Serum Osmolality in the Coelacanth, Latimeria chalumnae: Urea Retention and Ion Regulation

Abstract. *Samples of blood (hemolyzed) were obtained from the renal vein, the hepatic portal vein, and the heart of a freshly thawed specimen of* Latimeria chalumnae. *The coelacanth uses high concentrations of urea to maintain its serum osmolality at approximately that of sea water. The mean value for the total osmolality was 1181 milliosmoles per liter. The mean values (milliequivalents per liter) were: for sodium, 181; for potassium, 51.3; for calcium, 6.9; for magnesium, 28.7; for chloride, 199; and for bicarbonate, 4.7. The mean urea concentration was 355 millimoles per liter, and the mean nonprotein nitrogen was 1343 milligrams percent. Heart blood showed significantly lower values for osmolality (921 milliosmoles per liter) and nonprotein nitrogen (1030 mg percent) and was probably less severely contaminated with products of protein breakdown. Fluid from the anterior chamber of the eye showed values of 952 milliosmole/liter; the urea value for this fluid was 303 mmole/liter, and the magnesium was 7.3 meq/liter. The magnesium value for the aqueous humor was used to correct the abnormally high concentrations in the hemolyzed serum. The high level of serum potassium also was attributed to hemolysis.*

Studies of the regulation of the blood and body fluids have contributed physiological information that is of crucial importance to our understanding of the origin and evolution of the vertebrates. Subsequent to the discovery of the living coelacanth, Smith (*1*) pointed out that if the coelacanths originated in freshwater, as seems probable, a study of the blood would reveal the mechanism of adaptation to sea water, whether by way of urea retention (as in elasmobranchs) or in a manner similar to that employed by marine teleosts. The former hypothesis was favored, partly on account of the presence of a well-developed glomerular kidney. It was therefore of compelling interest to study the blood of the specimen that was received in frozen condition at the Peabody Museum, Yale University (*2*). In that only one specimen was available, statistical treatment of the data we present was not possible. However, we provide the first direct evidence that urea occurs in the blood of the coelacanth at a concentration comparable with that of elasmobranchs. The only previously published investigations of the chemical properties of the blood of *Latimeria* are those of Prailauné (*3*) who studied the properties of the hemoglobin and of Beck (*4*) who reported a high level of copper in the whole blood (1.01 mg/liter).

The specimen (a male) was allowed to thaw slowly and uniformly during 60 hours at 2° to 10°C. On 30 May 1966, the abdomen was opened, and we were permitted to collect blood samples. The condition of the organs was good, firm and without evident decomposition; there were a few remaining ice crystals in the depths of the body cavity, indicating an optimum thawing. Blood was taken from the hepatic portal (0.4 ml) and renal (0.8 ml) veins in hypodermic syringes (no heparin was used); the samples were free of tissue contamination. A third sample was collected from the pericardial chamber when the heart was cut (0.5 ml). All samples were completely hemolyzed, and only the ghosts of red blood cells and other fine debris could be removed by centrifugation. The supernatants were frozen for subsequent study.

Total osmolality, in reference to standard NaCl, was estimated with a Mechrolab vapor-pressure osmometer equipped with Hamilton microsyringes; sodium and potassium were determined with an Instrumentation Laboratory flame photometer, chloride with an Amineo-Cotlove chloride titrator, and bicarbonate with the Natelson microgasometer after equilibration with 7 percent CO_2 in nitrogen saturated with water vapor. For serum calcium we used an adaptation (ultramicromethod) of the neutral-fast-red method, and for magnesium a similar adaptation of the titan yellow method (*5*). Serum urea was determined several times by two different methods: a modification (ultramicromethod) of the diacetyl monoxime method, and standard urease hydrolysis followed by direct nesslerization. The two procedures gave similar results. Total nonprotein nitrogen (NPN) was determined by direct nesslerization after acid digestion (micro-Kjehldahl). Total protein, including hemoglobin, was estimated from the Beckman Microzone electrophoretic strip; crystalline bovine serum albumin was used as a standard. We did not make determinations of constituents that are known to be rapidly and severely affected by changes in samples from dead animals (glucose, phosphate, and others). Urea is not so affected (*6*).

On a sample (15 to 20 μl) of the aqueous humor (provided by D. F. Cole) we were able to determine total osmolality, urea, and, for reasons explained below, magnesium.

Osmolality was higher in the hepatic portal and renal samples than in blood taken from the pericardial chamber (Table 1). At first it was suspected that the heart blood was diluted, perhaps from the melting of an ice crystal that had remained in the pericardial cavity. Such an interpretation is not supported by the data on Na and Cl, both of which had similar concentrations in the renal and heart serum. The difference appears to be due to the NPN fraction, which was considerably lower in the heart blood. It is suspected that high amounts of nonprotein nitrogen, exclusive of urea nitrogen, in the renal and portal blood can be attributed to a greater degree of decomposition of the serum proteins. The heart and renal samples remained clear, but the hepatic portal sample, clear when first centrifuged, became cloudy and brownish after freezing and thawing. This sample gave the highest NPN. The electrophoretic strip indicated protein breakdown, especially in this sample.

It has been suggested to us that urea in the blood could have arisen from the breakdown (enzymatic or nonenzymatic) of arginine in the blood through hydrolysis. This possibility seems to be excluded by the following considerations. If urea were formed from arginine, an equimolecular amount of ornithine would also be formed. Hence the total NPN less that due to urea would be at least as great as that of urea NPN. This is clearly not the case, as inspection of Table 1 will show. As much as 80 percent or more of the total NPN is accounted for by urea in the renal and heart samples, leaving only a maximum of 20 percent for ornithine.

Contamination of the blood by urea generated from the action of liver and kidney arginase is excluded by the work of Brown and Brown (*7*), who report stoichiometry for the ornithine carbamoyltransferase in whole homogenates of the liver of the specimen from which the blood was taken. Stoichiometry could not have been shown if L-ornithine had been present in the liver

at even one-tenth of the molar concentration of the urea. This argues that urea in the liver must have been derived from arginine while the fish was alive, not during cold storage. We are aware of reports of the action of enzymes in the frozen state (*8*), but if such action had taken place to any appreciable extent for the hydrolysis of arginine it would have appeared in our non-urea NPN, which it did not. Of course we cannot rule out the possibility that a small amount of urea (say 10 percent or less) arose during storage. However, we feel confident that the large amounts of urea we report are not the result of freezing and deterioration of the specimen.

The osmolality of equatorial water of the Indian Ocean is of the order of 1090 milliosmole/liter near the site, and at the probable depth of capture (*9*). If we accept a mean value for serum osmolality (1181 milliosmole/liter), it is possible that the blood is slightly hypertonic to the external medium, as it is in elasmobranchs. However, it is more probable that the lower value reported for the heart blood (921 milliosmole/liter) should be accepted, and, in this case, the blood is slightly hypotonic. Only studies on freshly drawn blood can resolve this problem.

In respect to the constituents contributing to the osmolality of the serum of elasmobranchs and teleosts, key references are listed (*10*). Serum Na is lower in *Latimeria* than it is in typical marine elasmobranchs, and it is in the normal range for marine teleosts. The high concentration of K in the serum can be attributed to hemolysis and may be disregarded. Serum Ca is at the upper limit, but within the range for marine elasmobranchs and teleosts. The measured value for serum Mg is very high, but this may result from hemolysis. Platner (*11*) gives values for Mg of 2.02 mg percent (milligrams per 100 ml of serum) and 12.34 mg percent, respectively, for the serum and whole blood of goldfish—a ratio of 1:6. We therefore determined the value for the aqueous humor in which, at least in elasmobranchs (*12*), Mg is present in the same concentration as in the serum. On this basis the serum Mg of *Latimeria* would be of the order of 7.3 meq/liter, giving a ratio of 1:4 for hemolyzed serum Mg to aqueous-humor Mg. Even then the concentration is high, either for an elasmobranch or for a teleost, although very high values have been reported in stressed specimens of the goosefish *Lophius piscatorius (13)*.

Table 1. Summary of data on the blood serum of *Latimeria chalumnae*. R.V., renal vein; H.P.V., hepatic portal vein. Numbers of determinations are indicated in parentheses.

Source	Osmolality	Ions (meq/liter)						Urea* (mmole/liter)	NPN		Total protein (g %)	Hemoglobin † (%)
		Na	K	Ca	Mg	Cl	HCO_3		Total (mg %)	Urea fraction (%)		
						Blood						
R.V.	1289 ± 5 (6)‡	168 ± 1 (4)	48.5 (2)	7.0 (1)	25.4 (2)	187 (2)	4.5 (1)	381 ± 18 (7)	1372 (2)	77.8	7.1	68
H.P.V.	1333 ± 8 (6)	196 ± 2 (4)	66.5 (2)	7.3 (1)	32.9 (2)	226 (2)		361 ± 10 (6)	1626 (2)	62.2	3.2	44
Heart	921 ± 3 (4)	179 ± 4 (4)	39.0 (2)	6.3 (1)	27.8 (1)	185 (2)	4.8 (1)	324 ± 12 (7)	1030 (2)	88.1	5.1	66
Average	1181	181	51.3	6.9	28.7	199	4.7	355	1343	76.0	5.1	59
						Aqueous humor						
	952 (1)				7.3 (1)			303 (1)				

*Urea values determined by urease and diacetyl monoxime methods were averaged. A single, low urea value (246 mmole/liter) cited previously (*20*) has been excluded because it was not confirmed in subsequent analyses. † Ratio of the area under the hemoglobin peak to the total area of the electrophoretic tracing. ‡ Mean ± standard error of replicate determinations on the same sample.

In respect to serum ions, Cl is higher than Na, perhaps because of hemolysis. However, the tabulation by Bernard, Wynn, and Wynn (*10*) reveals that some investigators have reported large amounts of Cl in elasmobranchs, although most investigators give lower values. In contrast, all recent and reliable studies of teleosts show that serum Cl is lower, in milliequivalents, than serum Na. The carbon dioxide content is low, possibly as the result of an accumulation of lactic acid during the stress of capture, but low values are prevalent in elasmobranchs (*10, 14*).

Urea concentration is in the range for that of marine elasmobranchs. Clearly *Latimeria* employs this metabolite to maintain serum osmolality at a high level. Low values for nonurea NPN in heart blood, believed to be the least-deteriorated sample, suggest that other nitrogenous substances play only a minor role. Urea accounts for 88 percent of the total NPN fraction, compared with about 96 percent for elasmobranchs.

The results of electrophoresis of serum proteins are of value only in assessing the state of deterioration of the blood: degradation was not complete, as evidenced by four to eight discernible zones; some protein deterioration had occurred, as is indicated by reduced resolution of peaks in the smear of lost zonal margins. Total serum protein, estimated after substraction of the hemoglobin, appears to be in the low normal range for elasmobranchs and teleosts. The estimated value for hemoglobin (3.3 g percent) in the heart blood is in line with the low value reported by Beck (*4*) for the blood of a female (2.8 g percent). The renal blood gave a slightly higher value (4.8 g percent), but the presence of serum protein may have increased the apparent level in both instances.

Ureotelic mechanisms have evolved in other vertebrates. Hibernating toads (*Scaphiopus couchi*) and the tortoise (*Testudo hermanni*), and the estivating lungfish *Protopterus* retain urea as an end product of nitrogen metabolism, to be eliminated when the animal awakens (*15*). The euryhaline frog *Rana cancrivora* employs urea in the osmoregulation of the blood (*16*), although this mechanism develops only after metamorphosis.

If the blood of *Latimeria* is slightly hypotonic to sea water, the fish must drink. The low levels of Na and Cl suggest the presence of an efficient method for salt excretion, imperative

if the fish drinks, but nothing is known in regard to "chloride cells" or a possible salt-secreting function of the nodular gland associated with the cloacal pouch (*17*).

Some information is available regarding the composition of the aqueous and vitreous humors in the eye of teleosts, but Cl is stated to be lower in both chambers than in the blood (*18*). More is known regarding the ocular fluids of elasmobranchs: the total osmolality of the aqueous humor is slightly below that of the blood; the major inorganic ions and urea follow the same pattern (*12, 19*). Our data on urea are compatible with these results. If comparison is made with heart blood, the aqueous humor appears to be hypertonic, as is true of elasmobranch vitreous humor (*19*).

The work reported above was essentially completed (*20*) independently of that of Brown and Brown (*7*), and our mutual findings are complementary. They found urea concentrations of the order of 280 mmole per kilogram of liver (wet weight), and we report an average of 355 mmole per liter of serum. Clearly the tissues are in approximate equilibrium with the blood.

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References and Notes

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21. Supported by NSF grant GB-0132.

12 December 1966

Urea and Its Formation in Coelacanth Liver

Abstract. *Urea occurs in liver of the coelacanth* Latimeria chalumnae *to the extent of about 1.7 percent by weight. It was determined quantitatively by reaction with 1-phenyl-1,2-propanedione-2-oxime (Archibald reagent) and by measurement of ammonia released upon treatment with urease. Arginase and ornithine carbamoyltransferase, enzymes instrumental in the formation of urea in typical ureotelic vertebrates, occur in homogenates of coelacanth liver. Formed in part by the ornithine-urea cycle, urea may have an osmoregulatory function in the coelacanth as it has in elasmobranchs.*

Anatomical studies (*1*) suggest a close relation between the coelacanth *Latimeria chalumnae* and the fossil rhipidistian fishes. The latter are supposed to be closely related to the antecedants of Amphibia and subsequently emerging higher vertebrates (*2*). Our biochemical studies on liver of the coelacanth

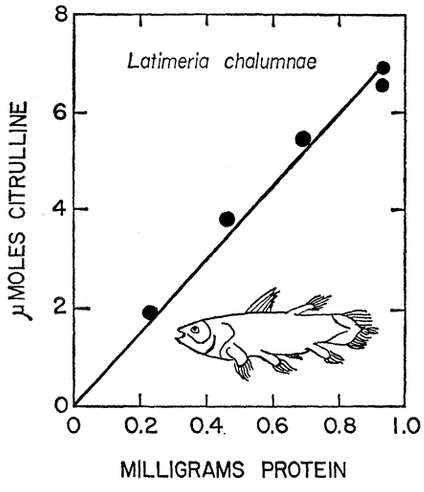

Fig. 1. Ornithine carbamoyltransferase of coelacanth liver: protein concentration study. Conditions as given in Table 3 but with varying amounts of protein (30 minutes).

(*3*) suggest that *Latimeria* has an intermediary nitrogen metabolism more closely akin to that of the Elasmobranchii (sharks, rays, and skates), Dipnoi (lungfish), and Amphibia than to that of the Teleostei (higher bony fishes).

Elasmobranchs, lungfish (*Protopterus*), and amphibians have the functional ornithine-urea cycle described by Krebs and Henseleit (*4*). Hence members of these three classes of vertebrates can synthesize enzymically, *de novo*, urea from carbon dioxide, ammonia, and amino groups of amino acids. While elasmobranchs maintain high concentrations of urea in tissues and plasma (1 to 3 percent by weight) (*5*), the estivating lungfish *Protopterus aethiopicus* (*6*) and the marine frog *Rana cancrivora* (*7*) accumulate correspondingly large amounts of urea in tissues and in the blood. The osmotic importance of elasmobranch blood urea is well recognized (*5*). On the other hand, teleostean fishes have been supposed to lack the functional ornithine-urea cycle (*8*), and there is no evidence to suggest that they ever build up appreciable quantities of urea in tissues or blood (*5*).

We have found that (Table 1) coelacanth liver contains approximately 17 mg of urea per gram of liver (wet weight) (1.7 percent), a value comparable to that of the livers of many sharks (*5*). The quantity of urea per gram of liver (Table 1) was estimated on 10-percent water homogenates (*9*) of coelacanth liver in two ways: (i) by reaction with 1-phenyl-1,2-propanedione-2-oxime (*10*), and (ii) by the amount