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Alkaline Phosphatase Activity in the Developing Pronephros and Mesonephros of the Frog *Bombina orientalis*

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발생중의 무당개구리 前腎 및 中腎의 Alkaline Phosphatase활성

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摘 要

발생중의 무당개구리에서의 전신 및 중신 alkaline phosphatase활성을 Gomori 변법을 써서 관찰하였다.

전신 분비세관의 분화와 함께 세관 刷子緣에 출현한 alkaline phosphatase 활성은 鰓蓋완성기에 최고도에 달하였다가 제3후지 형성기에 전신의 퇴화와 함께 효소활성은 감소 소실되었다.

鰓蓋완성기에 출현한 중신세관에서의 alkaline phosphatase활성은 변태기를 통하여 강한 양성반응을 보였다.

INTRODUCTION

Since the work of Gomori (1939) and Takamatsu (1939) the distribution of alkaline phosphatase activity in the kidney has been investigated by morphological and cytochemical methods. The localization of the alkaline phosphatase in the developing chick was observed with a light microscope by Moog (1944). Desalu (1966) reported morphogenetic changes associated with the localization of alkaline phosphatase during the development of the rat kidney. Hah (1969) also observed alkaline phosphatase activity in the undifferentiated mesenchymal tissue during the early stages of the chick kidney, and reported that, as the kidney tubule grew differentiated, its activity was found to disappear in the mesenchymal tissue surrounding the tubule and was absorbed at the brush borders of differentiated secretory tubules of mesonephros and metanephros.

On the other hand, the neonatal mammalian kidney does not possess the same functional capacity as that of the adult (McCane and Widdowson, 1960). Histochemical evidence has accumulated that there is less enzymatic activity in newborn

than in adult kidney (Longley and Fisher, 1956; Pinkstaff *et al.* 1962; Ivemark, 1959). Such investigations have been carried out for the most part in rat with special reference to renal cortex.

The present report deals with the alkaline phosphatase activity in the developing frog kidney. Histochemical examinations are not confined to kidney cortex but are extended to medulla as well.

MATERIALS AND METHODS

Materials used were the developing pronephros and mesonephros of the frog *Bombina orientalis*. The larvae, tadpoles and young frogs were sacrificed on successive days. Whole kidney of small pieces of tissue were removed immediately and fixed in cold 85% ethanol at 4°C for 16-25 hours for the preparation of paraffin sections. After the tissue was made into 6μ thick sections Gomori's medium(1952), containing sodium β-glycerophosphate as substrate, was used for the demonstration of alkaline phosphatase activity at pH 9.4. Incubation was carried out for 3-4 hours at 37°C. Kidney sections of the frogs studied as control were fixed in 10% formaldehyde and stained with hematoxylin and eosin.

RESULTS

The pronephros

The pronephros of the *Bombina* frog was first noticed at the tail bud stage (stage 17) as the pronephric ridge just posterior to the gill plate. This was developed in the somatic wall of the nephrotomal region at the level of the II to IV somites. Within the nephrotomal masses there appear cavities, the nephrocoels. This grew posteriorly along the lateral border of the nephrotomes to form the paired pronephric ducts. Posterior to the IV somite this duct developed independently of the nephrotomal tissue and became joined to the cloaca at the stage of muscular response (stage 18). The pronephric tubule appeared between the pronephric duct and coelom at the stage 18. By the gill bud stage (stage 19) the pronephric tubule acquired an opening, the nephrostome, into the adjacent coelom. The pronephric tubules lengthened and became convoluted and at the hatching stage (stage 20) pronephros are large and conspicuous, and are embedded in the mesenchyme. By the operculum complete stage (stage 25) the pronephros were attained its maximum development, but by the third stage of posterior limb formation (stage 28) they have begun degenerate and neither the tubules at the level of somites II to IV nor the related glomi remained.

A summary of the results of the histochemical study of the pronephros is shown in Table 1. Alkaline phosphatase activity of pronephros was first found in the

pronephrogenic cord at the stage 17. After the pronephric tubule formed from the nephrogenic cord the weak enzyme activity appeared in the brush border of the tubule. By the stage of transition from larva to tadpole this activity increased gradually as the brush border differentiated, and disappeared in the pronephrogenic tissue at about the hatching stage. After the hatching stage the enzyme activity on the brush border of the tubule became highly positive, but as pronephros have reached their maximum development and then begun to degenerate, the enzyme activity decreased and finally disappeared. The weak alkaline phosphatase activity was observed in the glomus, but no activity was observed in association with the collecting tubule, pronephric duct and other tissues.

Table 1. Distribution of alkaline phosphatase in the developing pronephros

Tissues \ Stages	17	18	19	20	21	22	23	24	25	26	27	28
Secretory tubule			+	++	+++	++	+++	+++	+++	+++	++	+
Collecting tubule			−	−	−	−	−	−	−	−	−	−
Glomus			−	±	+	+	+	+	+	+	+	+
Pronephric duct			−	−	−	−	−	−	−	−	−	−
Nephrogenic cord	+	++	++	++	+	−	−	−	−	−	−	−

+++, strongly positive; ++, moderately positive; +, weakly positive; ±, faintly positive; and −, negative to Gomori's Na β-glycerophosphate reaction.

The mesonephros

The nephrotomal mass posterior to the pronephros gave rise to the mesonephros which is the functional kidney of the adult frog. It extended from the level of somite VII through XII and begun to develop the continuous mesonephrogenic cord at stage of operculum over right gills (stage 24) to the operculum complete stage (stage 25). At the level of each of these somites there developed several nephrotomes, each with a seperate nephrocoel, mesonephric vesicles. The mesonephric vesicles became greatly coiled and constricted so as to give rise to primary, secondary, and tertiary units. The mesonephric vesicles became continuous and convoluted to form the mesonephric tubules. They formed spherical masses around the developing blood capillaries emanating from both the renal artery and renal portal vein. These formed capillary networks which are both arterial and venous and constituted true glomeruli. Each glomerulus is surrounded by the thin-walled Bowman's capsule of the fully formed renal corpuscle within the mesonephros. The pronephric duct from the level of somite VII posteriorly is now known as the Wolffian duct. By the time of complete metamorphosis (stage 35) this mesonephric kidney was fully formed.

A summary of the results of the histochemical experiments of the mesonephros is shown in Table 2. By the stage 25, before definite appearance of the secretory tubules, alkaline phosphatase activity appeared moderately in the undifferentiated

mesonephrogenic cord. After mesonephric tubules were formed the alkaline phosphatase activity of the tubules was gradually increased and showed the highest activity at the second stage of posterior limb formation (stage 27) to the time of complete metamorphosis. As the mesonephric tubules fully differentiated, the alkaline phosphatase activity was found to have disappeared in the mesonephrogenic cord. The weak alkaline phosphatase activity was found in the glomeruli; however, no activity was observed in association with Bowman's capsule, collecting tubule and Wolffian duct throughout the developing and metamorphosis stages.

Table 2. Distribution of alkaline phosphatase in the developing mesonephros

Tissues \ Stages	24	25	26	27	28	29	30	31	32	33	34	35
Secretory tubule		+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Collecting tubule		−	−	−	−	−	−	−	−	−	−	−
Glomerulus		−	+	+	−	+	+	+	+	+	+	+
Bowman's capsule		−	−	−	−	−	−	−	−	−	−	−
Wolffian duct		−	−	−	−	−	−	−	−	−	−	−
Nephrogenic cord	+	++	++	++	++	++	+	+	+	−	−	−

DISCUSSION

In the present study, Gomori's modified technique (Gomori, 1952) was used to gain an insight into the histochemistry of the frog in the developing pronephros and mesonephros. Different techniques have been employed for the microscopic visualization of alkaline phosphatase activity. These are based on direct or indirect precipitation of calcium phosphate which is transformed into cobalt phosphate at pH 9.4. The existence of alkaline phosphatase on the brush border of kidney has been demonstrated previously by histochemical and biochemical methods. Recently, Mizutani and Barrnett (1965) have introduced a method based on the use of calcium as capturing agent and obtained excellent localization in the brush border of kidney tubule. Reale and Lucians (1967) have emphasized the importance of fixation on the intracellular localization of the alkaline phosphatase activity in the mouse proximal convoluted tubule. In a slight modification of Gomori's method, Hugon and Borgers (1966) used lead nitrate instead of cobalt nitrate and obtained better results. In the kidney they were able to observe visible deposits of lead phosphate in the brush borders. By Fishman and Watanabe (1964) the positive enzyme reaction, which is obtained using the calcium-cobalt method with β-glycerophosphate as a substrate, was largely confined to the region of the brush border and visualized as a purple dye deposit. The present study showed the alkaline phosphatase reaction appeared in the brush border of the developing pronephric and mesonephric tubules. However, the enzyme reaction was not observed in the

collecting tubule and Wolffian duct of the frog kidney. These differences in localization of the enzyme in the kidney permit one to suggest that the enzyme plays some definite roles in the tissue metabolism. Danielli (1952) suggested that phosphatase plays some part in molecular transport and metabolism. Novikoff *et al.* (1964) and Sobel (1964) discussed the role of various phosphatases in cellular metabolism and cytomembranes. Alkaline phosphatase activity in the pronephric tubule of the frog appeared at first very weakly by the stage 19. After the hatching stage the enzyme activity on the brush border of the pronephric tubule showed highly positive, but as the pronephros have reached maximum development and then begun to degenerate the enzyme activity decreased and disappeared at last. At about the time of maximum development of the pronephros the mesonephros begun to develop and took over the excretory functions. As mesonephric tubule was formed the alkaline phosphatase activity gradually increased and showed highly positive at the stage 27 through the time of metamorphosis. According to Wachstein and Bradshaw (1964) there is no activity in the neogenic zone of cortex in newborn rat and rabbit. Functional insufficiency of neonatal kidney has been observed by several investigators. The different functional capacities of proximal convoluted tubules of fetal and newborn animals have been illustrated strikingly by Baxter and Yoffey (1948). The maximum rate of excretion in the kidney of newborn is only 25% of that in adult (Barnett and Vesterdal, 1953) and the maximum rate of glucose reabsorption by tubules of neonatal kidney is only 20% of that in adult kidney (Tuvdad, 1949). Baxter and Yoffey (1948) injected trypan blue into newborn rat and found that the dye accumulated in mature tubules of the inner cortex only and not in immature tubules of the neogenic cortical zone. Storage of dye varied with developmental age of tubules, more mature tubules store more dye than those less mature. Alkaline phosphatase activity, in general, reflects the degree of maturity found in the kidney of developing frogs in present study. In pronephros and mesonephros of frog there showed highly positive for alkaline phosphatase in the mature tubules. However, immature tubules and degenerated ones showed little or no enzymatic activity.

Although there exists, in general, good correlation between morphologic and enzymatic maturity and functional adequacy of the growing kidney, many findings remain unexplained. Further studies dealing with the significance of many of observed enzyme activities in specific cellular structures, not only in kidney but in other organs also, are necessary to gain the variety of detailed findings in this field.

SUMMARY

The cobalt capture method of Gomori's modified technique (Gomori, 1952) was employed to study the histochemistry of the developing frog kidney.

Alkaline phosphatase activity was observed in association with the brush borders of pronephros and mesonephros. By the stage of transition from larva to tadpole alkaline phosphatase activity was gradually increased on the brush border of pronephros, and as the pronephros begun to degenerate the enzyme activity was decreased and disappeared.

By the time of maximum development of the pronephros the mesonephros began to develop and alkaline phosphatase activity of the mesonephric tubules showed highly positive throughout the stage of metamorphosis. No activity was observed in association with the collecting tubules and ductal elements.

REFERENCES

Barnett, H.L. and J. Vesterdal. 1953. The physiological and clinical significance of kidney function in young infants. *J. Pediat.* **42** : 99—119.

Baxter, J.J. and J.M. Yoffey, 1948. The postnatal development of renal tubules in the rat. *J. Anat.* **82** : **189—197.**

Danielli, J. F., 1952. Structural factors in cell permeability and secretion. *Symp. Soc. Exp. Biol.* **6** : 1—16.

Desalu, A.B.O., 1966. Correlation of localization of alkaline and acid phosphatases with morphological development of the rat kidney. *Anat. Rec.* **154** : 253—260.

Gomori, G., 1939. Microtechnical demonstration of phosphatase in tissue sections. *Proc. Soc. Exp. Biol. Med.* **42** : 23—26.

___, 1952. Microscopic Histochemistry, Principle and Practice. The University of Chicago Press. Chicago.

Fishman, W.H. and K. Watanabe. 1964. Application of the streospecific inhibitor L-phenylalanine to the enzymorphology of intestinal alkaline phosphatase. *J. Histochem. Cytochem.* **12** : 252—260.

Hah, J.C., 1969. Distribution of alkaline phosphatase, PAS-positive materials and phospholipid in the kidney of the developing chick. *Korean. J. Zool.* **12** : 77—84.

Hugon, J. and M. Borgers, 1966. A direct lead method for the electron microscopic visualization of alkaline phosphatase activity. *J. Histochem. Cytochem.* **14** : 429—431.

Ivemark, B.I., 1959. Histochemical studies on the human fetal kidney(metanephros) glucose-6-phosphatase and 5-nucleotidase. *Acta Pathol. Microbiol. Scand.* **45** : 1—6.

Longley, J.B. and E.R. Fisher, 1955. A histochemical basis for changes in renal tubular function in young mice. *Quart. J. Micr. Sci.* **97** : 187—195.

McCane, R. A. and E. Widdowson, 1960. Aspects of renal function before and after birth. *Biblio. Pediat.* **74** : 137—144.

Mizutani, A. and R.J. Barrnett, 1965. Fine structural demonstration of phosphatase activity at pH 9. *Nature (London)* **206** : 1001.

Moog, F., 1944. Localization of alkaline phosphatase and acid phosphatase in the early embryogenesis of the chick. *Biol. Bull.* **186** : 51—80.

Novikoff, A.B., E. Essner, S.J. Goldfischer and M. Heus, 1964. Nucleotide phosphatase activities of cytomembranes. *Symp. Int'l. Soc. Cell Biol.* **1** : 149—192.

Pinkstaff, C.A., M. Sander and G.H. Bourne, 1962. Phosphatase studies on prenatal, neonatal and adult rat kidney. *J. Geront.* **17** : 267—271.

Reale, E. and L. Lucians. 1967. Effect of fixation on the alkaline phosphatase activity of mouse proximal convoluted tubules. *J. Histochem. Cytochem.* **15** : 413.

Sobel, H.J., 1964. Phosphatase of rat thyroid and anterior pituitary glands during various phases of secretory activity. A cytochemical study. *J. Endocrinol.* **30** : 323—336.

Takamatsu, H., 1939. Histologische und biochemische Studien über die Phosphatase. Histochemische Untersuchungmethodik der Phosphatase und deren Verteilung in Verschiedenen Organen und Geweben. *Trans. Soc. Path. Jap.* **29** : 492—498.

Tuvdad, F., 1949. Sugar reabsorption in premature and full term babies. *Scand. J. Lab. Invest.* **1** : 281—283.

Wachstein, M. and M. Bradshaw, 1964. Histochemical localization of enzyme activity in kidneys of three mammalian species during their postnatal development. *J. Histochem. Cytochem.* **12** : 44—56.

EXPLANATION OF FIGURES

Fig. 1. Pronephric kidney of stage 22 showing positive alkaline phosphatase activity at brush borders of secretory tubules. ×100.

Fig. 2. Pronephric kidney of stage 23 showing positive alkaline phosphatase activity at brush borders of tubules, but no activity in immature tubules. ×200.

Fig. 3. Pronephric kidney of stage 25 showing positive alkaline phosphatase activity at brush borders of tubules. ×100.

Fig. 4. Mesonephric kidney of stage 25 showing moderate alkaline phosphatase activity in mesonephrogenic cord (arrow). ×100.

Fig. 5. Mesonephric kidney of stage 28 showing strong alkline phosphatase activity in secretory tubules, but weak activity in glomerulus (arrow). ×100.

Fig. 6. Mesonephric kidney of stage 35 showing no activity in immature tubules of neogenic cortical zone. ×100.

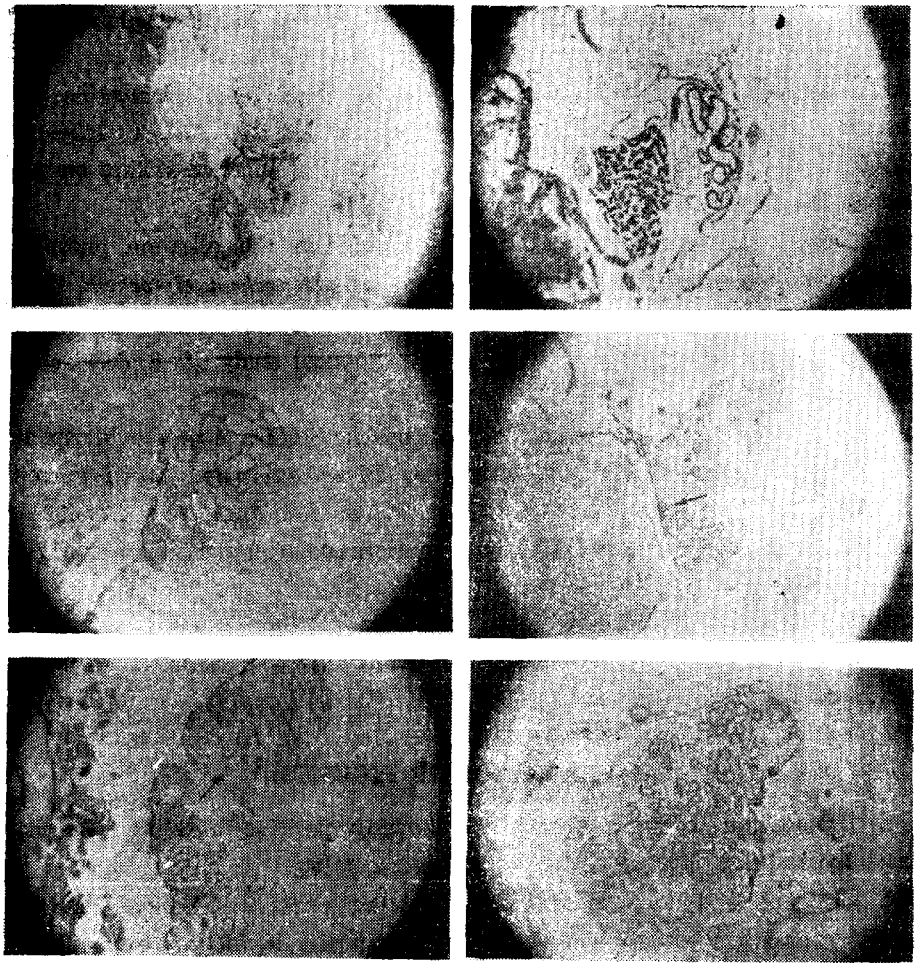

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.