyl CoA may be derived from any of the potential aerobic metabolic fuels, which include glucose, lactate, fatty acids, and ketone bodies. Maximal in vitro activity of CS correlates with power development in heart from a wide spectrum of vertebrate animals when both parameters are expressed per gram of tissue (90). There are significantly higher levels of CS in endotherms than in ectotherms, implying that in the transition from ecto- to endothermy there was a substantial expansion in the capacity for aerobic metabolism. This is in marked contrast to the decrease in HK activity and reinforces the concept that capacities of different metabolic pathways may be independently regulated.

2. Glycogen phosphorylase

In goldfish subjected to anoxia for 24 h at 7°C, the percentage of glycogen phosphorylase in the active *a* form decreased from 49 to 8% of the total. The total activity of glycogen phosphorylase did not change (326). This finding implies a decrease in the rate of glycogenolysis at a time when it is probable that glycogen reserves are exhausted (246).

Glycogen phosphorylase activity has been assessed in both intact turtles (49) and perfused hearts (291). No change in total glycogen phosphorylase was noted in either experiment. In turtles that had been subjected to 5-h anoxic submergence at 18°C, relative phosphorylase *a* activity decreased from 25 to 14% of the total activity (49). Measurement of enzyme activity in the anoxic animals was probably made ~2 h after glycogen mobilization had ceased. In isolated perfused hearts, 1 h after an aerobic-anaerobic transition there was an increase in relative phosphorylase *a* activity from 25 to >50% of the total activity (291). Under these perfusion conditions

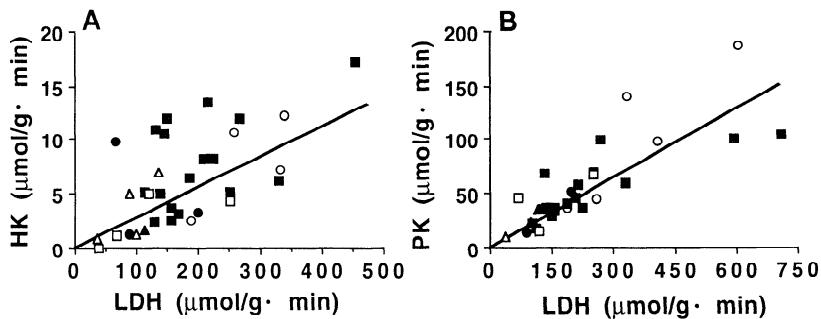


FIG. 2. Relationships between maximum in vitro enzyme activities from hearts of ectothermic vertebrates. Activities are plotted at reported assay temperatures to avoid assumptions of Q_{10} values. Assay temperature for enzymes from Antarctic teleosts was 0–1°C, and for all other species it ranged from 10 to 25°C. A: hexokinase (HK) vs. lactate dehydrogenase (LDH). B: pyruvate kinase (PK) vs. LDH. Regression equations were as follows: HK = (0.03)LDH + 1.27, $r = 0.62$, $n = 33$; PK = (0.20)LDH + 10.6, $r = 0.82$, $n = 30$. Solid triangle, agnathid: *Myxine glutinosa* (153, 310); open square, elasmobranchs: *Potamotrygon magdalenae* (313), *Raja erinacea* (250, 310), *Squalus acanthias* (310); open triangle, Antarctic teleosts: *Chænocephalus aceratus* (310), *Notothenia gibberifrons* (70), *Notothenia rossi* (310), *Trematomus newtoni* (70); solid square, temperate-zone teleosts: *Anguilla rostrata* (20, 233), *Cyprinus carpio* (310), *Dicentrarchus labrax* (310), *Esox niger* (19, 310, 369), *Gadus morhua* (153, 310), *Gaidropsarus vulgaris* (310), *Hemitripterus americanus* (91, 302), *Lophius piscatorius* (310), *Macrozoarcetes americanus* (91), *Makaira nigricans* (91), *Micropterus dolomieu* (303), *Morone americanus* (303, 310), *Morone saxatilis* (310), *Myoxocephalus octodecemspinosis* (70), *Oncorhynchus mykiss* (48, 112), *Perca flavescens* (303), *Propoterus aethiopicus* (93), *Salmo salar* (101), *Salvelinus fontinalis* (90, 101, 213), *Scomber scombrus* (310), *Tautoga onitis* (70); solid circle, amphibians: *Necturus maculosus* (87), *Rana pipiens* (81, 87); open circle, reptiles: *Pseudemys* sp. (90, 233), *Iguana iguana* (233), *Scleroporus undulatus* (233), *Mabuya striata* (151), *Chameleo jacksonii* (151).

heart performance was maintained and glycogen mobilization was active (289, 290). These experiments are consistent with enhanced activity levels of glycogen phosphorylase *a* under conditions of active glycogen utilization.

3. Hexokinase

The maximal in vitro activity of HK measured in total homogenates of skeletal muscles from a variety of species matches in a 1:1 stoichiometry carbon flux through glycolysis predicted on the basis of oxygen consumption measurements (67). Measurements of in vitro HK and oxygen consumption by isolated fish (18, 302), turtle (289, 292), and rat heart (288) suggest maximal rates of carbon flow through HK that are substantially higher than maximal flux required to support aerobic metabolism. Similarly, cardiac uptake of 2-deoxyglucose in swimming rainbow trout suggests that glucose oxidation accounts for <10% of energy production in vivo under aerobic conditions (372). Calculated anaerobic ATP yield based on in vitro HK activity matches total ATPase activity (i.e., ATP use) in heart homogenates from many ectothermic and endothermic vertebrates (90, 310). Therefore, in heart, in vitro activity of HK is not quantitatively predictive of the use of glucose as a fuel to support aerobic metabolism but, in light of its key position in the glycolytic pathway, is probably a qualitative indicator of maximal anaerobic glucose utilization.

The maximal rate of exogenous glucose utilization by perfused turtle hearts under anoxia was ~3

$\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. The maximal activity of HK in total crude homogenates was $8 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ with approximately one-half of the activity associated with a fraction that was pelleted by high-speed centrifugation, suggesting that the enzyme was bound to a subcellular fraction. None of the other glycolytic enzymes exhibited this behavior. There is a remarkably close match up between the maximal rate of anaerobic use of glucose and the maximal activity of HK, implying that this enzyme is a rate-limiting step in anaerobic glucose utilization. Further support for this concept was obtained from assessment of lactate production in cell homogenates where at high protein concentrations the addition of HK alone to the media enhanced lactate production (292).

4. Phosphofructokinase

Phosphofructokinase was activated during the early stages of oxygen limitation as indicated by cross-over analysis (Fig. 3) in intact Atlantic hagfish (153), African lungfish (94), goldfish (307), and turtles (202) and in perfused isolated turtle hearts (278). In this experimental approach the concentrations of glycolytic intermediates are assessed before and after a transition period. Identification of control sites is made by expressing the tissue content of each intermediate, after the onset of anoxia, as a percentage of the aerobic value. When glycolytic flux increases, there will be a relative depletion of the reactants and accumulation of the products of the rate-limiting reactions. The control sites are identified by crossovers in the sequence of glycolytic intermediates. Crossover points only identify control sites in the glycolytic sequence and do not necessarily imply

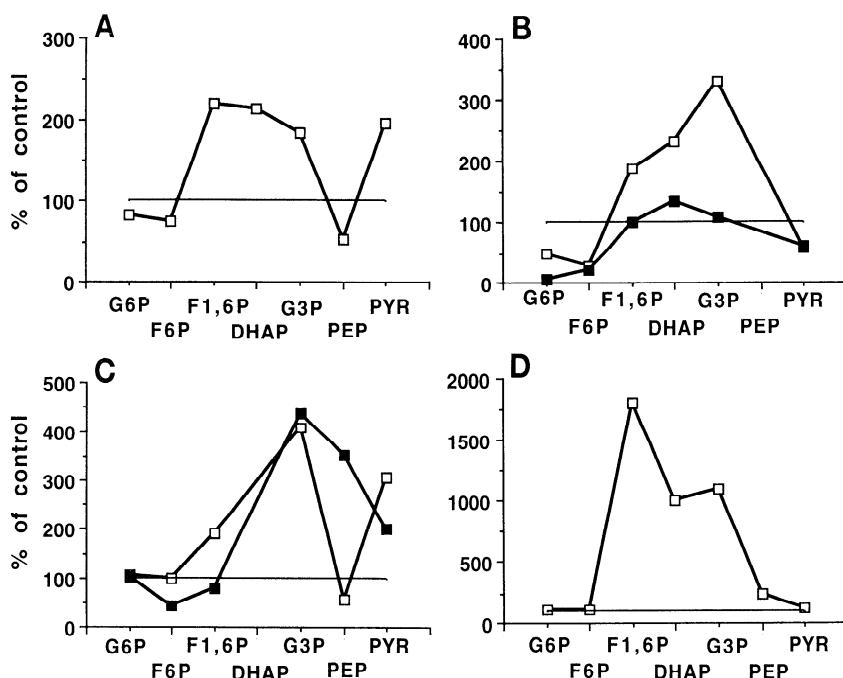


FIG. 3. Crossover plots of glycolytic intermediates in heart after transition from aerobic to anoxic conditions. *A*: Atlantic hagfish were exposed to anoxic water for 20 h (153). *B*: African lungfish, which are air breathers, were subjected to forced dives at 22°C. Open symbols, 3 h; solid symbols, 12 h (94). *C*: turtles were subjected to forced dives at 18°C. Open symbols, 1 h; solid symbols, 5 h (202). *D*: isolated rat hearts were perfused for 1 min at 37°C (375). G6P, glucose-6-phosphate; F1,6P, fructose-1,6-diphosphate; G3P, glucose-3-phosphate; PYR, pyruvate; F6P, fructose-6-phosphate; DHAP, dihydroxyacetone phosphate; PEP, phosphoenolpyruvate.

that such sites control the flux. For instance, HK could still control the overall glycolytic rate, even though PFK was maximally activated. In the goldfish heart PFK activation was still indicated after 60 h of anoxia at 4°C (309), whereas in turtle heart, after 5 h of anoxic submergence at 18°C, crossover analysis no longer indicated PFK activation (202).

Phosphofructokinase kinetics have been assessed on partially purified preparations from a number of fish species including flounder, trout, cod, and goldfish. Fish heart PFK, similar to other systems, is inhibited by low pH, high ATP, and citrate; activators include AMP, P_i , and fructose-2,6-diphosphate ($F\text{-}2,6\text{-P}_2$). Flounder heart PFK is also inhibited by CP (195). Information on CP effect in other species is not available. There are no obvious differences between hypoxia-tolerant and -intolerant myocardia with respect to potential regulation by pH and the adenylates (191). Of the regulators, $F\text{-}2,6\text{-P}_2$ may be especially important with a Michaelis activation constant (K_a) of $\sim 0.16 \mu\text{M}$ for the goldfish enzyme. After 24 h of anoxia at 7°C, $F\text{-}2,6\text{-P}_2$ levels in goldfish heart increased from 0.13 to 0.47 nmol/g. Under anoxia, a rise from about the K_a level to a saturating level would stimulate a large increase in PFK activity by increasing the affinity for fructose-6-phosphate (F-6-P) to a concentration closer to the physiological range of the substrate (326).

An alternative view on long-term regulation of PFK is offered by Rahman and Storey (286). Goldfish were subjected to 24 h of anoxia at 7°C. Under these conditions the maximal *in vitro* velocity of PFK was decreased in concert with an increase in the K_a for $F\text{-}2,6\text{-P}_2$ and the K_a for AMP. The authors (286) argue that this could indicate a covalent modification of the enzyme via a dephosphorylation which results in a less

active PFK and that this is consistent with a curtailment of glycolysis following long-term anoxia. However, crossover plots indicate an activation of PFK even after 60 h of anoxia at low temperature (307), and heart performance studies do not support the concept of a substantial metabolic depression in hearts of teleost fish. The significance of anoxia-induced alterations in kinetic constants for PFK remains to be elucidated.

The kinetic properties of purified or partially purified turtle heart PFK have been assessed many times (49, 190, 191, 229, 327). The enzyme is generally inhibited by low pH and high concentrations of ATP, citrate, and CP. Activators or deinhibitors include P_i , AMP, fructose-1,6-diphosphate ($F\text{-}1,6\text{-P}_2$), and $F\text{-}2,6\text{-P}_2$. Of the known metabolic alterations during an aerobic-anaerobic transition, changes in CP and P_i are likely candidates as prime activators of PFK *in vivo*.

Control at the level of PFK following long dive times is more difficult to resolve. After 5 h of anoxic submergence, the Michaelis constant (K_m) for ATP was decreased from 31 to 19 μM , and the effector concentration resulting in half-maximal inhibition (I_{50}) (citrate) was increased from 0.65 to 1.13 mM, changes indicative of the induction of a more active enzyme. Kinetic constants for F-6-P, P_i , AMP, and $F\text{-}2,6\text{-P}_2$ were not altered. It was proposed that the enzyme was phosphorylated under anoxia (49) as occurs with epinephrine treatment of rat hearts when glycolysis is activated (254). However, it is not clear that glycolytic activity in these turtle hearts is enhanced relative to the aerobic situation at this time into the dive. Moreover, levels of $F\text{-}2,6\text{-P}_2$ are reduced relative to controls (49), and pH_i would be lower. Both of these features could contribute to an inhibition of PFK. In turtle heart, as is the case with goldfish, despite extensive work the regulatory properties

of PFK with respect to actual glycolytic flux under anoxia are yet to be fully resolved.

5. Pyruvate kinase

Flounder heart PK is inhibited by ATP and alanine (195). Goldfish enzyme is activated by F-1,6-P₂ and inhibited by alanine (286). After 24 h of anoxia at 7°C, there was a marked reduction in I₅₀ (alanine) from 12 to 3 mM. Maximum velocity and kinetic constants for phosphoenolpyruvate (PEP), ADP, and F-1,6-P₂ were unaltered. Large increases in F-1,6-P₂ under anoxia (307) suggest stimulation of PK via feed-forward activation.

Turtle heart PK has an acidic pH optimum (\approx 6.5), F-1,6-P₂ is a potent positive effector with a concentration of \sim 0.1 μ M resulting in half-maximal activation (A₅₀), and ATP and alanine are inhibitors (49, 328). No alterations in kinetic constants were observed following an anoxic period (48). Crossover plot analysis indicated an activation of PK during the early stages of anoxia followed by an inhibition at this site after 5 h of forced submergence at 18°C as PEP accumulated and pyruvate levels fell (Fig. 3) (202). Fructose-1,6-diphosphate is a prime candidate for in vivo control, since its concentration increased early in a dive period and later fell below control levels (202). Maximal in vitro activity of PK was not altered following the 5-h dive (48); however, after 48 h of submergence, a significant decrease in PK was noted (312). Until the turnover time of this protein is known, the possibility that metabolic control via a decrease in enzyme content should not be ruled out as suggested by Simon et al. (312).

6. Lactate dehydrogenase

A single B₄(H₄) LDH homotetramer is expressed in Atlantic hagfish heart. The enzyme is inhibited by high concentrations of pyruvate; however, K_m for pyruvate is higher than values for B₄ isozymes from a number of teleosts and endothermic vertebrates. The isozyme may be functionally similar to a A₄(M₄) skeletal muscle type isozyme (308). The hagfish (*Epatretus cirrhatus*) also has a B₄ LDH with a relatively high K_m for pyruvate (21).

The heart of many teleosts usually exhibits one primary B₄(H₄) LDH isozyme. In most cases, kinetics of purified enzyme exhibit a low K_m for pyruvate and inhibition at high pyruvate concentrations (21, 138, 227, 232, 280, 301). In other species, though, the heart isozyme resembles a skeletal muscle type A₄(M₄) LDH with a high K_m for pyruvate and a lack of pyruvate inhibition in the pyruvate to lactate direction (88, 116, 195, 325). Within this group there is no obvious correlation between the tolerance of isolated preparations to anoxia and either maximal in vitro LDH activity or LDH isozyme activity ratios (135).

Turtles have electrophoretically distinct heart and

skeletal muscle LDH isozymes that display similar kinetic properties, showing weak pyruvate inhibition at high pyruvate concentrations. In this sense the turtle heart isozyme is intermediate between mammalian A₄ and B₄ isozymes (6).

7. Metabolic regulation in relationship to performance

Transitions from aerobic to oxygen-limiting conditions where cardiac power output is maintained relatively high must be associated with an activation of glycogenolysis and glycolysis. Given a phosphate-to-oxygen ratio of 2-3 and lactate production from both glycogen and exogenous glucose, the requirement is for about a 10-fold increase in carbon flow through glycolysis to maintain balance between ATP generation and utilization. If cardiac energy demand is reduced to a value less than \sim 10% of normoxic rates, then a curtailment of glycolysis even under hypoxia is feasible.

I) FISH. In hagfish heart, requirements of the energy-utilizing systems are similar under both aerobic and anaerobic conditions. Low resting energy demands are met under anoxia through an activation of anaerobic glycolysis. This does not imply glycolytic rates are exceptionally high in this species but only that ATP supply can meet demand.

Cardiac power output may decrease to 50% in some teleosts and elasmobranchs under oxygen-limiting conditions. Under anoxia/hypoxia, glycogen and blood-borne glucose serve as metabolic fuels, and glycolytic flux remains elevated during the entire period. The use of exogenous glucose is consistent with high activities of HK in this group of animals. Glycolysis is activated at the level of PFK, and at least in goldfish F-2,6-P₂ appears to be an important regulator. There may also be feed-forward activation at PK via increases in F-1,6-P₂. Adenosine 5'-triphosphate production via anaerobic glycolysis keeps pace with the decreased ATP demand. Hearts fail for reasons other than ATP availability. Alterations in CP levels are still in question and require further investigation, since CP is a potential inhibitor of PFK and P_i directly impairs contractility (see sect. II E2).

II) TURTLES. Performance of turtle hearts under anoxia is dependent on glycogen and exogenous glucose as fuel sources. The capacity for anaerobic glycolysis is adequate to sustain resting levels of performance, but in vivo there is a gradual depression of performance under anoxia that is under neural control. High rates of glycolysis/glycogenolysis are essential during the early stages of anoxia, but when cardiac energy demand is reduced greater than \sim 10-fold, glycolysis may actually be curtailed during the anoxic period relative to rates that occur under air-breathing conditions. Tissue levels of total ATP are held relatively constant under anoxia, but levels of CP decrease. Hexokinase and PFK are both important rate-controlling enzymes, with carbon flow through HK proceeding at close to maximal velocity

when power output is high. Phosphofructokinase and PK are activated during the aerobic-anaerobic transition. Pyruvate kinase may play a role in decreased rates of glycolysis once demand for ATP is reduced well into a dive.

8. Comparison of ectotherms and endotherms

Isolated mammalian heart preparations subjected to anoxia invariably fail. Performance of perfused isolated rat and squirrel hearts was reduced by ~50% after 2 min of anoxia at 35–37°C. Lactate production was linear at rates between 4 and 9 $\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. Under these conditions CP and ATP were decreased, so glycolysis was probably running at maximal velocity (51, 375). Rates of carbon flow through glycolysis equalled 2–5 $\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. Flow through HK would be less than this dependent on the degree of glycogenolysis. These values are comparable to those found in isolated turtle hearts maintaining performance at 23–25°C. The point is that anaerobic glycolysis is the same in mammalian and turtle hearts at their respective body temperatures provided that ATP demand is high enough to drive the catabolic process. When energy demand is decreased, rates of lactate production are decreased. Rabbit papillary muscle working at 20°C loses 50% of initial force after ~10 min of anoxia (Fig. 1). Under these conditions CP falls, ATP remains constant, and lactate is produced at a rate of 0.6 $\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ (238). The rate of force decay and the rate of lactate production are very similar to that which occurs in trout ventricle strips at the same temperature. These studies further illustrate the common finding that contractile failure is not due to total tissue ATP limitation by itself and that glycolytic activity ensures that ATP demand and ATP supply are matched. It should be emphasized that energy demand in terms of contractility is usually curtailed in ectothermic myocardia under O₂-limiting conditions.

In mammalian heart, as in ectothermic vertebrates, glycolysis is activated at the level of PFK as evidenced by crossover plots (Fig. 3) (375). Control of PFK in rat heart is considered to be due to a large extent to activation via F-2,6-P₂ (254). Control of PFK in ectothermic hearts is probably also under control of this effector, since the K_a values in turtles and goldfish are in the micromolar range. There is no evidence that CP is an effector of mammalian heart PFK. The possibility of this metabolite playing an important role in hearts of ectothermic vertebrates needs to be more fully elucidated.

E. Determinants of Calcium-Activated Force Under Hypoxia

In multicellular preparations, oxygen shortage or inhibition of cellular respiration typically results in a drop in contractility. Notably, this is true for coronary

perfused mammalian hearts, where washout of lactate and other waste products should be like those *in vivo*. In this respect the ectothermic myocardia lacking or with a poorly developed coronary system should be well mimicked by perfused multicellular preparations. Oxygen lack depresses aerobic energy liberation. This depression in some circumstances is not fully compensated for by anaerobic glycolysis. Hence, an imbalance between demand and liberation of energy occurs, with a lowering of the energy state as a consequence. This together with the change from aerobic to anaerobic metabolism tends to alter the acid-base balance and metabolite composition of the cytoplasm. The mechanisms linking these changes to changes in contractile performance are the topic of this section.

1. Cytoplasmic energy state and contractility under hypoxia

Usually, inhibition of cell respiration causes a drop in CP, while the ATP level remains almost intact (e.g., Refs. 160, 199). Therefore, the free energy of ATP hydrolysis instead of ATP itself was suggested to be the main determinant of contractility (199). Tentatively, a decreasing free energy (G) of ATP hydrolysis may lower the efficiency of myosin ATPase and of transport ATPases such as Na⁺-K⁺-ATPase and Ca²⁺-ATPase (e.g., Ref. 149). The energy liberated by ATP hydrolysis is given by

$$\Delta G = \Delta G^0 - RT \ln (ATP/ADP \cdot P_i)$$

(e.g., Ref. 198). This negative quantity is often used with the opposite sign and referred to as affinity of ATP hydrolysis. In the cytoplasm of muscle cells, this parameter is assessed by the use of the creatine kinase-catalyzed reaction: CP + ADP + H⁺ = Cr + ATP. With the assumption of equilibrium and an unchanged pH_i, the concentration of CP provides a good approximation of the logarithmic term of the expression above (247).

Short-lasting stimulations of anoxic force development by elevations of extracellular Ca²⁺ in the perfused rat heart were taken as evidence against a strict restraint of anaerobic force development by the affinity of ATP hydrolysis (198). This conclusion was supported by experiments with “skinned” preparations, which allow a direct control of the phosphorylation potential at the myofilaments. The lowered contractility seems rather to be due to the single factors defining this affinity (e.g., Refs. 143, 205).

On balance, it seems unlikely that the losses of contractility following oxygen lack are caused primarily by a concomitant drop in the affinity of ATP hydrolysis acting on the cross bridges. The situation is, however, less clear in regard to other ATPases.

2. Contractile proteins, inorganic phosphate, and adenosine 5'-diphosphate under hypoxia

The contractile system is depressed substantially by the elevations of P_i associated with reductions of the

phosphorylation potential (143, 173, 204). Both the force at saturating levels of Ca^{2+} and the Ca^{2+} sensitivity of the myofilament are depressed by P_i (174, 204).

In, for example, trout myocardium, P_i may increase 3–10 times under anaerobic conditions (160). According to the model of Pate and Cooke (275), the inhibition of force depends more on relative than on absolute changes in P_i . The effect of P_i is difficult to predict quantitatively, since changes in P_i are associated with other changes that also affect contractility. Hence, increases in ADP favor formation of “strong” bonds and thereby force development (275). In accordance with this, the negative effect of P_i on force generation in skinned myocardial tissue is diminished at low CP levels, where ADP and the formation of strong cross bridges may be elevated (245). Regarding intact heart tissue, increases in P_i are a main cause for the loss of myocardial contractility during hypoxia according to the predominant opinion (e.g., Ref. 235), but opposing views are expressed (197).

Adenosine 5'-triphosphate and CP are coupled through the creatine kinase reaction, such that a given drop in ATP is accompanied by about a 100 times larger drop in CP. Furthermore, P_i varies inversely with CP (e.g., Ref. 247). Therefore, an inhibition of contractility by P_i may serve as a sensitive mechanism for adjusting energy demand to energy liberation. Tentatively, the efficiency of this mechanism will vary with the levels of CP and the potential for P_i liberation (e.g., Ref. 363). Therefore, the observation that the dimensions of the myocardial creatine-creatin phosphate (Cr-CP) system seem to differ among species (90) should be considered in regard to species-dependent differences in hypoxic contractility.

3. Contractility and intracellular pH under hypoxia

The inhibition of contractility by hypoxia may, in addition to P_i , also depend on pH_i . Changes in pH_i during hypoxia depend mainly on the following reactions. Anaerobic glycolysis tends to lower pH_i , as does hydrolysis of ATP to ADP. Rephosphorylation of ADP at the cost of CP does the opposite by binding H^+ (e.g., Refs. 97, 209). The common observation with multicellular preparations is that pH_i falls or stays unchanged. Elevations of the H^+ activity tend to inhibit both the contractile system (see sect. III C2) and the glycolytic energy liberation (e.g., Ref. 320).

Experiments with skinned preparations (e.g., Ref. 143) strongly suggest that the decreases in pH_i , measured in intact cell preparations (e.g., Ref. 3), explain a substantial part but not all of the decrease in contractility. Hence, other factors such as P_i must be involved in addition to pH_i (e.g., Ref. 143). In some studies pH_i even appeared as rather unimportant. Twitch force of trout myocardial tissue declined by ~70% during anaerobic conditions, although no change in pH_i could be recorded (264). In toad myocardial strips, inhibition of oxidative

phosphorylation imposed a drop first in contractility and then in pH_i (217). Intracellular pH as measured with ^{31}P -NMR in the isolated heart of turtle subjected to anoxia for 4 h declined by ~0.2 units and did not relate in time to the changes in contractility (365).

Apart from its effects on the contractile proteins and glycolysis, an acid load may also alter the cellular Ca^{2+} balance. The E-C coupling and the role of Ca^{2+} during hypoxia are dealt with in section II E4. Here, it is sufficient to state that an acid load may counteract its direct negative action on the contractile system by enlarging the amount of Ca^{2+} in the E-C coupling. This effect probably implicates a removal of cytoplasmic H^+ . Therefore, an acidotic challenge may influence mechanical performance to an extent that is not predicted by the change in pH_i (see sect. III C3).

The large differences in myocardial contractility under hypoxia among ectothermic vertebrates may relate to the size of the acidotic challenge and its different effects. This possibility is suggested by the maintenance of a high contractility both under hypoxic and acidotic conditions in myocardial tissue of turtle (133, 281) and eel (265). Regarding passive buffering, interspecies comparisons do not suggest any positive relationship between tolerance to hypoxia and tissue nonbicarbonate buffering capacity as measured in homogenates (71). Active pH regulation involving sarcolemmal Na^+-H^+ exchange and Na^+-H^+ cotransport or mitochondria may be important but cannot be assessed at present due to lack of information about differences in the active pH regulation among myocardia of ectothermic species.

To conclude, hypoxia may impose an acid load, which by effects on the contractile system and the cellular Ca^{2+} balance may have a complex influence on mechanical performance. These aspects should be considered with regard to the large species-dependent differences in myocardial contractility under hypoxia.

4. Excitation-contraction coupling under hypoxia

The negative inotropic effects of P_i and acidosis involve a decreased Ca^{2+} sensitivity of the contractile system. In accordance with this, anaerobic force development in frog (359) and trout myocardium (131, 264, 265) varies with the Ca^{2+} available for excitation, which was altered experimentally by epinephrine and changes in extracellular Ca^{2+} . In particular, twitch force falls together with the energy state as expressed by CP, but even at very low values in trout myocardium, elevation of extracellular Ca^{2+} increased force at a given CP (285) (Fig. 4). The response to changes in Ca^{2+} availability is much less expressed for the rat myocardium (198, 263). It is not clear if this represents a more general difference between ecto- and endotherms. Regardless, these observations emphasize the possibility that hypoxic restrictions of myocardial contractility in ectothermic species might be mediated by the E-C coupling.

Lowering of ATP may diminish the activation of

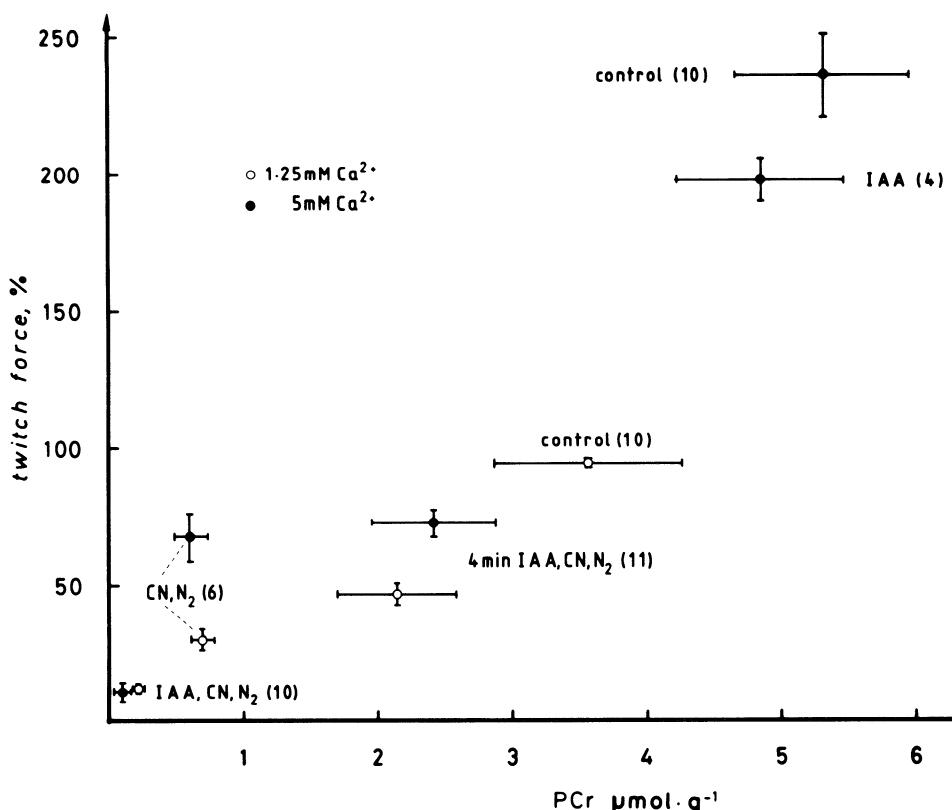


FIG. 4. Extracellular Ca^{2+} and relationship between twitch force and creatine phosphate (PCr) in isometric ventricular strips of trout paced at 0.2 Hz and subjected to different metabolic inhibitions. Iodoacetic acid (IAA; 1 mM) was used to block glycolysis, and cyanide (CN; 5 mM) and N_2 were used to block cell aerobic respiration. [From Purup Hansen and Gesser (285).]

the "slow" channels carrying Ca^{2+} (318). Furthermore, there exist sarcolemmal K^+ channels (258) that are activated by lowering of ATP and increases in ADP with a shortening of the action potential in consequence. The relationship between sarcolemmal Ca^{2+} influx and the drop in contractility during oxygen deprivation is, however, obscure. An aerobic block decreased the contractile force, the amplitude, and duration of the action potential and the slow inward current in frog cardiac tissue, but the changes in mechanical and electrical parameters were not correlated. In particular, addition of glucose prohibited the fall in the electrical activity, whereas the drop in force remained almost unchanged (359). Similar to this, an aerobic block on trout myocardial tissue imposed a large decrease in contractile force, but only a transient change of the action potential (132). In a study on rabbit papillary muscle, extracellular accumulation of K^+ appeared as a main reason for the action potential shortening observed during hypoxia (381). Therefore, caution should be exercised in comparing the effects of hypoxia on action potential duration, since this effect should depend on the rate by which interstitial K^+ equilibrates with that of the bath solution.

Direct measurements of the intracellular Ca^{2+} activity showed that the Ca^{2+} transient remained unchanged in spite of decreases of isometric twitch force in papillary muscles of rat, cat, and ferret (4). In later experiments with ferret papillary muscle, cyanide was even found to enhance the Ca^{2+} transient in the face of a decrease in force (2). Such an enhancement was also re-

corded for a significant fraction of isolated ventricular cells of the rat (97).

The lack of corresponding data for cardiac tissue of ectotherms is unfortunate, since the Ca^{2+} transient may be implicated in the large differences among species in mechanical performance during hypoxia. As already noted, changes in Ca^{2+} available for activation Ca^{2+} appear to have a large freedom to act in hypoxic myocardia of ectotherms. Possible changes in the Ca^{2+} transient may, apart from the influx through Ca^{2+} -specific channels, also involve other sources of Ca^{2+} . Sarcoplasmic reticulum appears to be of crucial importance to the cellular Ca^{2+} regulation in mammalian myocardial cells, whereas its function in the ectotherm heart is less clear. Although the SR of trout heart appears to be well developed (see sect. I E4), the mechanical response to an aerobic block was not significantly affected by inhibitors of the SR function (132). Because anaerobic conditions lower the H^+ gradient across the inner mitochondrial membrane and, thus, the driving force of mitochondrial Ca^{2+} uptake, Ca^{2+} should be shifted from mitochondria to cytoplasm (see sect. I E5 and Refs. 128, 196). As a result, the diastolic Ca^{2+} activity might be elevated and shift the Ca^{2+} transient and the twitch force upward (27). The Ca^{2+} transient may also be modified by intracellular Na^+ acting on the sarcolemmal $\text{Na}^+-\text{Ca}^{2+}$ exchange (see sect. I E2). Increases in intracellular Na^+ following inhibition of the aerobic energy liberation have been recorded in sheep Purkinje fibers and were tentatively ascribed to a depression of the Na^+-K^+ pump activity (46). As already discussed, the sarcolemmal ex-

change of Na^+ for H^+ and of Na^+ for Ca^{2+} in combination with a cellular acidosis may also be of significance in this respect.

5. Regulations of hypoxic contractility

Apart from factors directly associated with a decreased energy state and an enhanced glycolysis, oxygen lack often implicates hormones or hormonelike substances that modify contractility. The levels of catecholamines (e.g., Refs. 339, 366) and Ca^{2+} (see sect. I $E7$) may increase during oxygen lack. Furthermore, and probably due to hypoxia, frog cardiac tissue has been found to release ATP, which by acting on purinergic receptors stimulates contractility (83). The multiple effects of extracellular ATP, which may differ between atrial and ventricular tissue, have recently been reviewed (356). The effects of ATP may be modulated by other local effectors such as arachidonic acid (72). In myocardial tissue of mammals, inhibitions of cellular respiration strongly attenuate the positive inotropic actions of catecholamines or elevations of extracellular calcium (e.g., Refs. 198, 263). For ectotherms, however, large species-dependent differences in the corresponding response have been reported. The stimulatory effect of epinephrine on the force developed by frog (359) was reduced by an aerobic block. Such a reduction occurred for flounder myocardium, too, and was tentatively ascribed to a myocardial release of adenosine (222). In isolated myocardial tissue of trout and eel, the aerobic block did not affect the maximal response of force to epinephrine as measured in relative terms, although the affinity for epinephrine was reduced in the eel heart (131). It should again be emphasized that the stimulation of contractility by epinephrine, attenuated or not, indicates the maintenance of a reserve capacity for mechanical performance and energy liberation under an aerobic block (264).

Myocardial cells of mammals (189) and of ectotherms as exemplified by flounder (225) may be irreversibly damaged by hypoxia. Here, the turtle heart should be noted as a probable exception (365, 367). Regarding irreversible damages, glycolytic metabolites such as lactate may be critical (256). Therefore, the attenuation of the response to catecholamines by adenosine may represent a protective mechanism. This may of course be true for the negative action of P_i and cellular acidosis as well. The turtle heart should be noted as an example of a very efficient tissue protection by downregulative mechanisms (365, 367; see sect. III D). Arguments for an active downregulation of force following restriction of energy liberation have also been provided by studies of mammalian myocardia (198, 274).

The variation among species in the sensitivity to catecholamines maintained under even severe oxygen lack may reflect two conflicting tendencies. The maintenance of strong response to catecholamines by the trout myocardium may help the fish escape the hypoxic expo-

sure, whereas the attenuated response of the flounder myocardium may represent a downregulation to better sustain hypoxic exposure.

6. Contracture under a depressed metabolism

Prolonged oxygen deprivation is frequently associated with elevations of resting tension and/or shortening of resting length. Formation of rigor complexes seems to be a main cause for this (80). An inefficient removal of cytoplasmic Ca^{2+} during diastole may also contribute, however. Total metabolic inhibition caused isolated rat heart myocytes to go into a contracture that was preceded by an augmented cellular Ca^{2+} activity as measured with fura 2 (97). At the beginning of its development (but not later), the contracture was diminished, when extracellular Ca^{2+} was removed. Similar results have been recorded with cultured chick myocardial cells (23).

For the trout myocardium subjected to a full metabolic block, twitch force was stimulated by an elevation of extracellular Ca^{2+} from 1.25 to 5 mM (Fig. 4). This indicates that more Ca^{2+} enters and has to be handled by the cell. Therefore, it should be noted that the difference in extracellular Ca^{2+} did not affect the substantial increases in resting tension following a full metabolic block (285). Tentatively, the Ca^{2+} regulation of the myocardial cell at a severely depressed energy state is better maintained in trout and perhaps in other ectothermic species than in mammals. Such differences may relate to differences in the E-C coupling mechanisms. This result might suggest that the E-C coupling in ectothermic species such as the trout is more resistant to lowering of the cellular energy state.

7. Conclusions

Typically, hypoxia induces an elevation of P_i that appears to be the main cause for the concomitant loss of myocardial force. Moreover, pH_i may decrease, and there may be a further decrease in contractility. These direct effects of an inhibited aerobic metabolism and a stimulated glycolysis are likely to be confounded with effects of mechanisms in the cellular pH homeostasis, intracellular Ca^{2+} stores, and substances acting as local effectors. With regard to the large species differences in myocardial mechanical activity under hypoxia, several factors are involved in addition to the energy-liberating capacity. Hence, the size of the Cr-CP system is probably one determinant of the increase in P_i . The activity of the Na^+-H^+ exchange influences the change in pH_i as well as cellular Na^+ , with probable consequences on the amount of activator Ca^{2+} . Mitochondria deserve particular interest as to changes in activator Ca^{2+} , as does the ability of the E-C coupling to work at a lowered energy state. Local effectors such as extracellular ATP and adenosine have also to be considered.

III. CONTRACTILITY UNDER ACIDOTIC CONDITIONS

A. Range of Hydrogen, Carbon Dioxide, and Bicarbonate

1. General remarks

Cardiac muscle may be exposed to acid loads, which vary largely both in frequency and severity among vertebrate species. Several reviews deal with general aspects of acid-base regulation in ectothermic vertebrates (fish, Refs. 166, 167; amphibia, Ref. 343; reptiles, Ref. 185). Only some points of specific relevance to cardiac acidosis are treated here. Of importance to ectotherms is that tissue and blood pH generally tend to vary inversely with temperature. For example, in the elasmobranch *Scyliorhinus stellaris* $\Delta\text{pH}/\Delta t$ in plasma is -0.0115 in the temperature range of $10\text{--}20^\circ\text{C}$. The corresponding value in the myocardial cells is -0.007 (169).

Two main types of acidosis occur. Elevations of PCO_2 as the prime event result in respiratory or hypercapnic acidosis. As an immediate and passive result, HCO_3^- increases to an extent determined by the non- HCO_3^- buffer value. The second type of acidosis is due to production of acid metabolites other than CO_2 , predominantly lactic acid. As a direct consequence HCO_3^- is lowered in being transformed to H_2CO_3 and CO_2 . With an inhibited gas exchange, as during diving, PCO_2 will, therefore, be augmented.

The acid-base balance tends to differ between obligate water breathers such as cyclostomes, elasmobranchs, and teleosts on the one hand, and obligate air breathers such as reptiles on the other. Amphibians and facultative air-breathing fishes, for which gas exchange may take place in both water and air, are in an intermediate position.

2. Obligate water breathers

The absolute values of PCO_2 and HCO_3^- in blood and tissues are lower in water breathers than in air breathers. Typically, PCO_2 ranges between 0.1 and 0.5 kPa and HCO_3^- from <5 to 15 mM in plasma of fishes (166).

In general, challenges of acid-base balance appear to be smaller in water breathers than in air breathers. It should be noted, though, that the passive buffering capacity of blood relating to both HCO_3^- and non- HCO_3^- substances is five to six times smaller in water breathers than in mammals and birds. Also, the buffer value of skeletal muscle tissue is only 50–70% of that recorded for mammals (166). The PCO_2 and HCO_3^- values may vary largely in relative terms. Thus fish may be exposed to elevated environmental PCO_2 as, for instance, in lakes rich in vegetation and microorganisms when during the night respiration is high in the absence of photosynthesis. Water PCO_2 values as high as 8 kPa have

been recorded (168). Many flatfishes burrow into the bottom mud. This is likely to depress exchange of water around the gills so that the excretion of PCO_2 and the supply of O_2 may be impeded (223). Increases of PCO_2 may also be imposed by hyperoxia, which suppresses gas exchange and removal of CO_2 . Hyperoxia occurs in waters with low convection and high photosynthetic activity. Here, PO_2 may attain values beyond 67 kPa (79).

Typically, the drop in pH induced by hypercapnia is counteracted over several hours by elevations of HCO_3^- . For example, exposure of *Conger conger* and *Scyliorhinus stellaris* to an environmental PCO_2 of 1 kPa lowered plasma pH by ~ 0.4 units as plasma PCO_2 rose from ~ 0.25 to ~ 1.3 kPa. Over 5–10 h, pH was partially recovered by an elevation of HCO_3^- from <10 to ~ 20 mM (164).

After exhausting muscular activity, H^+ is released from the skeletal muscles. For instance, in *S. stellaris*, the arterial pH fell sharply from 7.8 to 7.2 at the same time as PCO_2 rose from ~ 0.25 to 0.67 kPa and HCO_3^- fell from 7 to 3 mM (180). The anatomy of the circulatory system and the frequently poorly developed coronary system (see sect. IC) may in addition to acidosis impose a shortage of O_2 on the cardiac muscle of the strenuously active fish.

3. Air-breathing fish and amphibians

In the facultative air breather *Symbranchius marmoratus*, a shift from water to air breathing changed plasma PCO_2 from ~ 0.75 to 3.5 kPa with an associated fall in pH of ~ 0.6 units. No long-term (4–5 days) change in cardiac pH_i was recorded, whereas intracellular HCO_3^- rose by fourfold (165). In their larval stage, amphibians are obligate water breathers. This is also more or less true for adult amphibians dwelling in water due to gas exchange across the skin and sometimes also rudimentary gills. Plasma PCO_2 tends to increase as the animal shifts from water to air breathing. Notably, though, there is no clear relationship between the mode of breathing and blood total CO_2 (343). Both metabolic and respiratory acidosis have been recorded upon forced diving though (100). *Siren lacertina* and *Amphiuma means* are exposed to extreme environmental hypercapnia when dwelling in waters with PCO_2 values around 8 kPa. Directly upon exposure of *A. means* to this PCO_2 , arterial PCO_2 rose to ~ 5.0 kPa from ~ 2.3 kPa. pH fell from 7.8 to <7.4 . Similar changes occurred for *S. lacertina* (168) and for the toad *B. marinus* (344). The effects of exercise resemble those described for water breathers.

4. Reptiles

Reptiles are exclusively lung breathers. Many species, for example, turtles, alligators, water snakes, and iguanas, may stay submerged under water for long periods with both hypercapnic and respiratory acidosis in combination with oxygen deprivation as a consequence

(185). In turtles, plasma pH fell from ~7.75 to ~7.0 during 6 h of forced diving. The PCO_2 rose from ~3.3 to ~12 kPa. Exposure of the same length of time to N_2 breathing resulted in a considerably attenuated drop in plasma pH, the values being ~7.75 and 7.4, respectively, most likely because of an uninhibited gas exchange and an almost constant plasma PCO_2 . Under both conditions blood lactate rose from ~0 to ~20 mM, indicating an increased dependence on anaerobic energy liberation. Relative to plasma pH, pH_i of different tissues (for heart, see sect. III B) was affected less and to more similar extents by the two conditions (368). Activity also produces acidosis. This is predominantly of the metabolic type, since the animal hyperventilates to counteract elevations of PCO_2 (185). As an example, blood pH of the rattlesnake was found to fall from 7.45 to 6.82 following intense exercise (295).

B. Acidosis and Cardiac Intracellular pH

1. In vivo

Existing information about cardiac pH_i during acidosis *in vivo* does not include short-term effects. Values have been obtained with the 5,5-dimethoxyazolieno-2,4-dione (DMO) method, which might require one-half an hour or so for equilibration (266). Cardiac pH_i so assessed was generally less affected than plasma pH by acidotic challenges and was essentially the same during water and air breathing in the facultative air-breathing fish *Symbranchus marmoratus* (165). A moderate decline from 7.1 to 6.85 was found for turtle myocardial pH_i during forced diving or N_2 exposure (368). Cardiac pH_i fell by ~0.2 units in the toad *B. marinus* during exposure to ~5 kPa of CO_2 (344). The cardiac cell in the living animal, thus, appears to possess efficient mechanisms to resist acidotic challenges by passive buffering or active pH regulation. A study of single myocardial cells from guinea pig suggests that passive buffering has been overestimated in the past, when measured in multicellular preparations (41). Probably, the ability to resist changes in pH_i *in vivo* depends mainly on active pH regulation. In cardiac muscle, this seems to rely primarily on an extrusion of H^+ in exchange for Na^+ (e.g., Refs. 1, 78) and on an $\text{Na}^+-\text{HCO}_3^-$ cotransport (213, 228). In evaluating the impact of these two mechanisms, it is important to note that they seem to be modulated by pH_i , such that the upward shift in pH_i stops before equilibration is attained (213).

Due to methodological problems, short time effects of acidosis on cardiac pH_i *in vivo* seem not to have been recorded. These effects should be expected to be significantly enlarged relative to those after active pH restitution has had time to act. Active regulation of cellular pH seems to occur over considerable time (32). Furthermore, the drop in cardiac pH_i as measured with the DMO method may be underestimated when cells produce acid metabolites and an outward acid gradient in

their close vicinity. As a result, the concentration of undissociated DMO around and in the cell may be underestimated and pH_i overestimated (266).

2. In vitro

In isolated myocardial preparations acidosis may impose long-term lowering of pH_i . Thus, upon elevation of CO_2 of the perfusing gas from 5 to 10%, pH_i fell rapidly then more slowly by ~1.5 units in isolated toad myocardium as recorded continuously by the light absorbance of an intracellular applied dye (315). In resting myocardial tissue from turtle bathed in 30 mM HCO_3^- , pH_i as assessed with the DMO method fell from 7.13 to 6.74 30–60 min after an elevation of CO_2 from 3 to 13%. In trout myocardium exposed to similar treatment, the corresponding pH_i values were 7.34 and 6.99 (133). Two studies of isolated turtle heart with ^{31}P -NMR provide information about effects of hypercapnic and lactic acidosis under both oxygenated and anoxic conditions. Acidosis imposed by 35 mM lactic acid (extracellular pH 7.0) caused pH_i to fall from 7.42 to 6.88 over the first hour. Then, a weak recovery occurred so that pH_i increased to 7.14 at the fourth hour of acidosis (365). No such recovery occurred during acidosis imposed by elevations of CO_2 in the range of 3–60% (186).

The data presented seem to indicate that the ability to avoid a cellular acidosis is lower in isolated preparations than in the cardiac muscle *in vivo*. This difference may relate to factors outside the myocardial cell. Hence, intra- and extracellular compartments were acidified in the cited studies. Drops in extracellular pH are typically counteracted by different mechanisms in the living animal. This may protect cellular H^+ excretion. It should be recalled that sarcolemmal Na^+-H^+ exchange is inhibited by lowering of extracellular pH (357).

C. Acidosis and Contractility

1. Influence of alterations in partial pressure of carbon dioxide or bicarbonate

Because CO_2 relative to HCO_3^- easily crosses the sarcolemma, a given decrease in extracellular pH due to an elevation of PCO_2 causes a more rapid and larger drop in pH_i than one due to a lowered HCO_3^- (98). Accordingly, it also inflicts larger and more rapid changes in contractility, as has been shown for turtle atrial (382) and flounder and cod ventricular tissue (136, 284). Hypercapnic acidosis with the low values of PCO_2 and HCO_3^- typical of fish had negative contractile effects in lowering both the single contraction and contraction frequency in hearts perfused *in situ* of ocean pout (*M. americanus*) and sea raven. Only short-term effects were recorded, since the acidotic exposure lasted for 5 min (113).

The immediate effect of acidosis is to depress myo-

cardial contractility, but during acidosis obtained by elevations of CO_2 , subsequent increases in force have been observed. This recovery varies largely among species and is one important aspect of the large species-dependent differences in myocardial performance at least during hypercapnic acidosis. Thus performance is high in turtle and viper (*V. berus*) myocardia but low in several fish such as trout and cod mainly due to the different ability to recover force. This was interpreted as an adaptation to the higher levels of blood and tissue CO_2 associated with air breathing (135, 283). Flounder heart tissue, however, exhibits a recovery potential as high as that of turtle (e.g., Ref. 284). Tentatively, this may be of adaptational value in this fish, which frequently is burrowed in bottom mud, so that water exchange and excretion of CO_2 is likely to be impeded (222). Caution should be exercised, however. The levels and changes of PCO_2 and HCO_3^- applied in many of the studies on isolated tissue are within the physiological range for air breathers, but often far above those encountered by water breathers. This raises questions about the importance *in vivo* of the reactions observed with isolated preparations as pointed out by Farrell (108). Conceivably, the recovery recorded under a hypercapnia, not likely to be encountered *in vivo*, may represent mechanisms for maintaining performance under cellular acidosis in general. For instance, such mechanisms may be part of the response to hypoxia (see sect. II E4), which is likely to imply an acidotic challenge.

Apart from influencing the single contraction, acidosis also tends to diminish the frequency of contraction (113, 382). As assessed with turtle cardiac preparations, this effect was about the same regardless of the type of acidosis applied. It seems, therefore, to be mediated by extracellular sites (186, 382).

2. Acidotic depression of contractility

Acidosis primarily seems to depress myocardial contractility by effects on the contractile system, but decreases in energy state may contribute. A ^{31}P -NMR study of isolated turtle heart (365) indicates that the cellular levels of ATP and CP are well preserved during lactic acidosis despite a decrease, although small, in contractility. In fact, the recorded decrease in "free" P_i should improve the phosphorylation potential. However, when turtle heart was exposed to severe acidosis, CP and in the most extreme cases also ATP fell. The decrease in pH_i for this to occur was less for hypercapnia than for lactic acidosis (186).

Under full oxygenation, however, the decreased contractility during acidosis seems mainly to be due to direct effects of acidosis on the contractile proteins. In skinned preparations, lowering of pH depresses force at saturating levels of Ca^{2+} as well as shifting the force- Ca^{2+} relation toward higher Ca^{2+} activities (106). Different reasons for the latter effect have been proposed. Hydrogen ions compete for the Ca^{2+} sites (35), shield the binding sites electrostatically (142), or affect the bind-

ing properties of the Ca^{2+} sites indirectly by affecting some other site on the troponin complex (316).

Simultaneous measurements of force and Ca^{2+} transient in ferret papillary muscle showed that acidosis in intact cells primarily acts by decreasing the Ca^{2+} sensitivity of the contractile proteins. The depression of maximal force that also occurs is probably due to a decrease in the number of mobilizable actin-myosin bridges rather than to a weakening of the single bridges (269). Acidosis also tends to diminish the amount of activator Ca^{2+} by reducing the Ca^{2+} current (208). This has been explained in terms of Ca^{2+} binding at the exterior of the sarcolemma (220).

Studies on mammalian myocardia have further demonstrated effects on the SR. The Ca^{2+} loading and the Ca^{2+} -induced Ca^{2+} release of the SR were both depressed by acidosis (105). As discussed in section I E4, the function of the SR in heart muscle of ectothermic vertebrates is unclear as, consequently, is its response to acidosis. Apart from these effects, acidosis may impair the cooperation of cardiac cells by its tendency to depress gap junctions (319).

3. Recovery of contractility during acidosis

As first noted for the cat (117), myocardial tissue is able to recover initial lost contractility during ongoing hypercapnic acidosis. Within ectotherms, this ability seems to vary largely and to be very high for some species. Some examples are given in Figure 5, which depicts twitch force development of ventricular preparations of flounder (*Platichthys flesus*), cod, turtle, and trout. Upon an increase in CO_2 and a drop of extracellular pH from 7.6 to 6.9 in 30 mM extracellular HCO_3^- , twitch force fell for all preparations, but for flounder and turtle myocardia a subsequent increase occurred after a few minutes, bringing force back to or above the prehypercapnic level (133). Simultaneous measurements on isolated toad myocardium of force and pH_i indicate that this increase in force is not due to a recovery of pH_i , which remained lowered (315). Similar results were obtained for turtle and trout myocardial tissue (133). Moreover, the interspecific differences in force of the cardiac muscle during acidosis did not relate to the large differences in tissue non- HCO_3^- buffer value measured on homogenates (71).

The depression of contractility under acidosis appears mainly to be due to a decreased Ca^{2+} sensitivity of the contractile proteins. Conceivably, the recovery process could involve an enlarged amount of activator Ca^{2+} . In agreement with this hypothesis, cellular acidosis enhanced the resting Ca^{2+} activity and the transient elevation of Ca^{2+} following excitation as measured with aequorin in ferret and rat myocardial tissue. Furthermore, some evidence was obtained that the source for these increases in Ca^{2+} was intracellular (268, 269). Because it was removed by inhibitors of the SR function, the enlarged Ca^{2+} transient was suggested to be due to the elevated resting Ca^{2+} activity, causing an increase in the Ca^{2+} taken up and released by the SR (268).

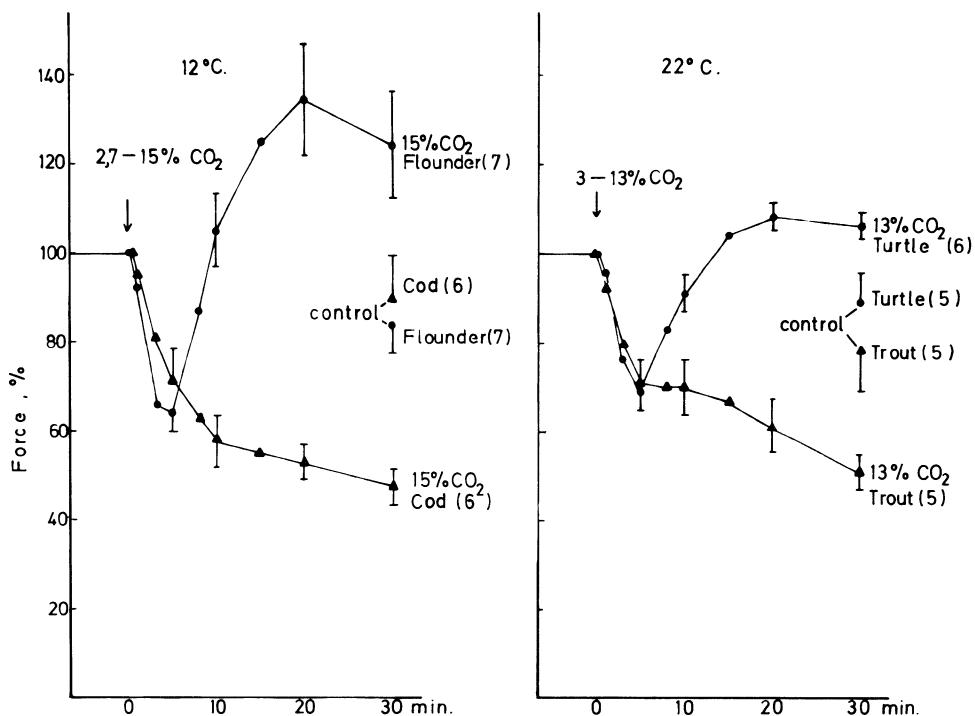


FIG. 5. Isometric twitch force with increased PCO_2 of ventricular strips of 4 ectothermic vertebrates paced at 0.2 Hz. [From Gesser and Jørgensen (133).]

Corresponding direct measurements of the effect of acidosis on the cellular Ca^{2+} are not available for ectothermic myocardia. The contention of an increased cellular Ca^{2+} activity as a cause of the observed recovery is supported by indirect evidence, though, including measurements of Ca^{2+} efflux. According to current models of sarcolemmal transport mechanisms, this efflux should covary with the intracellular Ca^{2+} activity. The Ca^{2+} efflux was enhanced in ventricular tissue upon exposure to an elevation of PCO_2 to a variable extent among species. Notably, this response was not seen for myocardium of trout, which does not exhibit any significant force recovery during acidosis (133). The enhancement of Ca^{2+} efflux occurred in resting as well as contracting preparations. It was recorded also in a nominally Na^+ - and Ca^{2+} -free solution and seems, therefore, not to be due to a stimulation of sarcolemmal exchange of either Na^+ for Ca^{2+} or Ca^{2+} for Ca^{2+} . Moreover, no effect of acidosis on Ca^{2+} uptake could be detected (137). Like the recovery of contractility (136), the enhanced Ca^{2+} efflux seems to depend on an intra- rather than an extracellular acidification in being significantly less, when the concentration of HCO_3^- was decreased than when PCO_2 was elevated (137).

An augmented Ca^{2+} activity may be transformed into an increased Ca^{2+} transient by a stimulation of the excitation-dependent Ca^{2+} influx through the sarcolemma. This possibility is suggested by the effect of changes in the Ca^{2+} activity on channel-dependent influx recorded in frog heart (see sect. IE4) and on the $\text{Na}^+-\text{Ca}^{2+}$ exchange, which may bring Ca^{2+} into the cell during excitation (see sect. IE2). Furthermore, an augmented diastolic Ca^{2+} activity shifts the start value and thus probably the peak value of the Ca^{2+} transient upward (27).

The SR is an obvious candidate in regard to cellular Ca^{2+} stores, which may be released by acidosis. The resting tension of the viper myocardium bathed in a Na^+ - and Ca^{2+} -free solution resembles twitch tension in exhibiting a biphasic response upon an elevation of PCO_2 . This response was not significantly affected by caffeine (135). Moreover, a strong recovery of twitch force during acidosis occurred in myocardia of frog and flounder (135, 136), in which SR appears to be poorly developed (99, 102, 298). Therefore, the recovery does not seem to rely primarily on the SR. This was also concluded in a study of ferret and rat myocardia, in which the SR, however, appears to transform the increase in cellular Ca^{2+} activity into an increased Ca^{2+} transient and contractility (268).

The role of the negatively charged groups at the cytoplasmic leaflet of the sarcolemma as a cellular Ca^{2+} store is unclear (e.g., Ref. 219). They have, however, been suggested to bind Ca^{2+} in a pH-dependent way (231), such that Ca^{2+} is released by acidosis. Although no significant increase in Ca^{2+} uptake in terms of tracer flux could be detected (137), an increase in cellular Ca^{2+} uptake might occur and contribute to the force recovery. As already noted, cellular acidosis is counteracted by sarcolemmal Na^+-H^+ exchange and $\text{Na}^+-\text{HCO}_3^-$ cotransport. Both processes tend to augment cellular Na^+ . As a result, cellular Ca^{2+} tends to increase via the $\text{Na}^+-\text{Ca}^{2+}$ exchange (8, 335, 357). For instance, in rat myocardial cells, a CO_2 -induced acidosis resulted in increases in intracellular Na^+ probably mediated by the sarcolemmal change and in both systolic and diastolic Ca^{2+} . These changes were accompanied by a weak recovery of contractility (159). In many situations, however, these effects may be attenuated by an extracellular decrease in pH, which exceeds the intracellular one. This is because

the $\text{Na}^+ - \text{H}^+$ exchange is stimulated by drops in pH_i , but inhibited by drops in extracellular pH (357). Regarding the increase in Ca^{2+} accompanying that in Na^+ , the question arises as to what extent it is due to a sarcolemmal $\text{Na}^+ - \text{Ca}^{2+}$ exchange, since the mitochondrial $\text{Na}^+ - \text{Ca}^{2+}$ exchange may also contribute.

Mitochondria exchange Ca^{2+} for H^+ as well as for Na^+ (see sect. I E5). Results obtained with myocardium of the viper (*V. berus*) and frog comply with the hypothesis that the recovery of force during hypercapnic acidosis is caused by a shift of Ca^{2+} from mitochondria to cytoplasm (135). For example, the recovery process of frog myocardium was enhanced by an application of cyanide just before PCO_2 was increased. Tentatively, cyanide by inhibiting the respiratory chain facilitates the Ca^{2+} release (135).

The possible mechanisms suggested above for increasing the amount of Ca^{2+} in the E-C coupling all counteract a drop in cytoplasmic pH. The net effect of an acidotic challenge on contractility is, therefore, probably not related directly to the changes in pH_i .

4. Regulation of force under acidosis

Catecholamines are likely to be increased under conditions involving cellular acidosis (e.g., Refs. 108, 366). During acidosis catecholamines have a protective effect on cardiac function in mammals as assessed in open-chested dogs (321) and in isolated myocardial tissue (253). This seems to extend to all vertebrates. Thus epinephrine increased twitch force about twofold in both trout and eel ventricular preparations whether acidotic or not. This response does not relate to the capacity to maintain contractility under acidosis, which is markedly higher for the eel than for the trout myocardium (131). Furthermore, epinephrine diminishes the short-term negative effects of acidosis on contraction and frequency of contractions as demonstrated on *in situ* hearts of ocean pout and sea raven (113). Increases in stroke volume with preload were found to be depressed during short exposures to hypercapnic acidosis as shown with an *in situ* heart preparation of sea raven. This effect of acidosis was removed by α -stimulation (109). It is of interest to further investigations that catecholamines and extracellular ATP influence the mechanisms for sarcolemmal H^+ extrusion (214).

D. Acidosis and Hypoxia in Combination

As already noted in sections II E and III C, dealing with hypoxia and acidosis separately, ectotherms might be exposed to conditions involving oxygen lack and acid loads in combination. In turtles, the aerobic energy liberation is severely depressed during diving. Anaerobic metabolism results in a production of H^+ , which reacts with HCO_3^- and increases PCO_2 of blood and tissues, since CO_2 cannot be excreted by gas exchange (368). Water breathers inhabiting environments rich in plants

and microorganisms are likely to experience substantial lowering of PO_2 and increase in PCO_2 during the night, when respiration proceeds in the absence of photosynthesis (79).

The combined influence of acidosis and hypoxia on cardiac pH_i *in vivo* was discussed in section III B1. Regarding isolated preparations, simultaneous exposure to anoxia did not change the drop in pH_i of turtle heart due to increases in CO_2 . In contrast, the decreases in pH_i imposed by lactic acid were markedly enhanced by anoxia (186). The reason for this difference between lactic acid and CO_2 acidosis is unclear at present (186), and information about other ectotherms in this respect is lacking.

Application of hypercapnic acidosis (from 1 to 10% CO_2 in 30 mM HCO_3^-) together with anoxia as compared with either condition alone increased loss of force in ventricular preparations of trout and eel (265). As one exception already discussed (see sect. III C3), myocardium of frog transiently developed greater twitch force during a respiratory chain block combined with hypercapnic acidosis than during either condition alone (135). The proposal that acidosis preserves cellular integrity during hypoxia by lowering the energy demand (34) was not supported by measurements of contractility and energy state in turtle heart. Here, both parameters were more depressed during anoxia combined with acidosis than during anoxia alone (186, 365). The depression of glycolysis by H^+ should be recalled in this context (320). However, the turtle heart unlike that of mammals and, for instance, that of flounder (see sect. II E6) showed a complete recovery of contractility and energy state after exposure to hypoxia and acidosis in combination (365) and to ischemia (367) such that no irreversible damages were noted. With no renewal of the solution surrounding the cells, ischemia implies oxygen lack, substrate depletion, and acidosis due to accumulation of acid products, primarily lactic acid (367). The as yet unknown reason for this protection of the turtle heart may implicate an efficient downregulation of energy demand (367). Such protective mechanisms have already been discussed (see sect. II E6).

Protective mechanisms are likely to be mediated by cellular Ca^{2+} . Thus it should be noted that acidosis together with anoxia attenuates the influence of extracellular Ca^{2+} more than either anoxia and acidosis alone in ventricular tissue of the frog (212), trout and eel (265), and turtle (364). This result is to be expected from the depression of the contractile system by elevations of P_i due to anoxia and acidosis acting together.

E. Conclusions

In the living animal, short-term decreases in pH_i are probable, whereas pH_i appears to be well protected given enough time for extra- and intracellular pH regulatory mechanisms to act. Decreases in pH_i tend to depress contractility by affecting both the Ca^{2+} affinity and the maximal Ca^{2+} -activated force of the contractile

system. These negative effects may be opposed to an extent that varies among species by mechanisms that tend to enlarge the amount of Ca^{2+} in the E-C coupling at the same time as they also may counteract a drop in cytoplasmic pH. These mechanisms implicate the cellular Na^+ balance and intracellular Ca^{2+} stores in ways that remain to be identified. An acidotic challenge may, therefore, influence mechanical performance in a complex way that does not relate directly to the change in pH_i .

IV. IMPACT OF LOW TEMPERATURE ON CONTRACTILITY AND METABOLISM

A. Acute Temperature Transitions Above Freezing

1. Performance *in situ*

In fish and amphibians, decreases in temperature within the physiological range result in decreases in heart rate with a Q_{10} of ~ 2 (108, 129, 175, 239). Simultaneous information on pressure development and cardiac output is minimal. In winter flounder (*P. americanus*), arterial blood pressure is maintained between 5 and 15°C following both acute transitions and seasonal acclimatization; cardiac output decreases as temperature is decreased (57). Dorsal aortic blood pressure is constant in eel tested at seasonal temperatures of 8 and 16°C (279). In contrast, an acute transition from 10 to 5°C results in a marked decrease in ventral aortic pressure of sea raven from 4.2 to 0.18 kPa (17). This response may be associated with sea raven becoming quite torpid at low temperatures. In lingcod (*O. elongatus*), an acute temperature decrease from 15 to 4°C results in a decrease in cardiac output and heart rate as stroke volume remains constant (324). Similarly, decreases in temperature result in large decreases in cardiac output ($Q_{10} = 4$) of quiescent trout (22). Arterial blood pressure decreases in bullfrog (*R. catesbeiana*) with a decrease in body temperature (370). The general picture that emerges is that although blood pressure may be maintained in some species overall, heart power output declines in concert with lowering of environmental/body temperature.

2. Performance of isolated preparations

The rate of contraction of spontaneously beating fish (42, 146, 226, 239, 346, 347) and amphibian (156, 244, 323) heart preparations always decreases with a decrease in temperature. A decrease in temperature results in slowing of rate of the isolated endothermic heart as well; however, for nonhibernators, cardiac arrest occurs between 10 and 25°C. This temperature is much higher than that for either ectotherms or hibernating endotherms (130).

The impact of acute temperature transitions on the performance of electrically paced ventricle preparations has been assessed in a number of teleosts and leads to the following generalizations (18, 19, 183, 224, 239). A decrease in temperature results in a lengthening of the times to peak tension and relaxation. In teleost preparations contracting at low frequencies, temperature decreases within the physiological range have very little impact on the ability to develop force and in some cases actually result in an increase in force development. At elevated contraction frequencies, however, force development is compromised by low temperature due to either increases in resting tension or inability to follow imposed pacing regimes.

The situation with respect to tension development appears different in electrically paced ventricle strips from the frog (*R. temporaria* and *Xenopus laevis*; Ref. 211). Preparations induced to contract at a rate of 12/min and taken through a temperature transition from 25 to 5°C showed marked decreases in tension development. The difference in response between fish and amphibians may relate to the latter species entering torpor at low temperatures.

3. Contractile mechanism and excitation-contraction coupling

Temperature influences cardiac twitch contractility by several mechanisms. Lowering of temperature decreases the force generated by attached cross bridges. Thus the maximal Ca^{2+} -activated force was found to be decreased in myocardia of the frog (*R. pipiens*) (158, 293) and of two mammalian species (158). In addition, the Ca^{2+} sensitivity of the contractile system has been shown to decrease (77, 158), although exceptions have been recorded (103). Species differences indicating adaptation to the working temperature have been recorded. Thus, within the range of 29–1°C, the Ca^{2+} activity needed for generation of half-maximal force in skinned myocardial tissue is lower for frog than for rat and guinea pig (158). In spite of the decrease in maximal force and Ca^{2+} activity, lowering of temperature often causes increases in twitch tension in myocardial tissue of the usual experimental vertebrates (i.e., mammals and amphibian species). The increase in force is probably due to an enhanced Ca^{2+} availability to the contractile system. Hence, prolongations of the action potential have been recorded upon lowering of temperature in mammalian and amphibian species (e.g., Ref. 38) and in flounder (*P. flesus* L.) (224) and trout (249). As shown for bullfrog atrial tissue, this probably is due to a potentiated Ca^{2+} current, indicating an increased Ca^{2+} influx (145). Furthermore, a prolonged depolarization will favor an inward shift of Ca^{2+} by the electrogenic sarcolemmal $\text{Na}^+-\text{Ca}^{2+}$ exchange. This tendency will be further emphasized in that decreases in temperature may depress the Na^+-K^+ -ATPase activity and increase intracellular Na^+ as shown for cat heart muscle (272). Consistent with this, flux studies with radioactive Ca^{2+} on iso-

lated flounder heart tissue revealed that cellular Ca^{2+} uptake decreased as temperature was elevated above 10°C, taken to be the ambient temperature of the flounders studied (224).

Apart from affecting sarcolemmal Ca^{2+} fluxes, temperature affects the SR activity. As discussed in section I E 4, the importance of the myocardial SR in ectothermic species is unclear. For instance, it is strongly expressed under certain conditions in the force development of the trout myocardium (183, 184). In particular, a marked influence of temperature has been recorded in this tissue. Hence, the sensitivity to ryanodine, an inhibitor of SR function, fell from being strongly developed at 20–25°C to insignificance at 10–5°C (183). Similarly, lowering of temperature in the interval of 37–22°C diminished the relative importance of the SR to the E-C coupling in rat and rabbit ventricular muscle (304).

In conclusion, lowering of temperature tends to diminish actin-myosin interaction at a given Ca^{2+} activity. At the same time, however, it tends to increase both the amount and the time of action of activator Ca^{2+} . The net effect on twitch contractility varies and may be positive as well as negative.

4. Metabolism in isolated preparations

Simultaneous measurements of oxygen consumption and performance following abrupt changes in temperature have been obtained with a few species of teleost fish. Isolated spontaneously beating goldfish hearts immersed in Ringer solution have higher rates of contraction and oxygen consumption at 15°C than at 10°C (350). Studies have been conducted with perfused isolated hearts generating power outputs of ~1 mW/g, a level close to in vivo resting conditions. Sea raven (with glucose alone as a metabolic fuel), pickerel, and American eel hearts perfused with medium containing low levels of Ca^{2+} all showed lower rates of oxygen consumption in association with lower temperatures. Changes in overt power output were small but in the same direction as the alterations in oxygen consumption (17, 18). An elevation in extracellular Ca^{2+} resulted in a twofold increase in oxygen consumption by sea raven hearts but no change in pickerel and American eel hearts. At least for sea raven it appears that neither the electron transport system nor fuel utilization was the limiting factor in performance at the lower calcium level.

Perfusion of sea raven hearts with alternative fuel sources has revealed a complex and yet to be resolved role for fatty acids at low temperature (302). Hearts receiving glucose alone as an exogenous fuel showed a decrease in power output and oxygen consumption at 5°C relative to 15°C. The inclusion of palmitoleate (a 16-carbon fatty acid with 1 double bond) in the medium enhanced both oxygen consumption and performance at 5°C only, such that there was no difference in these parameters between the two temperatures. The simplest explanation for the finding is that fatty acid metabo-

lism is facilitated at low temperatures and provides the necessary energy to support contractility. Attempts to directly test this hypothesis with radiolabeled fuels were unsuccessful, since $^{14}\text{CO}_2$ production from either [6- ^{14}C]glucose or [1- ^{14}C]palmitate yielded only ~2% of predicted rates on the basis of oxygen consumption. Label was incorporated into glycogen and lipid pools at similar rates at both 5° and 15°C and also probably remains in the acid-soluble fraction as shown for rat tissue preparations (358).

5. Impact of temperature on enzyme activities

The impact of acute temperature transitions within the physiological range on maximal in vitro enzyme activity has been assessed for key enzymes of energy metabolism (18, 70, 267, 302, 303). A decrease in assay temperature always results in a decrease in HK activity which follows a Q_{10} of 2.63 ± 0.29 (mean \pm SE for 7 species of teleosts). Temperature transitions have a much lower impact on CS activity which follows a Q_{10} of 1.60 ± 0.08 (8 species of teleosts plus turtle).

The relationship between temperature and the aerobic oxidation of fatty acids as metabolic fuels is the subject of much interest. The oxidation of fatty acids is dependent on the sequential action of two mitochondrial membrane-bound carnitine fatty acyl CoA transferases, which facilitate the movement of fatty acid derivatives across the mitochondrial membrane to the site of β -oxidation. Because palmitate is the fatty acid most commonly investigated, the enzyme system is usually referred to as carnitine palmitoyl CoA transferase (CPT). Carnitine palmitoyl CoA transferase I converts palmitoyl CoA to palmitoyl carnitine, which is translocated across the inner mitochondrial membrane and subsequently reconverted to palmitoyl CoA by CPT II for entry into the β -oxidation spiral (355). In some muscle types, CPT is considered to be the rate-limiting step in fatty acid oxidation (68). The effect of temperature change on the in vitro activity of CPT appears to be quite species specific. In sea raven and smallmouth bass, the Q_{10} was very steep at 5.5 and 9.9, respectively. In longhorn sculpin (*Myoxcephalus octodecemspinosis*) it was 2.2, but in four other species of teleosts, a change in assay temperature did not alter the activity of CPT. In some species the direct impact of temperature may be very substantive at this locus and as such may be an important regulatory site not only for fatty acid oxidation but also for the control of intracellular levels of fatty acids that modify function of membrane channels (271).

B. Chronic Low-Temperature Exposure Above Freezing

Many ectotherms are exposed to large changes in temperature on a seasonal basis. In this section information from laboratory studies involving temperature

acclimation and acclimatization under field conditions is reviewed.

1. Nonpolar teleosts

Some (but not all) teleosts show an enhancement of swimming performance following acclimation to low temperature (297). Such behavior, which must be dependent on cardiac performance, may allow for active foraging and/or escape from predators at winter temperatures. Evidence for adaptive responses in heart which presumably underwrite additional demands of blood supply either during or following activity periods is summarized below.

One frequently observed response to low temperature is an increase in heart mass relative to body mass that is typically in the range of 10–25% following an acclimation period of a few weeks to a temperature 10–15°C lower (111, 144, 147, 203, 303, 350). Cardiac growth does not result in changes in protein content per gram heart weight (111, 203). An increase in heart size following acclimation to low temperature need not always occur, since no changes were noted in white perch (*Morone americanus*) or yellow perch (*Perca flavescens*) (303), species which are active at low temperatures. Moreover, an increase in heart mass may occur in species that are quite sedentary at low temperature such as smallmouth bass.

Maximal performance levels were determined for *in situ* perfused trout hearts. Hearts from animals acclimated to 5°C generated ~60% of maximal power output per gram as hearts from fish acclimated to 15°C when both were tested at their respective holding temperature (147, 148). The larger heart size of the cold-acclimated animals contributed to enhanced stroke volumes at low temperatures, and consequently, there was considerable overlap between the two groups in the maximal level of power output per heart. Acute effects were not assessed for either 5 or 15°C acclimated fish, so it is not known if additional adaptational responses occurred.

Positive thermal adaptation has been demonstrated in *Perca*, which remain active at winter temperatures. In *P. flavescens* acclimated to 20°C, the maximal rate of contraction of electrically paced ventricle strips was >48 beats/min at 20°C but only 30 beats/min at 5°C. Moreover, at 5°C, twitch force development decreased substantially with an increase in contraction frequency. After acclimation to 5°C, hearts could be paced at frequencies >48 beats/min, and twitch force development was enhanced. The enhanced chronotropic responsiveness was associated with about a 25% decrease in time to relaxation (19). Acclimation to low temperature did not result in changes in HK, CS, or carnitine acyl CoA transferase activities in *P. flavescens* (303). Atria from *P. fluviatilis* acclimated to 5°C had higher rates of spontaneous contraction at low test temperatures than atria from animals acclimated to 19°C, and similarly, noncontracting isolated hearts had

higher rates of oxygen consumption. Low temperature acclimation also resulted in an increase in the volume density of SR but no alteration in mitochondrial volume density or myoglobin content (42). The situation with respect to thermal acclimation in *Perca* seems to be both clear and consistent. Low-temperature acclimation results in alterations in ability to maintain higher rates of contraction and presumably cardiac output with no increase in heart mass. The increase in SR is possibly related to shorter relaxation times and subsequently higher maximal contraction frequencies. The metabolic machinery necessary to supply ATP is quite adequate and requires no further expansion as evident by constant mitochondrial volume density, constant activity of the mitochondrial marker enzymes, and myoglobin content.

Goldfish hearts also exhibit enhanced performance following low-temperature acclimation. Spontaneously contracting, isolated preparations from cold-acclimated goldfish had higher frequencies and/or amplitudes of contraction and higher rates of oxygen consumption per gram than hearts from warm-acclimated animals at low test temperatures (347, 349, 350). At low test temperatures, glucose had a protective effect on performance of hearts isolated from warm-acclimated but not cold-acclimated goldfish, suggestive of alterations in ability to utilize exogenous glucose as a function of thermal history (348).

Positive thermal adaptations in cardiac energy metabolism are evident in some species. Hearts from pickerel acclimated to 5° have higher levels of HK, CPT, CS, and cytochrome oxidase than hearts from animals acclimated to 25°C. Similarly, animals sampled in winter had higher enzyme activities than those sampled in summer (369). Carp (*C. carpio*) showed an increase in cytochrome oxidase following acclimation to low temperature (53), and white perch hearts exhibited a two- to fourfold increase in activity of CPT and carnitine oleoyl CoA transferase (oleate is a 18-carbon fatty acid with 1 site of unsaturation) following low-temperature acclimation (303). The latter finding suggests an enhanced ability to oxidize unsaturated fatty acids. Increases in enzyme activities are taken to mean an increase in the concentration of enzyme as opposed to the expression of specific isozymes at different temperatures. The fish species in which there are increases in cardiac enzyme activities as a function of cold temperature acclimation all show maintenance of whole animal swimming performance at low temperatures.

Positive thermal adaptation in cardiac performance and/or metabolism does not occur in all species. *In situ* performance of perfused sea raven hearts from summer and winter acclimation conditions was assessed (146). Inherent heart rate of summer fish was higher at both 14 and 4°C test temperatures. There was no evidence for a positive thermal compensation in power output, since hearts from summer animals tested at 4°C exhibited marginally higher levels than hearts from winter animals tested at 4°C. These findings are consistent with the very low activity patterns that sea raven in

captivity exhibit at low temperatures. Acclimation to low temperature did not result in changes in HK, CS, or CPT in white perch, yellow perch, or smallmouth bass (303).

2. Antarctic teleosts

Fish living in the Antarctic have evolved at temperatures close to 0°C. In icefish (*Channichthyidae*), which lack hemoglobin, oxygen delivery is achieved via immense cardiac outputs per kilogram body facilitated by especially large hearts (0.3% body wt) (115, 157, 179, 193). In *Chaenocephalus aceratus* at 1°C, heart rate is ≈16 beats/min, mean ventral aortic pressure is ≈2 kPa, and cardiac output is 20–40 ml · min⁻¹ · g heart⁻¹ (171, 179). Heart rate and cardiac output are similar to temperate zone fish operating at higher temperatures; ventral aortic pressure development is one-half that of a temperate zone teleost. Cardiac power output is ~1 mW/g in *C. aceratus*, which is similar to hemoglobin-containing Antarctic teleosts at 0°C (11). The particularly large heart size in the hemoglobinless icefish is not an adaptation to cold temperature alone, since other hemoglobin-containing Antarctic teleosts have heart sizes within the range typically encountered in nonpolar species (11, 179, 193). Aspects of energy metabolism relevant to a lack of myoglobin in heart are discussed in section II.B.

Maximal in vitro activities of enzymes of energy metabolism have been assessed for five species of hemoglobin/myoglobin-containing Antarctic fish and two species of icefish (70, 115, 116, 310). After adjustments for assay temperature, on the basis of a Q₁₀ of 2, activities (concentrations) of enzymes of carbohydrate metabolism (i.e., HK, PFK, PK) are similar in Antarctic fish and other ectotherms. However, activities of mitochondrial enzymes necessary for aerobic fatty acid catabolism (i.e., CPT, 3-hydroxyacyl CoA dehydrogenase, CS, cytochrome oxidase) and LDH are significantly higher in Antarctic fishes than in other ectotherms. This general contention is consistent with the comparison of enzyme activities in heart from two species of Antarctic fish with two temperate zone species of similar body form and behavior (70). Overall, the enzyme data suggest that in Antarctic species there is an increase in the concentration of enzymes necessary for a fatty acid-based aerobic metabolism to compensate for low temperature. This response is the same as that occurring in some species of temperature zone teleosts acclimated to cold temperature.

3. Amphibians

In contrast to some teleosts, amphibians enter states of dormancy when exposed to winter temperatures. Under these conditions whole animal activity, oxygen consumption, and cardiac performance are curtailed relative to levels at warmer temperatures. With

the exception of freeze-tolerant terrestrial frogs, oxygen availability should not be a factor in low-temperature survival, since air-breathing species may continue to ventilate lungs and aquatic animals may utilize cutaneous respiration.

The heart of the frog (*R. temporaria*) shows clear alterations in response to laboratory acclimation or seasonal acclimatization. Isolated perfused hearts from animals acclimated to low temperature have lower contraction frequencies over a wide range of test temperatures than hearts from warm-acclimated frogs (156). The same relationship is found for animals sampled directly from the field in winter or summer (156, 314).

In intact wood frogs (*Rana sylvatica*) and bullfrogs, there is a lengthening of the P-QRS interval of the electrocardiogram as temperature is acutely decreased from 15 to 5°C. After 48 h at the low temperature, the P-QRS interval becomes even longer (230).

Toads also exhibit seasonal changes in cardiac performance capabilities and metabolism. Perfused isolated hearts from winter animals had lower rates of contraction and lower stroke volumes (although maximum pressure development was higher) than hearts from summer animals when both were tested at 25°C. Hearts from winter animals, even when perfused with medium containing glucose, had a respiratory quotient of ~0.75, indicative of fatty acid oxidation. Hearts from summer animals had a respiratory quotient of ~0.95 (indicative of a carbohydrate metabolism) when glucose was available; however, in the absence of glucose this decreased to 0.75 (255).

Glucose uptake and ¹⁴CO₂ production from labeled glucose were higher in heart slices from summer toads than winter toads at a common assay temperature (124). The performance of ventricle strips, electrically paced at 6 beats/min, was assessed at 31°C (276). Tension development, velocity of contraction, and velocity of relaxation were all lowest in winter then increased gradually, reaching a maximum in summer. Elevation in extracellular Ca²⁺ had little effect on tension developed by heart from winter animals but had a marked positive inotropic effect on hearts from summer animals. As well, high extracellular Ca²⁺ reduced the length of action potential duration in summer but not in winter toads. A seasonal alteration in an extracellular Ca²⁺-modulated component of the myocardium is highly implicated by these data.

4. Reptiles

In aquatic turtles, low winter temperatures are always associated with oxygen limitation, since animals are denied access to air. Responses to hypoxia and acidosis have been discussed above. Studies that address the direct impact of temperature are limited. Maximal oxygen consumption per milligram protein, with some substrates, was lower with mitochondria isolated from cold- than warm-acclimated turtles at the same test temperature (200). This observation is consistent with

lower activities of mitochondrial enzymes following anoxic periods (267, 311). Acclimation of turtles to 3°C with access to air resulted in a decrease in heart levels of ATP and an increase in CP with respect to warm-acclimated animals (234). The decrease in temperature probably resulted in an increase in pH_i, which in turn would shift the equilibrium of the CK reaction in favor of CP. There was no difference in the expression of heart LDH isozymes between cold- and warm-acclimated turtles (25).

C. Low-Temperature Exposure of Freeze-Tolerant Frogs

At least four species of terrestrial frogs (*R. sylvatica*, *Hyla versicolor*, *Hyla crucifer*, *Pseudacris triseriata maculata*) survive freezing at temperatures of about -3 to -7°C. The heart does not beat in the frozen state and appears pale probably because of withdrawal of blood into distended large vessels above the heart (330). The exceptional ability of the wood frog (*R. sylvatica*) to maintain heart performance was evident by continued spontaneous contractility of isolated hearts down to -2°C and in intact animals the maintenance of amplitude of the QRS wave of the electrocardiogram between 15 and 0°C, conditions under which the QRS of the bullfrog deteriorated (230).

In some species, intracellular glucose accumulates and appears to act as a cryoprotectant and metabolic fuel. Acute transitions of intact wood frogs to -2.5°C resulted in an increase in intracellular glucose from ~0 to ~150 μmol/g. Heart glucose was derived from the plasma as glycogen levels remained constant. Under frozen conditions, oxygen delivery to the heart by diffusion alone must be very low, and lactate accumulates. Freeze-thaw cycles of 96 h resulted in oscillations in glucose levels such that glucose decreased when temperature was elevated and increased again in response to freezing. Lactate takes longer to clear than glucose, and levels as high as 70 μmol/g were noted after three cycles (329). Ventricle strips from wood frog survived freezing in vitro, in the presence of high levels of glucose, and could be stimulated to contract upon thawing. Hearts from grass frogs (*R. pipiens*) did not tolerate freezing in keeping with freeze intolerance at the whole animal level (54).

D. Conclusions

Isolated preparations from ectothermic myocardia reveal that an acute decrease in temperature usually results in decreases in heart performance associated with lower rates of contraction, longer time to peak tension, longer time to relaxation, a lengthening of the action potential, and a decrease in oxygen consumption. The effect of a decrease in temperature on contractility represents a compromise of two tendencies. Force development tends to fall due to a decrease in actin-myosin interactions at a given state of activation. This is coun-

teracted by an increase in both the amount and time of activator Ca²⁺. Decreases in temperature usually result in an impairment of maximal in vitro levels of enzyme activities, although the Q₁₀ for CS in particular may be very low. Energy metabolism is not the limiting factor in performance following acute thermal decrease; however, in some species there may be marked decreases in fatty acid catabolism.

Some nonpolar fish species exhibit positive thermal compensation in response to seasonal low temperatures. This is evident by increases in rates of contraction and ability to maintain force development at elevated frequencies. One component of this response is a decrease in time to relaxation. An increase in heart mass is a frequent response to low temperature. Both increases in maximal contraction frequency and heart size could contribute to enhanced maximal cardiac output. In some species there is an increase in metabolic potential due to elevated levels of some enzymes of energy metabolism. Most frequently observed are increases in mitochondrial enzymes necessary for aerobic fatty acid catabolism. In Antarctic species that live their entire life cycles at temperatures close to 0°C, there are higher concentrations of enzymes necessary for a fatty acid-based aerobic metabolism relative to other fish species.

In contrast to those fish that exhibit positive thermal compensation, cardiac performance in amphibians that do not freeze appears to be decreased following a period of low-temperature acclimation. This is based on rates of contraction, electrical properties, and tension development. A reduction in contractility is consistent with whole animal responsiveness and cardiac demands. In amphibians there is a shift in fuel preference from a carbohydrate- to a lipid-based metabolism as occurs in some fish species.

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