Studies on Mutarotases
IV. Mutarotase in Kidneys of Saltwater versus Freshwater Fish (33995)

J. MARTYN BAILEY, PETER H. FISHMAN, AND SALLY MULHERN
Department of Biochemistry, George Washington University, School of Medicine, Washington, D. C. 20005

Mutarotase is the enzyme which catalyzes the interconversion of the anomeric forms of aldoses (1–9). It has been found in all forms of life ranging from bacteria to mammals. Despite this widespread distribution of mutarotase no specific function for the enzyme has yet been defined. It has been shown that it is unlikely that the enzyme is involved in glucose metabolism since no anomeric specificity is displayed by any of the enzymes normally involved in glucose metabolism (10, 11). Furthermore, it has been shown that mutarotase levels in the developing embryonic kidney of the rat and rabbit parallel the development of the proximal segment of the kidney tubule (12). As sugar reabsorption in the kidney occurs in the proximal portion of the tubules this suggests that mutarotase may be involved in the transport of sugars. Since these findings demonstrate the correlation of mutarotase and kidney development, it seemed pertinent to study the enzyme from sources representing various evolutionary periods in the developing of the kidney.

The evolutionary development of the kidney is evident in freshwater and saltwater fish. Since freshwater fish are osmotically superior to their environment they had to evolve a kidney which would filter off excess water and retain salts, sugars, and amino acids. The glomerular kidney accomplishes this by allowing waste products to be filtered from the blood while permitting glucose, salts, and amino acids to be reabsorbed in the tubules. Conversely, saltwater fish must retain water and therefore have evolved a glomerulus which is varingly reduced in size and activity (13). Since only a minimum fluid is excreted in the saltwater fish, there is a minimum requirement for a glucose reabsorption mechanism in the tubules of the kidney.

An interesting contrast in levels of the enzyme in kidneys of the two types was noted. The results for both fish and mammals are consistent with the idea that the enzyme is related in some way to the reabsorptive function of the kidney.

Materials and Methods. Kidneys were obtained from freshly caught fish and the enzyme content was measured on the refrigerated specimens usually within 24 hr. It was shown that storage in this way did not materially affect the mutarotase content. The mammalian kidneys which were also sampled for comparative purposes (see Table I) were treated in a similar manner. The samples were homogenized in 10 vol of ice-cold EDTA buffer (5 m*M*, pH 7.4) and the solution was clarified by centrifugation. Portions of the supernatant were assayed for enzyme activity as described below. For kinetic studies, the enzymes from catfish (*Ictalurus punctatus*) and toadfish (*Opsanus*) were concentrated following homogenization in 10 vol of phosphate buffer (1 m*M*, pH 6.4). The supernatant was mixed with 5 g of hydroxyapatite, centrifuged, dialyzed against EDTA buffer (5 mM, pH 7.4), and the final clear solution used as the enzyme source.

Mutarotase was assayed by measuring acceleration in the rate of conversion of freshly-dissolved alpha- to beta-glucose. Measurements were carried out in a Bendix type 143A automatic polarimeter. Output from the polarimeter was displayed on a recorder fitted with logarithmic gears so that by suitable precalibration of the apparatus, the first order mutarotational rate constant could be read directly from the slope of the straight line plot (6). The rate of reaction was derived as the product of the rate constant (after correction for spontaneous mutarotation) and the alpha-glucose concentration. One unit of mutarotase was defined as the amount of enzyme which converts 1 μmole of alpha- to beta-glucose/min at 25°, pH 7.4,

TABLE I. Mutarotase Content of Some Vertebrate Kidneys.[a]

Species	Units/g of wet wt[b]
Mammals	
Hog	4200
Ox	3640
Lamb	3260
Mouse	3020
Rabbit	1670
Rat	1617
Human	1000
Freshwater fish	
Catfish (*Ictalurus punctatus*)	226
Carp (*Carpiodes*)	187
Rock bass (*Ambloplites rupestris*)	103
Largemouthed bass (*Micropterus salmoides*)	143
Crappie (*Pomoxis annularis*)	132
Pumpkin seed (*Lepomis gibbosus*)	182
Warmouth (*Crypticanthodes maculatus*)	384
Saltwater fish	
Croaker (*Micropogon undulatus*)	26
Flounder (*Paralichtys*)	10
Sea trout (*Cynoscian nebulosus*)	13
Sheepshead (*Archosargus probayocephalus*)	27
Toadfish (*Opsanus*)	54
Blow fish (*Sphaeroides maculatus*)	51
Sea robin (*Prionotus evolans*)	40
Ling (*Molva molva*)	20
Sand shark (*Carcharus taurus*)[c]	63

[a] Mammalian kidneys were from freshly slaughtered animals (hog, beef, lamb) or from laboratory animals (rabbit, rat, mouse). Human kidneys were obtained at autopsy 18–24 hr following death. We are indebted to the Virginia State Game and Fisheries Commission and to Dr. Frank Schwartz of the Natural Resources Institute, Solomons Island, Maryland, for their assistance in obtaining specimens of the fish studied. The results represent the mean of three determinations for each of the mammals and larger fish, and for the smaller fish, single or duplicate assay on pooled kidney tissues from a number of individuals as follows: crappie (5), pumpkin seed (5), warmouth (4), toadfish (8).

[b] One unit of mutarotase catalyzes the conversion of 1 μmole of alpha-glucose/min at pH 7.4, 25°, and optimum substrate concentration (6).

[c] Elasmobranch.

and at optimum substrate concentration.

Results and Discussion. The relative levels of the enzyme in kidneys of the different species are given in Table I. Of particular interest is the finding that the mutarotase levels in kidneys of freshwater fish are without exception higher than those in saltwater fish, averaging six times greater. This extends an earlier observation (14) by Keston that the enzyme was present in very low levels in toadfish kidney. The amount of enzyme in freshwater fish kidney is considerable. It may be calculated, for example, that 1 g of catfish kidney contains sufficient enzyme to convert approximately 2.5 g of alpha-glucose/hr under physiological conditions. This is far exceeded by the mammalian kidney however, the corresponding value for hog kidney is 96 g of alpha-glucose converted per gram per hour.

The mutarotase levels in other tissues and organs of some species were determined by the same method for comparative purposes. It was found that the higher levels of mutarotase in freshwater versus saltwater fish were confined to kidney tissue. The amounts of enzyme in liver, spleen, heart, and skeletal muscle were approximately the same for the two groups. A comparison of enzyme levels in tissues of mammals with those in fish was made (Table II). Most mammalian tissues contained only from 2 to 5 times more enzyme than the corresponding tissue in the fish whereas the mutarotase levels in the mammalian kidney were over 60 times higher than those in the fish.

The sugars which were substrates for the fish enzyme (D-glucose, D-galactose, D-xylose, and L-arabinose) are the same as those reported for the enzymes from *E. coli* (4), *P.*

TABLE II. Levels of Mutarotase in Rat and Croaker Tissues.[a]

Tissue	(units/g fresh wt) Rat	Fish (croaker)	Ratio (rat/fish)
Kidney	1617	26	64
Liver	605	185	3
Intestine	237	45	5
Spleen	128	82	2
Heart	88	76	1
Muscle	7	15	0.5

[a] In most cases, the results represent one determination per tissue.

notatum (5), green pepper (6), and kidney and liver from a number of mammalian species (3, 7–9, 14, 15). The common structural feature required for a substrate in all the species studied thus appears to be an equatorial–equatorial (*trans*) relationship of the hydroxyl groups at C-2 and C-3 of the sugar ring (15).

The only difference found in the properties of the enzyme from fish as compared to the mammalian enzyme is the K_M value for glucose. The K_M value for both the catfish and toadfish kidney enzymes was 12mM glucose and was thus significantly less than that for most mammals for which the K_M value for glucose usually is in the range of 19–25 mM (16).

Summary. The mutarotase content of kidneys from seven species of freshwater fish was measured and found to be uniformly higher than in nine species of saltwater fish. Kidney levels averaged six times higher whereas other tissues (liver, intestine, spleen, heart, and skeletal muscle) did not differ significantly. Substrate specificity of the fish enzyme (D-glucose, D-galactose, D-xylose, and L-arabinose) was the same as the mammalian enzyme. The K_M for enzyme from catfish and toadfish kidney was 12 mM glucose and was significantly less than that for most mammalian kidney enzymes.

This work was supported by grants AM 10082 and 5 K3 Ca 1630 from the U. S. Public Health Service.

1. Bentley, R. and Neuberger, A., Biochem. J. **45**, 584 (1949).

2. Keilin, D. and Hartree, E. F., Biochem. J. **50**, 331 (1952).

3. Keston, A. S., Science **120**, 355 (1954).

4. Wallenfels, K. and Herrman, K., Biochem. Z. **343**, 294 (1965).

5. Bentley, R. and Bhate, D. S., J. Biol. Chem. **235**, 1219 (1960).

6. Bailey, J. M., Fishman, P. H., and Pentchev, P. G., J. Biol. Chem. **243**, 4263 (1967).

7. Keston, A. S., Arch. Biochem. Biophys. **102**, 306 (1963).

8. Li, L. K. Arch. Biochem. Biophys. **110**, 156 (1965).

9. Chase, A. S. and Lapedes, S., Biochem. Biophys. Res. Commun. **31**, 967 (1968).

10. Salas, M., Vinuela, E., and Sols, A., J. Biol. Chem. **240**, 561 (1965).

11. Bailey, J. M., Fishman, P. H., and Pentchev, P. G., J. Biol. Chem. **243**, 4287 (1968).

12. Bailey, J. M., Fishman, P. H., and Pentchev, P. G., Federation Proc. **25**, 655 (1966).

13. Smith, J. W., "The Kidney." Oxford Univ. Press, London and New York (1951).

14. Keston, A. S., J. Biol. Chem. **239**, 3241 (1964).

15. Pentchev, P. G., Ph.D. thesis, George Washington University (1968).

16. Mulhern, S. A., M.Sc. thesis, George Washington University (1968).

Received Feb. 10, 1969. P.S.E.B.M., 1969, Vol. 131.