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AMINO ACID UPTAKE AND METABOLISM BY EMBRYOS OF THE BLENNY *ZOARCES VIVIPARUS*

BY BODIL KORSGAARD

Institute of Biology, Odense University, Campusvej 55, 5230 Odense M, Denmark

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Summary

The ability of blenny *Zoarces viviparus* (L.) embryos in early and late development to assimilate and metabolize ambient L-alanine was investigated *in vitro* and *in vivo* by means of autoradiographic and radiochemical methods. Autoradiograms showed that after 24 h of exposure to L-[^{14}C]alanine, label was distributed in the tissues of the embryos.

Uptake rates for ^{14}C-labelled L-alanine *in vitro* were estimated by measuring the disappearance of radioactivity from the medium. Net uptake rates were measured by high performance liquid chromatography of samples taken simultaneously from the medium. Uptake rates, based on uptake of the tracer ($0.60\,\mu mol\,g^{-1}\,h^{-1}$), were similar to net uptake rates ($0.54\,\mu mol\,g^{-1}\,h^{-1}$) in embryos in late development in the *in vitro* incubations in $60\,\mu mol\,l^{-1}$ alanine. *In vivo*, the injected tracer was completely cleared from the ovarian fluid over 24 h. After intraovarian and *in vitro* incubation of the embryos with labelled alanine, there was evidence for both catabolic and anabolic metabolism of the amino acid by production of $^{14}CO_2$ and by incorporation of radioactivity into molecules insoluble in trichloroacetic acid, respectively.

The data provide evidence for uptake and metabolism of amino acids by embryos *in vitro* and *in vivo*. An increase in the capacity for uptake and metabolism of L-alanine may occur during development of the embryos in the ovary.

Introduction

Previous studies have shown that embryos, larvae and juveniles from different teleost species have the ability to take up low molecular weight compounds such as glucose and amino acids from their ambient medium (Siebers and Rosenthal, 1977; Lin and Arnold, 1982; Fauconneau, 1985). By autoradiographic methods, it was recently shown that turbot (*Scophthalmus maximus*) larvae are able to ingest amino acids from sea water during their yolk-sac stage (Korsgaard, 1991). The significance of these different observations in relation to larval nutritional physiology is at present very uncertain. It has been suggested that for fish larvae,

Key words: embryos, *Zoarces viviparus*, amino acid uptake, metabolism.

which encounter a metabolic deficit during the transition period up to first feeding, the ability to assimilate low molecular weight compounds may have nutritional importance (Heming and Buddington, 1988). Considering the high mortality of marine fish larvae during early post-yolk-sac life, nutritional requirements and metabolic capacity during early larval development are of particular interest in the field of fish physiology.

Maternal–foetal trophic relationships in viviparous teleosts offer a unique opportunity for the study of nutritional physiology of developing fish embryos (Wourms *et al.* 1988; Korsgaard and Weber, 1989). Heming and Buddington (1988) suggested that, by rearing embryos from viviparous teleosts, before and after hatching on defined media, it may be possible to obtain valuable information on the nutritional requirements of early life stages.

In the blenny *Zoarces viviparus*, the embryos are retained in the ovarian cavity for 4–5 months. During this period they undergo extensive growth and development, from a fertilized egg of approximately 20 mg through yolk-sac and post-yolk-sac stages into a young fish of approximately 400 mg at parturition (Korsgaard and Andersen, 1985; Korsgaard, 1986). During most of this time they lie freely in the ovarian lumen surrounded by ovarian fluid, which contains dissolved organic substances such as glucose at a concentration of approximately $1000\,\mu mol\,l^{-1}$ (Korsgaard, 1983) and amino acids at approximately $400\,\mu mol\,l^{-1}$ (Kristoffersson *et al.* 1973). These substances are believed to serve as nutritional material for the growing embryos (Kristoffersson *et al.* 1973; Korsgaard, 1983) In the present work, the capacity of the developing embryos after hatching to assimilate and metabolize L-[^{14}C]alanine from the ambient medium *in vitro* or *in vivo* was investigated. Since uptake, evaluated by a tracer technique only, may not indicate the occurrence of net uptake, medium depletion of labelled L-alanine was compared with net changes in ambient alanine concentrations as assessed by high performance liquid chromatography (HPLC) measurements. In addition, autoradiograms were prepared and studied by dark-field illumination to detect the presence of label in the embryonic tissue. Metabolic conversion of tracer L-alanine was evaluated by the production of $^{14}CO_2$ and by distribution of labelled carbon in the trichloroacetic acid (TCA)-soluble and TCA-insoluble (protein) fractions of embryonic tissue.

Materials and methods

Pregnant female blennies *Zoarces viviparus* were caught in the Little Belt of Denmark. The fish weighed between 120 and 155 g. In the laboratory they were kept in recirculated, aerated sea water (20‰) at 11 °C and allowed an acclimation period of 6 days before experiment.

In vitro *experiments*

In the *in vitro* experiments the embryos were dissected out of a quickly decapitated female, weighed and placed in Ringer's solution for 1 h at 11 °C.

Standard incubations consisted of 1.5 g of embryos in 10 ml of Ringer's solution with 60 or 600 μmol l^{-1} unlabelled carrier L-alanine to which 10 μl (50 μCi) of tracer was added. Labelled L-[U-^{14}C]alanine (New England Nuclear) with a specific activity of 172 mCi $mmol^{-1}$ was used in the experiments. The low concentration of unlabelled alanine was chosen because it is similar to the natural concentration found in the ovarian fluid, which is within the range of 51.6±20.3 μmol l^{-1} (N=5) measured in November (Kristoffersson *et al.* 1973) to 64.2±7.2 μmol l^{-1} (N=6) as measured in October by HPLC in the present work. The high concentration (600 μmol l^{-1}) was chosen to investigate the effect of a different carrier concentration on the uptake rate and turnover time of the labelled L-alanine. The Ringer's solution consisted of iso-osmotic saline, 170 mmol l^{-1} NaCl, 4 mmol l^{-1} KCl, 4 mmol l^{-1} $CaCl_2$, 10 mmol l^{-1} $NaHCO_3$, pH 7.5, with a final osmotic concentration of 340 mosmol kg^{-1}, in accordance with values obtained by Korsgaard (1983) in ovarian fluid. All glassware used in the experiments was carefully acid-cleaned, and all solutions were freshly made with double-distilled water. No antibiotics were used in the experimental samples. However, identical samples (two in each experiment) containing penicillin and streptomycin (5000 i.u. and 50 mg l^{-1}) were used as controls for microbial activity in the experiments. These samples were not included in the results since uptake and turnover rates and production of labelled CO_2 in the antibiotic-treated control samples were found to be similar to those of the experimental samples to which no antibiotics were added. Samples were taken at intervals to follow the time course of depletion of labelled and total L-alanine from the medium. 2 ml of 0.02 mol l^{-1} HCl was added to the 0.1 ml sample and the mixture was shaken for 20 min to remove $^{14}CO_2$ produced by respiration of the labelled substrate. Scintillation fluor (2 ml of Hydrocount) was added and the radioactivity was counted in a Searle Mark III liquid scintillation counter with automatic quench correction. The quench correction was based on a quench curve constructed on the basis of a quench series and enclosed in the memory of the scintillation counter. The samples were counted after 5 h to avoid chemiluminescence in the CO_2 extracts. The concentration of L-alanine in the samples was measured by high performance liquid chromatography (HPLC) according to the method of Godel *et al.* (1984) using *o*-phthaldehyde (OPA) as the derivatizing agent and a Waters Novapack C18 (3.9 mm×15 cm) stainless-steel column. HPLC-graded, filtered NaH_2PO_4 (12.5 mmol l^{-1}) buffer and acetonitrile/NaH_2PO_4 (1:1) were used as the solvent systems at pH 7.5. The L-alanine peak in the samples was identified on the basis of retention time established with an L-alanine standard (Sigma), using internal standards as controls. The concentration of L-alanine was calculated from the peak area of the samples on the basis of the standard peak area.

After 24 h of labelling in the tracer medium, the embryos were rinsed, blotted on absorbent tissue paper and placed in tracer-free Ringer's solution (5 ml) in closed flasks. The effect of the rinsing procedure was tested by taking a sample of the Ringer's solution immediately after transfer of the embryos and counting it in the scintillation counter. 0.2 ml of 10 % KOH was added to paper wicks as the

$^{14}CO_2$-trapping system in the flasks. The release experiment was stopped after 2 h by removing the embryos and the $^{14}CO_2$ and dissolved organic carbon (DOC) were measured as described by Korsgaard and Andersen (1985). Embryonic tissue was disrupted, extracted overnight in 10 % TCA, washed twice in 5 % TCA made up in 50 % ethanol and digested in Lumasolve. Radioactivity in the resulting TCA-insoluble and TCA-soluble fractions of the embryonic tissue was determined by scintillation counting.

The kinetic variables uptake rate (V_{up}) and turnover time (T_t) were calculated on the basis of the initial linear part of the uptake curve as follows:

$$V_{up} = \frac{\text{disints min}^{-1}\text{ taken up} \times \text{amount of substrate added } (\mu\text{mol})}{\text{disints min}^{-1}\text{ added} \times \text{experimental time (h)} \times \text{fish mass (g)}},$$

$$T_t = \frac{\text{disints min}^{-1}\text{ added} \times \text{experimental time (h)}}{\text{disints min}^{-1}\text{ taken up}}.$$

Linear curve fitting was also used in calculations of rates and turnover time of L-alanine as measured by HPLC.

In vivo *experiments*

In the *in vivo* experiments, 70 μl of the radioactive compound was injected into the ovary of the pregnant fish simultaneously with 1 ml of 60 or 600 μmol l^{-1} carrier alanine solution. Samples of the ovarian fluid were collected by syringe at intervals. The disappearance of labelled L-alanine was measured by scintillation counting. After 24 h of intraovarian incubation, the embryos were quickly dissected out, rinsed and blotted. They were then immediately placed in unlabelled Ringer's solution in closed flasks as described above. The production of $^{14}CO_2$ and DOC was measured and calculated as a percentage of the radioactivity in the embryos at the beginning of the release experiment. Radioactivity in the TCA-soluble and TCA-insoluble fractions of the embryos was determined as described above.

For autoradiography, embryos were taken after 24 h of exposure to labelled L-alanine and prepared according to the methods described by Korsgaard (1991). Briefly, the embryos were dehydrated then embedded in paraffin and serially sectioned in 5-μm sections. Sections were coated under darkroom conditions with Kodak fine-grain stripping film, placed in black boxes with silica gel desiccant and stored for 3 weeks. After development of the autoradiograms, embryonic sections were photographed using dark-field illumination. Control sections from embryos which had not been exposed to radiolabelled alanine were prepared to evaluate positive and negative artefacts. Results were subjected to statistical analysis by Student's *t*-test.

Results

Fig. 1 shows that after 24 h L-[^{14}C]alanine is distributed throughout the tissues of

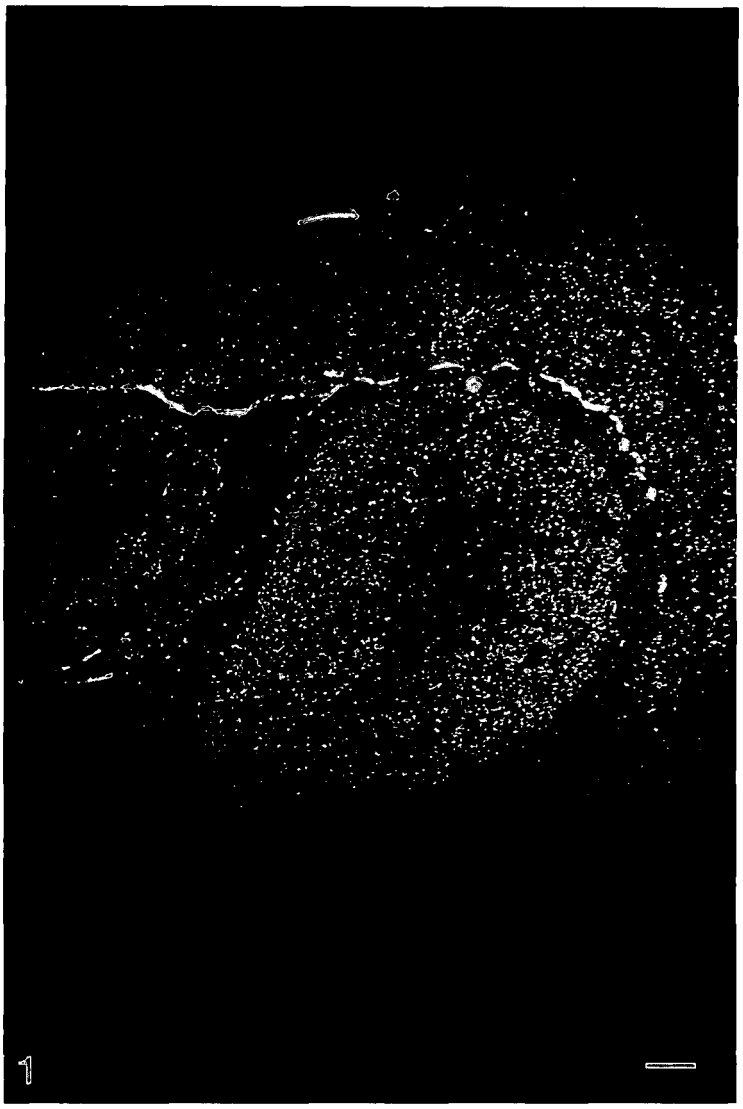

Fig. 1. Autoradiogram of a cross section from a yolk-sac embryo exposed to L-[^{14}C]alanine for 24 h *in vitro*. Scale bar, 40 μm.

the embryo. In Fig. 2B,D the autoradiograms represent sections of intestinal and muscular tissue from embryos in late development exposed for 24 h to labelled L-alanine. Fig. 2A,C shows corresponding sections from embryos not exposed to radiolabelled alanine, but otherwise prepared by the same autoradiographic method. A careful analysis of the spread of grain densities over background areas was performed on several sections to evaluate the difference between label and background. The autoradiograms in the figures, viewed by dark-field illumination, confirm that label is incorporated into the embryonic tissues well above back-

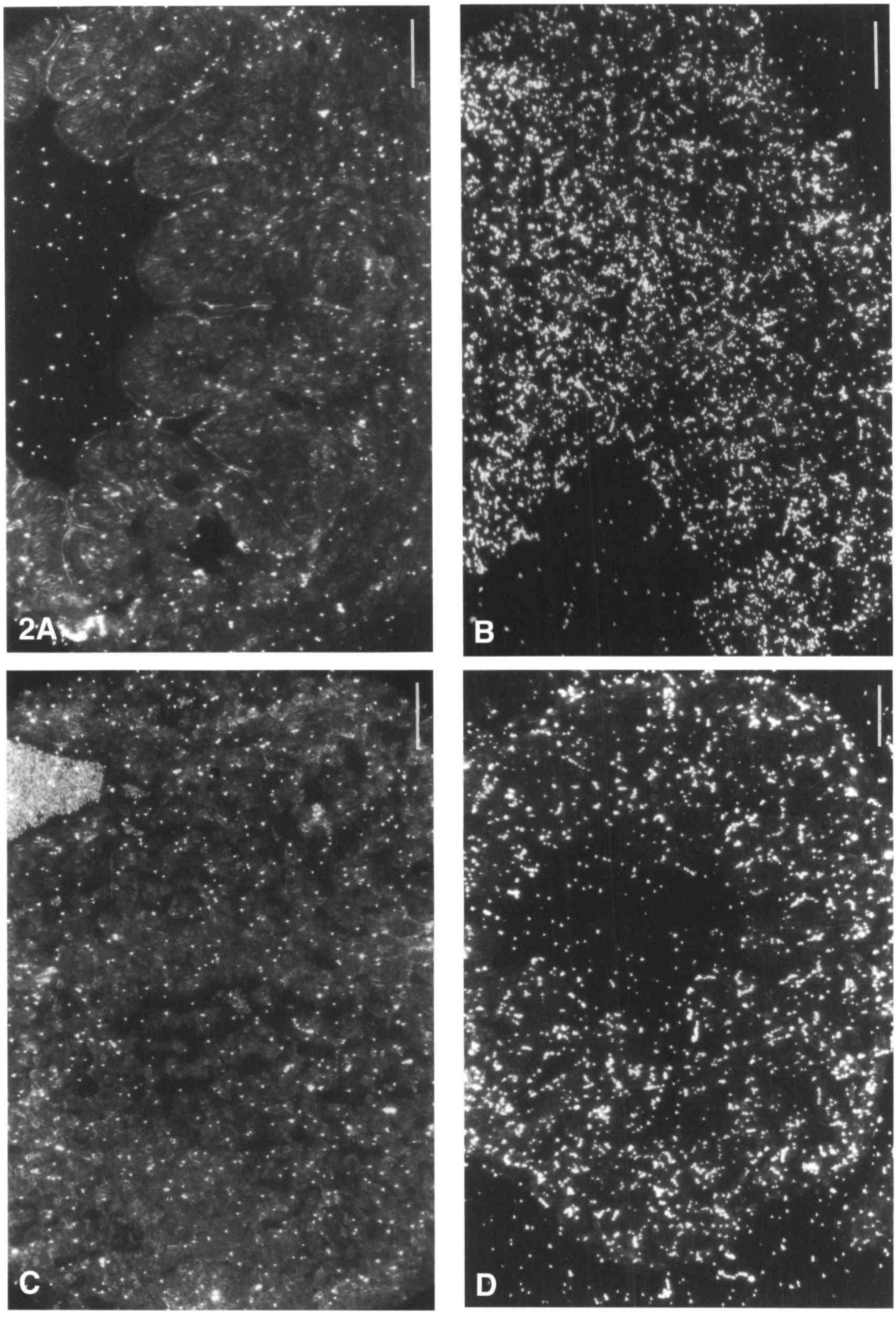
2A
B
C
D

Fig. 2. Autoradiograms of embryonic intestinal (A and B) and muscular (C and D) tissue. B and D are sections from embryos in late development exposed to labelled L-alanine for 24 h. A and C represent unlabelled controls for positive and negative artefacts. Scale bars, 40 μm.

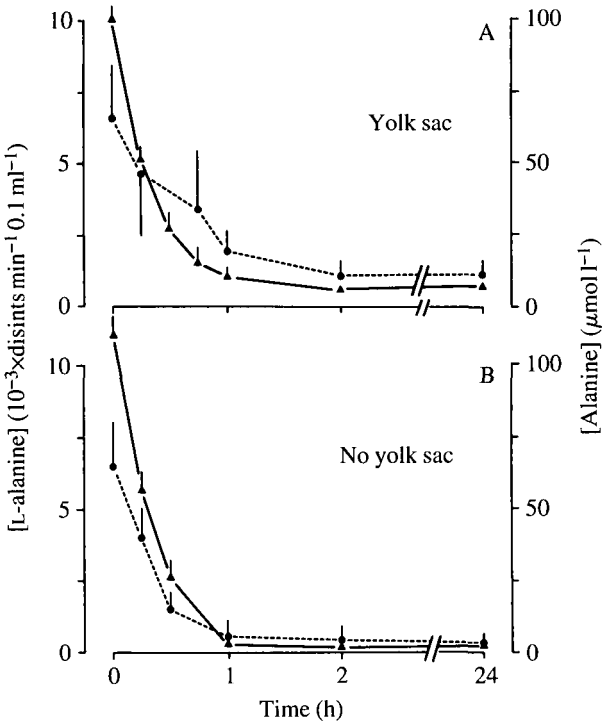

Fig. 3. (A,B) Time course of depletion of ^{14}C-labelled (▲) and total (●) L-alanine during 24 h of *in vitro* incubation. The experiments were performed on yolk-sac embryos (October) and on embryos without a yolk-sac in December. Mean values (+S.E.M.) of five different samples.

ground levels. Thus, the experiment clearly demonstrates that the embryos are able to assimilate alanine from ambient micromolar solutions.

From Fig. 3A it appears that the embryos during their yolk-sac stage already have the ability to take up alanine from a 60 μmol l^{-1} ambient solution, which is approximately the concentration normally found in the ovarian fluid (an average of 64 μmol l^{-1} as measured by HPLC in the present experiment). From Table 1, however, it appears that the disappearance rate of the tracer (0.52 μmol g^{-1} h^{-1}) is significantly higher than the net disappearance rate of L-alanine (0.33 μmol g^{-1} h^{-1}) as measured by HPLC. The difference may be due to a concomitant release of cold alanine from the embryos. That the embryos do not use exogenous

Table 1. *Uptake of* L-*alanine* (V_{up}), *turnover time* (T_t) *and release of* $^{14}CO_2$ *and dissolved organic carbon (DOC) by* Zoarces viviparus *embryos* in vitro *during late yolk-sac stages*

	V_{up}		T_t			
Substrate	^{14}C (μmol g^{-1} h^{-1})	HPLC (μmol g^{-1} h^{-1})	^{14}C (h)	HPLC (h)	$^{14}CO_2$ (% per 2 h)	DOC (% per 2 h)
Alanine (60 μmol l^{-1})	0.52±0.04	0.33±0.10*	0.71±0.07	1.75±0.39*	1.46±0.14	0.74±0.02

The experiment was performed during early pregnancy in October.
Values are means±S.E.M. of five determinations with approximately 1.5 g of embryos per determination.
* Indicates a significant difference ($P>0.05$) from values measured by the ^{14}C technique.

Table 2. *Uptake of* L-*alanine* (V_{up}), *turnover time* (T_t) *and release of* $^{14}CO_2$ *and dissolved organic carbon (DOC) by embryos without a yolk-sac* in vitro *at two different carrier concentrations*

	V_{up}		T_t			
Substrate	^{14}C (μmol g^{-1} h^{-1})	HPLC (μmol g^{-1} h^{-1})	^{14}C (h)	HPLC (h)	$^{14}CO_2$ (% per 2 h)	DOC (% per 2 h)
Alanine (60 μmol l^{-1})	0.60±0.03	0.54±0.03	0.62±0.04	0.73±0.05	2.36±0.08	0.19±0.05
Alanine (600 μmol l^{-1})	2.40±0.17*	2.78±0.19*	1.65±0.13*	1.40±0.11*	2.52±0.14	0.06±0.01

The experiment was performed during late pregnancy (December). Five determinations with approximately 1.5 g per determination.
* Indicates a significant difference ($P>0.001$) from values measured at 60 μmol l^{-1}.

alanine as efficiently during their yolk-sac stage is also reflected by a smaller production of labelled CO_2 and a larger production of dissolved organic carbon (DOC) (Table 1), when compared with later stages of development (Table 2)

Fig. 3B shows substrate depletion in the ambient medium *in vitro* by embryos in late development, approximately 2 months later, when the yolk-sac has been absorbed. It appears that net flux measured by HPLC closely follows the uptake of the tracer. The calculated rates of uptake (Table 2) measured by the tracer method ($0.60\,\mu mol\,g^{-1}\,h^{-1}$) and the HPLC method ($0.54\,\mu mol\,g^{-1}\,h^{-1}$) at a carrier concentration of $60\,\mu mol\,l^{-1}$ show a close correspondence. The production of labelled CO_2 at $60\,\mu mol\,l^{-1}$ alanine appears to be larger (2.36%) than that observed during the yolk-sac stages (1.46%). The results indicate that there is a higher metabolic conversion of alanine during late development of the embryos and that their capacity for uptake of amino acids increases with increasing metabolic demands during development.

In vivo *experiments*

The *in vivo* experiments were carried out to investigate whether the embryos take up and metabolize L-[^{14}C]alanine when they are enclosed in the ovarian sac. The tracer alanine was completely cleared during 24 h in both carrier groups. However, different time courses were found for label in the ovarian fluid at the two carrier concentrations (Fig. 4). The labelled alanine was taken up faster at the low carrier concentration as indicated by the initial part of the curve (at 0.25 and 0.5 h). After 24 h of intraovarian labelling, production of labelled CO_2 and DOC by the embryos was measured for 2 h (Table 3). The production of $^{14}CO_2$, calculated as a percentage of the radioactivity in the embryos immediately after dissection from the ovary, lies within the same low range as was observed *in vitro*.

Anabolic metabolism of L-alanine was indicated by the large incorporation of radioactivity into molecules insoluble in TCA (protein) (Table 4). Results from the *in vitro* experiments were included in Table 4 for comparative reasons. The fraction of the tracer L-alanine that is precipitated as protein in post-yolk-sac embryos appears to be of the same magnitude at $60\,\mu mol\,l^{-1}$ alanine both *in vitro* and *in vivo*. The TCA-insoluble fraction of the embryonic tissue amounts to approximately 35% of the total radioactivity in the two $60\,\mu mol\,l^{-1}$ alanine carrier

Table 3. *Release of labelled CO_2 and dissolved organic carbon (DOC) by post-yolk-sac embryos* in vitro *after intraovarian labelling with* L-*[^{14}C]alanine at two different concentrations of carrier*

Substrate	$^{14}CO_2$ (% per 2 h)	DOC (% per 2 h)
Alanine ($60\,\mu mol\,l^{-1}$)	3.97±0.31	1.73±0.93
Alanine ($600\,\mu mol\,l^{-1}$)	2.59±0.45	1.26±0.30

Mean values (±S.E.M.) of five samples.

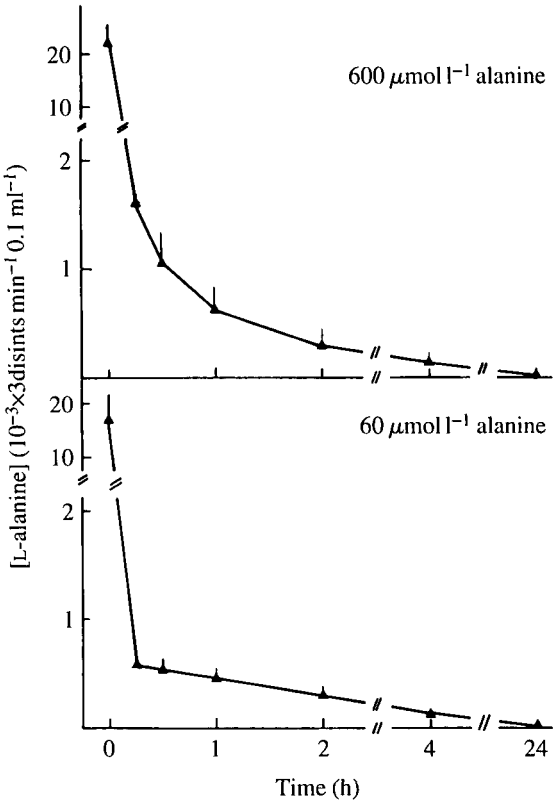

Fig. 4. Time course of depletion of labelled L-alanine in the ovarian fluid during 24 h after injection of the tracer into the ovary. The experiment was performed by simultaneous injection of a carrier solution of 600 μmol l^{-1} or 60 μmol l^{-1} L-alanine in December. Mean values (+S.E.M.) of five samples.

Table 4. *Radioactivity determined in the ethanol/TCA-soluble and -insoluble (protein) fractions of* Zoarces viviparus *embryos labelled* in vitro *or* in vivo *with* L-[^{14}C]*alanine*

Stage	Substrate	Soluble (%)	Insoluble (%)
In vitro			
Yolk-sac	Alanine (60 μmol l^{-1})	78±3	22±3
Post-yolk-sac	Alanine (60 μmol l^{-1})	64±7	36±2
	Alanine (600 μmol l^{-1})	77±3	23±4
In vivo			
Post-yolk-sac	Alanine (60 μmol l^{-1})	67±5	33±9
	Alanine (600 μmol l^{-1})	74±13	26±9

Values were calculated as the percentage of total disints min^{-1} found in the soluble and insoluble fractions.

Mean±S.E.M. of five samples each with 1.5 g of embryos.

groups and 24 % in the 600 μmol l^{-1} alanine groups and in embryos in early development.

Discussion

In the present work the term 'embryo' has been used for the entire posthatch development in the ovary, as it normally is in viviparous fish (Wourms *et al.* 1988). The embryos of *Zoarces viviparus* are not provided with any specific external structures for absorption as are embryos from many other viviparous fish. The alimentary canal is therefore believed to be the principal pathway for uptake of nutrients from the ovarian fluid, at least during later stages of development, when the skin of the embryos is relatively thick and non-vascularized (Kristoffersson *et al.* 1973). These authors describe the greatly expanded and richly folded hindgut as the only exceptional structure for nutrient uptake in the developing embryos.

Drinking activity by embryos of *Z. viviparus* may thus be one of the prerequisites for uptake of nutritional substances such as amino acids, which can be detected in the ovarian fluid. This has also been suggested by Kristoffersson *et al.* (1973), who observed that red blood cells, which may be found in the ovarian fluid as a result of minor bleeding, could also be found in the alimentary tract of the embryos during later development. Their observations are confirmed by the present autoradiographic studies, which show that label can be observed in the intestinal tissue of the embryos (Fig. 2) exposed to [^{14}C]alanine for 24 h. Similarly, autoradiographic studies performed on the teleosts *Clinus superciliosus* and *C. dorsalis* during intrafollicular gestation showed that embryos absorbed nutrients from the embryotrophe secreted by the follicular wall and that most of the nutrient absorption took place through the alimentary canal (Veith, 1980; Cornish, 1985). Drinking activity has also been shown to occur in yolk-sac and first-feeding larval stages of various oviparous teleosts and is normally related to osmoregulation (Mangor-Jensen and Adoff, 1987; Tytler and Blaxter, 1988). Drinking may, however, also represent a way of supplying nutrients or other substances *via* the gut, as suggested by Tytler *et al.* (1990) and Korsgaard (1991). However, during early development of *Z. viviparus* embryos, the skin and gills may also be of importance as absorptive surfaces.

Net uptake rates for L-alanine by embryos *in vitro* are considerably lower during their yolk-sac stages than rates found after absorption of the yolk-sac. This observation indicates that the yolk-sac embryos do not utilize ambient substrate as effectively as they do later in development. This difference may simply be due to an increased surface area for absorption in the gut or an increased drinking rate in the post-yolk-sac embryos. In contrast, Fauconneau (1985) found the highest rate of amino acid incorporation into body protein in yolk-sac larvae of the coregonid fish (*Coregonus scinzii pallea*) when he compared different developmental stages of the fish after immersion in solutions containing [^{14}C]arginine. In *Zoarces viviparus*, the rate of embryonic growth during early development is probably a function of mixed feeding, defined as an uptake of yolk nutrients from the yolk

mass and a simultaneous uptake of low molecular weight compounds from the ambient ovarian fluid. Thus, mixed feeding, in viviparous as well as in oviparous teleost larvae, may prevent any potential metabolic deficit prior to complete yolk absorption and may enhance growth and survival during the terminal phase of the yolk-sac stages (Eldridge *et al.* 1981, 1982; Heming *et al.* 1982; Wiggins *et al.* 1985; Yin and Blaxter, 1987).

During late embryonic development, the rate of disappearance of [^{14}C]alanine from the ambient medium seems to be an accurate measurement of net flux, when compared with the medium depletion of alanine measured by HPLC. The considerable increase in net uptake rates from 0.33 μmol g^{-1} h^{-1} during yolk-sac stages to 0.54 μmol g^{-1} h^{-1} during late development and the concomitant increase in the production of $^{14}CO_2$ from 1.46 % to 2.36 % per 2 h indicate that the importance of amino acids for nitrogen anabolism and catabolism has increased in relation to the increasing weight of the embryos. Similarly, the amount of nitrogen per embryo was shown to increase linearly with time after hatching of the *Z. viviparus* embryos, indicating that nitrogenous energy sources are involved in the maternal nutrition of the embryos in the ovary (Korsgaard, 1986). According to observations by Terner (1968), the capacity of fertilized eggs from rainbow trout (*Oncorhynchus mykiss*) to oxidize ^{14}C-labelled acetate, pyruvate and glucose also increased continuously during development.

The release of labelled CO_2 and dissolved organic carbon in the present work was in general very low, indicating that amino acids are used mostly for synthetic activities. This agrees well with previous studies on embryos from *Zoarces viviparus*, which showed that glucose contributed approximately 14 % of the average total aerobic respiration, whereas the amino acid glycine contributed only insignificantly to respiration (Korsgaard and Andersen, 1985). The oxidation rate of L-alanine in the present work is in accordance with the oxidation rate observed in turbot larvae during their yolk-sac stage (Korsgaard, 1991). Fyhn and Serigstad (1987) found free amino acids in developing eggs and early yolk-sac stages of the larvae in cod (*Gadus morhua*) to be the main substrate for aerobic energy production during the first 19 days after hatching. The mass of body protein did not change significantly during this period, whereas the pool of free amino acids was reduced by 80 % over the period of investigation.

Complete clearance of tracer L-alanine from the ovarian fluid was observed during 24 h *in vivo*. These experiments were performed to compare anabolic and catabolic metabolism of embryos labelled under natural conditions in the ovarian fluid with metabolism of embryos labelled under *in vitro* conditions. Calculated on the basis of total label in the embryos after 24 h, catabolism evaluated by production of $^{14}CO_2$ was higher in the embryos labelled *in vivo*, whereas incorporation of radioactivity into TCA-soluble and TCA-insoluble fractions was surprisingly similar. It is difficult to obtain turnover times for alanine in the ovarian fluid because the volume of fluid and number of embryos are highly variable in different mother fish. Also, the ovarian fluid is not a static pool, but undergoes a rapid circulation and exchange of metabolites with the maternal organs (Kors-

gaard, 1983). Thus, under natural conditions, turnover of alanine in the ovary may be much faster, since the number of embryos per unit volume of ovarian fluid is higher in the ovary than in the *in vitro* incubation systems. The experiments performed in the present work with simultaneously injected tracer and carrier solutions indicate, however, that uptake mechanisms can operate efficiently at the natural concentration (60 μmol l^{-1}) as well as at a higher concentration of carrier alanine.

In conclusion, the present data indicate that embryos from the blenny *Z. viviparus* have the ability in their early and late development to assimilate ambient L-alanine *in vitro* and *in vivo*. The capacity for uptake of L-alanine may increase during embryonic development in the ovary. The results show that label is distributed in the tissues of the embryos and that the labelled amino acid contributes to the general metabolism of the embryos in the ovarian cavity.

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