THE JOURNAL OF EXPERIMENTAL ZOOLOGY 233:347–358 (1985)

Amino Acid Metabolism in Euryhaline Bivalves: Regulation of Glycine Accumulation in Ribbed Mussel Gills

LEHMAN L. ELLIS, JAMES M. BURCHAM, KENNEDY T. PAYNTER, AND STEPHEN H. BISHOP
Department of Zoology, Iowa State University, Ames, Iowa 50011

ABSTRACT Glycine levels in isolated ribbed mussel (*Modiolus demissus*) gill tissue increased slightly and decreased markedly when incubated at high and low salinities, respectively. Low levels of the enzymes involved in the biosynthesis of serine from triose phosphate intermediates, the serine hydroxymethyltransferase, and serine dehydrase were detected in gill tissue homogenates. Experiments using gill tissue incubated with (U-^{14}C)-glycine and (U-^{14}C)-serine indicated interconversion between serine and glycine and transfer of label to alanine, asparate, glutamate, CO_2, organic acids, and protein. Glyoxylate was metabolized more slowly than glycine and was probably converted to glycine for catabolism. Studies using (1-^{14}C)-glycine and (2-^{14}C)-glycine with isolated gill tissue and mitochondria indicated that the mitochondrial glycine cleavage enzyme was the major route of glycine catabolism. Metabolic controls activating or inhibiting the glycine cleavage enzyme regulate tissue glycine accumulation and catabolism during hypersalinity or hyposalinity stress.

High levels of intracellular amino acids aid in the control of the intracellular osmotic pressure in most marine and estuarine molluscs. The concentrations of free amino acids in the tissues increase and decrease with the increase and decrease of the blood osmotic pressure during environmental salinity changes. With mussels, particularly ribbed mussels (*M. demissus*), the concentrations of alanine, glycine, and taurine added together comprise about 90% of the total intracellular free amino acid pool of most tissues (Pierce and Greenberg, '72; Baginski and Pierce, '75,'77; Hoyaux et al., '76; Shumway et al., '77; Shumway and Youngson, '79; Livingstone et al., '79; Greenwalt and Bishop, '80).

During short-term changes (hours) in environmental salinity, the membrane-controlled processes seem to respond rapidly to adjust the intracellular amino acid concentrations (Pierce and Greenberg, '72; Shumway et al., '77; Shumway and Youngson, '79; Livingstone et al., '79; Strange and Crowe, '79a,b; Crowe, '81). With prolonged hypoosmotic stress, the amino acids are metabolized within the animals and the nitrogen is released as ammonia (Bartbarger and Pierce, '76; Livingstone et al., '79). Transfer of mussels adapted at low salinities to high salinities for extended periods (weeks) results in a rapid increase in tissue alanine levels and a slower, continuous increase in tissue glycine and taurine levels (Baginski and Pierce, '75,'77; Livingstone et al., '79). The metabolic component determines which of the amino acids accumulate. Experiments using ribbed mussel gill and heart tissues with transaminase inhibitors indicate that the metabolic events associated with the adjustment of the alanine levels are transaminase linked. There is no clear indication that the regulation of the glycine levels is entirely transaminase linked (Greenwalt and Bishop, '80; Bishop et al., '81) and glycine is not a substrate for the particulate L-amino acid oxidase in ribbed mussel tissues (Burcham et al., '80). The metabolic component regulating taurine accumulation is uncertain (see Bishop et al., '83).

Tracer studies using (U-^{14}C)-glucose with a number of molluscs indicate that the labeling of both glycine and serine from intermediates of glycolysis is weak compared to the labeling of the TCA cycle intermediates and the amino acids alanine, aspartate, and glutamate (Chen and Awapara, '69; de Zwaan and van Marrewijk, '73a; Campbell and Bishop, '70; Allen and Kilgore, '75; Baginski and Pierce, '78; Bishop et al., '83). From these limited data, it would appear that the pulmonate gastropods have a

Dr. Ellis's current address is Virginia Institute of Marine Science, College of William and Mary, Gloucester Pt., Virginia 23062.

greater de novo serine and glycine biosynthetic capacity than the bivalves.

In most organisms, glycine metabolism is closely coupled to serine and glyoxylate metabolism and to one carbon transfer (Campbell and Bishop, '70). The transamination of glyoxylate to glycine has been demonstrated in tissue homogenates of several bivalves including ribbed mussels (Read, '62; Greenwalt and Bishop, '80; Falany and Freidl, '81; Noguchi et al., '82). However, de novo biosynthesis of glyoxylate by either the dicarboxylic acid cycle (deZwaan and van Marrewijck, '73b) or the purine catabolic pathway (Andrews and Reid, '72; Campbell and Bishop, '70) is either unlikely or slow in most molluscs including mussels. The interconversion of serine and glycine by the serine hydroxymethyltransferase (SHMT) and the production of reactive one carbon derivatives of tetrahydrofolic acid (THFA) have been demonstrated in a number of marine invertebrates including molluscs (Whiteley, '60). The results of tracer studies using (3-^{14}C)-serine and (2-^{14}C)-glycine to evaluate uric acid biosynthesis by pulmonate land snails (Lee and Campbell, '65) are suggestive of the presence of both the SHMT and the glycine cleavage (synthase) complex (Kikuchi, '73; Kikuchi and Hiraga, '82). This enzyme complex has multiple subunits with lipoamide and pyridoxal phosphate bound as cofactors and catalyzes the following reaction: H^+ + glycine + NAD + THFA $\rightleftharpoons$ CH_2 = THFA + NADH + NH_3 + CO_2.

Considering that the glycine cleavage enzyme and the SHMT are reversible and that both might be fairly active in some species, the studies using (^{14}C)CO_2 to trace biosynthetic capacities in these molluscs are of interest. Little or no radioactivity from (^{14}C)CO_2 is incorporated into tissue glycine or serine in studies with oysters (Hammen and Wilbur, '59) and sea mussels (Ahmad and Chaplin, '79). The incorporation of radiolabel from (^{14}C)CO_2 into both glycine and serine with the tissues of pulmonate land snails (Campbell and Speeg, '68) supports data from a previous study (Lee and Campbell, '65) on uric acid biosynthesis.

Intracellular glycine can also participate in the biosynthesis of strombine (Fields et al., '80; Dando, '81), a dead-end product of anaerobic glycolysis in many bivalves (Zurburg et al., '82; de Zwaan et al., '83; Eberlee et al., '83; Korycan and Story, '83).

The purpose of this investigation is to define the basic metabolic processes regulating glycine and serine turnover in euryhaline bivalves.

MATERIALS AND METHODS

Mussels were purchased from Northeast Marine Environmental Institute (Monument Beach, Mass.) and maintained in the laboratory in 12 o/oo artificial seawater (ASW) as previously described (Greenwalt and Bishop, '80).

Biochemicals and chromatography solvents were of reagent grade and purchased from Sigma Chemical Co. (St. Louis, Mo) or Fisher Scientific Co., (Pittsburg, Pa). Aquasol, (U-^{14}C)-glycine, (U-^{14}C)-serine, ^{3}H-sucrose, (U-^{14}C)-glyoxalate, (^{14}C)$NaHCO_3$, and (U-^{14}C)-threonine were obtained from New England Nuclear (Boston, Mass.); (3-^{14}C)-serine, (1-^{14}C)-glycine, and (2-^{14}C)-glycine were purchased from ICN (Irvine, Cal.). Silicone oil was purchased from Aldrich Chem. Co. (Milwaukee, Wisc.). Thin layer chromatographic and radiographic supplies were obtained as described previously (Bishop et al., '81).

Metabolic studies

For metabolic studies, gill pieces were incubated at 22°C in 12 o/oo or 32 o/oo ASW as described by Bishop et al., ('81) with ^{14}C-labeled amino acids or (^{14}C)HCO_3^- in 3.0 ml sterile 12 o/oo or 32 o/oo ASW using 25 ml Erlenmeyer flasks fitted with rubber stoppers and plastic center wells (Kontes, Vineland, N. J.) containing 0.2 ml of 1 M hyamine hydroxide in methanol. Incubations were terminated by the addition of 0.5 ml 2.0 M $HClO_4$. Released (^{14}C)CO_2 was determined by the method of Cooley et al. ('76). Gill pieces and incubation medium were quantitatively transferred to a glass TenBroeck homogenizer and then homogenized. The homogenate was centrifuged at 1000 g for 10 min and the supernatant and pellet collected separately. The pellet was dissolved in 0.2 ml of 0.1 M NaOH, neutralized, and transferred to an Aquasol scintillation cocktail for counting. The supernatant was neutralized with 0.3 ml of 2.0 M KOH cooled in an ice-bath for 30 min and $KClO_4$ removed by centrifugation. The supernatant was poured onto a small Dowex 50W-X8 (100 mesh) column in acid form. The columns were washed with five bed volumes of distilled water and the eluate collected. This eluate was lyophilized and the lyophilate redissolved in 1.0 ml distilled water. An aliquot was added to Aquasol and counted (radioactivity). Following the water wash, 3 M NH_4OH was applied to the column to elute the amnio acid fraction. This eluate was repeatedly taken to dryness in a

rotary evaporator and redissolved in 0.5 ml distilled water. Radioactive amnio acids were separated using two-dimensional thin-layer chromatography on 20 × 20 cm silica gel plates with butanol:formic acid:water (75:15:10, v:v:v) and phenol:water (4:1, w:w) as developing solvents. Radioautography was accomplished using the procedure of Bishop et al. ('81). Unresolved amino acids, usually only serine and glycine, were scraped from the plates and redissolved in 0.1 ml distilled water. The amino acids were dansylated by the method of Hartley and Massey ('56) and the dansyl derivatives chromatographed on a silica-gel plate with chloroform:*t*-amyl alcohol:formic acid (70:30:3, v:v:v) as the chromatographic solvent. Dansyl-amino acid spots were scraped from the plate and the radioactivity counted in Aquasol.

Mitochondrial preparation and metabolism

Gill mitochondria were prepared by homogenizing gills in five volumes of a cold isolation buffer containing 400 mM sucrose, 1.0 mM EGTA, 1.0 mg/ml defatted bovine serum albumin, and 40 mM Tris pH 7.4. Homogenization was performed with an Ultra-Turrax model T45/N homogenizer (Tekmar, Cincinnati, O.) using three passes of 18 seconds each at a setting of 40. The homogenate was mixed with an equal volume of isolation buffer and filtered through cheesecloth. The filtrate was centrifuged at 700 g for 10 min to remove cells and nuclear material. The supernatant was collected and recentrifuged at 10,000 g for 20 min and the resulting supernatant used for some enzyme assays. The pellet was resuspended in isolation buffer and recentrifuged two times at 7000 g for 20 min. The washed pellet (mitochondria) was resuspended in isolation buffer at a concentration of 5 mg mitochondrial protein/ml.

Metabolism of glycine and glyoxalate by gill mitochondria was assessed using 3.0 ml of suspended mitochondria. Radioactive substrates and additives were incubated with the mitochondria for 1 hour. The $(^{14}C)CO_2$ evolution was analyzed as in the gill fragment metabolic experiments. In experiments with specifically labeled (^{14}C) glycine, the dimedon-aldehyde trap was employed in the manner described by Motokawa and Kukuchi ('74).

Mitochondrial metabolite transport

Mitochondrial uptake of glycine and glyoxalate was assessed using a modification of the silicone oil separation method of LaNoue et al. ('73). The acid layer was 2.4 M $HClO_4$ and the silicone oil layer was a density of 1.050. At timed intervals, a 1.0-ml aliquot of mitochondrial suspension was layered onto the silicone oil layer and centrifuged at 15,600 g for 3 min in an Eppindorf microfuge at 4°C. Extramitochondrial space was estimated from the distribution of ^{3}H-sucrose.

Enzyme assays

All enzyme assays were performed at 22°C. The activity of the following enzymes were measured in tissue extracts which had been desalted on a G-25 column using the following referenced methods: 3-phosphoglycerate dehydrogenase (Pizer, '64), 3-phosphohydroxy-pyruvate:L-glutamate aminotransferase (Grillo and Coghe, '66), 3-phosphoglycerate phosphatase in 50 mM Tris HCl pH 9.0 rather than barbital buffer (Fallon et al., '66) using the inorganic phosphate analytical procedure of Dryer et al. ('57), D-glycerate dehydrogenase (Willis and Sallach, '64), hydroxypyruvate:L-alanine aminotransferase (Fukushima et al., '78), L-serine dehydrase (Fallon et al., '66), L-serine hydroxymethyltransferase (SHMT) (Taylor and Weissbach, '65; Schirch et al., '77), L-threonine aldolase (Schirch and Gross, '68), and L-threonine dehydrase (Park and Datta, '79). The products of the hydroxypyruvate:L-alanine aminotransferase and SHMT were confirmed in the following manner. The reactions were allowed to proceed in the absence of NAD and coupling enzymes for 1 hour. The reactions were terminated by the addition of an equal volume of 10 mg/ml bovine serum albumin and immersion in a boiling water bath for 5 minutes. The protein precipitate was removed by centrifugation, the supernatant solution taken to dryness in a rotary evaporator, and the residue redissolved in 0.5 ml of 0.01 N HCl. This solution was subjected to dansylation and chromatographed as previously described.

Protein was determined using Miller's ('59) modification of the Lowry procedure. Mitochondrial protein was estimated using the biuret procedure (King, '67). Amino acid levels were determined using the previously described method (Greenwalt and Bishop, '80).

RESULTS

Specific enzymes

As a prelude to the ^{14}C-tracer experiments with glycine and serine, the activities of some

of the enzymes involved in serine and glycine biosynthesis and the effects of aminooxyacetic acid (AOA) and β-chloro-L-alanine (CA) on the activity of some of these enzymes were evaluated (Table 1). Except for phosphoserine phosphatatase, all of the enzymes required for the synthesis of serine by the phosphorylated and nonphosphorylated triose pathways were detected. The levels of most of these enzymes were relatively low. These results were in general agreement with previous tracer studies using ^{14}C-glucose or amino acids indicating only modest labeling of glycine and serine after extended incubation periods (see introduction). Some L-serine dehydrase but no L-threonine dehydrase was detected. Subcellular fractionation showed that the serine dehydrase was cytosolic and that the SHMT and the hydroxypyruvate:L-alanine aminotransferase activities were both cytosolic and mitochondrial. The levels of the glycine:pyruvate aminotransferase have been reported previously (Greenwalt and Bishop, '80).

Aminooxyacetic acid inhibited the 3-phosphopyruvate:L-glutamate aminotransferase, the hydroxypyruvate:L-alanine aminotransferase, the SHMT, the serine dehydrase, and the threonine aldolase (Table 1). AOA inhibition of the alanine aminotransferase, the aspartate aminotransferase, and the glycine:pyruvate aminotransferase has been reported previously (Greenwalt and Bishop, '80; Paynter et al., '84a,b). Previous work has shown that CA inhibited alanine aminotransferase (Paynter et al., '84b) but not the isoenzymes of aspartate aminotransferase (Paynter et al., '84a). β-Halogen substituted alanines have been shown to inhibit SHMT from mammalian liver (Wang et al., '81). CA inhibited hydroxypyruvate:L-alanine aminotransferase, SHMT, threonine aldolase, and serine dehydrase but not the 3-phosphohydroxypyruvate:L-glutamate aminotransferase from the gill tissue (Table 1).

Tissue experiments

The rate of oxidation of glycine to CO_2 was more than an order of magnitude slower in gills incubated at high salinity compared to gills incubated at low salinity (Table 2). During this time period (1 hr) the free glycine concentrations in the tissue preparations increased from about 10 μmoles/g dry wt to 12 μmoles/g dry wt with gills at 32 o/ooo and decreased from 10 μmoles/g dry wt to about 5 μmoles/g dry wt with gills held at 12 o/ooo. The incorporation of ^{14}C into $HClO_4$ insoluble components was the same at both salinities. The Dowex column partitioning of the radioactivity from the $HClO_4$ soluble fractions into the water eluate (acidic and neutral organics) and the ammonia water eluate (free amino acids) indicated considerable metabolism of glycine to other components. AOA inhibited CO_2 production from glycine at both high and low salinity with the most pronounced effect at low salinity. Arsenite (AsO_2) was more effective at inhibiting CO_2 production from glycine at low salinity than at high salinity.

Following thin-layer chromatography of the ammonia water eluate from the Dowex column, it was apparent that in the absence of inhibitors glycine was converted primarily to serine with some label appearing in alanine,

TABLE 1. Tissue levels of enzymes of serine metabolism in M. demissus *gill plus I_{50} levels for aminooxyacetic acid (AOA) and β-chloro-L-alanine (CA)*

Enzyme activity	Tissue level (μMol product/g/hr)	AOA (I_{50})[1]	CA (I_{50})[1]
3-Phosphoglycerate dehydrogenase	0.3	**	**
3-Phosphopyruvate:L-glutamate transaminase	0.2	3.6×10^{-6}M	> 1 mM
3-Phosphoserine phosphatase	ND*	**	**
3-Phosphoglycerate phosphatase	0.8	**	**
D-Glycerate dehydrogenase	2.4	**	**
Hydroxypyruvate:L-alanine transaminase[2]	2.1	6.8×10^{-8}M	1.7×10^{-5}M
Serine hydroxymethyltransferase[2]	6.2	3.2×10^{-7}M	4.9×10^{-4}M
Threonine aldolase	0.5	3.2×10^{-7}M	4.9×10^{-4}M
Serine dehydrase	0.8	1.5×10^{-4}M	7.3×10^{-3}M

*Below detectable limits (< 0.01 μMol product/g/hr).
**Not determined.
[1]I_{50} represents the concentration of inhibitor causing 50% inhibition under standard assay conditions.
[2]Products confirmed using thin-layer chromatography of dansyl derivatives (see Methods).

TABLE 2. Effect of salinity and metabolic inhibitors on the distribution of radioactivity from (U-^{14}C) glycine in isolated gill tissue[1]

Tissue (mg wet)	Salinity (o/oo)	Inhibitor (1mM)	Average dpm/mg tissue CO_2	Perchlorate ppt	Water eluate	NH_4OH eluate	Ser	Gly	Ala	Asp	Glu
(34.6,33.9)	12	None	7,809 (2.0)*	1468	15,832	4,772	1050	3,340	278	43	61
(32.3,33.0)	32	None	207 (0.12)*	1354	7,995	19,287	4436	12,925	1,545	87	114
(30.3,30.9)	12	AOA	351 (0.10)*	2214	2,549	18,838	**	18,491	**	**	**
(34.1,33.2)	32	AOA	128 (0.09)*	8679	2,917	17,718	**	16,893	**	**	**
(26.3,28.8)	12	AsO_2	2,600 (0.62)*	2937	4,769	15,226	3,512	9,594	1,840	65	**
(30.2,28.4)	32	AsO_2	62 (0.06)*	4893	2,955	17,301	2,976	13,675	1,659	**	**

[1]Incubations were for 1 hr with the indicated amount of tissue and 0.5 μc of (U-^{14}C)-glycine (carrier free, SA 113 μC/μmol).
*Values in parentheses are the specific rate of CO_2 evolution in nM/hr/10 mg wet tissue using the levels of tissue free amino acids in Greenwalt (1981).
**Radioactivity below detectable limits.

TABLE 3. Effect of salinity and metabolic inhibitors on the distribution of radioactivity from (U-^{14}C)L-serine in isolated gill tissue[1]

Tissue (mg wet)	Salinity (o/oo)	Inhibitor (1 mM)	Average dpm/mg wet tissue CO_2*	Perchlorate ppt	H_2O eluate	NH_4OH eluate	Ser	Gly	Ala	Asp	Glu
(31.6) (29.0)	12	None	7,048 (4.2)	2,135	3,464	9,047	5,727	637	1,219	538	756
(30.8) (32.0)	32	None	1,571 (1.5)	8,550	2,309	8,668	6,438	521	1,378	187	102
(32.6) (33.6)	12	AOA	49 (0.07)	3,784	1,629	16,593	15,818	**	**	**	**
(31.2) (25.8)	32	AOA	40 (0.06)	7,599	1,888	11,700	10,647	**	**	**	**
(27.3) (34.7)	12	ASO_2	2,477 (1.6)	3,693	3,947	11,377	7,264	1,287	1,579	497	416
(28.0) (30.6)	32	ASO_2	899 (0.8)	9,992	2,580	7,759	4,181	984	1,766	188	168
(27.8) (32.7)	12	CA	56	2,811	1,152	17,069	16,979	**	**	**	**
(26.7) (30.5)	32	CA	35	2,244	1,385	17,993	15,726	**	**	**	**

[1]Incubations were for one hr with the indicated amount of tissue and 0.5 μc of (U^{14}C)-serine (carrier free 181.8 μCi/μmol).
*Values in parentheses are the specific rate of CO_2, evolution in nM/hr/mg wet tissue using the levels of tissue free amino acids determined by Greenwalt ('81).
**Radioactivity below detectable limits above background (20 dpm).

glutamate, and aspartate. These results were consistent with the demonstrated presence of SHMT plus the serine dehydrase and the serine aminotransferase (Table 1). Addition of AOA to the incubation mixture caused a retention of the ^{14}C-label in glycine. The sensitivity of CO_2 production from glycine to AsO_2 and AOA inhibition indicated that lipoamide dehydrogenase (decarboxylating) and pyridoxal phosphate linked systems were involved in glycine catabolism.

When gill explants were then incubated with (U-^{14}C)-serine at different salinities (Table 3), (^{14}C)CO_2 production was slower at the high salinity compared to the low salinity. However, this reduction in serine metabolism was not as acute as that seen for glycine (Table 2). A significant portion of ^{14}C from serine appeared in alanine, glycine, aspartate, and glutamate with a larger fraction of the label appearing in alanine at the higher salinity. Addition of AOA or CA blocked serine catabolism at both salinities. Although AOA appeared to have no effect on the incorporation of ^{14}C into $HClO_4$ insoluble components, CA depressed incorporation into this fraction. Addition of AsO_2 caused a reduction in CO_2 production from serine at both low and high salinities and increased incorporation of label into glycine and alanine. These results were consistent with the view that the metabolism of serine was through both glycine (SHMT) and hydroxypyruvate plus pyruvate (aminotransferase and dehydrase).

An examination of threonine metabolism (Table 4) indicated that threonine was catabolized very slowly by gill tissue at both salinities.

When gill fragments were incubated with (^{14}C)-HCO_3 (Table 5), radiolabel incorporated into amino acids was primarily in aspartate and alanine. Glutamate was labeled at a much slower rate than aspartate or alanine indicating CO_2 fixation into the TCA cycle intermediates by the organic acid carboxyli-

TABLE 4. Effect of salinity on the distribution of radioactivity in gill tissue incubated with (U-^{14}C)-L-threonine[1]

Tissue (mg wet)	Salinity (o/oo)	Average dpm/mg wet tissue									
		CO_2	Perchlorate ppt	H_2O eluate	NH_4OH eluate	Thr	Ser	Gly	Ala	Asp	Glu
(27.8) (31.6)	12	50	1,478	847	15,406	15,047	*	*	*	*	*
(28.4) (27.1)	32	45	2,910	470	15,732	14,738	*	*	*	*	*

[1]Incubations were for one hr with the indicated amount of tissue and 0.5 μCi of (U-^{14}C)-threonine (carrier free, 234 μCi/μmol).
*Radioactivity below detectable limits above background (20 dpm).

TABLE 5. Fixation of $^{14}CO_2$ into amino acids and protein of M. demissus *gill tissue incubated in 12o/oo ASW: the effect of added amino acids and inhibitors (1 mM)*

Additions to incubation mixture*	Radioactivity incorporated (dpm/mg tissue)							
	Gly	Ser	Ala	Glu	Asp	Perchlorate ppt	NH_4OH eluate	H_2O eluate
$^{14}CO_2$	--	-	844	156	1,313	327	2,431	2,815
$^{14}CO_2$ + Glu	--	--	1,093	37	1,227	450	2,316	3,067
$^{14}CO_2$ + Gly	76	--	1,167	232	1,377	436	2,773	2,541
$^{14}CO_2$ + Ser	187	--	1,068	256	1,455	350	2,815	3,777
$^{14}CO_2$ + Gly + AOA	--	--	--	—	—	-	118	2,650
$^{14}CO_2$ + Gly + AsO_2	-	--	1,288	105	339	309	1,604	2,978
$^{14}CO_2$ + Ser + AOA	—	--	—	—	—		86	3,215
$^{14}CO_2$ + Ser + AsO_2	32	--	1,418	139	216	212	1,796	3,904

*Each value represents the mean of three determinations. The dashed lines indicate radioactivity below detectable limits (20 CPM/mg wet weight). Preparations had 40 mg of tissue in 3.0 ml buffered ASW at pH 7.2 (see Materials and Methods) containing 10 μCi of (^{14}C)HCO_3 in 2.2 mM of $NaHCO_3$. Incubations were for 1 hour.

gases (Baginski and Pierce, '78). If glutamate was added to the incubation medium, the amount of label incorporated into glutamate decreased, the labeling of alanine and aspartate increased, and the labeling glycine and serine was unchanged. When cold (^{12}C) glycine or serine were added to the incubation medium, ^{14}C was then observed in glycine suggesting the presence of a glycine-CO_2 exchange reaction and a trapping of the label by the increased cellular pool size of glycine. Incorporation of (^{14}C)CO_2into the amino acids was blocked by AOA and partially inhibited by AsO_2. The specific incorporation of radiolabel from (^{14}C)CO_2 into alanine was not appreciably affected by addition of AsO_2. The decrease in label in aspartate and glutamate in the presence of AsO_2 indicated that CO_2 fixation into both aspartate and glutamate was dependent on the cycling of intermediates in the TCA cycle. These results were consistent with other similar CO_2 tracer experiments with tissues from various molluscs (see introduction).

The results with (U-^{14}C)-glycine and (^{14}C)CO_2 tracers were indicative of a glycine cleavage enzyme in gill tissue. To test for the presence of the glycine cleavage enzyme, gill pieces were incubated with specifically labeled (1-^{14}C)-glycine and (2-^{14}C)-glycine (Table 6). It was apparent that the C-1 of glycine was the principle source of (^{14}C)CO_2 observed in the earlier experiments with (U-^{14}C)-glycine (Table 2). The production of CO_2 from the C-1 of glycine was sensitive to changes in salinity as shown by the two orders of magnitude decrease in CO_2 production at the increased salinity. The slow production of CO_2 from the C-2 of glycine was unaffected by salinity. When the dimedon trap for aldehydes was used with specifically labeled glycine, essentially no label was found as the dimedon derivative using (1-^{14}C)-glycine. On the other hand, label was found as the dimedon derivative using (2-^{14}C)-glycine. This result indicated that the labeled dimedon derivative contained only carbon from C-2 of glycine and that glyoxalate, the product of glycine transamination, was probably not significantly involved in glycine oxidation in the gill tissue. The incorporation of the C-2 of glycine into aldehyde equivalents was influenced by salinity in a fashion parallel to the influence on the production of CO_2 from the C-1 of glycine. A much lower level of radioactivity was found in the aldehyde trap with (2-^{14}C)-glycine compared the level of radioactivity in CO_2 with (1-^{14}C)-glycine. This

TABLE 6. Effect of salinity on the distribution of radioactivity from specifically labeled glycine into CO_2 and dimedon-derivative fractions by isolated gill pieces[1]

Additions to incubation mixtures	Average dpm/mg wet tissue			
	Salinity (12 o/oo)		Salinity (32 o/oo)	
	CO_2	Dimedon derivative	CO_2	Dimedon derivative
(1 - ^{14}C)glycine	10,885	33	237	35
(2 - ^{14}C)glycine	164	550	171	91

[1]The incubations were for 1 hr with 0.5 μCi (^{14}C)-glycine (carrier free) and 35 mg of wet gill tissue. The specific radioactivity of the (1-^{14}C)-glycine and (2-^{14}C) glycine were 52.9 μCi/μmol and 51 μCi/μmol, respectively.

TABLE 7. Distribution of radioactivity from specifically labeled glycine into CO_2 and dimedon-derivative fractions by gill mitochondria[1]

Additions to Incubation mixture	Average dpm/mg protein	
	CO_2	Dimedon derivative
(1 - ^{14}C)glycine	2,799	33
(2 - ^{14}C)glycine	114	480

[1]The incubations were in triplicate for 1 hr with 0.5 μCi of the indicated (^{14}C)-glycine, 30 μmoles of (^{14}C)-glycine as carrier (final SA for glycine = 36,700 dpm/μmole), and 3 ml of resuspended mitochondria (15 mg mitochondrial protein).

result was probably due to a dynamic turnover of the methylene groups in the THFA one carbon metabolic pools and accounted for the labeling of serine by reaction of glycine with CH_2=THFA in the SHMT reaction (Tables 1 and 2) (see Sato et al., '69b).

Studies with mitochondria

Subcellular fractionation was employed to determine the cellular location of glycine and glyoxalate metabolism. Only the mitochondrial fractions were found to produce (^{14}C)CO_2 from labeled glycine or glyoxalate (data not shown). The rate of production of (^{14}C)CO_2 from (U-^{14}C)-glycine was found to be proportional to the amount of mitochondria and to the incubation time (Fig. 1). Mitochondrial preparations (5 mg/ml mitochondrial protein) were then incubated with specifically labeled glycine (Table 7). The results were similar to the results using whole gill pieces (Table 2 and Table 6). The production of (^{14}C)CO_2 was primarily from the C-1 of glycine and aldehyde equivalents, trapped as the dimedon derivative, were derived from the C-2 of glycine.

To further evaluate mitochondrial glycine oxidation and the role of glyoxalate in glycine oxidation, a series of competition experiments were performed. To insure that the results were not due to a lack of mitochondrial transport of glyoxalate and glycine, metabolite uptake experiments were performed using the centrifugal silicone oil partitioning

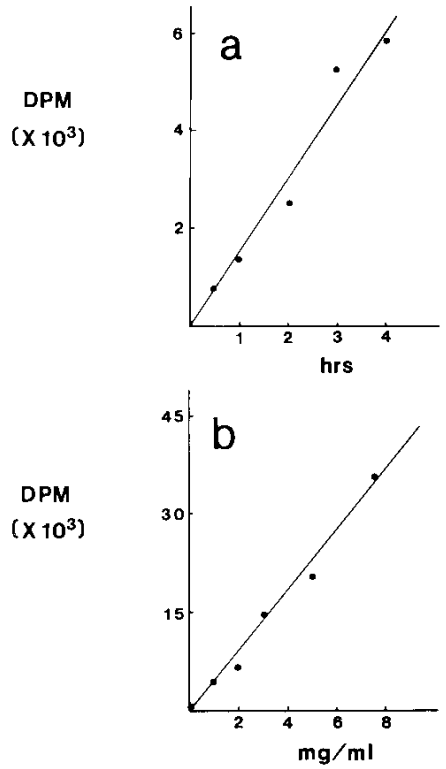

Fig. 1. a. Time course of (^{14}C)CO_2 release per mg mitochondrial protein from (1-^{14}C)-glycine (specfic activity 36,700 dpm/μmole) by gill mitochondria (5 mg mitochondrial protein/ml). b. Effect of mitochondrial concentration of the release of (^{14}C)CO_2 from (1-^{14}C)-glycine (specific acitivity 36,700 dpm/μmole) after 1 hr.

procedure (Figure 2). With both glycine and glyoxalate, the distribution of ^{14}C-label was near equilibrium after an hour. Although the rate of glycine uptake was slightly more rapid than the rate of glyoxalate uptake, glyoxalate did access the mitochondrial compartment at a rapid rate. Therefore, both compounds were used as the ^{12}C-derivatives (cold) to produce isotope dilution (competition) effects during some of the ^{14}C-tracer experiments.

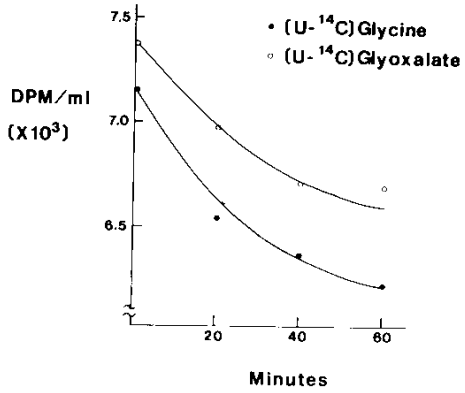

Fig. 2. Time course of removal of (^{14}C)-glycine or (^{14}C)glyoxalate specific activities 36,700 dpm/μmole) from incubation medium by gill mitochondria (5 mg mitochondrial protein/ml).

Glycine metabolism by the mitochondria was more rapid than glyoxylate metabolism (Fig. 3). The metabolism of both was sensitive to inhibition by AOA. Although AsO_2 inhibited glycine oxidation, it only slightly reduced the slow rate of glyoxylate oxidation. Addition of cold (^{12}C) glyoxalate did not reduce the amount of (^{14}C)CO_2 produced from (^{14}C)-glycine. However addition of cold (^{12}C)-glycine reduced the amount of (^{14}C) CO_2 produced from (^{14}C)-glyoxylate. Therefore, glycine showed an isotope dilution effect on glyoxylate catabolism but glyoxylate had no effect on glycine catabolism.

Mitochondria were then incubated with (^{14}C)-glycine in the presence of added pyruvate or a α-ketoglutarate or with (^{14}C)-glyoxalate in the presence of added L-alanine or L-glutamate to drive the glycine aminotransferase reactions (Fig. 3). The oxidation of both glycine and glyoxalate was reduced by these treatments. These results indicated that the glycine aminotransferases may be mitochondrial and function mainly in the thermodynamically favored glycine synthesizing direction at high glycine levels (Metzler et al., '53) and/or that the added ketoacids and amino

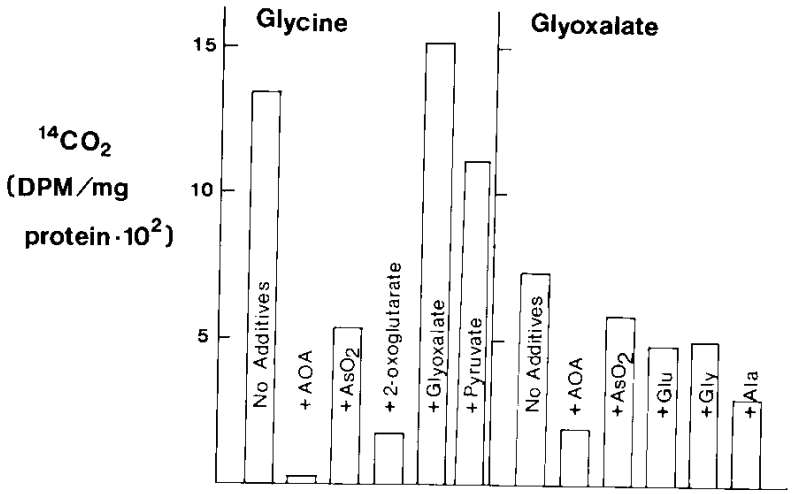

Fig. 3. Release of (^{14}C)CO_2 from (^{14}C)-glycine or (^{14}C)-glyoxalate (specific activities 36,700 dpm/μmole) by gill mitochondria (5 mg mitochondrial protein/ml) with and without added inhibitors or alternate unlabeled substrates. Additives were preincubated with mitochondria for 10 min prior to addition of labeled glycine or glyoxalate. Inhibitor concentrations (AOA,AsO_2) were 1 mM, and alternate substrates were equimolar to glycine or glyoxalate.

acids interfered with the utilization of glycine and glyoxalate in some other manner (See Hampson et al., '83,'84). Since 2-oxoglutarate failed to simulate glycine decarboxylation in gill mitochondria, it was unlikely that the aminoacetone or succinate-glycine cycles were primary pathways for glycine catabolism (Lewis et al., '67; Yoshida and Kikuchi, '72).

Overall, these studies (Fig. 3) indicated that glycine was oxidized directly and not primarily via glyoxalate and that most of the glyoxalate catabolism occurred after transamination to glycine.

DISCUSSION

The results indicate that the major route of glycine catabolism in ribbed mussel gill tissue is through the glycine cleavage enzyme reaction in the mitochondria. The sensitivity of glycine decarboxylation to AOA and AsO_2, the mitochondrial localization of the glycine catabolic activity, the specific release of CO_2 from the C-1 of glycine, the incorporation of the C-2 of glycine into aldehyde equivalents, and the pattern of CO_2 fixation into glycine are similar to results with other organisms that use the glycine cleavage enzyme as a major route of glycine catabolism (Yoshida and Kikuchi, '71, Kawasaki et al.,'66; Sato et al., '69a,b; Nakada et al., '55; Sinha and Cossins, '64; Sagers and Gunsalus, '61; Klein and Sagers, '66; Yoshida and Kikuchi, '72, de Boiso and Stoppani, '67; Moore et al., '77; Rathnam, '79; Hampson et al., '83, '84).

From the data in Table 2 and Table 7, one can estimate the relative rates of glycine decarboxylation by isolated gill tissue and by isolated gill mitochondria, respectively. Although the rate of glycine decarboxylation by the isolated tissue at low salinity was fairly rapid ($\sim$ 3 nmoles/g/min), this rate is considerably slower than the maximal rates (125 nmoles/g/min) observed for perfused rat liver preparations (Hampson et al., '84). If one considers the temperature difference of the assays and the low glycine levels in the low salinity adapted gill preparations then corrects for these factors using an apparent K_m for glycine of 2 mM (Hampson et al., '84), the estimated maximal glycine decarboxylation rate by the tissue is still five to ten times slower than the maximal rate with rat liver. On the other hand, the calculated rate with isolated gill mitochondria (Table 7) of about 76 nmoles/mg protein/hr is comparable to the rates (60–120 nmoles/mg protein/hr) calculated for isolated rat liver mitochondria (Hampson et al., '83). These results would seem to indicate that the glycine cleavage enzyme in the gill mitochondria is just as active as the enzyme in rat liver mitochondria but that the gill tissue must have fewer mitochondria than rat liver resulting in a slower rate of glycine oxidation at the tissue level. The very strong inhibition of glycine oxidation in gills incubated at high salinities indicates an acute regulation of the gill glycine cleavage enzyme that is apparently not found with the rat liver enzyme.

The presence of alanine:glyoxalate aminotransferase suggests that glycine could be transaminated to glyoxalate for oxidation (Bishop et al., '81). However, the slow rate of glyoxalate oxidation compared to glycine oxidation suggests that this is not the case. Further, in competition experiments, glycine inhibited glyoxalate oxidation but glyoxalate had no effect on glycine oxidation. This result suggests that when glyoxalate is available it is primarily converted to glycine by the gill transaminases and agrees with observations on glyoxalate metabolism in the tissues of other organisms (Roswell et al., '72, Yokota et al., '78, Rofe and Edwards, '78, Noguchi et al., '82).

Most of the glycine appears to be derived from serine through the SHMT reaction or from protein through the release of preformed glycine during protein turnover (Greenwalt and Bishop, '80; Bishop et al., '81). At low salinities the relative amounts of carbon transferred from serine to glycine compared to carbon transferred to pyruvate or hydroxypyruvate through the dehydrase or transaminase reactions seem similar. The conversion of serine to pyruvate and alanine is favored at high salinity. Quantitative glycine biosynthesis through reversal of the glycine cleavage enzyme seems unlikely considering the tissue levels of glycine and the results of the $(^{14}C)CO_2$ exchange experiments (Table 5).

The biosynthesis of serine and glycine from glucose by ribbed mussel gill tissue (^{14}C-glucose tracer studies) seems to be slow (Baginski and Pierce, '78; Greenwalt, '81). All the enzymes of the nonphosphorylated pathway for serine biosynthesis from triose phosphate glycolytic intermediates are present in the gill tissue. Phosphoserine phosphatase was the only enzyme of the phosphorylated pathway that was not detected. The lack of this

phosphatase has also been shown in ammonotelic vertebrate livers that produce serine by the phosphorylated pathway (Grillo and Coghe, '66; Grillo et al., '66). The absence of this phosphatase activity may be irrelevant because a variety of alkaline and acid phosphatases are known to occur in mussel tissues (Koehn et al., '73). In any event, it appears that ribbed mussels have the capacity for slow serine biosynthesis from glucose via the glycolytic intermediates.

The catabolism of threonine is slow and a weak threonine aldolase activity (SHMT, data not shown) is present. This result is similar to results obtained with vertebrate liver where threonine aldolase is probably the primary route of threonine catabolism (Schirch and Gross, '68; Bird and Nunn, '79). However, because no label from the (^{14}C)-threonine was detected in glycine, the in vivo role of the threonine aldolase (SHMT) in threonine catabolism in gill tissue is speculative.

In conclusion, it appears that, metabolically, the glycine levels in these tissues are controlled by an acute regulation of the mitochondrial glycine cleavage enzyme, the major route of glycine catabolism. At high salinities, glycine derived from a variety of sources including serine, glyoxylate, or protein appears to accumulate behind a severely blockaded, mitochondrial, glycine cleavage enzyme. At low salinities, this blockade is removed and glycine turnover increases about 20-fold. These results account for the rather slow accumulation of tissue glycine as the ribbed mussels are adapted to high salinities (Baginski and Pierce, '75,'77) and for the rapid metabolism of glycine by ribbed mussel tissues at low salinities. Studies on the specific mechanisms regulating the glycine cleavage enzyme in ribbed mussel tissues are in progress.

ACKNOWLEDGMENTS

This work was supported in part by grants from the ISU Research Foundation and the NSF (PCM 80-22606). We thank Dr. J. M. Viles and the Houston Endowment for the use of some equipment and Ms. M. Nims for assistance in the preparation of the manuscript. This paper is contribution 222 from the Tallahassee, Sopchoppy, and Gulf Coast Marine Biological Association. L.L.E. is a Fellow of the USPHS (National Research Service Award).

LITERATURE CITED

Ahmad, T. A., and A. E. Chaplin (1979) Seasonal variation in the anaerobic metabolism of the mussel *Mytilus edulis* (L.). Comp. Biochem. Physiol., *64B*:351–356.

Allen, W. V., and T. Kilgore (1975) The essential amino acid requirements of the red abalone, *Haliotis rufescens*. Comp. Biochem. Physiol., *50A*:771–775.

Andrews, T. R., and R. G. B. Reid (1972) Ornithine cycle and uricolytic enzymes in four bivalve molluscs. Comp. Biochem. Physiol., *42B*:475–491.

Baginski, R. M., and S. K. Pierce (1975) Anaerobiosis: A possible source of osmotic solute for high-salinity acclimation in marine molluscs. J. Exp. Biol., *62*:589–598.

Baginski, R. M., and S. K. Pierce (1977) The time course of intracellular free amino acid accumulation in tissues of *M. d. demissus*. Comp. Biochem. Physiol., *57A*:407–412.

Baginski, R. M., and S. K. Pierce (1978) A comparison of amino acid accumulation during high salinity adaptation with anaerobic metabolism in the ribbed mussel, *Modiolus demissus demissus*. J. Exp. Zool., *203*:419–428.

Bartberger, C. A., and S. K. Pierce (1976) Relationship between ammonia excretion rates and hemolymph nitrogenous compounds of a euryhaline bivalve during low salinity adaptation. Biol. Bull., *150*:1–14.

Bird, M. I., and P. B. Nunn (1979) Glycine formation from L-threonine in intact isolated rat liver mitochondria. Biochem. Soc. Trans., *7*:1276–1277.

Bishop, S. H., L. L. Ellis, and J. M. Burcham (1983) Amino acid metabolism in molluscs. In: The Mollusca. K. M. Wilbur, ser. ed., (Metabolic Biochemistry and Molecular Biomechanics. P. W. Hochachka, ed.) Academic Press, New York, Vol. I, pp. 243–327.

Bishop, S. H., D. E. Greenwalt, and J. M. Burcham (1981) Amino acid cycling in ribbed mussel tissues subjected to hyperosmotic shock. J. Exp. Zool., *215*:277–287.

Burcham, J. M., D. E. Greenwalt, and S. H. Bishop (1980) Amino acid metabolism in euryhaline bivalves: the L-amino acid oxidase from ribbed mussel gill tissue. Mar. Biol. Lett., *1*:329–340.

Burcham, J. M., A. Ritchie, and S. H. Bishop (1984) Preparation and some respiratory properties of coupled mitochondria from ribbed mussel (*Modiolus demissus*) gill tissue. J. Exp. Zool., *229*:55–67.

Campbell, J. W., and S. H. Bishop (1970) Nitrogen metabolism in molluscs. In: Comparative Biochemistry of Nitrogen Metabolism. J. W. Campbell ed. Academic Press, Inc., N.Y. Vol. 1, pp. 103–206.

Campbell, J. W., and K. V. Speeg, Jr. (1968) Arginine biosynthesis and metabolism in terrestrial snails. Comp. Biochem. Physiol., *23*:3–32.

Chen, C., and J. Awapara (1969) Effect of oxygen on the end-products of glycolysis in *Rangia cuneata*. Comp. Biochem. Physiol., *31*:395–401.

Cooley, L., D. R. Crawford, and S. H. Bishop (1976) Urease from the lugworm *Arenicola cristata*. Biol. Bull., *151*:96–107.

Crowe, J. H. (1981) Tranport of exogenous substrate and cell volume regulation in bivalve molluscs. J. Exp. Zool., *215*:363–370.

Dando, P. R. (1981) Strombine [N-(carboxymethyl)-D-alanine] dehydrogenase and alanopine [*meso*-N-(1-carboxyethyl)-alanine] dehydrogenase from the mussel *Mytilus edulis* L. Biochem. Soc. Trans., *9*:297–298.

de Boiso, J. F., and A. O. M. Stoppani (1967) Metabolism of serine and glycine in baker's yeast. Biochim. Biophys. Acta, *148*:48–59.

de Zwaan, A., A. M. T. de Bont, W. Zurburg, B. L. Bayne,

and D. R. Livingstone (1983) On the role of strombine formation in the energy metabolism of adductor muscle of a sessile bivalve. J. Comp. Physiol., *149*:557–563.

de Zwaan, A., and W. J. A. van Marrewijk (1973a) Anaerobic glucose degradation in the sea mussel *Mytilus edulis* L. Comp. Biochem. Physiol., *44B*:429–439.

de Zwaan, A., and W. J. A. van Marrewijk (1973b) Intracellular localization of pyruvate carboxylase, phosphoenolpyruvate carboxykinase and "malic enzyme" and the absence of glyoxylate cycle enzymes in the sea mussel (*Mytilus edulis* L.). Comp. Biochem. Physiol., *44B*:1057–1066.

Dryer, R. L., A. R. Tammes, and J. I. Routh (1957) The determination of phosphorous and phosphatase with N-phenyl-β-phenylenediamine. J. Biol. Chem., *255*:177–183.

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

Falany, C. N., and F. E. Freidl (1981) Amino acid transamination in the fresh water clams *Anodonta couperiana* and *Popenaias buckleyi*. Comp. Biochem. Physiol., *68B*:119–123.

Fallon, H. J., E. J. Hackney, and W. L. Byrne (1966) Serine biosynthesis in rat liver. Regulation of enzyme concentration by dietary factors. J. Biol. Chem., *241*:4157–4167.

Fields, J. H. A., A. K. Eng, W. D. Ramsden, P. W. Hochachka, and B. Weinstein (1980) Alanopine and strombine are novel imino acids produced by a dehydrogenase found in the adductor muscle of the oyster, *Crassostrea gigas*. Arch. Biochem. Biophys., *201*:110–114.

Fukushima, M., Y. Aihara, and A. Ichiyama (1978) Immunochemical studies on induction of rat liver mitochondrial serine:pyruvate aminotransferase by glucagon. J. Biol. Chem., *253*:1187–1194.

Greenwalt, D. E. (1981) Role of amino acids in cell volume control in the ribbed mussel: alanine and proline metabolism. PhD Thesis, Iowa State University, Ames, Iowa, pp. 1–127.

Greenwalt, D. E., and S. H. Bishop (1980) Effect of aminotransferase inhibitors on the pattern of free amino acid accumulation in isolated mussel hearts subjected to hyperosmotic stress. Physiol. Zool., *53*:262–269.

Grillo, M. A., and M. Coghe (1966) Phosphoserine phosphatase of vertebrates. Comp. Biochem. Physiol., *17*:169–173.

Grillo, M. A., T. Fossa, and M. Coghe (1966) Synthesis of serine in the liver of vertebrates. Comp. Biochem. Physiol., *19*:589–596.

Hammen, C. S. and K. M. Wilbur (1959) Carbon dioxide fixation in marine invertebrates. I. The main pathway in the oyster. J. Biol. Chem., *234*:1268–1272.

Hampson, R. K., L.L. Barron, and M. S. Olsen (1983) Regulation of the glycine cleavage system in isolated rat liver mitochondria. J. Biol. Chem., *258*:2993–2999.

Hampson, R. K., M. L. Taylor, and M. S. Olsen (1984) Regulation of the glycine cleavage system in the isolated perfused rat liver. J. Biol. Chem., *259*:1180–1185.

Hartley, B. S. and V. Massey (1956) The active center of chymotrysin. I. Labelling with a fluorescent dye. Biochim. Biophys. Acta, *21*:58–70.

Hoyaux, J., R. Gilles, and Ch. Jeuniaux (1976) Osmoregulation in molluscs of the intertidal zone. Comp. Biochem. Physiol., *53A*:361–365.

Kawasaki, H., T. Sato, and G. Kikuchi (1966) A new reaction for glycine biosynthesis. Biochem. Biophys. Res. Commun., *23*:227–233.

Kikuchi, G. (1973) The glycine cleavage system: Composition, reaction mechanism, and physiological significance. Mol. Cell. Biochem., *1*:169–187.

Kikuchi, G., and K. Hiraga (1982) The mitochondrial glycine cleavage system: Unique features of the glycine decarboxylation. Mol. Cell. Biochem., *45*:137–149.

King, T. E. (1967) The Keilin-Hartree heart muscle preparation. In: Methods in Enzymology. R. W. Estabrook and M. E. Pullman, eds. Academic Press, New York, Vol. X, pp. 202–208.

Klein, S. M., and R. D. Sagers (1966) Glycine metabolism. I. Properties of the system catalyzing the exchange of bicarbonate with the carboxyl group of glycine in *Peptococcus glycinophilus*. J. Biol. Chem., *241*:197–205.

Koryean, S. A., and K. B. Storey (1983) Organ-specific metabolism during anoxia and recovery from anoxia in the cherrystone clam, *Mercenaria mercenaria*. Can. J. Zool., *61*:2674–2681.

Koehn, R. K., F. J. Turano, and J. B. Mitton (1973) Population genetics of marine pelecypods. II. Genetic differences in microhabitats of *Modiolus demissus*. Evolution, *27*:100–105.

LaNoue, K. F., E. I. Walajtys, and J. R. Williamson (1973) Regulation of glutamate metabolism and interactions with the citric acid cycle in rat heart mitochondria. J. Biol. Chem., *248*:7171–7183.

Lee, T. W., and J. W. Campbell (1965) Uric acid synthesis in the terrestrial snail, *Otala lactea*. Comp. Biochem. Physiol., *15*:457–468.

Lewis, M., G. R. Lee, G. E. Cartwright, and M. M. Wintrobe (1967) Glycine decarboxylation in the porcine erythrocyte: Its relation to amino-levulinic acid synthesis. Biochim. Biophys. Acta, *141*:296–309.

Livingstone, D. R., J. Widdows, and P. Fieth (1979) Aspects of nitrogen metabolism of the common mussel *Mytilus edulis*: Adaptation to abrupt and fluctuating changes in salinity. Mar. Biol., *53*:41–55.

Metzler, D. E., J. Olivard, and E. E. Snell (1953) Transamination of pyridoxamine and amino acids with glyoxylic acid. J. Am. Chem. Soc., *76*:644–648.

Miller, G. L. (1959) Protein determination for large numbers of samples. Anal. Chem., *31*:964.

Moore, A. L., C. Jackson, B. Halliwell, J. E. Dench, and D. O. Hall (1977) Intramitochondrial localization of glycine decarboxylase in spinach leaves. Biochem. Biophys. Res. Commun., *78*:483–491.

Motokawa, Y. and G. Kikuchi (1974) Glycine metabolism by rat liver mitochondria. Reconstitution of the reversible glycine cleavage system with partially purified protein components. Arch. Biochem. Biophys., *164*:624–633.

Nakada, H. I., B. Friedman, and S. Weinhouse (1955) Pathways of glycine catabolism in rat liver. J. Biol. Chem., *216*:583–592.

Noguchi, T., S. Fujiwara, Y. Takada, T. Mori, and M. Nagano (1982) Metabolism of urea and glyoxalate, degrative products of purines in marine animals. J. Biochem. (Tokyo), *92*:525–529.

Park, L. S. and P. Datta (1979) The role of glyoxalate in the regulation of biodegrative threonine dehydratase of *Escherichia coli*. J. Biol. Chem., *254*:7927–7934.

Paynter, K. T., R. J. Hoffmann, L. L. Ellis, and S. H. Bishop (1984a) Partial characterization of the cytosolic and mitochondrial aspartate aminotransferases from ribbed mussel gill tissue. J. Exp. Zool. *231*:185–198.

Paynter, K. T., L. L. Ellis, and S. H. Bishop (1984b) Cellular localization and partial characterization of the alanine aminotransferase in ribbed mussel gill tissue. J. Exp. Zool., *232*:51–58.

Pierce, S. K., and M. J. Greenberg (1972) The nature of cellular volume regulation in marine bivalves. J. Exp. Biol., *57*:681–692.

Pizer, L. I. (1964) Enzymology and regulation of serine biosynthesis in cultured human cells. J. Biol. Chem., *239*:4219–4226.

Rathnam, C. K. M. (1979) Metabolic regulation of carbon flux during C_4 photosynthesis. II. In situ evidence for refixation of photorespiratory CO_2 by C_4 phosphoenolpyruvate carboxylase. Planta, *145*:13–23.

Read, K. R. H. (1962) Transamination in certain tissue homogenates of the bivalved molluscs *Mytilus edulis* L. and *Modiolus modiolus* L. Comp. Biochem. Physiol., *7*:15–22.

Rofe, A. M., and J. B. Edwards (1978) Oxalate synthesis in isolated rat hepatocytes: The effects of hydroxypyruvate and aminooxyacetate. Biochem. Med., *20*:323–335.

Rowsell, E. V., K. Snell, J. A. Carnie, and K. V. Rowsell (1972) The subcellular distribution of rat liver L-alanine-glyoxylate aminotransferase in relation to a path way for glucose formation involving glyoxalate. Biochem. J., *127*:155–165.

Sagers, R. D. and I. C. Gunsalus (1961) Intermediary metabolism of *Diplococcus glycinophilus*. J. Bacteriol., *81*:541–549.

Sato, T., H. Kochi, N. Sato, and G. Kikuchi (1969a) Glycine metabolism by rat liver mitochondria. III. The glycine cleavage and the exchange of carboxyl carbon of glycine with bicarbonate. J. Biochem. (Tokyo), *65*:77 83.

Sato, T., H. Kochi, Y. Motokawa, H. Kawasaki, and G. Kikuchi (1969b) Glycine metabolism by rat liver mitochondria. I. Synthesis of two molecules of glycine from one molecule each of serine, bicarbonate and ammonia. J. Biochem. (Tokyo), *65*:63–70.

Schirch, L., and T. Gross (1968) Serine transhydroxymethylase. Identification as the threonine and allothreonine aldolases. J. Biol. Chem., *243*:5651–5655.

Schirch, L., C. M. Tatum, and S. J. Benkovic (1977) Serine transhydroxymethylase: Evidence for a sequential random mechanism. Biochemistry, *16*:410–419.

Shumway, S. E., P. A. Gabbott, and A. Youngson (1977) The effect of fluctuating salinity on the concentrations of free amino acids and ninhydrin-positive substances in adductor muscles of eight species of bivalve mollusca. J. Exp. Mar. Biol. Ecol., *29*:131–150.

Shumway, S. E., and A. Youngson (1979) The effects of fluctuating salinity on the physiology of *Modiolus demissus* (Dillwyn). J. Exp. Mar. Biol. Ecol., *40*:167–181.

Sinha, S. K., and E. A. Cossins (1964) The metabolism of ^{14}C–glycine by plant tissues. Biochem. J., *93*:27–34.

Strange, K. B., and J. H. Crowe (1979a) Acclimation to successive short term salinity changes by the bivalve *Modiolus demissus*. I. Changes in hemolymph osmotic concentration, hemolymph ion concentration and tissue water content. J. Exp. Zool., *210*:221–226.

Strange, K. B., and J. H. Crowe (1979b) Acclimation to successive short term salinity changes by the bivalve *Modiolus demissus*. II. Nitrogen metabolism. J. Exp. Zool., *210*:227–236.

Taylor, R. T., and H. Weissbach (1965) Radioactive assay for serine transhydroxymethylase. Anal. Biochem., *13*:80–84.

Wang, E. A., R. Kallen, and C. Walsh (1981) Mechanism-based inactivation of serine transhydroxymethylases by D-fluoroalanine and related amino acids. J. Biol. Chem., *256*:6917–6926.

Whiteley, H. R. (1960) The distribution of the formate activating enzyme and other enzymes involving tetrahydrofolic acid in animal tissues. Comp. Biochem. Physiol., *1*:227–247.

Willis, J. E., and H. J. Sallach (1964) The occurrence of *D*-3-phosphoglycerate dehydrogenase in animal tissue. Biochim. Biophys. Acta, *81*:39–54.

Yokota, A., Y. Nakano, and S. Kitaoka (1978) Metabolism of glycolate in mitochondria of *Euglena gracilis*. Agric. Biol. Chem., *42*:121–129.

Yoshida, T., and G. Kikuchi (1971) Significance of the glycine cleavage system in glycine and serine catabolism in avian liver. Arch. Biochem. Biophys., *145*:658–668.

Yoshida, T., and G. Kikuchi (1972) Comparative study on major pathways of glycine and serine catabolism in vertebrate livers. J. Biochem. (Tokyo), *72*:1503–1516.

Zurburg, W., A. M. T. de Bont, and A. de Zwaan (1982) Recovery from exposure to air and the occurrence of strombine in different organs of the sea mussel *Mytilus edulis* L. Mol. Physiol., *2*:135–147.

NOTE ADDED IN PROOF

Glycine (10 mM) added to coupled, respiring gill mitochondria prepared according to the procedure of Burcham et al. ('84) stimulated ADP-dependent oxygen consumption and showed a P:O ratio of 2.8. This result indicates that the oxidation of NADH produced in the mitochondrial glycine cleavage reaction is coupled through the mitochondrial respiratory chain at Site 1 in the same fashion as is found with rat liver mitochondria (Hampson et al., '83).