Two major hepatic pathways exist for the production of urea in vertebrates. Urea is formed from \( \text{NH}_4^+ \) (or glutamine) and \( \text{HCO}_3^- \) in the ornithine–urea cycle (OUC, six enzymes: carbamoyl phosphate synthetase (CPSase), ornithine transcarbamylase (OTCase), argininosuccinate synthetase and lyase, arginase and the accessory enzyme glutamine synthetase (GSase)). The uricolytic pathway, by which uric acid is converted to urea, involves three enzymes: uricase, allantoinase and allantoicase. The OUC is found in marine elasmobranch and coelacanth fish but, until recently, was thought to be absent from the largest group of living vertebrates, the teleost fish (Brown and Cohen, 1960). In previous studies, on a variety of teleosts, OUC enzyme activities ranged from barely detectable to moderate, while substantial uricolytic enzyme activity for GSase was several-fold lower than in whole larval trout and OTCase and CPSase III (glutamine- and N-acetylglutamate-dependent CPSase catalysing the first step of the OUC) activities were essentially absent in adult liver. We conclude that embryonic and larval trout are primarily ammoniotelic. Urea is synthesized immediately after fertilization, but is not excreted until after the embryo is hatched. The results provide evidence for the presence of the OUC in larval rainbow trout, since four of the OUC enzymes are induced just after hatching and the levels of activity are relatively high compared with those in adult liver tissue. Furthermore, we suggest that all teleosts have retained the OUC genes, which are expressed only during certain stages of development (embryogenesis), and in a few rare species expression is maintained throughout the life cycle to cope with unusual environmental conditions (e.g. alkaline water, air exposure).

Key words: ammonia, urea, enzyme activities, embryo, larvae, carbamoyl phosphate synthetase, liver, trout, \textit{Oncorhynchus mykiss}.

\[ \text{Summary} \]
et al., 1989b). Furthermore, studies on the marine toadfish Opsanus beta (Read, 1971; Mommsen and Walsh, 1989; Walsh et al., 1990) and several freshwater, air-breathing teleosts from India (e.g. Heteropneustes fossilis, Saha and Ratha, 1987, 1989) have demonstrated the presence of a functional OUC and enhanced urea excretion rates. It is now clear that the full complement of OUC enzymes is present in a few teleost species that are either adapted to unusual environmental conditions (O. a. grahami) or are periodically air-exposed (O. beta, H. fossilis). Taken together, these findings suggest that all teleosts have retained the genes for the ornithine–urea cycle, but expression of a functional pathway is relatively rare.

Why is the OUC functional in a few teleost species, but inoperative in the majority of species? It is highly unlikely that the genes for the OUC would have been conserved during fish evolution, if they no longer code for functional proteins. The observation that many teleost species express all or most of the OUC enzymes, even if some of the activities are very low, suggests (1) that some or all of the OUC enzymes may be involved in other metabolic pathways, or (2) that the OUC may be functional and expressed at higher levels during the early life stages. In addressing the first possibility, it should be noted that arginase, the last enzyme in the OUC, is widely found in teleost tissues. Arginase is thought to be directly involved in urea synthesis via the degradation of dietary arginine (CVancara, 1969b; Wright, 1993). The fact that the remaining OUC enzymes have relatively low activities probably discounts a significant involvement in other metabolic reactions. The second possibility warrants further attention. Ammonia is formed when yolk protein is metabolised during embryonic and larval rainbow trout, but that urea was not detected in larval trout (Rice and Stokes, 1974). Rainbow trout fingerlings (25–40g) were reported to have the full complement of liver OUC enzymes (Chiu et al., 1986); however, as in the Rice and Stokes (1974) study, the assay conditions for CPSase activity may not have been appropriate. The first step in the OUC is catalysed by CPSase I in mammalian ureotelic species. Until recently, OUC capability in fish was assessed by measuring enzyme activities, assuming that CPSase activity would be due to a mitochondrial ‘mammalian-type’ CPSase I, i.e. utilized only ammonia as the nitrogen-donating substrate and required the presence of N-acetyl-l-glutamate (AGA) for activity (Campbell and Anderson, 1991). In ureosmotic elasmobranchs, however, the CPSase catalysing the first step of the OUC is a mitochondrial CPSase III, which is similar to CPSase I except that it utilizes the amide group of glutamine in preference to ammonia as the nitrogen-donating substrate (Anderson, 1980, 1991). It is likely, therefore, that CPSase III rather than CPSase I fulfils this role in all fish (Mommsen and Walsh, 1989, 1991; Anderson, 1991; Campbell and Anderson, 1991; Cao et al., 1991). When measuring very low levels of CPSase activity, it is also necessary to be sure that the activity measured is not due to a CPSase II, which is probably present in most tissues (Cao et al., 1991). CPSase II catalyses the first step in the pyrimidine nucleotide pathway; this enzyme utilizes either glutamine or ammonia (at high concentrations) as a substrate, is subject to allosteric inhibition by uridine triphosphate (UTP), does not require AGA for activity and is localised in the cytosol (Campbell and Anderson, 1991).

The present study was initiated to determine whether OUC enzymes are induced in embryonic and/or larval trout and whether significant urea formation and/or excretion occurs. Ammonia and urea excretion rates, ammonia and urea tissue levels, and four ornithine–urea cycle enzyme activities (CPSase, OCTase, GSase and arginase) were measured in embryonic and larval rainbow trout (Oncorhynchus mykiss) up to 93 days after fertilization. In addition, an effort was made to confirm that the CPSase activity detected was due to a CPSase III.

Materials and methods

Animals and experimental protocol

Fertilized rainbow trout (Oncorhynchus mykiss (Walbaum)) embryos were obtained from Blue Springs Fish Hatchery (Hanover, Ontario, Canada). Eggs were maintained in continuous-flow incubating troughs (11–13°C) for up to 93 days (water pH 8.0, water hardness 125mg l⁻¹). Approximately 2 weeks after hatching (i.e. at day 54), larval fish were fed starter feed (Martin Mills Starter) several times per day.

Each experiment (days 4, 8, 16, 30, 37, 40, 45, 50, 53, 58, 64, 71 and 93) was performed on 180 embryos or larval fish randomly selected from the incubation trough. Thirty animals were placed in each of six chambers containing 50ml of aerated water. To measure nitrogen excretion rates, water samples (10ml) were collected at 0h and 4h and immediately frozen (−20°C) for later determination of urea and ammonia levels. Excretion rates (nmol N g⁻¹ h⁻¹) were calculated as the difference in concentration (0–4h) in nmol N l⁻¹ multiplied by the volume of the chamber (l) divided by the mass of the tissue (g) and the time period (4h) (1nmol N⁻¹ of urea=2nmol N⁻¹ urea and 1nmol N⁻¹ ammonia=1nmol N⁻¹ ammonia, where N=nitrogen). At the end of the 4h experiment, animals were
blotted and weighed. To determine water content, 10 animals were dried to constant mass in a drying oven at 50°C and the difference between wet and dry mass was recorded. The remainder of the animals were frozen in liquid N₂ (stored at −80°C for up to 2 months) for later analysis of tissue ammonia and urea concentrations and enzyme activities.

Water and tissue ammonia and urea analysis

Water samples were analyzed for ammonia and urea levels using colorimetric assays described by Verdouw et al. (1978) and Rahmatullah and Boydé (1980), respectively. For tissue determinations, six subsamples of frozen embryos or larvae (0.25–0.50g) were ground to a fine powder in liquid N₂, deproteinized in 1 volume of ice-cold perchloric acid (8%), and centrifuged at 12000g for 10min (4°C). The supernatant was removed, neutralized with saturated KHC₃O₃ and centrifuged again (12000g for 4min, 4°C). The final supernatant was analyzed for ammonia levels using a Sigma diagnostic kit (171-C) and urea levels with Sigma reagents and centrifuged at 12000g for 10min (4°C). The reaction supernatant was deproteinized in 1 volume of ice-cold perchloric acid (8%), and centrifuged at 12000g for 10min (4°C). The supernatant was analyzed for ammonia levels using a Sigma diagnostic kit (171-C) and urea levels with Sigma reagents (procedure no. 535). Ammonia and urea tissue concentrations were corrected for tissue water content and expressed as mmol N⁻¹ tissue water.

Preparation of extracts for enzyme analysis

Tissues were stored at −80°C for up to 6 weeks. All procedures were carried out at 4°C. Extracts were prepared by homogenization of 1.5–2g of the whole animal (except for adult trout, where only liver tissue was used) with 4 volumes of extract buffer (0.05mol l⁻¹ KCl, 0.05mol l⁻¹ Hepes buffer, pH7.5, 0.5mmol l⁻¹ EDTA, 0.01mol l⁻¹ ATP, 0.015mol l⁻¹ MgCl₂, 5mmol l⁻¹ NaHCO₃ and 1mmol l⁻¹ dithiothreitol, DTT), with a Potter–Elvijehem homogenizer followed by brief sonication. ATP, MgCl₂ and NaHCO₃ were included in the extract buffer for CPSase measurements only to maximize the stability of the extracted enzyme (Anderson, 1981). The supernatant (5ml) obtained after centrifugation at 12000g for 10min was passed through a 25ml Sephadex G-25 column equilibrated with 0.1mol l⁻¹ Hepes, pH7.5, 0.1mol l⁻¹ KCl, 0.5mmol l⁻¹ EDTA and 1mmol l⁻¹ DTT. Fractions containing most of the protein were pooled. Protein concentration was measured before and after the gel filtration chromatography step as a measure of the dilution of the extract; calculations of μmolmin⁻¹ g⁻¹ tissue (units g⁻¹) were corrected for this dilution. Enzyme activities in each extract were measured immediately following gel filtration chromatography.

Enzyme assays

OTCase, arginase, GSase and CPSase activities were measured at 26°C, essentially as previously described (see below). Reaction mixtures for OTCase contained 10mmol l⁻¹ ornithine, 5mmol l⁻¹ carbamoyl phosphate, 0.05mol l⁻¹ KCl, 0.05mol l⁻¹ Hepes, pH7.4, 0.5mmol l⁻¹ DTT, 0.25mmol l⁻¹ EDTA and 0.5ml of extract (prepared as above); the reaction was terminated and protein precipitated after 0, 10, 20 and/or 40min by addition of 75 μl of 2mol l⁻¹ HClO₄ and citrulline concentration in the supernatant obtained after centrifugation was determined as previously described (Xiong and Anderson, 1989). Reaction mixtures for arginase contained 0.06mol l⁻¹ glycine, pH9.7, 4.8mmol l⁻¹ MnCl₂, 15.2mmol l⁻¹ l-[guanido-¹⁴C]arginine; the reaction was terminated after 0, 30 and 60min (0, 3 and 6min for adult liver) and [¹⁴C]urea formed was determined as described previously (Casey and Anderson, 1982; Ruegg and Russell, 1980). Reaction mixtures for measuring the γ-glutamyl transferase reaction of GSase was as described previously (Shankar and Anderson, 1985); γ-glutamyl hydroxamate formation was determined after reaction for 0, 10, 20 and 40min. Reaction mixtures for CPSase contained 10mmol l⁻¹ ATP, 14mmol l⁻¹ MgCl₂, 5mmol l⁻¹ [¹⁴C]bicarbonate, 0.055mol l⁻¹ Hepes, pH7.4, 0.055mol l⁻¹ KCl, 0.5mmol l⁻¹ DTT, 0.25mmol l⁻¹ EDTA and, where indicated, 10mmol l⁻¹ glutamine, 0.5mmol l⁻¹ N-acetyl-L-glutamate (AGA), 100mmol l⁻¹ NH₄Cl and/or 2.5mmol l⁻¹ uridine triphosphate (UTP); [¹⁴C]carbamoyl phosphate formed after 60min was determined as previously described (Anderson et al. 1970). Mitochondrial CPSase III (an OUC enzyme) requires glutamine as a substrate and AGA as a positive effector. Cytosolic CPSase II (involved in pyrimidine nucleotide biosynthesis) can use either ammonia or glutamine as a substrate, does not require AGA, and is inhibited by UTP. To differentiate between CPS II and III, duplicate experiments were performed to determine the effects of substrates and effectors on enzyme activity (Table 1). The estimated limits of detection for each of the four enzymes assayed, expressed in terms of μmolmin⁻¹ g⁻¹ tissue, were 0.0003 (CPSase), 0.01 (OTCase), 0.08 (GSase) and 0.001 (arginase).

It was not possible to measure argininosuccinate synthetase and lyase activities in this study because the levels of activities are typically very low (even in mammals with a very active OUC) and insufficient tissue was available.

Protein measurement

Protein was determined by the dye-binding method with Bio-Rad Laboratories reagents as described by Bradford (1976), using bovine serum albumin as a standard.

Results

Urea and ammonia excretion rates and tissue levels

Both ammonia and urea concentrations in the embryos increased several-fold in the first 40 days after fertilization, peaking at 1.7mmol N⁻¹ and 2.5mmol N⁻¹, respectively (Fig. 1). Following hatching, ammonia levels increased by a further twofold and then decreased, as did urea concentrations, to 1.3mmol N⁻¹ (ammonia) and 0.4mmol N⁻¹ (urea) by day 93. Ammonia excretion was detected immediately (day 4) in embryonic rainbow trout, while urea excretion was not detected until after hatching (Fig. 2). Ammonia excretion increased linearly until day 30 and then remained constant until after hatching. Immediately after hatching (day 44), ammonia excretion rates increased relatively rapidly and were 17-fold higher than pre-hatch rates by day 93. Until day 57, urea excretion rates remained relatively low but, thereafter, rates
increased several-fold. The percentage of nitrogenous wastes (ammonia+urea) excreted as urea increased over the 93 day period: days 4–40, 0%; days 45–64, 7%; and days 71–93, 14%.

Water content remained relatively constant in embryos until just before hatching (approximately 65%; Fig. 3). Starting at day 37, there was a linear increase in water content until the yolk sac was completely consumed (day 63); water content remained constant thereafter (approximately 84%; Fig. 3).

**Enzyme activities**

CPSase activity (AGA and glutamine present in assay mixtures=CPSase II+CPSase III) was very low, but could be detected in whole animal homogenates beginning at day 40 (Fig. 4A). Activity peaked at day 64 and then declined by day 93 to levels comparable to those in adult liver tissue. The ratio of glutamine-dependent CPSase activity with AGA present to that without AGA present is plotted in Fig. 4B. A ratio of more than 1.0 indicates CPS III activity. The ratio peaked at day 53 and declined gradually to day 93. The ratio in adult liver tissue was close to 1.0, indicating an absence of CPS III and the presence of CPSase II only.

The effect of different substrates and effectors on CPSase activity in 64-day-old larvae and in adult liver tissue is shown in Table 1. In the absence of substrate, glutamine or ammonia, there is some activity that may be due to endogenous substrate (including, perhaps, side-chain glutamine amide groups in protein) in the tissue homogenate. The results clearly show a stimulation of glutamine-dependent CPSase activity by N-acetylglutamate (AGA) in larval tissue, indicating the presence of CPSase III activity. This stimulation was not observed in adult liver and indicates an absence of CPSase III. There is apparently a mixture of CPSase II and CPSase III in larval tissue (CPSase II activity is inhibited by uridine triphosphate, UTP). Activity in the presence of ammonia is low in larval tissue but relatively high in adult tissue, which is consistent with the presence of CPSase III in larval tissue and its absence in adult liver.

The time course of changes in OTCase activity was very similar to that of total CPSase activity: activity increased after day 40, peaked at day 71 and then declined before day 93, with little or no activity in adult liver (Fig. 4C). GSase activity was low initially (day 16–40), but increased rapidly after day 40 (Fig. 4D). GSase activities in adult liver (units g$^{-1}$ liver) were

<table>
<thead>
<tr>
<th>Substrate and/or effector present</th>
<th>Day 64</th>
<th>Adult liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Glutamine</td>
<td>1.2</td>
<td>2.0</td>
</tr>
<tr>
<td>Glutamine + N-acetyl-L-glutamate</td>
<td>2.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Glutamine + uridine triphosphate</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Ammonia</td>
<td>1.0</td>
<td>5.8</td>
</tr>
<tr>
<td>Ammonia + N-acetyl-L-glutamate</td>
<td>1.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Ammonia + uridine triphosphate</td>
<td>0.3</td>
<td>0.7</td>
</tr>
</tbody>
</table>

CPSase activity, carbamoyl phosphate synthetase activity, nmol min$^{-1}$ g$^{-1}$ tissue.

Duplicate measurements are represented by experiment 1 and 2.

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![Fig. 1](image1.png)

**Fig. 1.** Ammonia-N (filled circles) and urea-N (open circles) concentrations in whole trout embryos and larvae up to 93 days after fertilization. Solid vertical bars indicate period of hatching and dashed vertical bar indicates when the yolk sac disappeared. Values are means ±1 S.E.M. of six measurements.

![Fig. 2](image2.png)

**Fig. 2.** Ammonia-N (filled circles) and urea-N (open circles) excretion rates by trout embryos and larvae up to 93 days after fertilization. Other details are as in Fig. 1.
18-fold lower than in larval tissue (Fig. 4D). There was a dramatic increase in arginase activity after day 45 and, in contrast to GSase and OTCase, the level of activity in adult liver was 20-fold higher than in 93-day-old larvae (Fig. 4E). It should be noted that enzyme activities were measured in whole-animal homogenates and that, prior to day 63, the yolk sac was a component of the embryo and fry. After the yolk sac had been consumed (day 63), the absolute enzyme activities were more representative of whole-body levels.

Discussion

The results presented here indicate that relatively high levels of all four of the OUC enzymes measured out of the six that are required for glutamine-dependent urea synthesis, appear at about the time of hatching. Significant OUC enzyme activity in the early life stages is somewhat surprising, since activity in adult trout is very low (Huggins et al. 1969; Wilkie et al. 1993), as it is for most teleost species (Huggins et al. 1969; Anderson, 1980; Cao et al. 1991; Wright, 1993). Our findings may help to clarify a perplexing question. Why have teleosts apparently retained the genes for the OUC enzymes when only a few species express a functional pathway (Oreochromis alcalicus grahami, Randall et al. 1989; Opsanus beta, Mommsen and Walsh, 1989; Heteropneustes fossilis, Saha and Ratha, 1987, 1989)? We suggest that all teleosts utilize the OUC pathway during embryogenesis, but in the later developmental stages the genes are repressed, except in a few rare species. This hypothesis must be tested in a wide group of teleost species.

CPSase activities in larval tissue appear to be due to the presence of both CPSase II (required for pyrimidine nucleotide biosynthesis) and CPSase III (required for urea synthesis). The apparent absence of CPSase III in adult liver (no stimulation of glutamine-dependent CPSase activity by AGA) is consistent with the absence of OTCase activity and, therefore, OUC-dependent urea synthesis. The levels of CPSase II activity in adult liver and those estimated for larval and embryonic tissue are comparable to those reported in liver extracts of other fish species (Anderson, 1980; Cao et al. 1991). The levels of CPSase activity reported here are an order of magnitude lower than can be detected by the more commonly used colorimetric assay procedure, which explains in part why Rice and Stokes (1974) were not able to detect CPSase activity in their study of trout eggs or alevins. The latter study also did not utilize glutamine as a substrate (see Introduction). Chiu et al. (1986) reported unusually high levels of ammonia-dependent CPSase activity (approximately 0.05 μmol min⁻¹ g⁻¹ liver) in trout fingerlings; AGA was included in their assay mixtures, but activity with glutamine as a substrate was not measured. Furthermore, they injected fish with [¹⁴C]bicarbonate, but were unable to recover labelled arginine, implying that the OUC was not operative. Hence, the presence of a functional OUC in rainbow trout fingerlings remains unproved.

Except for adult liver, enzyme activities were measured from whole-animal preparations. It is reasonable to assume that the majority of these enzyme activities, especially CPSase III and OTCase, are localized in the liver, which may represent only 1–2% of the mass of the whole animal. Thus, the levels of these enzymes in developing liver may actually be quite high. The absolute enzyme activities will also be underestimated in embryo and fry prior to the disappearance of the yolk sac (day 63) while, after day 63, the activity values are truly representative of whole-animal tissue.

We could not detect OUC enzymes prior to day 40, but this may reflect the limit of the sensitivities of the enzyme assays (see Materials and methods) and the need to use whole-animal homogenates. Dépêche et al. (1979) found that the highest rate of urea synthesis from [¹⁴C]bicarbonate occurred in 8-day-old trout embryos. Moreover, we found that urea synthesis in embryos was initiated a few days after fertilization (Fig. 1). These results imply that the OUC may be functional very early in embryonic development, but our assay methods were not sensitive enough to detect lower levels of activity (see Materials and methods). Alternatively, uricolysis, the pathway by which urea is synthesized from uric acid, may be functional in early embryos. To our knowledge, uricolytic enzymes (uricase, allantoinase and allantoicase) have not been measured in embryonic or larval trout, although uricolyis is an important pathway in adult teleosts (see below).

Urea synthesis in the embryo may function as a mechanism to detoxify very high ammonia levels in the embryo and early larva (see Griffith, 1991). The yolk, composed mostly of protein and some fat, is the main source of fuel during the endogenous feeding period (Smith, 1947; for a review, see Kamler, 1992). Although ammonia is excreted during these early embryonic stages, ammonia concentrations still accumulate to 1.7 mmol N l⁻¹ in the egg. Tissue ammonia levels are even higher in newly hatched trout (3.0 mmol N l⁻¹ at day 44; Fig. 1), despite an acceleration in the rate of ammonia excretion (Fig. 2). As a comparison, in resting adult trout, plasma ammonia concentrations are between 0.05 and 0.50 mmol l⁻¹ (depending on the feeding history, among other factors), whereas intracellular levels are approximately 2 mmol l⁻¹ (Wright and Wood, 1988). Ammonia is compartmentalized in adult tissues according to pH gradients.
and/or electrical potential gradients across cell membranes (Wright and Wood, 1988; Wright et al. 1988; Wood et al. 1989a). It is probable, therefore, that ammonia is also compartmentalized in the embryo and larvae. Nevertheless, urea synthesis during early development may be essential to prevent the toxic accumulation of ammonia, while nitrogen stored as urea is far less harmful at equivalent concentrations. Indeed, when recently hatched trout were exposed to conditions that favour ammonia retention (0.2mmol l\(^{-1}\) ammonia or pH9.0 water for 4h), urea excretion increased sixfold (P. A. Wright and M. Land, unpublished data). It has also been demonstrated that external water ammonia concentrations as low as 0.15mmol l\(^{-1}\) can have severe effects on the survival of rainbow trout eggs (Solbé and Shurben, 1989). Clearly, excessive accumulation of ammonia can be extremely toxic to salmonids in the early life stages, just as it is to adult fish (Lumsden et al. 1993).

The elevation of tissue ammonia concentrations just after hatching may be associated with the exhaustive muscular activity required for the larvae to escape from the egg capsule. In exercising muscle, ammonia is produced anaerobically from the breakdown of adenylates (Mommsen and Hochachka, 1989). Exercising fish have much higher plasma ammonia levels than fish at rest (Wright and Wood, 1988; Wright et al.
The observation that tissue ammonia levels remain elevated for about 10 days after hatching (days 45–55), indicates that ammonia excretion is limited by the gills, which are initially underdeveloped (Rombough, 1988). Urea levels increase in parallel with ammonia levels (Fig. 1), suggesting that ammonia detoxification plays an important role in the developing larvae.

Urea accumulation in the embryo prior to hatching may signify a role for urea in osmoregulation. In marine elasmobranchs and the coelacanth, urea is retained in relatively high concentrations (approximately 800mmol N1−1) to balance the osmotic pressure of sea water. In a freshwater environment, however, it is hard to imagine why an accumulation of osmolytes within the organism would be necessary. There is also no evidence that urea plays a role in osmoregulation in adult teleost fish, either marine or freshwater species (Wood, 1993).

It has previously been reported that embryonic and larval trout did not excrete urea, only ammonia (Smith, 1947; Rice and Stokes, 1974). Using very sensitive water assay methods, we have presented new evidence that urea is excreted just after the embryos hatch (day 45). Once larval fish have escaped from the egg capsule, urea can be excreted across the gills. Immediately post-hatching (day 45), urea excretion was very low (Fig. 2), although the urea tissue concentration was relatively high (Fig. 1). At the end of the experiments (day 93), urea excretion had increased considerably (Fig. 2), but the urea tissue concentration was reduced compared with that in 45-day-old embryos (Fig. 1). This large increase in the urea diffusing capacity in larval fish probably relates to the rapid expansion of the gill surface area that occurs in the first half of the alein stage (endogenously feeding larvae, age 42–53 days) (Rombough, 1988).

The observation that urea is excreted only after the embryos hatch is not surprising. The embryo is surrounded by an acellular layer called the chorion or egg capsule. The permeability of the chorion is relatively low because it functions to protect the developing embryo from the external environment (Cottelli et al. 1988). Oxygen, carbon dioxide and ammonia permeate many biological membranes relatively easily and also diffuse across the chorion (Rombough, 1988). Both anions (Cl−) and cations (H+, K+, Ca2+) can also permeate the chorion (Peterson and Martin-Robichaud, 1987). In recent years, the universal permeability of urea has been questioned, as urea transport in some tissues has been shown to be dependent on a specific carrier protein (mammalian kidney tubules, Chou and Knepper, 1989; mammalian erythrocytes, Brahm, 1983; toad bladder. Levine et al. 1973). Our results imply that the chorion lacks a specific urea transport carrier and that urea cannot diffuse passively across the chorion membrane.

Adult trout excrete approximately 15% of their nitrogenous wastes as urea, although this percentage varies with environmental conditions, such as water pH and water ion composition (McGeer et al. 1994; Wilkie and Wood, 1991; Wright et al. 1993). The major pathway for urea synthesis in teleost fish is uricolysis and/or arginolysis, as most species lack a functional OUC (Goldstein and Forster, 1965; Huggins et al. 1969; Wright et al. 1993; Wright, 1993). CPSase III was not detected and OTCase was close to the limit of detection in adult liver tissue in the present study, indicating the absence of a complete OUC. Although the genes for the OUC enzymes are obviously present in rainbow trout, expression of CPSase III and OTCase was suppressed in adults. It appears that suppression of CPSase III and OTCase was initiated by day 93 (Fig. 4B,C); however, further studies are necessary to confirm this observation. Arginase and GSase are, however, expressed in adult liver tissue. Both enzymes are involved in other non-OUC metabolic pathways. Arginase is ubiquitous in fish tissues where it converts dietary arginine to urea (see Introduction). GSase catalyses the conversion of glutamate and ammonia to glutamine and is important in a number of tissues for ammonia detoxification and glutamine synthesis (Ritter et al. 1987; Chamberlin et al. 1991). GSase is also essential in the teleost liver to supply glutamine for the synthesis of purines which, in turn, are converted to urea via uricolyis. The regulation of OUC enzyme expression or suppression in teleost fish is not understood. The cloning of the OUC enzyme, CPSase III, from fish tissue (J. Hong, W. L. Salo, and P. M. Anderson, in preparation) and the availability of a specific CPSase III probe for measuring expression of mRNA will be helpful in addressing this question.

In summary, this study presents evidence for the induction of the OUC enzymes, including CPSase III, in larval rainbow trout. Urea synthesis occurred in embryos almost immediately after fertilization, but urea excretion to the environment was not initiated until after the embryos had hatched. Ammonia levels increased during embryonic development, despite excretion of ammonia to the environment. The observation that urea tissue levels parallel the increases in ammonia tissue concentrations provides evidence that urea synthesis is important in the prevention of ammonia toxicity during the early life stages in trout.

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