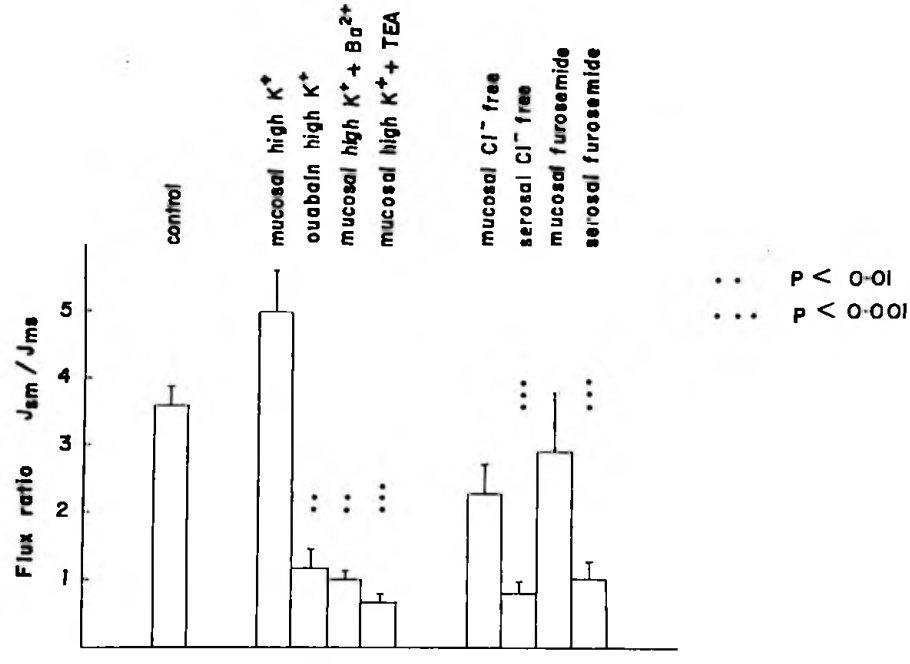

Figure 2.--2-Deoxy-D-galactose secretion by flounder intestinal mucosa. Effect of electrolytes and inhibitors on the flux ratio (J_{sm}/J_{ms}). For experimental details see Naftalin & Kleinzeller, 1981.

The above results, as well as preliminary experiments concerning effects of 2-dGal on the transepithelial P.D., and I_{sc} in the flounder mucosa, raise several possibilities as to the coupling between 2-dGal secretion and ionic fluxes. One such possibility involves, directly or indirectly, the furosemide-sensitive Na^+-Cl^- cotransport system localized at the mucosal cell face (c.f. Field et al., J. Memb. Biol. 41:265, 1978). This work was supported in part by NIH grant AM12619 and a grant from the Whitehall Foundation to Dr. Kleinzeller. R.J. Naftalin was a recipient of a NATO Research Fellowship.

THE METABOLIC PATHWAY FOR OXIDATION OF β-ALANINE IN THE LIVER OF RAJA ERINACEA AND ITS REGULATION DURING ENVIRONMENTAL DILUTION

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Free amino acids play a major role in cell volume regulation in the little skate. During acclimation of the fish to 50% seawater, the solute concentration in the tissues is reduced due to the release of certain amino acids into the plasma. The amino acid released depends on the tissue: β-alanine from muscle and erythrocytes, sarcosine from muscle and taurine from brain and myocardium (Goldstein, J. Exp. Zool. 215:371, 1981). In order to achieve osmoregulation, these amino acids must be eliminated from the plasma. In the case of taurine, this is achieved by excretion via the kidney but β-alanine is oxidised (to carbon dioxide and water) in the liver (King et al., J. Exp. Zool. 212:69, 1980). These workers also showed that liver slices from fish acclimated to 50% seawater oxidised β-alanine 1.7 times faster than comparable slices from fish maintained in seawater.

Comparatively little work has been done on the pathway of β-alanine oxidation in any organism but a transminase able to catalyse the transfer of an amino group from β-alanine to 2-oxo-glutarate has been described from mouse brain and liver (Roberts & Bregoff, J. Biol. Chem. 201:393, 1953) and from the bacterium Pseudomonas

fluorescens (Hayaishi et al., J. Biol. Chem., 236:781, 1961). In Pseudomonas the resulting malonate semialdehyde is oxidatively decarboxylated to acetyl-CoA in a reaction catalysed by a malonate semialdehyde dehydrogenase (Yamada & Jakoby, J. Biol. Chem., 235:589, 1960 and Hayaishi et al., loc. cit.).

In the study described below, the activities and properties of β-alanine transaminase (β-AT) and malonate semialdehyde dehydrogenase (MSDH) were determined in skate liver homogenates in order to provide information about the pathway and its regulation. This necessitated developing a new radiochemical assay procedure for MSDH due to the inavilability of its substrate, malonate semialdehyde.

METHODS

Skate were kept in normal seawater except for those exposed to environmental dilution. The environment of the latter was gradually adjusted to 50% seawater over 5 days and the fish maintained at this concentration for a further 2 days.

Fish were killed by spinal section and their liver removed onto ice. Small pieces of this tissue were homogenized in a pestle homogenizer in ten volumes of triethanolamine-HCl buffer (pH 7.5) containing EDTA (5mM) and $MgCl_2$ (10mM). (For the assay of MSDH, 50mM 2-mercaptoethanol was added to the extraction medium.) All homogenates were briefly sonicated to rupture mitochondria.

β-AT was assayed by incubation with β-alanine (24mM) and 2-oxoglutarate (6 mM) in tris/phosphate buffer at pH 8.1. The amount of glutamate generated was measured spectrophotometrically, using NAD^+ and glutamate dehydrogenase (Bernt & Bergmeyer, in Methods of Enzymatic Analysis (ed. Bergmeyer), 1704, 1974).

The assay developed for MSDH involved the generation of ^{14}C-malonate semialdehyde from β-[1-^{14}C]-alanine (New England Nuclear) in a preliminary incubation with skate liver homogenate and 2-oxoglutarate. Conditions and concentrations for this incubation were as described above for the β-AT assay but with the addition of NAD^+ (2 mM) and glutamate dehydrogenase to regenerate glutamate and so displace the equilibrium towards malonate semialdehyde synthesis. Approximately 7% conversion of β-alanine was achieved. After deproteinisation with perchloric acid and neutralization, the reaction mixture was used as a source of ^{14}C-malonate semialdehyde without further purification. The amount present was determined using aniline citrate to decarboxylate an aliquot (Krebs & Eggleston, Bloch, J., 39:408, 1945) and estimating the $^{14}CO_2$ released.

To measure MSDH activity the homogenate was incubated with ^{14}C-malonate semialdehyde (approx. 0.4 mM), NAD^+ (1.7 mM), coenzyme A (0.5 mM) and aminooxyacetate (2.4 mM, to inhibit β-AT) in tris/phosphate buffer (pH 8.1, 33 mM) in a sealed Erlenmeyer flask. After 30 min the reaction was stopped by addition of 6N sulfuric acid and the $^{14}CO_2$ displaced into an absorbent for scintillation counting. Controls were carried out in which acid was added before the homogenate.

All determinations of $^{14}CO_2$ release, including those from β-alanine incubated with liver slices, were carried out as described by King et al. (loc. cit.).

RESULTS

Neither the activity of β-alanine transaminase nor malonate semialdehyde dehydrogenase showed a significant increase in fish adapted to 50% seawater (Table 1).

The transaminase had a K_m for β-alanine of 1.11 mM and for 2-oxoglutarate of 0.22 mM. The pH optimum was pH 8.25, with approximately 50% activity at pH 7.0, γ-Aminobutyric acid was also transaminated, apparently by the same enzyme, but at a lower rate (64% when γ-aminobutyrate concentration was 24 mM). Activity was undetectable in the presence of 2 mM aminooxyacetate and showed no increase with added pyridoxal phosphate (10^{-4}M).

TABLE 1

Changes in the activities of β-AT and MSDH in the livers of little skate acclimated to 50% seawater.

ENZYME		SEAWATER	50% SEAWATER
β-alanine transaminase	activity*	17.89	17.00
	n	8	6
	S.E.M.	1.65	1.44
malonate semialdehyde dehydrogenase	activity*	2.20	1.70
	n	8	7
	S.E.M.	0.56	0.21

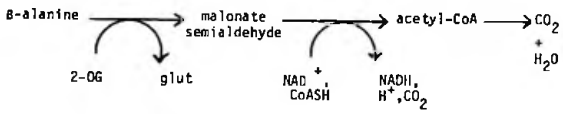
* $\mu mol.g^{-1}.hr^{-1}$ at 15°C

Linear timecourses were obtained for the dehydrogenase assay up to 20% conversion of substrate to product over 60 minutes. The activity was reduced to 13% and 8% in the absence of added NAD^+ and coenzyme A respectively but no pyridoxal phosphate requirement could be demonstrated. No decarboxylation of β-alanine occurred when it was incubated with liver homogenate in the presence of aminooxyacetate.

Inhibitors of electron transfer or oxidative phosphorylation reduced the rate of oxidation of β-alanine by liver slices. Rotenone was least effective and azide most effective.

DISCUSSION

The demonstration of a β-alanine transaminase and an enzyme catalysing the decarboxylation of malonate semialdehyde which is dependent on NAD^+ and coenzyme A strongly support the proposed pathway for β-alanine oxidation, which can be represented:



It is likely that the small $^{14}CO_2$ production in the absence of added NAD^+ and coenzyme A is due to the presence of these metabolites in the homogenate.

In principle, regulation of this pathway could be achieved by allosteric means or by changes in the amount of enzyme present. Although no significant change was found in the latter, the wide variation in MSDH activity in control fish (possibly due to variations in the concentration of malonate semialdehyde in the assay) make it difficult to eliminate this possibility. On theoretical grounds, regulation is predicted at the enzyme whose maximal activity is close to the largest flux through the pathway. The flux measured in slices taken from fish acclimated 50% seawater is 0.25 μmol β-alanine, g^{-1}, hr^{-1} (King et al., loc. cit.). This is less than the activity of either of the enzymes measured above but was measured at a concentration of β-alanine (0.1 mM) well below the K_m of β-AT for β-alanine. When this is taken into account it reveals MSDH as a likely site of control, assuming that the activities of this enzyme reported above are close to maximal.

The similarity between the K_m of β-AT for β-alanine (1.11 mM) and the concentration of this amino acid in the liver (0.97 mM) suggests that the flux through the pathway would be sensitive to changes in the concentration of β-alanine and this could be regulatory significance.

36 EFFECT OF RENAL PELVIC CONTRACTIONS ON FLUID COMPARTMENTS IN THE MAMMALIAN RENAL PAPILLA

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It has been shown in rodents that the renal pelvis milks the renal papilla, and that the peristaltic contractions