Ornithine Carbamoyltransferase in Liver of the Dipnoan Protopterus aethiopicus

Abstract. *Ornithine carbamoyltransferase, an enzyme that occurs typically in vertebrates having a ureotelic metabolism, is present in the liver of the African lungfish* Protopterus aethiopicus. *The reaction studied depends on* L-*ornithine, carbamoylphosphate, and unboiled homogenate of liver.*

In a continuing survey of the occurrence and nature of enzymes of the ornithine-urea cycle (*1*), several specimens of the lungfish, *Protopterus aethiopicus*, have been investigated (*2*). I now report evidence for the occurrence of the enzyme ornithine carbamoyltransferase (*3*),

$$\text{L-ornithine} + \text{carbamoylphosphate} \xrightarrow[\text{carbamoyltransferase}]{\text{ornithine}} \text{citrulline} + P_i,$$

in the liver of this lungfish in the aquatic habitat.

A water homogenate (10 percent) of liver of one specimen (body weight, 28.5 g; length, 16 cm) was prepared with a glass homogenizer (*4*). The homogenate was tested for ornithine carbamoyltransferase activity (*5*) in a system containing (the complete system): 20 μmole of L-ornithine, *p*H 8.1; 20 μmole of dilithium carbamoylphosphate; 90 μmole of glycylglycine (Na) buffer, *p*H 8.3; 13.3 μl of homogenate containing 141 μg of protein; and water to a final volume of 2.0 ml, *p*H 8.1. Incubation was for 15 minutes at 37°C. Citrulline content was determined at the end of incubation.

The following amounts (micromoles) of citrulline were found (mean value and mean deviation of duplicate incubations): the complete system, 1.76 ± 0; the complete system minus ornithine, 0.16 ± .05; the complete system (boiled homogenate), 0.13 ± .02; the complete system minus homogenate, 0.04 ± .04; the complete system minus carbamoylphosphate, 0 ± 0. Other experiments established that the rate of the reaction studied was directly proportional to protein concentration and that the amounts of citrulline produced were linear with respect to time.

The activity found with the complete system corresponds to 88.0 μmole of citrulline formed per minute per gram of liver (wet weight), or to a specific activity of 0.830 μmole of citrulline formed per minute per milligram of protein. Another specimen yielded a value of 56 μmole of citrulline per minute per gram of liver (wet weight). Such values are comparable with those obtained with liver homogenates of various elasmobranchs (*6*), a group of fishes with a ureotelic type of metabolism.

Janssens (*7*) demonstrated that arginase was present in liver of *P. aethiopicus*. However, arginase occurs in the liver of numerous fishes—both teleosts (*8*) and elasmobranchs (*8*); demonstration of this enzyme of the ornithine-urea cycle cannot be taken to indicate a ureotelic type of metabolism. Dipnoi, ammonotelic in the aquatic habitat, have long been known to store large quantities of urea during aestivation; such urea is excreted relatively rapidly upon resumption of the aquatic habitat (*9*). The metabolic pathway for the production of this urea is unknown.

My findings indicate a potential route for the synthesis *de novo* of urea from carbon dioxide and ammonia in the lungfish: ornithine carbamoyltransferase occurs in the liver of ureotelic vertebrates (mammals, amphibia, certain or, perhaps, all turtles, and elasmobranchs), while it has not yet been shown to occur in liver of typical uricotelic (birds, lizards, snakes) or ammonotelic (teleosts) vertebrates (*10*). The finding of this enzyme of the ornithine-urea cycle in lungfish liver is not a trivial observation inasmuch as the urea stored during aestivation could be formed, conceivably, by routes not including this enzyme: for example, from uric acid (by way of allantoin and allantoic acid) or from tissue arginine (by action of arginase).

Surprisingly, another enzyme of the cycle, carbamoylphosphate synthetase (*11*), could not be demonstrated with liver homogenates of the lungfish; it may be active only when the lungfish aestivates, at which time it would serve the economy of the lungfish to "turn on the cycle" adaptively, so that toxic ammonia would not accumulate.

The data of Janssens (*7*) on the production of urea from ammonia (and bicarbonate) by liver of *P. annectens* (or *aethiopicus*), although informative, do not provide conclusive evidence for the operation of the ornithine-urea cycle; compelling evidence is obtainable by demonstration of the activities of enzymes of the ornithine-urea cycle and by experiments showing the conversion of isotopic carbon dioxide to urea.

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References and Notes

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Schlieren Technique for Studying Water Flow in Marine Animals

Abstract. *A Schlieren optical technique has been developed for studying the flow of water through certain mollusks and brachiopods in sea-water environments. Optical contrast is accomplished by changing either the temperature or salinity of the water. A temperature difference of 0.1°C or a change in salinity of 0.5 per thousand over a path 1 mm long is visible. The technique may be applied* in situ *in the ocean or through windows in aquaria.*

During development of aquaria to study living deep-ocean animals under conditions controlled as to chemistry, temperature, and pressure, the need arose for a technique to detect and measure metabolic activity. These aquaria must consist of heavy-walled