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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12. Supported by PHS grant GM-9988.

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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

metal chambers with windows capable of withstanding internal pressures up to 1000 atm. Such windows are necessarily quite small, and the possibility of mechanically manipulating the animals inside is very limited.

Since most of the animals of interest in this study (Brachiopoda and Pelecypoda) are filter feeders, a technique for detecting or measuring water flow would indicate the condition of the animal. Many techniques have been used (*1*) to study water transport in mollusks and brachiopods, but none are usable in high-pressure, limited-access environments.

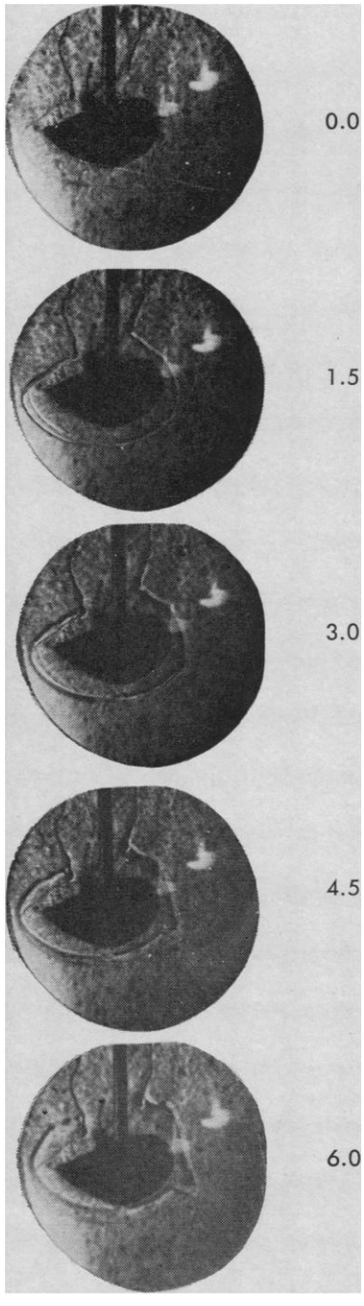

Fig. 1. The pumping action of *Mytilus edulis* at 1.5-second intervals (see text). The fixed, large, light-colored shapes are reflections from the window in the aquarium.

Schlieren optical techniques (*2*) were developed many years ago to detect changes in the refractive indexes of gases passing over high-velocity projectiles. We have applied these techniques to fluid-filled optical paths to detect small changes in index due to heating or variation in salinity; we can now look through a quite small window in a high-pressure aquarium and measure the water flow through and around a growing animal.

Any spatially nonuniform change of the refractive index in the light path in a Schlieren system will direct rays either behind the knife edge or completely free from the edge, causing the local area of the mirror behind the disturbance to appear either darker or lighter than the surroundings. A very small change in index creates a marked local brightening or darkening of the mirror: 0.1°C change in temperature or 0.5-per-mille change in salinity over a 1-mm path causes an easily visible change in brightness.

Figure 1 shows a small blue mussel, *Mytilus edulis*, supported on a plastic rod and encircled by a fine nickel wire that could be electrically heated to produce a small toroid of slightly heated water around the animal. The five photographs, in sequence at 1.5-second intervals, show successively the quiescent system (0), the heating water (1.5), distortion of the toroid by water exhausted from the mussel (3.0), further distortion (4.5), and convection of the whole toroid, plus two distortions due to exhaust from the animal (6.0).

Although the technique was developed for two-dimensional use, it is possible to use three orthogonal systems simultaneously to obtain a three-dimensional map of the water flow around an animal. It is also possible to drop individual grains of table salt around an animal to make a curtain of "threads" similar to the smoke streamers used in a wind tunnel; a single grain falling through the water produces a very fine opaque thread, which persists much longer than heated water. Details of the flow may also be studied by producing very small "blobs" of heated water locally by use of a very short heated wire.

Major advantages of this technique are absence of foreign material from the water circulated by the animal, least disturbance of the environment, suitability for use *in situ* or in very hostile environments, and vivid visual and photographic visibility in three dimensions.

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Triploidy in a Human Cell Line

Abstract. *Cytogenetic studies of a human amnion cell line (strain RA) indicate that the major stem cell was triploid. Consistently present in the triploid cells were two marker chromosomes, a small centric fragment, and an apparently telocentric D. At various times triploid cells constituted 40 to 80 percent of the cell population. The possible origin of the triploid cell is a diploid/triploid mosaic amnion.*

Studies of chromosomal evolution of cells grown in vitro indicate that chromosomal change seems to follow a pattern of diploidy to tetraploidy to heteroploidy followed by emergence of stem cells and further selection among stem cell derivatives (*1*).

This report describes the karyotype of strain RA ("recovered amnion"), a continuous epithelioid line derived by spontaneous alteration from clinically normal, term, human amnion (*2*). Strain RA is of interest because its major stem line is essentially triploid (*3*).

Strain RA was used entirely for virological purposes during its early history and was not studied cytogenetically until the third year of culture. No data are available on the karyotype of the original culture.

In the present study, cells were grown on slides in wide-mouthed French-square bottles, on Eagle's medium (*4*) with 10 percent calf serum. After 48 hours of incubation, colchicine ($10^{-6}M$) was added to the medium for 6 to 16 hours. The medium was then removed, and a hypotonic Hanks solution (1 to 6 dilution) was introduced for 20 to 30 minutes. The cells were fixed in acetic acid–methanol (1 : 3),