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# Activity of Phosphatases and Cellular Contents in Glomeruli and Tubules Isolated From Growing Rat Renal Cortex

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**Summary.** The activity of acid and alkaline phosphatases was determined in glomeruli and tubules isolated from 14-day old to adult rat renal cortex. Within a nephron fraction, each phosphatase activity produced a specific pattern which was not similar to that in other fractions. In the glomeruli the acid phosphatase activity increased between 14 and 21 days of life and was always higher than in the tubules where it did not significantly change with age. By using tartrate sodium and formaldehyde it was shown that the acid phosphatase isoenzymes are distributed according to a specific pattern which does not change during maturation. The alkaline phosphatase activity in glomeruli and in tubules increases with age but is always lower in the former fraction than in the latter. Furthermore, in young as well as in adult rats, glomeruli were characterized by a higher DNA content and lower RNA and protein/DNA ratios than the corresponding tubular fragments.

**Key words:** Kidney maturation, Glomeruli, Tubules, Acid and alkaline phosphatases, DNA, RNA, Protein.

## Introduction

The localization and the activity of acid and alkaline phosphatases have been studied in healthy and diseased kidneys. Both enzymes are present in the glomeruli and in the tubules [4, 13, 30]. Changes in activity occur during natural as well as experimental renal disorders [11, 19, 20, 25–27, 31]. According to some authors such changes precede morphological damage [11, 31]. Changes in activity levels during kidney development have been observed by staining and biochemical means [6, 7, 10, 18, 28, 32, 33]. However, results obtained from biochemical analysis of either whole kidney or total cortex homogenates essentially reflect the properties of the tubules, because those related to the glomeruli are masked. Therefore no information on age-related changes in glomeruli is available.

The present work was undertaken to examine the evolutionary pattern of the activity of the two phosphatases in rat glomeruli and cortical tubules during a period extending from the 14th day of postnatal life to adulthood. We also determined the DNA, RNA and protein contents in the two nephron compartments throughout the same period.

## Material and Methods

Fourteen to 30-day old rats of both sexes and adult male rats of the Wistar strain were used in this study. The infant rats were weaned at the age of 21 days and maintained on standard feeding and tap water thereafter.

Isolation of adult glomeruli and cortical tubules was performed according to a method developed in our laboratory [12, 23]. This method was slightly modified when using young rats. A collagenasic digestion of the renal cortex at 37 °C in Hanks' modified medium [15] was carried out for three 20 min periods each followed by a filtration step through a stainless-steel sieve of 110 $\mu$ pore size. The glomerulo-tubular suspension was then centrifuged on a step gradient made up with 65, 57 and 30% sucrose layers. The centrifugation was performed at 0 °C and at 5,000 g for 12, 15 or 20 min when 14, 21 or 30-day old rats were used respectively. The glomeruli and tubules were recovered from the surface of the 65 and 57% sucrose layers respectively.

For light microscopy the fractions were fixed with 2% glutaraldehyde in Millonig buffer, postfixed in 1% osmium tetroxide, dehydrated and embedded in epoxy resin (Epon). Slices of 0.5 $\mu$ thickness were cut and stained with methylene blue.

### *Determination of the Activity of the Acid and Alkaline Phosphatases*

The acid phosphatase activity was determined at ph 4.8 [1] and the alkaline phosphatase activity at ph 10.5 [3]. In order to study the distribution of the acid phosphatase isoenzymes we used sodium tartrate and formaldehyde as inhibitors of the enzyme activity [13, 14]. In all cases 15–20 $\mu$g of proteins of either cortex, glomeruli or tubules, previously homogenized in 0.9% NaCl using a Potter Elvehjem device, were incubated in the presence of p-nitrophenyl-phosphate (pNPP). Hydrolysis of the substrate was measured after a 30 min incubation at 37 °C.

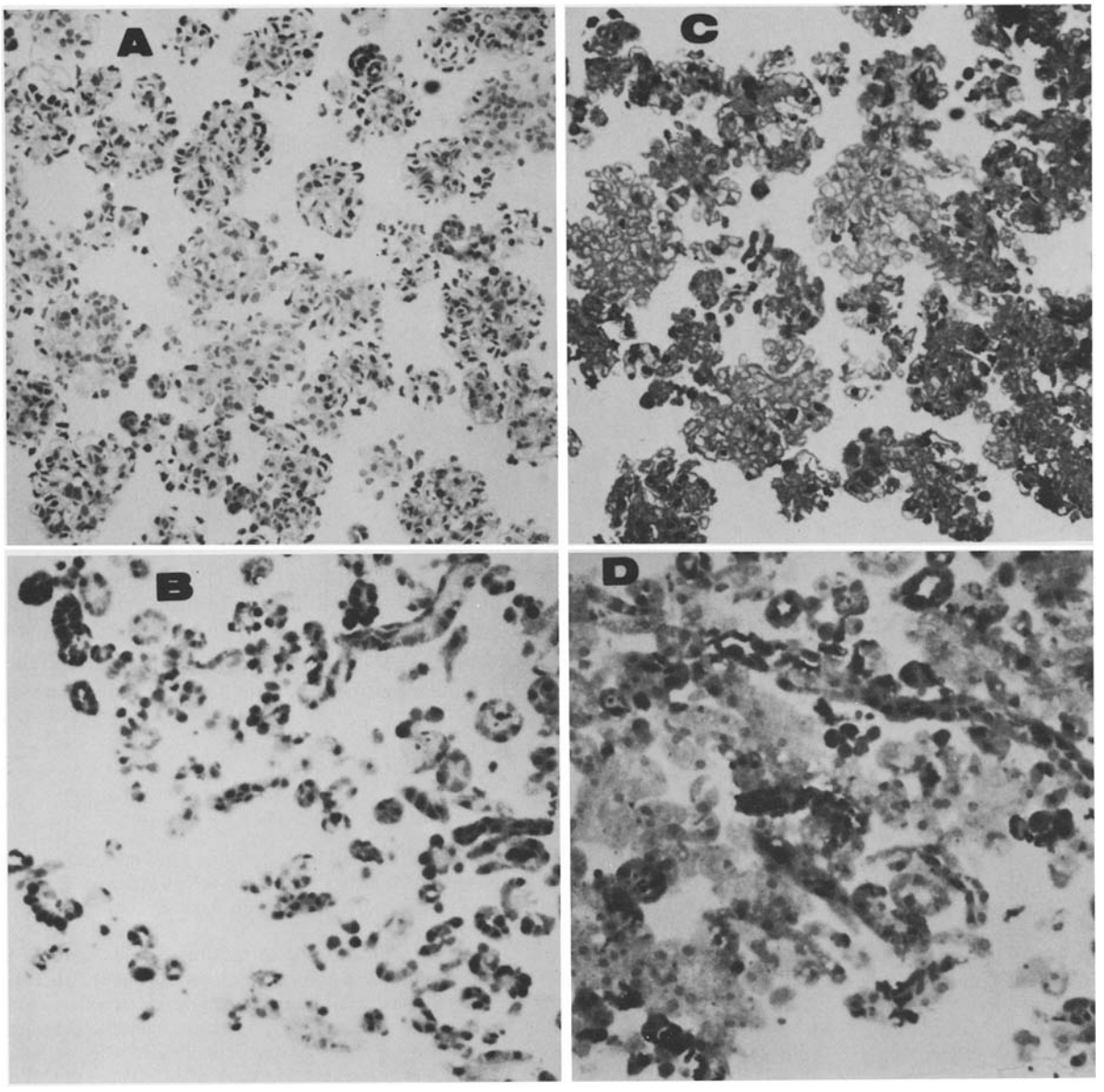


**Fig. 1A–D.** Glomerular and tubular preparations from 14 (**A** and **B**) and 30-day old (**C** and **D**) rat renal cortex. Magnification x250

*Quantitative Determination of DNA, RNA and Protein Contents*

Defatted tissue (DFT) was prepared according to a method previously described for chicken cerebral hemispheres [17]. The acid-soluble material was removed by three extractions with cold 0.6 N $HClO_4$ and the pellet was defatted using boiling ethanol and ether. The DFT was dried overnight at 37 °C, weighed and used for further fractionation of nucleic acids [29]. Samples were hydrolysed for 18 h at 37 °C using 0.3 N KOH and then acidified with 2 N $HClO_4$. After centrifugation the pellet was washed twice with 0.2 N $HClO_4$. The supernatant and the washings were used for the determination of ribose by the orcinol method [24] using cupric instead of ferric ions [16]. Purified RNA was submitted to the same treatment and used as a reference. DNA was extracted from the RNA-free insoluble fraction with 2 N $HClO_4$ for 20 min at 80 °C. More than 90% of the DNA, as determined by colorimetry [5], was recovered in the supernatant after centrifugation. Purified calf thymus DNA treated in the same way as the tissue served as a control.

The protein content of DFT was determined according to Lowry et al. [22], using bovine serum albumine as a standard.

*Statistical Methods*

Analysis of variance was used to compare data of the two groups and give a perspective of the changes with age. The Student's t test was used either with unpaired data to compare the results from different ages within the same fraction or with paired data to compare glomeruli and tubules of the same age. In all cases significance was defined as a $P$ value less than 0.05.

## Results

The typical appearance of glomeruli and cortical tubules from 14 and 30-day old rats as obtained in the present study is shown in Fig. 1.

Table 1. Absence of collagenase effect on the activity of phosphatases in rat kidney cortex

| Rat age (days) | 14 | 21 | 30 | Adult |
|---|---|---|---|---|
| Acid phosphatase | | | | |
| NC | 4.62 | 4.36 | 4.36 | 5.28 |
| DC | 4.69 | 4.55 | 4.33 | 5.67 |
| Alkaline phosphatase | | | | |
| NC | 6.27 | 11.38 | 19.52 | 18.66 |
| DC | 5.81 | 12.68 | 18.10 | 18.56 |

The results shown at each age ($\mu$M pNpp/h/mg protein) are the average of two separate experiments performed each in triplicate. *NC*, untreated cortex; *DC*, collagenase treated cortex

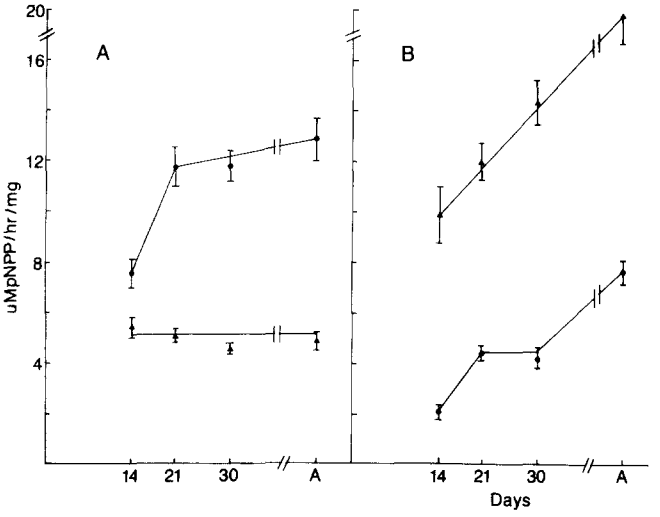


Fig. 2A, B. Age-related phosphatases activity in isolated glomeruli and tubules. For each age the results are mean ± SEM of five experiments performed in duplicate. A Acid phosphatase; B Alkaline phosphatase. ●—● Glomeruli ▲—▲ Tubules

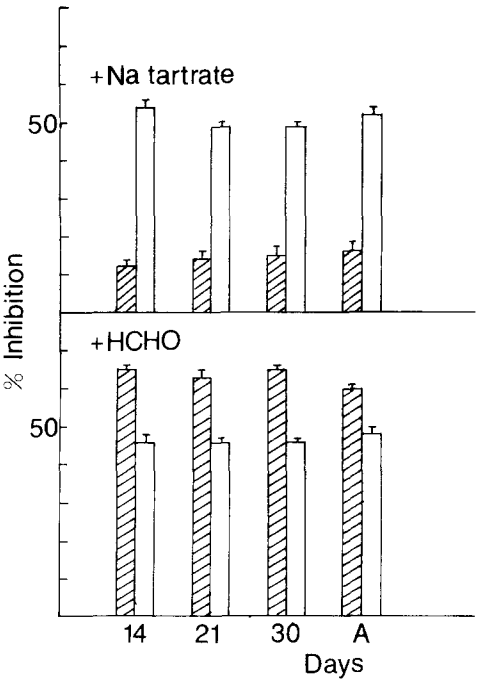


Fig. 3. Relative effect of inhibitors on glomerular and tubular acid phosphatase activity. 20 mM sodium tartrate (Na tartrate) or 1.85% formaldehyde solution (HCHO) is added to the incubation mixture. The percentage of inhibition is calculated, the results are mean ± SEM of five experiments running in duplicate at each age. *Hatched columns*, glomeruli. *White columns*, tubules

### *The Activity of the Acid and Alkaline Phosphatases in Isolated Glomeruli and Cortical Tubules*

Before evaluating the enzyme activities in the nephron compartments it was important to know more of the effect of collagenase on phosphatases and particularly whether this effect was similar in all age groups. Therefore we compared the activity of both phosphatases in cortical homogenates before and after the collagenase digestion. As shown in Table 1, collagenase had practically no effect upon both phosphatases undependant of the age of the rat.

The enzymes were present in both fractions at all ages tested although large differences in the absolute values and evolutionary pattern were found between glomeruli and tubules (Fig. 2). Even on day 14, the acid phosphatase activity was higher in glomeruli than in tubules, differences being more pronounced in the oldest rats (Fig. 2A). In the glomeruli a 55% increase occured between 14 and 21 days but there were no further significant changes later on. In the tubules the activity was constant, slight variations were insignificant. The alkaline phosphatase activity followed a different pattern. As shown in Fig. 2B the glomerular activity was lower than the tubular activity whatever the age. Indeed there was more than a twofold increase between 14 and 21-old glomeruli, followed by a constant value up to 30 days and a further 75% increase between 30 days and adulthood. In contrast the tubular activity constantly increased and reached the maximum value in the adult.

The effect on enzyme activity of two acid phosphatase inhibitors was examined in both fractions (Fig. 3). None of the inhibitors was specific for either fraction but their efficiency was different. The tubular enzyme was more sensitive to sodium tartrate than the glomerular enzyme. On average a 50% inhibition with no significant changes with age occured in the tubules. Inhibition did not reach 20% in the glomeruli and was not age dependant. The results were reversed when formaldehyde was used as an inhibitor. Throughout, the tubular enzyme activity was less effected than the glomerular activity. The sensitivity did not significantly change with age in either fraction.

### *Age-Related Changes in the Cellular Contents*

Whatever the age of the rat the DNA content was higher in the glomeruli than in the tubules although it decreased in both fractions (Table 2, Part A). In order to estimate the

Table 2. Comparison of DNA content and RNA and protein/DNA ratios in isolated glomeruli (*G*) and cortical tubules (*T*)

| Rat age (days) | | 14 (5) | 21 (5) | 30 (5) | Adult (4) | *P* |
|---|---|---|---|---|---|---|
| A) Results per 100 mg of defatted tissue | | | | | | |
| DNA mg | G | 3.66 ± 0.19 | 2.51 ± 0.20 | 2.48 ± 0.10 | 1.98 ± 0.05 | <0.01 |
| | T | 1.93 ± 0.09 | 1.70 ± 0.26 | 1.01 ± 0.10 | 0.81 ± 0.03 | <0.001 |
| B) Results per mg DNA | | | | | | |
| RNA | G | 1.14 ± 0.08 | 1.28 ± 0.11 | 1.20 ± 0.09 | 1.22 ± 0.06 | NS |
| | T | 2.09 ± 0.12 | 2.12 ± 0.22 | 2.97 ± 0.28 | 2.91 ± 0.13 | <0.01 |
| Protein | G | 5.41 ± 0.44 | 6.12 ± 0.42 | 6.05 ± 0.51 | 10.54 ± 0.42 | <0.01 |
| | T | 13.34 ± 0.97 | 17.45 ± 1.37 | 28.66 ± 1.56 | 40.64 ± 1.92 | <0.001 |

The results are mean ± SEM of the number of experiments indicated in parentheses
*NS*, non significant

average composition per cell, we expressed the glomerular and tubular RNA and protein content per mg DNA (Table 2, Part B). The RNA/DNA ratio did not change with age in the glomeruli and was always lower than in the corresponding tubules where a significant 40% increase occured between 21 and 30 days. The glomerular protein/DNA ratio changed after the age of 30 days whereas the ratio constantly increased in the tubules from the 14th day after birth to adulthood.

## Discussion

In the recent literature, there are relatively little biochemical data on glomeruli during kidney maturation whereas several reports exist on adult glomeruli. It has been shown, however, that glomeruli and tubules grow at a different rate in human kidney [9]. Although the time required for completion of nephrogenesis in rat is controversial, it has been reported that fully differentiated glomeruli are present two weeks after birth [8], while the development of the renal tubules continues up to 28 days post-partum [2]. It appears therefore that a biochemical study on kidney maturation requires that the experiments must be performed, as soon as possible and simultaneously, on isolated glomeruli and tubules.

We made several unsuccessful attempts to fractionate the nephron in rats younger than 14 days, However the results reported here show that age-related changes in glomeruli are not similar to those in the corresponding tubular fragments. In addition the evolutionary pattern of the activity of the acid and alkaline phosphatases in tubules reflects the activity in the whole cortex (Table 1 and Fig. 2) in which our results can be compared with previous reports [32, 33]. These findings indicate that, at least throughout the period of maturation studied, it was impossible to draw any conclusion about glomeruli when the experiments were performed using whole renal cortex. The present study brings some biochemical indices at the glomerular level during the period extending from the 14th day after birth to adulthood.

Although acid and alkaline phosphatases both catalyse the hydrolisis of phosphoesters, it is of interest to note that their activity did not produce the same pattern during kidney growth in glomeruli and tubules (Fig. 2). This may suggest that these enzymes are different gene products and/or their activity is regulated by different molecular mechanisms probably related to their specific role in renal function. This suggestion is supported by a report on the decrease of the activity of the alkaline phosphatase and the increase of the acid phosphatase activity in different areas of pathological and damaged kidneys [20].

The activity of the phosphatases and the distribution of the acid phosphatase isoenzymes in glomeruli and tubules isolated from infant rats and those from adulthood are closely related. Indeed at every age of the glomeruli the acid phosphatase activity is higher and the alkaline phosphatase activity lower than in the corresponding tubules (Fig. 2). These data are in agreement with reports on adult kidneys from our laboratory [13, 14] and from others [4, 21] with one exception [30]. The absence of significant changes with age in the specific distribution of acid phosphatase isoenzymes (Fig. 3) suggests, in this case, that maturation to adulthood involves quantitative rather than qualitative changes.

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