human nasal and bronchial submucosa and submaxillary salivary gland (*11*). Crabbé *et al.* reported that fluorescein-labeled antiserums against human IgA and IgG produced staining of the mucus of intestinal goblet cells (*1*). They considered these findings nonspecific because they could be extinguished after absorption of the conjugated antiserum with washed, mixed A, B, and O erythrocytes. We have not observed staining of the mucus of intestinal goblet cells. Our control staining experiments established the immunospecificity of the intraepithelial IgA. Further, the findings on IgA were identical when goat antiserum to human IgA from a different source was used (*12*).

Recently, IgA was demonstrated within human nasopharyngeal tonsillar epithelium. This immunofluorescence was attributed to the presence of lymphoid cells (containing IgA) within the epithelial cells (*4*). We occasionally observed IgA cells in the overlying surface mucus, but not within intestinal mucosal epithelial cells. There is good evidence for the presence of lymphocytes within intestinal epithelial cells, although the exact source, nature, function, and fate of these lymphocytes remain uncertain (*13*).

While we have used an antiserum to 7*S* IgA, positive staining in the surface mucus within the gastrointestinal lumen suggests that this antiserum also is reactive against secretory IgA. Supplementary investigations of the intraepithelial IgA are in progress to define further the immunochemical form of this immunoglobulin. It is unknown whether IgA enters the epithelial cells (i) derived from local lymphoid cells, (ii) by intraepithelial synthesis, or (iii) by absorption from the overlying surface mucus. It is probable that IgA enters epithelial cells from the adjacent lymphoid cells (containing IgA) of the lamina propria (*5*), but the mechanism by which this takes place remains unclear.

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Ornithine-Urea Cycle Activity in Xenopus laevis: Adaptation in Saline

Abstract. *The concentration of urea in the blood and the rate of urea excretion were markedly elevated in* Xenopus *maintained in hypertonic saline for 2 to 3 weeks. These changes were accompanied by a twofold increase in the activity of the ornithine-urea cycle as measured in liver slices. The activity of carbamoyl phosphate synthetase rose threefold in frogs adapted to saline. These results suggest that changes in activities of urea cycle enzymes may be important in the adaptation of aquatic organisms to environments of varying salinities.*

The South African frog, *Xenopus laevis*, is normally aquatic and, like many water-dwelling animals, excretes the major fraction of its waste nitrogen as ammonia (*1*). *Xenopus*, however, can live for extended periods out of water or in hypertonic (0.9 percent) saline (*2*). In such situations, where the availability of water is restricted, the animals accumulate urea, but not ammonia, in their tissues (*2, 3*), thus simultaneously avoiding toxic amounts of ammonia and improving their osmotic status with respect to the environment. Accumulation of urea in response to water deprivation has been recorded in a number of other amphibia (*4, 5*). For example, the concentration of urea in the plasma of *Rana cancrivora* increased nearly tenfold when these frogs were transferred from freshwater to 80 percent seawater (800 mosmole/liter, *4*). An elevated concentration of urea in the tissues appears to serve the same purpose in these animals as it does in elasmobranchs (*6*), that is, to decrease water loss by reducing the osmotic gradient between the animal and its environment.

The manner in which the urea concentration of the tissues is elevated in amphibia placed in hypertonic media is not fully understood. One contributing factor is a lowered rate of urine flow such as that observed in *R. cancrivora* (*7*) and *X. laevis* (*8*) when the ambient osmolarity is increased. Janssens (*3*), however, calculated that the rate of urea production increased in *Xenopus* after its transfer to saline. The nature of this adaptation in urea synthesis is unknown. We have examined the possibility that the activity of the ornithine-urea cycle is elevated in *Xenopus* kept in hypertonic saline.

Male frogs (*Xenopus laevis*) weighing about 35 g (*9*) were maintained in tap water and were not fed for 18 days before use. At the beginning of the experiment, each frog was placed in 0.5 liter of either tap water or a saline (300 mosmole/liter) solution (Na^+, 150 meq/liter; K^+, 3.4 meq/liter; Ca^{2+}, 6.8 meq/liter; Cl^-, 160 meq/liter; HCO_3^-, 0.6 meq/liter). This saline solution is hypertonic to normal *Xenopus* plasma, which was estimated on the basis of plasma electrolyte content to have an osmolarity of 235 mosmole/liter. Solutions were changed daily. The water temperature was held at 24° to 26°C. On the 14th or 21st day of the experiment, we measured the nitrogen excretion by replacing the solutions with 500 ml of either sodium phosphate buffer (*p*H 6.5, 0.01*M*; freshwater frogs) or similarly buffered saline (saline-adapted frogs) and analyzing samples of the media taken at

Table 1. Effect of a hypertonic environment on ammonia and urea metabolism in *Xenopus laevis*. Frogs were fasted for 18 days in freshwater. They were then divided into two groups of eight frogs each. One group was kept in freshwater for an additional 2 to 3 weeks, and the other group was maintained in saline (300 mosmole/liter) for a similar period. Excretion rates and blood values were measured on the last day of the experiment. Values are means ± standard errors of the means; probabilities are given in parentheses.

Group	Blood N concentrations (μatom/ml)		Nitrogen excretion (μatom/100 g of body weight per hour)		
	Ammonia	Urea	Ammonia	Urea	Ammonia + Urea
Freshwater	0.21 ± 0.03	5.0 ± 0.72	24 ± 2.6	9.5 ± 2.3	33 ± 4.2
Saline	0.20 ± 0.04	79 ± 6.8	14 ± 4.2	43 ± 9.9	57 ± 14
		($P<.001$)	($.05<P<.1$)	($P<.01$)	($.1<P<.2$)
		Ratio of saline to freshwater values			
	1.0	16	0.7	4.5	1.7

the beginning and end of a timed period (about 20 hours). The animals were then killed by decapitation. Blood and water samples were analyzed for ammonia (*10*) and urea. Urea was determined as ammonia after its conversion by urease (*11*). We assayed urea cycle activity in liver slices by measuring the rate of incorporation of ^{14}C-labeled bicarbonate into urea (*12*). The activity of carbamoyl phosphate synthetase was estimated in liver homogenates by the method of Brown and Cohen (*13*). There was no significant difference between the results for the 2- and 3-week groups of frogs, and the values were therefore combined.

Although there were no significant changes in the concentrations of ammonia in the blood or in the excretion of ammonia after the period in hypertonic saline, blood urea increased about 16-fold, and the rate of urea excretion increased almost fivefold (Table 1). There was a tendency (not significant, $P > .05$) for the output of ammonia to decline and for that of total urea and ammonia to rise. As a consequence of these changes, 2 to 3 weeks after the frogs were transferred to saline, urea constituted 75 percent of the eliminated nitrogen, compared to 30 percent when the frogs were in freshwater. These results, for unfed animals, are similar to those found by Janssens (*3*) for animals fed twice weekly after transfer to 0.9 percent NaCl.

The mean increase in the concentration of urea in the blood of 37 mmole/liter (74 μatom of urea nitrogen per milliliter, Table 1) would elevate the plasma osmolarity of frogs adapted to saline from about 235 mosmole/liter, to approximately 270 mosmole/liter, thus necessitating a rise of only 15 meq of Na^+ per liter to achieve osmotic equilibrium with the saline environment (300 mosmole/liter). The observed increase in plasma Na^+ (mean of values from two frogs) was 18 meq/liter.

Maintenance of frogs for 2 to 3 weeks in a hypertonic medium produced a twofold increase in urea synthesis by liver slices (Table 2). The results were similar whether urea cycle activity was expressed per gram of liver, per 100 g of body weight, or per gram of liver protein (not shown in table). These results suggest that at least part of the increased urea synthesis in saline-adapted *Xenopus* is due to an increase in the activity of urea cycle enzymes, a conclusion supported by the concomitant threefold increase in the activity of carbamoyl phosphate synthetase (Table 2). The stimulus that induces the increase in the activities of these enzymes in saline-adapted frogs is not ammonia, since the concentrations of this compound in blood (see Table 1) and liver (*8*) are unchanged.

In at least two vertebrate groups, the amphibians (*4*, *5*) and the elasmobranchs (*6*), the level of tissue urea is correlated with the need to conserve water. Although renal mechanisms participate in the regulation of the concentration of urea in tissues of these forms (*6*, *7*) our studies indicate that the adaptation of ornithine-urea cycle enzymes may also play an important role in this process.

Table 2. Ornithine-urea cycle activity of *Xenopus laevis* in a hypertonic environment. We measured incorporation of ^{14}C-labeled bicarbonate into urea by *Xenopus* liver slices by incubating approximately 100-mg slices in 3.0 ml Krebs-Ringer bicarbonate solution (diluted with water to a final sodium concentration of 115 meq/liter) containing 18 m*M* sodium lactate, 0.8 m*M* L-ornithine, 3.7 m*M* ammonium chloride, and ^{14}C-bicarbonate (5.3×10^5 count/min). The mixtures were shaken for 1 hour in 95 percent O_2 and 5 percent CO_2 at 30°C. Values are means ± standard errors of the means of eight frogs per group.

Group	Liver weight (% body weight)	Hepatic urea synthesis		Carbamoyl phosphate synthetase (μmole g^{-1} hr^{-1})
		(μmole g^{-1} hr^{-1})*	(μmole/100 g of body weight per hour)†	
Freshwater	1.5 ± 0.06	3.9 ± 1.0	6.0 ± 1.4	83 ± 16
Saline	1.7 ± 0.10	7.7 ± 0.48‡	13.3 ± 1.5‡	250 ± 44‡
		Ratio of saline to freshwater values		
	1.1	2.0	2.2	3.0

* Calculated by dividing rate of incorporation of ^{14}C bicarbonate into urea (count/min per gram of liver per hour) by specific activity of ^{14}C-bicarbonate in medium (1×10^4 count min^{-1} $\mu mole^{-1}$).
† Product of micromoles per gram of liver per hour and liver weight as percentage of body weight.
‡ Significantly different from freshwater group ($P < .01$).

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