J. Exp. Mar. Biol. Ecol., 1988, Vol. 121, pp. 165–176
Elsevier

JEM 01125

Tissue-specific biochemical responses during anoxia and recovery in the channeled whelk

John C. Eberlee and Kenneth B. Storey
Institute of Biochemistry and Department of Biology, Carleton University, Ottawa, Ontario, Canada

(Received 24 February 1988; revision received 24 May 1988; accepted 3 June 1988)

Abstract: Anaerobic metabolism was monitored in five tissues of the channeled whelk *Busycon canaliculatum* (L.) over a time course of 24 h of experimental anoxia followed by 6 h of aerobic recovery. All tissues accumulated succinate and alanine as metabolic end products during anoxia and utilized glycogen and aspartate as substrates. Total end product accumulation was highest in ventricle (succinate + alanine = 32.5 μmol $\cdot$ g^{-1} wet weight), lowest in hepatopancreas (1.4 μmol $\cdot$ g^{-1}), and intermediate in other tissues, suggesting differing rates of metabolism in individual tissues during anaerobiosis. Patterns of end product accumulation also varied between tissues. Gill and foot muscle depended upon the coupled conversion of glycogen to alanine and aspartate to succinate for ATP production throughout anoxia. Ventricle and radular retractor muscle, however, largely limited alanine accumulation and aspartate utilization to the first 8 h of anoxia; subsequently, the conversion of glycogen to succinate became the dominant ATP-producing pathway. Tissues also varied in their metabolic responses during recovery in aerated seawater. Ventricle and gill rapidly cleared accumulated end products and restored aspartate reserves within the 6-h recovery period. Foot and radular retractor, however, showed little or no change in metabolite levels over the recovery period. Imino acids (octopine, alanopine, strombine) were not accumulated in significant amounts in any tissue during anoxia but all three accumulated rapidly in the ventricle during the aerobic recovery period.

Key words: Anaerobiosis; *Busycon canaliculatum*; Glycolysis; Organ-specific metabolism

Introduction

Numerous species of molluscs, particularly those inhabiting the marine intertidal zone, have well-developed capacities for survival in the absence of environmental oxygen. Anaerobic metabolism has been well studied in a number of bivalve species, most extensively in *Mytilus edulis* (for review, see de Zwaan, 1983). In general, succinate and alanine are the major products of anaerobic fermentation with other compounds including propionate, acetate, octopine, alanopine, and strombine accumulating in some species. The fuels for anaerobic metabolism are glycogen and aspartate. This pattern is largely the same for gastropod species although fewer and less thorough studies have been made. Accumulation of succinate and alanine has been noted in several species (Wieser, 1980; Ellington, 1981, 1982; Storey *et al.*, 1982; Livingstone & de Zwaan,

Correspondence address: K.B. Storey; Institute of Biochemistry and Department of Biology, Carleton University, Ottawa, Ontario K1S 5B6, Canada.

1983; Klutymans & Zandee, 1983; Rao & Rao, 1983) but limited attention has been given to tissue-specific metabolism or to metabolic changes over time in the anoxia state. Exceptions to this are studies of anaerobic metabolism in the isolated ventricle and radula retractor of the whelk (Ellington, 1981, 1982) and an examination of the time course of whole animal substratum utilization and product accumulation in two cerithiid snails (Rao & Rao, 1983).

The present study is a comprehensive examination of tissue-specific metabolism in the channeled whelk *Busycon canaliculatum* (L.) over a time course of anoxia and aerobic recovery. The very large size of the whelk makes this species particularly useful for studies of individual tissue responses. Indeed, the species has already become a valuable model system in our laboratory for analysis of tissue-specific enzyme control during anoxia (Plaxton & Storey, 1982, 1983, 1984a,b, 1985; Eberlee & Storey, 1984; Storey, 1984, 1985, 1988). The results show marked differences in the metabolic patterns and metabolic rates of individual tissues of *B. canaliculatum* in response to environmental anoxia.

Materials and Methods

Animals and Chemicals

Whelks were purchased from the Marine Biological Laboratory, Woods Hole, Massachusetts, and were held without feeding in aerated, recirculating seawater at 18 °C for 3–6 wk before use. Experiments were conducted during September and October. All biochemicals were purchased from Sigma Chemical, St. Louis, Missouri, or Boehringer Mannheim, Montreal, Canada. Alanopine and strombine dehydrogenases were prepared as previously described (Storey *et al.*, 1982). Purified succinyl-CoA synthetase was a gift from W. Bridger, University of Alberta, Edmonton, Canada.

Anoxia and Recovery

Control, aerobic whelks were sampled directly from the seawater tank. To impose anoxia, animals were removed from the tank and placed in large jars in air. These were flushed with nitrogen gas for 20 min and then sealed. Anoxic animals were held at room temperature (20 °C). At intervals of 4, 8, 12, and 24 h, animals were samplied. Shells were quickly removed and tissues were dissected out, blotted, and immediately frozen in liquid nitrogen. For recovery experiments, whelks were given 24-h anoxia exposure and were then returned to the aerated seawater tank and sampled after 2 and 6 h. Frozen tissues were stored at − 80 °C until use.

Sample Preparation

Samples of frozen tissues (up to 0.5 g) were ground to a powder under liquid nitrogen and then transferred to preweighed, polypropylene centrifuge tubes stored on dry ice.

After a second weighing to determine tissue weight, tubes were transferred one at a time to a dry ice–methanol bath at −8 °C. Four vol (w/v) of 6% perchloric acid containing 1 mM EDTA (chilled at −8 °C) were quickly added and the tissue was then immediately homogenized using a Polytron PT 10 homogenizer. A 50-μl aliquot of homogenate was removed at this point to use in glycogen determination. The remainder of the homogenate was centrifuged at 6000 × *g* for 15 min at 4 °C to pellet precipitated protein. The acid supernatant was removed and neutralized by addition of 3 N KOH/0.3 M imidazole/0.4 M KCl. Precipitated $KClO_4$, was removed in a second centrifugation. Neutralized samples were then stored at −80 °C until analysis.

METABOLITE ASSAYS

All metabolites were determined spectrophotometrically using coupled enzyme assays and monitoring the oxidation or reduction of NAD(P)(H) using a Pye Unicam SP 8–100 spectrophotometer. Glycogen was determined by the method of Keppler & Decker (1974) involving hydrolysis to glucose by amyglucosidase and subsequent quantification of glucose yield. ATP, alanine, aspartate, and glucose were determined by the methods of Lowry & Passonneau (1972). Succinate was determined as in Williamson & Corkey (1969) with the substitutions of ATP- and ATP-dependent succinyl-CoA synthetase from *E. coli*. Alanopine and strombine were determined as in Storey *et al.* (1982) using alanopine and strombine dehydrogenases purified from *Littorina littorea* and *Mercenaria mercenaria* foot muscles, respectively. The method for octopine was as in Storey *et al.* (1979) without the addition of EDTA to the assay and using the commercially available octopine dehydrogenase from scallop muscle.

RESULTS

Metabolite levels in *B. canaliculatum* tissues over the course of 24 h of anoxia followed by 6 h of aerobic recovery are shown in Figs. 1–5 for ventricle, foot muscle, gill, radular retractor muscle, and hepatopancreas, respectively.

VENTRICLE

Succinate and alanine accumulated as end products during anoxia in the ventricle, each with a distinct time-dependent pattern (Fig. 1). Alanine was the major product over the initial hours of anoxia, the ratio alanine : succinate accumulated being 1.8 : 1 after 4 h. Aspartate was rapidly depleted early in anoxia, 9.3 $\mu mol \cdot g^{-1}$ wet weight being lost over the first 4 h. As anoxia was extended, alanine production and aspartate utilization slowed and stopped but succinate accumulation continued. Net accumulation of succinate was 25.3 ± 5.2 $\mu mol \cdot g^{-1}$ after 24 h, the highest amount accumulated by any tissue during anoxia. Glycogen content of ventricle declined steadily throughout anoxia, the net decrease in content (33.5 $\mu mol \cdot g^{-1}$ in glucose units) being sufficient to

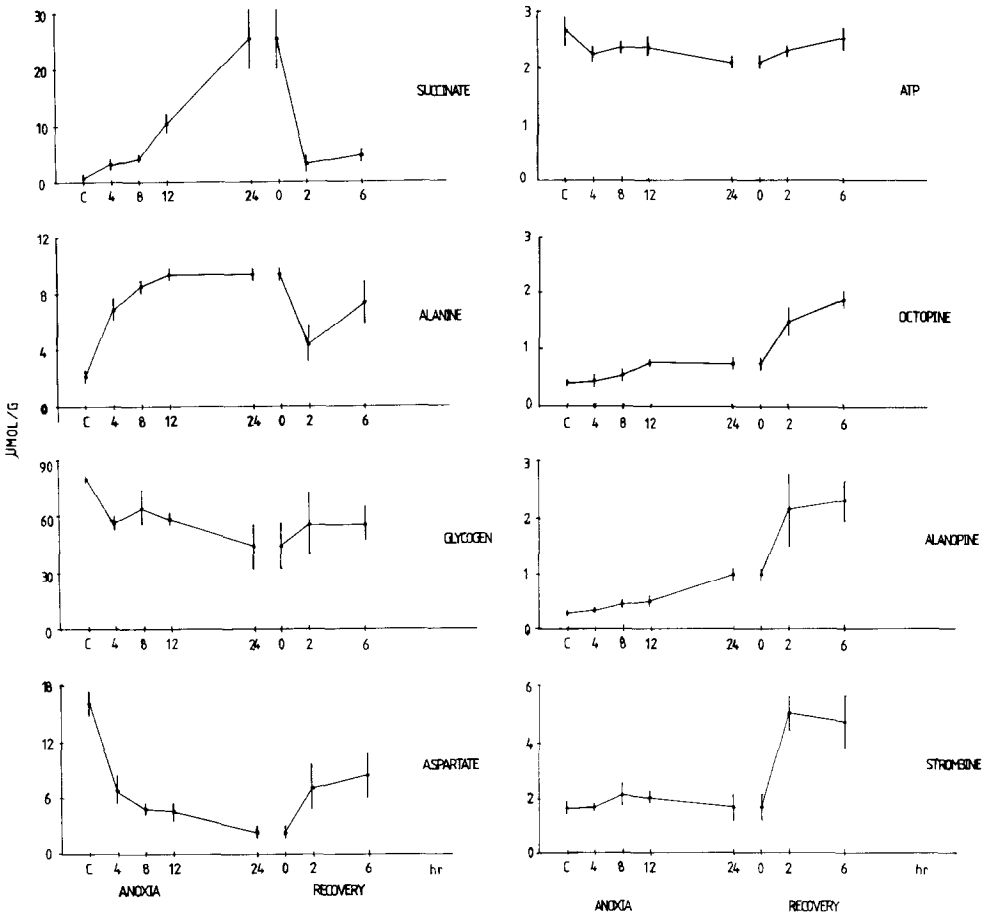

Fig. 1. Changes in the concentrations of selected metabolites in whelk ventricle over a time course of 24 h of environmental anoxia followed by 6 h of aerobic recovery. Data are μmol $\cdot$ g^{-1} wet weight, means $\pm$ SEM, n = 5 individuals at each point. Glycogen is expressed in glucose units. C, control animals taken directly from the seawater tank.

account for the accumulated products. Imino acids were minor products of anaerobiosis, alanopine and octopine contents increasing by only 0.7 and 0.35 μmol $\cdot$ g^{-1}, respectively, whereas strombine was not produced. ATP content of the ventricle declined by $\approx$20% over the 24 h course of anoxia.

Recovery from anoxia was accomplished rapidly in ventricle. Eighty-seven percent of accumulated succinate was cleared over the first 2 h after whelks were returned to aerated seawater. Alanine was partially cleared and aspartate content was restored to $\approx$40% of the control value over the 6 h of recovery. Aerobic recovery initiated a rapid accumulation of all three imino acids in ventricle, with production being highest over the first 2 h and total accumulations of 1.2, 3.4, and 1.1 μmol $\cdot$ g^{-1} for alanopine, strombine, and octopine, respectively.

FOOT MUSCLE

Foot muscle showed a somewhat different metabolic pattern during anoxia (Fig. 2). Both succinate and alanine contents increased in a linear fashion over the entire 24-h anoxic period with net accumulations of 6.4 and 5.5 μmol $\cdot$ g^{-1}, respectively. Aspartate content in foot decreased by 8 μmol $\cdot$ g^{-1} during anoxia, an amount sufficient to account for the succinate accumulated, although the time courses of aspartate depletion and succinate production were not mirror images. Glycogen content dropped by over 50 μmol $\cdot$ g^{-1}. No significant change in ATP or imino acid content of foot muscle occurred during anoxia.

During the 6 h of recovery in aerated seawater, metabolites concentrations in foot muscle were not significantly changed from those seen after 24 h of anoxia. Alanine and succinate levels remained high, glycogen and aspartate remained low, and imino acid levels were not altered.

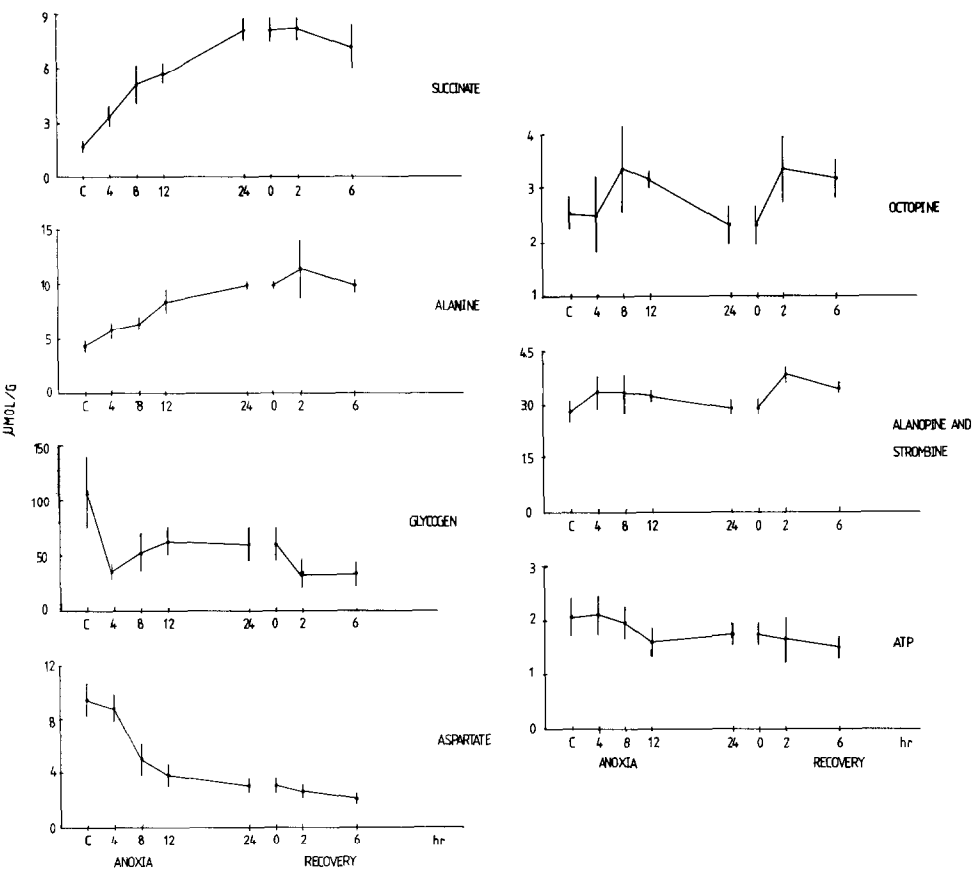

Fig. 2. Changes in the concentrations of selected metabolites in whelk foot muscle over a time course of 24 h anoxia and 6 h aerobic recovery. Further details are as in Fig. 1.

GILL

Over the first 12 h of anoxia, gill showed only minor accumulations of anaerobic products despite a decrease in aspartate content of 4 μmol $\cdot$ g^{-1} and a rapid depletion of the entire glycogen reserve (Fig. 3). Between 12 and 24 h of anoxia, however, alanine content rose by 2.7 μmol $\cdot$ g^{-1} and succinate by 3.9 μmol $\cdot$ g^{-1}. Alanopine and octopine were produced in minor amounts (0.8 and 0.6 μmol $\cdot$ g^{-1}, respectively) whereas strombine was not detected. ATP content dropped by 65% during anoxia.

Upon return to aerobic water, succinate was rapidly cleared from the gill, with an inverse rise in aspartate content occurring. Alanine was partially cleared over the 6 h but ATP, alanopine, and octopine contents did not change. Glycogen was not resynthesized.

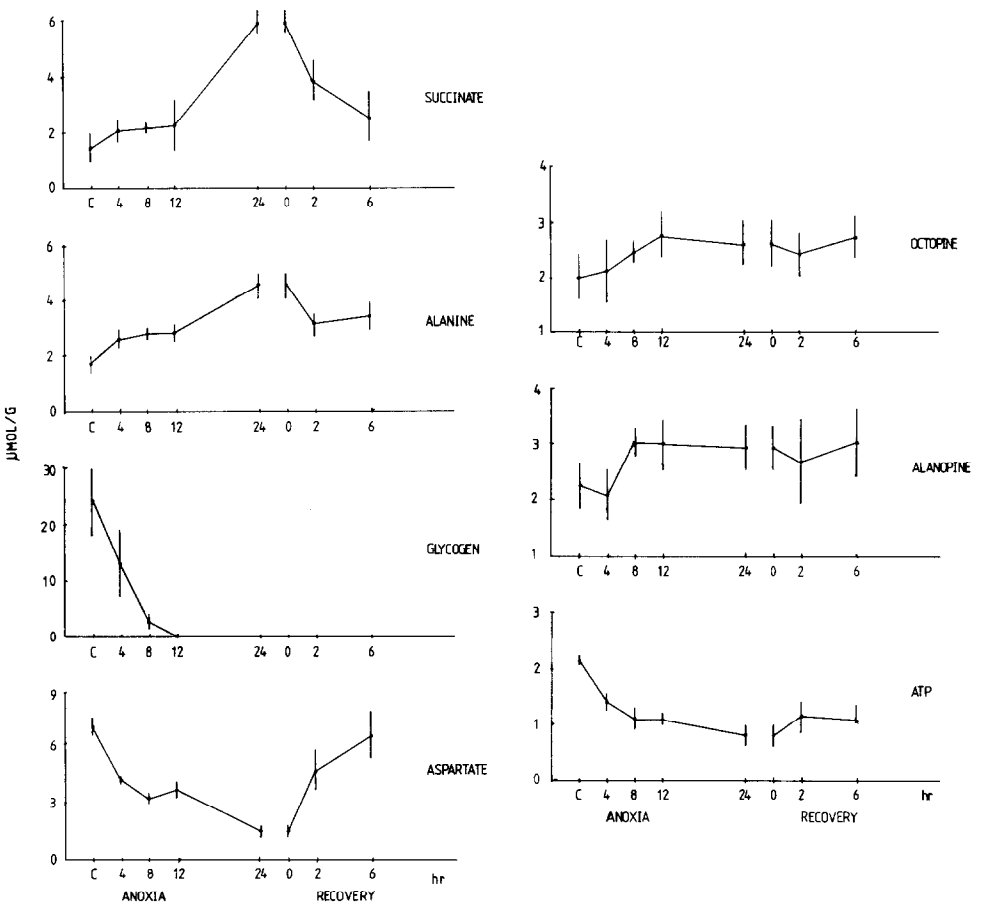

Fig. 3. Changes in the concentrations of selected metabolites in whelk gill over a time course of 24 h anoxia and 6 h aerobic recovery. Further details are as in Fig. 1.

RADULAR RETRACTOR MUSCLE

Alanine concentration rose rapidly (net accumulation 6.9 μmol $\cdot$ g^{-1}) in retractor muscle over the first 8 h of anoxia but remained constant thereafter (Fig. 4). Succinate content increased progressively over the entire anoxic period with a total accumulation of 3.5 μmol $\cdot$ g^{-1}. Aspartate content of the muscle decreased during the early hours of anoxia but levels were again elevated, and not different from control values, at 12 and 24 h of anoxia. Glycogen in retractor muscle decreased by $\approx$30 μmol $\cdot$ g^{-1} over the 24 h. A minor accumulation of strombine was seen but alanopine and octopine were not produced. ATP levels remained high and constant throughout anoxia.

As in foot muscle, recovery from anoxia was slow in retractor muscle. Over the 6 h

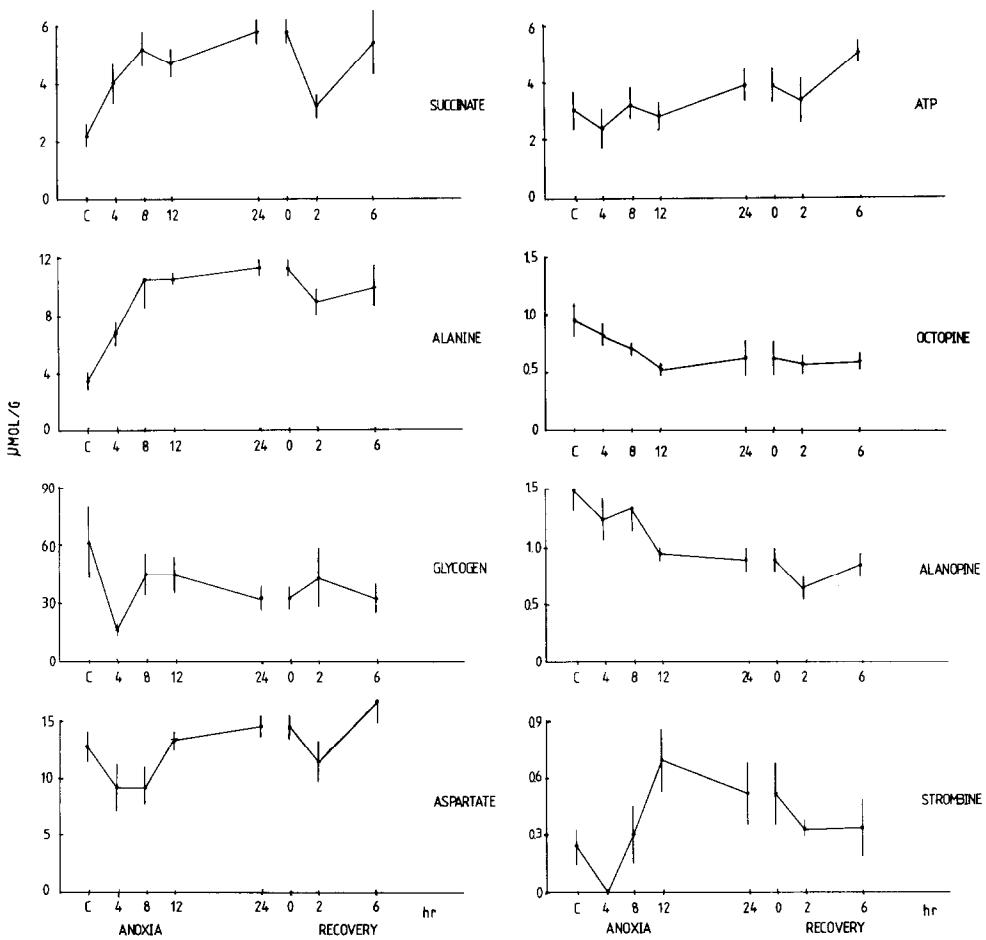

Fig. 4. Changes in the concentrations of selected metabolites in whelk radular retractor muscle over a time course of 24 h anoxia and 6 h aerobic recovery. Further details are as in Fig. 1.

period, there were no significant changes in the levels of anaerobic products or glycogen. However, aspartate content rose by 4 μmol $\cdot$ g^{-1} and ATP levels increased by 31%.

HEPATOPANCREAS

Anoxia had a minimal effect on metabolite levels in hepatopancreas. Succinate content increased by only 1.4 μmol $\cdot$ g^{-1} over the 24 h and aspartate decreased by an equal amount (Fig. 5). Contents of alanine and imino acids did not change over the course of anoxia.

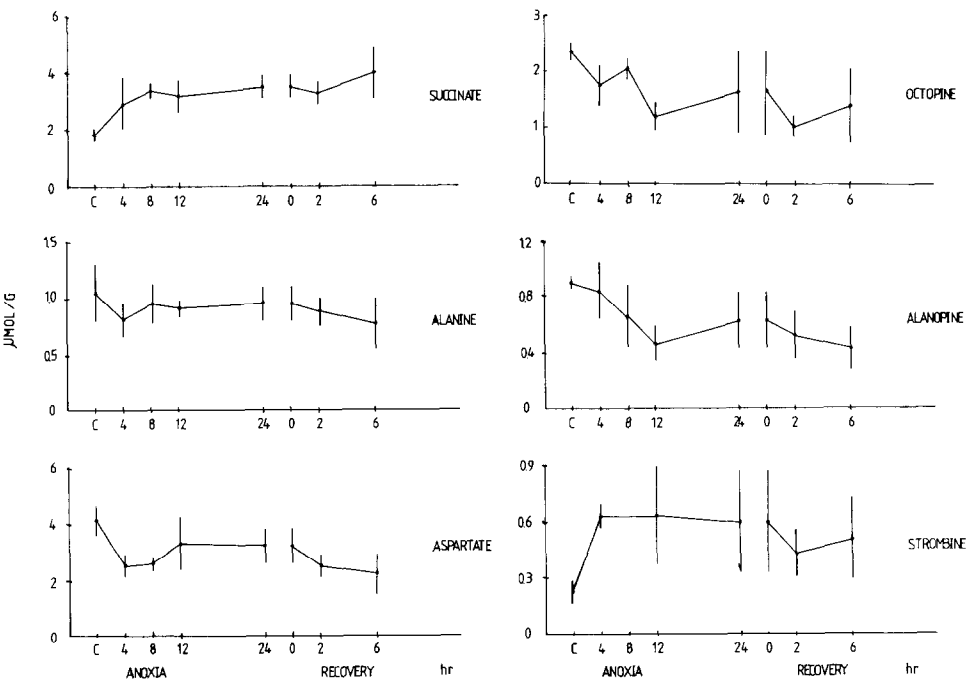

Fig. 5. Changes in the concentrations of selected metabolites in whelk hepatopancreas over a time course of 24 h anoxia and 6 h aerobic recovery. Further details are as in Fig. 1.

Recovery was slow in hepatopancreas; none of the compounds measured changed significantly in concentration over the 6-h period.

Discussion

Numerous studies of marine molluscs have shown that the basic pattern of anaerobic metabolism involves the coupled use of glycogen and aspartate as substrates with the formation of alanine and succinate as end products (de Zwaan, 1983; Livingstone &

de Zwaan, 1983). This is also the basic pattern of the whelk. However, the present study also shows strong tissue-specific and time-specific variations in anaerobic metabolism, representing the first such complete studies on a gastropod species. Evidence of tissue-specific rates of metabolism during anoxia is also indicated.

Recent studies have established that metabolic rate depression (to levels 5–10% of aerobic control values) is a key component of facultative anaerobiosis in bivalves (Famme *et al.*, 1981; Shick *et al.*, 1983). This is presumably due to a strong suppression of rates of non-essential processes. Data in the present study can imply that there are substantial variations in anaerobic metabolic rates between individual tissues. Based on total end product accumulation over the anoxic excursion, tissues can be grouped into three categories. The sum of succinate + alanine accumulated was 32.5 $\mu mol \cdot g^{-1}$ wet weight in ventricle, 6.6, 11.9, and 10.4 $\mu mol \cdot g^{-1}$ in gill, foot, and radular retractor muscle, and only 1.4 $\mu mol \cdot g^{-1}$ in hepatopancreas. This suggests a 20-fold difference in anoxic metabolic rate between ventricle and hepatopancreas. Such differences may be made up of two components: normal differences in metabolic functions (and ATP consumption) between organs as well as differential effects of anoxia on the metabolic rates of individual organs.

Tissues of the whelk showed some variations in the contents of anaerobic substrates maintained in control animals. Glycogen content was highest in foot muscle, intermediate in ventricle and radular retractor muscle, and low in gill. For foot this is not unexpected; foot muscle has a high glycolytic capacity (high activities of glycolytic enzymes, few mitochondria) and muscle work is probably largely dependent on glycolytic ATP production under all circumstances. The low carbohydrate content in gill, on the other hand, is consistent with a tissue that is normally well oxygenated; indeed, gill was the only tissue that exhausted its glycogen reserves during the anoxic exposure (no glycogen remained at 12 h). Carbohydrate, lipid and protein contents of whelk tissues are known to vary seasonally, being highest in autumn (the season in which the present study was performed). Seasonal variation is largely related to the reproductive cycle (Stickle, 1975; Belisle & Stickle, 1978) although carbohydrate contents might also respond to changes in the dependence on anaerobic pathways for energy needs as seasonal temperature changes. Whereas glycogen can be either an aerobic or an anaerobic substrate, aspartate pools are largely preserved for anaerobic function. Tissue-specific variation in aspartate levels may, therefore, provide another indication of anaerobic capacity and/or relative tissue metabolic rates during anaerobiosis. Thus, aspartate content was highest in ventricle and radular retractor muscle, intermediate in gill and foot, and lowest in hepatopancreas.

In marine bivalves, two patterns of anaerobic succinate synthesis have been described (de Zwaan, 1983). The first utilizes aspartate as the carbon source; after transamination, the resulting oxaloacetate is reduced to succinate. These reactions are coupled to the simultaneous fermentation of glycogen to alanine through two links, redox balance and amino group transfer. This pattern appears to be the initial metabolic response to anoxia in all species and all tissues and, in many instances, is also the sole pattern utilized

(Eberlee *et al.*, 1983; de Zwaan *et al.*, 1983a). The second pattern of succinate synthesis utilizes glycogen as the carbon source, forms P-enolpyruvate via glycolysis, and then carboxylates P-enolpyruvate to form oxaloacetate. When this route is taken, alanine synthesis from glycogen is strongly suppressed due to tight regulatory control over carbon flow at the "P-enolpyruvate branchpoint". Pyruvate kinase is inactivated to permit P-enolpyruvate carboxykinase function. In general, this route of succinate synthesis is initiated only when anaerobiosis becomes prolonged and aspartate reserves have been depleted although some tissues of bivalves appear to lack the ability to carboxylate P-enolpyruvate altogether (de Zwaan *et al.*, 1983a). The present study shows that these two routes of succinate synthesis are also available in the whelk and that individual tissues differ in their ability to convert glycogen to succinate. Ventricle, for example, shows two phases of anaerobic metabolism. Initially, alanine and succinate levels rise together, whereas glycogen and aspartate reserves fall; in this phase succinate carbon is derived from aspartate. When aspartate reserves are exhausted, however, alanine production is curtailed, and the second phase, glycogen fermentation to succinate, then permits continued ATP production. Radular retractor muscle shows the same pattern of anaerobic metabolism. Both of these muscles, then, have the ability to control carbon flow at the P-enolpyruvate branchpoint. The mechanism of this control is anoxia-induced phosphorylation of pyruvate kinase that converts the enzyme to a much less active form (Plaxton & Storey, 1984a,b, 1985) and, thereby, facilitates P-enolpyruvate conversion to oxaloacetate.

Gill and foot of the whelk, by contrast, appear to rely throughout anaerobiosis on the coupled conversion of glycogen to alanine and aspartate to succinate. Kinetic studies of pyruvate kinase in these tissues indicates that they should have the facility to redirect glycogen fermentation into succinate synthesis (Plaxton & Storey, 1985). In practise, however, the capacity for anaerobic conversion of glycogen to succinate does not appear to be used within the 24-h anoxic period. This was apparently due to two factors: (1) the relatively large size of the tissue aspartate reserve; and (2) the relatively low rate of aspartate utilization during anoxia (a low tissue metabolic rate).

Tissues of the whelk also differed in their metabolic responses during the aerobic recovery period. Ventricle and gill responded very rapidly to the return of oxygenated conditions, clearing most of the accumulated succinate and largely restoring aspartate pools with 2–6 h. Other tissues, however, showed little or no progress in reestablishing conditions characteristic of the control, aerobic state within the 6-h period. These differences in the pattern of metabolic recovery may reflect both the speed with which tissues are reoxygenated and the aerobic metabolic rate during recovery. Reoxygenation of gill and ventricle is likely to be particularly fast and it is not surprising, therefore, that these tissues are able to rapidly clear accumulated anaerobic end products.

Imino acids (alanopine, strombine, octopine) were not major products of anaerobic metabolism in any tissue of the whelk, and, indeed, accumulated only in minor amounts in ventricle and gill during anoxia. These findings agree with other studies of both gastropods (Ellington, 1981, 1982; Storey *et al.*, 1982) and bivalves (Eberlee *et al.*, 1983;

Gade, 1980, 1983; Korycan & Storey, 1983; de Zwaan *et al.*, 1983b). These products appear instead to be associated with metabolic situations in which tissue ATP demand temporarily outstrips the capacity for ATP synthesis by oxidative phosphorylation. Thus, octopine often accumulates during burst muscle work (in an inverse relationship to arginine phosphate hydrolysis) (Gade, 1980, 1981; Koorman & Grieshaber, 1980) whereas alanopine or strombine have been found to accumulate during the metabolic recovery period following anoxia exposure (Eberlee *et al.*, 1983; de Zwaan *et al.*, 1983b). Not surprisingly, then, levels of all three products rose dramatically in ventricle over the first 2 h of aerobic recovery. This indicates that a glycolytic component to muscle ATP production was required to support the return to an aerobic heart rate and work load (anoxia induces bradycardia; Ellington, 1981). Tissues of the whelk display both alanopine and octopine dehydrogenase activities with the ratio in ventricle being 1.6 : 1 (Plaxton & Storey, 1982). Differential functions for these two dehydrogenases might be predicted to explain the presence of the two enzymes. However, the present results indicate that both products accumulate in a parallel manner and to similar amounts in ventricle during aerobic recovery. With respect to this metabolic function, then, the two enzymes appear to serve the same purpose.

Acknowledgements

We thank J. M. Storey for critical reading of the typescript. Supported by an operating grant from N.S.E.R.C. Canada to K. B. Storey.

References

Belisle, B.W. & W.B. Stickle, 1978. Seasonal patterns in the biochemical constituents and body component indexes of the muricid gastropod, *Thais haemastoma*. *Biol. Bull. (Woods Hole, Mass.)*, Vol. 155, pp. 259–272.

Eberlee, J.C., J.M. Storey & K.B. Storey, 1983. Anaerobiosis, recovery from anoxia and the role of strombine and alanopine in the oyster, *Crassostrea virginica*. *Can. J. Zool.*, Vol. 61, pp. 2682–2687.

Eberlee, J.C. & K.B. Storey, 1984. Buffering capacities of tissues from marine molluscs. *Physiol. Zool.*, Vol. 57, pp. 567–572.

Ellington, W.R., 1981. Energy metabolism during hypoxia in the isolated perfused ventricle of the whelk *Busycon contrarium* Conrad. *J. Comp. Physiol.*, Vol. 142, pp. 457–464.

Ellington, W.R., 1982. Metabolism at the pyruvate branchpoint in the radula retractor muscle of the whelk, *Busycon contrarium*. *Can. J. Zool.*, Vol. 60, pp. 2973–2977.

Famme, P., J. Knudsen & E.S. Hansen, 1981. The effect of oxygen on the aerobic–anaerobic metabolism of the marine bivalve, *Mytilus edulis* L. *Mar. Biol. Lett.*, Vol. 2, pp. 345–351.

Gade, G., 1980. The energy metabolism of the foot muscle of the jumping cockle, *Cardium tuberculatum*: sustained anoxia versus muscular activity. *J. Comp. Physiol.*, Vol. 137, pp. 177–182.

Gade, G., 1981. Energy production during swimming in the adductor muscle of the bivalve *Lima hians*: comparison with data from other bivalve mollusks. *Physiol. Zool.*, Vol. 54, pp. 400–406.

Gade, G., 1983. Energy production during anoxia and recovery in the adductor muscle of the file shell, *Lima hians*. *Comp. Biochem. Physiol.*, Vol. 76B, pp. 73–77.

Keppler, D. & K. Decker, 1974. Glycogen: determination with amyloglucosidase. In, *Methods of enzymatic analysis*, edited by H.U. Bergmeyer, Academic Press, New York, pp. 1127–1131.

KLUYTMANS, J.H. & D.I. ZANDEE, 1983. Comparative study of the formation and excretion of anaerobic fermentation products in bivalves and gastropods. *Comp. Biochem. Physiol.*, Vol. 75B, pp. 729–732.

KOORMAN, R. & M. GRIESHABER, 1980. Investigations on the energy metabolism and on octopine formation of the common whelk, *Buccinum undatum* L. during escape and recovery. *Comp. Biochem. Physiol.*, Vol. 65B, pp. 543–547.

KORYCAN, S.A. & K.B. STOREY, 1983. Organic-specific metabolism during anoxia and recovery in the cherrystone clam, *Mercenaria mercenaria*. *Can. J. Zool.*, Vol. 61, pp. 2674–2681.

LIVINGSTONE, D.R. & A. DE ZWAAN, 1983. Carbohydrate metabolism in gastropods. In, *The Mollusca, Vol. 1*, edited by K.M. Wilbur, Academic Press, New York, pp. 177–242.

LOWRY, O.H. & J.V. PASSONNEAU, 1972. *A flexible system of enzymic analysis*. Academic Press, New York, 291 pp.

PLAXTON, W.C. & K.B. STOREY, 1982. Tissue specific isozymes of alanopine dehydrogenase in the channeled whelk, *Busycotypus canaliculatum*. *Can. J. Zool.*, Vol. 60, pp. 1568–1572.

PLAXTON, W.C. & K.B. STOREY, 1983. Purification and properties of alanopine dehydrogenase isozymes from the channeled whelk, *Busycotypus canaliculatum*. *Comp. Biochem. Physiol.*, Vol. 76B, pp. 321–326.

PLAXTON, W.C. & K.B. STOREY, 1984a. Purification and properties of aerobic and anoxic forms of pyruvate kinase from red muscle tissue of the channeled whelk, *Busycotypus canaliculatum*. *Eur. J. Biochem.*, Vol. 143, pp. 257–265.

PLAXTON, W.C. & K.B. STOREY, 1984b. Phosphorylation *in vivo* of red muscle pyruvate kinase from the channeled whelk, *Busycotypus canaliculatum*, in response to anoxic stress. *Eur. J. Biochem.*, Vol. 143, pp. 257–265.

PLAXTON, W.C. & K.B. STOREY, 1985. Tissue specific isozymes of pyruvate kinase in the channeled whelk, *Busycotypus canaliculatum*: enzyme modification in response to environmental anoxia. *J. Comp. Physiol.*, Vol. 155, pp. 291–296.

RAO, Y.P. & D.G.V.P. RAO, 1983. End products of anaerobic metabolism in *Cerithidea (Cerithideopsilla) cingulate* (Gmelin 1790) and *Cerithium coralium* Kiener 1841. *Can. J. Zool.*, Vol. 61, pp. 1304–1310.

SHICK, J.M., A. DE ZWAAN & A.M.T. DE BONT, 1983. Anoxic metabolic rate in the *Mytilus edulis* L. estimated by simultaneous direct calorimetry and biochemical analysis. *Physiol. Zool.*, Vol. 56, pp. 56–63.

STICKLE, W.B., 1975. The reproductive physiology of the intertidal prosobranch *Thais lamellosa* (Gmelin). II. Seasonal changes in biochemical composition. *Biol. Bull. (Woods Hole, Mass.)*, Vol. 148, pp. 448–460.

STOREY, K.B., 1984. Phosphofructokinase from foot muscle of the whelk, *Busycotypus canaliculatum*: evidence for covalent modification of the enzyme during anaerobiosis. *Arch. Biochem. Biophys.*, Vol. 235, pp. 665–672.

STOREY, K.B., 1985. A re-evaluation of the Pasteur effect: new mechanisms in anaerobic metabolism. *Mol. Physiol.*, Vol. 8, pp. 439–461.

STOREY, K.B., 1988. Mechanisms of glycolytic control during facultative anaerobiosis in a marine mollusc: tissue specific analysis of glycogen phosphorylase and fructose-2,6-bisphosphate. *Can. J. Zool.*, in press.

STOREY, K.B., D.C. MILLER, W.C. PLAXTON & J.M. STOREY, 1982. Gas-liquid chromatography and enzymatic determination of alanopine and strombine in tissues of marine invertebrates. *Anal. Biochem.*, Vol. 125, pp. 50–58.

STOREY, K.B., J.M. STOREY, K. JOHANSEN & P.W. HOCHACHKA, 1979. Octopine metabolism in *Sepia officinalis*: effect of hypoxia and metabolite loads on the blood levels of octopine and related compounds. *Can. J. Zool.*, Vol. 57, pp. 2331–2336.

WIESER, W., 1980. Metabolic end products in three species of marine gastropods. *J. Mar. Biol. Assoc. U.K.*, Vol. 60, pp. 175–180.

WILLIAMSON, J.R. & B.E. CORKEY, 1969. Assays of intermediates of the citric acid cycle and related compounds by fluorometric enzyme methods. *Methods Enzymol.*, Vol. 13, pp. 434–513.

ZWAAN, A. DE, 1983. Carbohydrate catabolism in bivalves. In, *The Mollusca, Vol. 1*, edited by K.M. Wilbur, Academic Press, New York, pp. 137–175.

ZWAAN, A. DE, A.M.T. DE BONT & J. HEMELRAAD, 1983a. The role of phosphoenolpyruvate carboxykinase in the anaerobic metabolism of the sea mussel *Mytilus edulis* L. *J. Comp. Physiol.*, Vol. 153, pp. 267–274.

ZWAAN, A. DE, A.M.T. DE BONT, W. ZURBURG, B.L. BAYNE & D.R. LIVINGSTONE, 1983b. On the role of strombine formation in the energy metabolism of adductor muscle of a sessile bivalve. *J. Comp. Physiol.*, Vol. 149, pp. 557–563.