ALKALINE PHOSPHATASE ACTIVITY IN KIDNEYS OF GLOMERULAR AND AGLOMERULAR MARINE TELEOSTS

MARIE J. BROWNE, MARJORIE W. PITTS AND ROBERT F. PITTS [1]

The Department of Physiology, Syracuse University College of Medicine, the Marine Biological Laboratory, Woods Hole, Mass., and the Mt. Desert Island Biological Laboratory, Salisbury Cove, Maine

It is generally accepted that glucose undergoes phosphorylation in the process of absorption from the glomerular filtrate (Shannon, 1942). Formation within the tubular cells of a hexose phosphate ester is presumed to create the diffusion gradient which permits a continuous flow of glucose across the lumenal membrane (Kalckar, 1941). Subsequently the ester is hydrolyzed and the glucose discharged into the peritubular blood stream. Presumably phosphorylation is effected at the expense of the labile phosphate bond energy of adenosine triphosphate. In favor of this concept is the presence within the cells of the proximal segments of the renal tubules of rich stores of ATP, hexokinase, and alkaline phosphatase (Kalckar, 1937), the latter two enzymes being necessary, respectively, for phosphorylation of glucose and hydrolysis of the ester. Furthermore, glucose is known to be absorbed solely by cells of the proximal segment (Walker *et al.*, 1941; Walker and Hudson, 1937) which alone contain the enzyme phosphatase in its characteristic location in the brush border (Gomori, 1941). In addition, the absorptive mechanism is an energy consuming one of limited transfer capacity (Shannon and Fischer, 1938) which can be partially or completely blocked by such enzyme inhibitors as cyanide (Bayliss and Lundsgaard, 1928), phlorizin (Lundsgaard, 1933) and mercury (Weston *et al.*, 1949).

Were alkaline phosphatase to be concerned only with the hydrolysis of the hexose ester formed within the tubular cells in the reactions outlined above, it should be absent in those forms which possess no glomeruli, filter no glucose and hence need and presumably have no glucose absorptive mechanism. According to Wilmer (1944) the renal tubules of the toadfish, an aglomerular marine teleost, are devoid of alkaline phosphatase, whereas those of fresh water glomerular teleosts exhibit an abundance of the enzyme. Kalckar (1941) in a similar vein states "aglomerular kidneys (toadfish) contain much less phosphatase than glomerular kidneys from closely related species." If these observations are correct, they constitute strong evidence that renal alkaline phosphatase, at least in the fish kidney, is largely concerned with the absorption of glucose, for the urines of glomerular and aglomerular forms do not differ appreciably in composition (Marshall, 1930).

To test the above hypothesis, the distribution of alkaline phosphatase has been studied in a representative series of marine fish. Because it has been found that the enzyme is present in the brush border of the "proximal segment" of both aglomerular and glomerular forms, it must be concerned with renal tubular functions in addition to, or other than, the absorption of glucose from the glomerular filtrate.

[1] Aided by a grant from the United States Public Health Service.

Methods

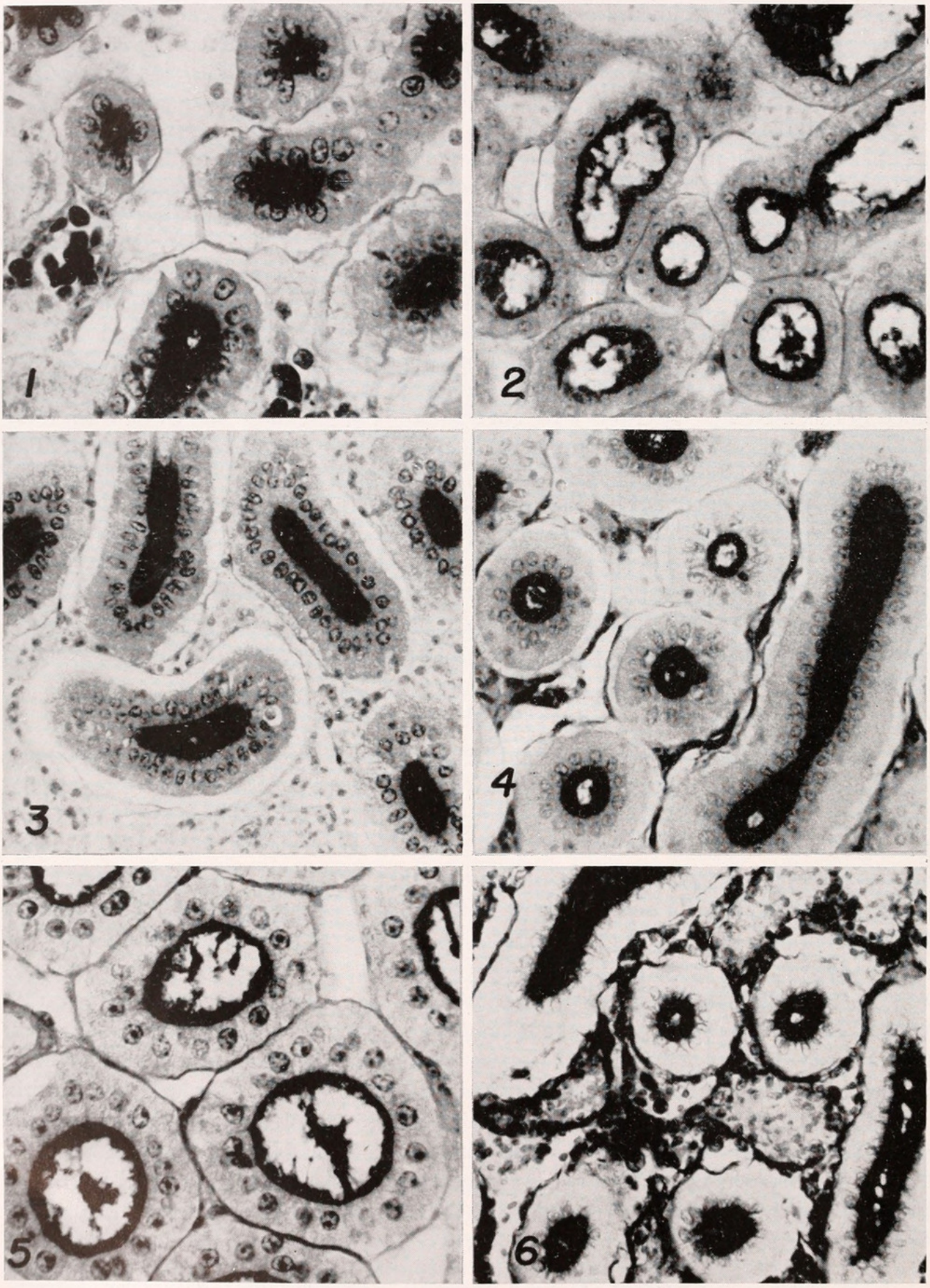
At least three specimens of each of the following fish were caught in traps or seines by the collecting crew of the Marine Biological Laboratory or by commercial dredgers off Menemsha Bight: sea robin (*Prionotus evolans strigatus*), puffer (*Tetraodon maculatus*), eel (*Anguilla rostrata*), dogfish (*Mustelus canis*), skate (*Raja erinacea*), toadfish (*Opsanus tau*), goosefish (*Lophius piscatorius*), and pipefish (*Syngnathus fuscus*). The latter three are aglomerular fish (Marshall, 1934). In addition, specimens of the daddy sculpin (*Myoxycelphalus scorpius*), the long horn sculpin (*Myoxycephalus octodecimspinosus*) and the flounder (*Pseudopleuronectes americanus*) were caught by hook and line at the Mt. Desert Island Biological Laboratory. The daddy sculpin, when full grown, is essentially aglomerular (Grafflin, 1933). All were killed by a blow on the head; the kidneys were promptly removed, and small blocks of tissue were fixed in 95 per cent alcohol for 14 to 20 hours according to the method of Gomori (1939). Further handling, including dehydration and embedding of the tissue in paraffin, was performed as outlined in this method. Sections were cut 6 microns in thickness and those from three to six different species were mounted on a single slide to compare enzymatic activity under identical conditions of treatment. The modified method of Krugelis (1946) was employed to demonstrate the presence of alkaline phosphatase and the slides were subsequently counterstained with light green. Incubation in alkaline glycerophosphate was carried out at 37° C. for ½ to 21 hours. Two types of controls were run: (a) preformed calcium deposits were visualized by cobalt exchange and precipitation by potassium sulfide; (b) the entire incubation and development procedure was carried out, except that glycerophosphate was omitted from the reaction mixture.

Results

Alkaline phosphatase activity could be demonstrated readily in its characteristic location in the brush border of the "proximal" tubular segments of ten of the eleven species of marine fish examined, including all of the aglomerular forms. The only fish in which no activity could be demonstrated in this location, in any one of three specimens, was the eel. In view of the fact that fixation in all blocks from all of these specimens was, for some unknown reason, very poor, we attach no significance to this finding. Plate I, Figures 1 through 6, illustrates in photomicrographs the distribution of the enzyme in six fish: two glomerular teleosts (sculpin and sea robin), two aglomerular teleosts (toadfish and pipefish), one nearly aglomerular teleost (daddy sculpin) [2] and one glomerular selachian (skate). It is apparent that activity is demonstrable in the brush border, in the nuclei of the tubular cells, in the interstitial tissue (so called pseudolymphatic tissue, Marshall and Smith, 1930), in the glomerular capillaries and in some parts of the peritubular network of vessels. Little or none is demonstrable in the cytoplasm of the tubular cells. Preformed deposits of calcium of any magnitude could be demonstrated only in the glomeruli of the eel and in the connective tissue of the renal capsule of the pipefish. When all steps of the procedure were carried out in the usual fashion, except that

[2] This daddy sculpin weighed 570 gms. According to Grafflin (1933), glomerular degeneration should be essentially complete.

PLATE I



glycerophosphate was omitted from the incubation mixture, no precipitate was observed other than that of preformed calcium in the instances noted above.

Discussion

We doubt that quantitative comparisons of enzyme activity (based on incubation time necessary to obtain a given density of precipitate) have, in this material, much cytochemical significance. Although the reaction is without caprice when applied on different occasions to sections from a given block of tissue, sections from different specimens of a given species vary considerably in reactivity. Even sections from different blocks of a given specimen vary somewhat, indicating that minor differences in fixation may account for variability. However, from the limited numbers of specimens in our series, it would appear that the sea robin, skate, pipefish, and toadfish give the most intense brush border reaction. Nevertheless, in one specimen of toadfish it was necessary to incubate for 21 hours at 37° C. to demonstrate enzyme activity adequately. We infer that the failure of Wilmer (1944) and Kalckar (1941) to observe the enzyme in the brush border of the toadfish may be related to inadequacy of fixation, and/or insufficient time of incubation.

Although our results do not in any sense negate the possibility that the alkaline phosphatase of the brush border of the proximal segment of glomerular kidneys functions in the absorption of glucose, they do suggest strongly that the enzyme is concerned in other tubular functions shared by aglomerular and glomerular forms.

Summary

The kidneys of 35 specimens of 11 marine fish have been studied by histochemical methods for the presence and distribution of alkaline phosphatase. All fish examined, with the exception of the eel, exhibited alkaline phosphatase in the brush border of the "proximal" segment. Since the ten species which exhibited the characteristic location of the enzyme included three aglomerular teleosts (toadfish, pipefish, and goosefish), one equivocal aglomerular teleost (daddy sculpin), four glomerular teleosts (sea robin, puffer, flounder, and long horn sculpin), and two

---

Plate I

Photomicrographs of sections of the renal tubules of a series of marine fish. All sections 6 micra in thickness; all photomicrographs 650 ×. Alkaline phosphatase activity was demonstrated by precipitation of black cobaltous sulfide and the sections were subsequently counterstained with light green. Variations in time of incubation (noted below) were used to clarify histological structure and localize activity rather than to quantify the amount of enzyme present. Evidence of alkaline phosphatase activity is seen in the brush border and nuclei of the tubular cells and in the interstitial tissue, but only to a negligible degree in the cytoplasm of the tubular cells. Figures 1 and 2 are from aglomerular fish; Figure 3 is from an essentially aglomerular fish; Figures 4–6 are from glomerular fish.

Figure 1. Renal tubules of the toadfish. Incubation time 4 hours.
Figure 2. Renal tubules of the pipefish. Incubation time 6 hours.
Figure 3. Renal tubules of the daddy sculpin. Incubation time 21 hours.
Figure 4. Renal tubules of the long horn sculpin. Incubation time 2 hours.
Figure 5. Renal tubules of the skate. Incubation time 4 hours.
Figure 6. Renal tubules of the sea robin. Incubation time 1 hour.

glomerular selachians (skate and dogfish), it is concluded that the enzyme of the brush border must be concerned in tubular processes shared by forms with and without glomeruli. It cannot, therefore, function solely in the absorption of glucose from the glomerular filtrate.

The authors are indebted to Miss Stella Zimmer for the preparation of the photomicrographs.

LITERATURE CITED

BAYLISS, L. E. AND E. LUNDSGAARD, 1928. The action of cyanide on the isolated mammalian kidney. *J. Physiol.*, **74**: 279.

GOMORI, G., 1939. Microtechnical demonstration of phosphatase in tissue sections. *Proc. Soc. Exp. Biol. and Med.*, **42**: 23.

GOMORI, G., 1941. The distribution of phosphatase in normal organs and tissues. *J. Cell. and Comp. Physiol.*, **17**: 71.

GRAFFLIN, A. L., 1933. Glomerular degeneration in the kidney of the daddy sculpin. *Anat. Record*, **57**: 59.

KALCKAR, H., 1937. The significance of phosphorylation in kidney tissue. *Skand. Arch. f. Physiol.*, **77**: 46.

KALCKAR, H., 1941. The nature of energetic coupling in biological synthesis. *Chem. Rev.*, **28**: 71.

KRUGELIS, EDITH J., 1946. Distribution of intracellular alkaline phosphatase. *Biol. Bull.*, **90**: 220.

LUNDSGAARD, E., 1933. Hemmung von Esterifizierungsvorgängen als Ursache der Phlorrhizinwirkung. *Biochem. Zeitschr.*, **264**: 209.

MARSHALL, E. K., JR., 1930. A comparison of the function of the glomerular and aglomerular kidney. *Amer. J. Physiol.*, **94**: 1.

MARSHALL, E. K., JR., 1934. The comparative physiology of the kidney in relation to theories of renal secretion. *Physiol. Rev.*, **14**: 133.

MARSHALL, E. K., JR., AND H. W. SMITH, 1930. The glomerular development of the vertebrate kidney in relation to habitat. *Biol. Bull.*, **59**: 135.

SHANNON, J. A., 1942. Kidney. *Ann. Rev. Physiol.*, **4**: 297.

SHANNON, J. A. AND S. FISCHER, 1938. The renal tubular reabsorption of glucose in the normal dog. *Amer. J. Physiol.*, **122**: 765.

WALKER, A. M., P. A. BOTT, J. OLIVER AND M. C. MACDOWELL, 1941. The collection and analysis of fluid from single nephrons of the mammalian kidney. *Am. J. Physiol.*, **134**: 580.

WALKER, A. M. AND C. L. HUDSON, 1937. The reabsorption of glucose from the renal tubule of the amphibian kidney and the action of phloridzin upon it. *Am. J. Physiol.*, **118**: 130.

WESTON, R. E., J. GROSSMAN, I. S. EDELMAN, D. J. W. ESCHER AND L. HELLMAN, 1949. Renal tubular action of diuretics; effects of mercurial diuresis on glucose absorption. *Federation Proc.*, **8**: 164.

WILMER, H. A., 1944. Renal phosphatase; a correlation between functional activity of the renal tubule and its alkaline phosphatase content. *Arch. Pathology*, **37**: 227.