QUANTITATIVE HISTOCHEMISTRY OF THE NEPHRON. II. ALKALINE PHOSPHATASE ACTIVITY IN MAN AND OTHER SPECIES *

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The classical experiments of Richards and his collaborators (1), which were started in 1920, laid the foundations for our understanding of renal function in terms of nephron anatomy. By direct puncture of renal tubules they demonstrated accurately the sites at which a variety of substances is absorbed or secreted by the nephron. This technique, which is continuing to provide useful knowledge in the hands of investigators such as Gottschalk and Mylle (2), and Wirz (3), cannot provide exact data on the enzymatic and other cellular mechanisms involved in the processes of tubular reabsorption and secretion. Detailed knowledge of the activities of specific enzymes in the individual anatomical and functional units of the nephron is necessary in order to understand these mechanisms, i.e., a knowledge of the "enzyme topography of the nephron."

Although conventional biochemical techniques using kidney slices or homogenates provide data on the enzyme activity of the kidney as a whole, they do not yield information of specific physiological importance because the individual functional units of the nephron cannot be studied by these techniques. Histochemical staining techniques are useful for purposes of orientation but do not yield quantitative results. In addition, when staining techniques are used to study enzyme distribution, the possibility always exists that artifacts might be caused by diffusion and absorption.

For these reasons the quantitative ultra-microchemical techniques of dissection, weighing, and assay of microscopically small fragments of tissue developed by Lowry and co-workers (4–6) for the brain, were adapted for study of the kidney (7, 8). In the present paper, the alkaline phosphatase distribution in the nephron of adult man, monkey, dog, rat, rabbit, frog, and toadfish is reported.

METHODS

The methods employed will be described only insofar as they differ from those developed by Lowry and associates (4–6). Slices of renal tissue from healthy animals were obtained as rapidly as possible after death. Human renal tissue was obtained by percutaneous renal biopsy (9) in four cases, and at autopsy in two (within 3 hours of death). The human tissue was obtained from persons with no clinical or histological evidence of renal disease.

Preparation of tissue for dissection. Immediately upon removal from the body the tissue was placed on a thin layer of a 5.0 per cent (weight/volume) tragacanth gel in 0.85 per cent NaCl [500 mg Mysteclin (Squibb) added per 500 ml to prevent fungal growth], which covered the top of a microtome sample holder (International Equipment Co., no. 3010). It was then immersed in liquid nitrogen and frozen rapidly.

Serial sections, 16 μ thick, were cut on a Minot rotary microtome (International Equipment Co., no. 3007 with safety razor holder no. 3017) in a cryostat (Harris Refrigeration Company, Cambridge, Mass.) at $-20°$ C. The first and third sections of every three cut were placed in a numbered well of a section holder (Acme Model Works, Chicago, Ill). The section holder containing the slices of tissue was inserted into a lyophilizing tube, and the sections were frozen-dried overnight in the cryostat at $-20°$ C. The evacuated tube was stored in a deep-freeze at $-35°$ C. The sections for enzyme assay were removed from the evacuated tube in the cryostat after temperature equilibration to $-20°$ C. They were placed in a second tube containing Drierite and brought to room temperature.

The middle section of every group of three cut was placed on a clean microscope slide, taken out of the cryostat, and allowed to thaw by warming the underside of the slide with the finger. The section was dried for 15 minutes in room air, immediately fixed for 5 minutes in 80 per cent ethyl alcohol, and stained by the periodic

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acid-Schiff (PAS) technique (5 minutes in 1 per cent HIO_4 in H_2O; rinsed in distilled H_2O; 10 minutes in 1 per cent fuchsin-1.9 per cent $Na_2S_2O_5$ in 0.5 N HCl; rinsed twice in 0.6 per cent $NaHSO_3$ in 0.05 N HCl; finally rinsed in H_2O). It was then counterstained with hematoxylin (5 seconds in hematoxylin-Harris, containing 4 volumes per cent CH_3COOH), rinsed for 30 seconds in tap water, passed through ethanol and xylene, and mounted in Permount.

Dissection of the frozen-dried tissue sections. The dissecting, weighing and insertion of tissue fragments into the substrate were all done in an air-conditioned laboratory maintained at a temperature of 23 ± 2° C and a relative humidity of less than 40 per cent, in order to prevent condensation of moisture on the specimens.

The stained section was studied under a Galileo LGT/2 microscope with projection viewer (Sanders Laboratories, E. Rutherford, N. J.) and a map was drawn of the whole section, glomeruli and blood vessels serving as landmarks. The various anatomical parts of the kidney were identified (see below). When this was done, one of the two adjacent frozen-dried sections was placed on the stage of an A. O. Spencer Cycloptic dissecting microscope (type 53M-D2) and orientated with respect to the image of the stained section seen on the projection viewer. Each of the anatomical units of the kidney which had been identified in the stained section was then located in the adjacent lyophilized section, and each unit was dissected out freehand with microscalpels. From 5 to 10 specimens of each specific unit of the nephron were dissected out in this manner and their location in the section was recorded on the map.

TABLE I

Properties of alkaline phosphatase in the human kidney

Michaelis-Menten constant	0.35 mM
Optimal substrate concentration	5.0 mM
Optimal pH	10.0
Activity vs incubation time (37° C)	Linear 0 to 60 minutes
Activity vs enzyme concentration	Linear between 0.0 and 0.4 m moles/hour/L of incubation mixture
$V_{2°\,C}/V_{37°\,C}$*	0.17
Effect of 2 mM Mg^{++}	23% Increase at 37° C
Effect of 10 mM CN^-	99.4% Inhibition at 37° C

* V = velocity of the enzyme reaction at the temperatures given.

Weighing of tissue fragments. Each of the dissected specimens was transferred from the dissection microscope to a specimen holder by means of a hair point (4). The specimen holder was a chemically clean 10 × 25 mm glass

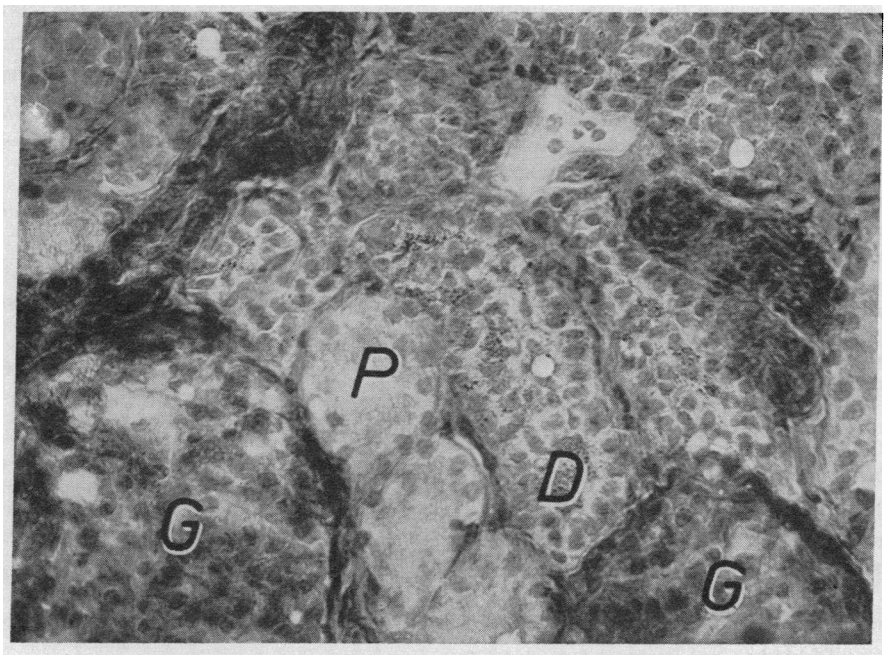

FIG. 1. SIXTEEN μ FROZEN SECTION OF RENAL CORTEX STAINED BY THE PERIODIC ACID–SCHIFF (*PAS*) TECHNIQUE AND COUNTERSTAINED WITH HEMATOXYLIN. Portions of two glomeruli are seen below (G). The proximal convoluted tubules (P) are broad and contain comparatively few nuclei in cross section. The distal convoluted tubules (D) are narrower, and many more nuclei are seen on cross section. Note the granules stained with PAS in the lumen.

slide, mounted on one end of a brass block 65 mm long × 12 mm wide × 10 mm high. Before weighing, the specimen holder, containing the fragments of tissue, was mounted on a manipulator, which was made from the mechanical stage of a microscope. The manipulator was used to load and unload the balance pan, and was placed on the left side of a horizontal stereoscopic microscope (A. O. Spencer, type 27K) which was used to read the displacement of the pan. This microscope was fitted with a vertical fine adjustment to facilitate the setting of the zero point.

The specimens were then weighed on a quartz fiber "fishpole" balance (4), with a sensitivity and reproducibility of a 0.4 mμg and a useful range of 10 to 100 mμg. The balance was calibrated with quinine hydrobromide crystals and the displacement of the quartz fiber was found to be related linearly to the sample weight over the entire useful range (10).

Enzyme assay technique. Each weighed specimen was inserted into 3 μl of substrate reagent contained in a microtest tube (ID 3 mm). This was done under the dissection microscope using a fine glass needle. Assays to determine alkaline phosphatase activity were then made using 3 μl of substrate reagent containing 0.5 M 2-amino-2-methylpropanol-1 (pH 10.0), 8 mM *p*-nitrophenylphosphate (Sigma), 2 mM $MgCl_2$, and 0.05 per cent bovine serum albumin (Armour). The conditions used for the assay were those we have found to be optimal for the human kidney (Table I). Omission of the 2 mM Mg^{++} from the assay medium decreased the enzyme activity by 19 per cent. Addition of 10 mM CN^- to the assay medium inhibited activity by 99.4 per cent.

After incubation for one hour at 37° C, the reaction was stopped and color was developed by adding 50 μl of 0.1 N NaOH. The optical density of the solution was read in Lowry-Bessey microcuvets at 410 mμ in the Beckman DU spectrophotometer. In each experiment suitable reagent blanks and standards were included. Tissue blanks were included in the initial experiments, but they were omitted subsequently after it had been found that the tissue, in the amounts used, did not increase the resulting optical density. The results were expressed in moles of substrate split per kilogram dry weight per hour (MKH units).

RESULTS

A. Morphology (Figure 1). To ensure that the individual anatomical and functional units of the nephron were dissected out accurately prior to chemical analysis, a detailed study of the morphology of the nephron was made both in stained and in unstained frozen-dried sections. As a result of these morphologic studies we were able to dissect out separately glomeruli, single proximal convolutions or single distal convolutions of the tubules, medullary rays, tubules from the outer and inner medullary zones, and collecting tubules from the base and apex of the papilla. In a few instances pars recta of proximal tubules, descending and ascending loops of Henle, and collecting tubules were separated out from the medullary rays and from the outer and inner zone of the medulla.[1] The various structures were identified and dissected out as follows.

The *glomeruli* were easily identified and the glomerular tufts dissected out free of Bowman's capsule. In the rabbit they were small and fragile. In the frog they occurred in clusters and appeared very light gray in the dry section where they were identified more easily than in the stained section.

The *proximal convolutions of the tubules* were broad, contained few nuclei in cross section and were composed of large cuboidal cells, which stained lightly with PAS (Figure 1). A distinct "brush border" was seen in the stained sections. In the dry sections they appeared gray and had no distinct lumen except in the rat, where the lumen was clearly visible. They formed the majority of the cortical tubules.

The *distal convolutions of the tubules* were usually narrower than the proximal, and contained many more nuclei on cross section (Figure 1). Some were identified by their intimate relationship to the macula densa. The cells were flat and the cytoplasm stained very lightly with PAS. PAS-stained granules of a uniform size were seen in the lumen in all species studied except the dog. In the dry sections the distal convolutions usually appeared darker than the proximal tubules, and a distinct lumen was visible.

The *medullary rays,* in all species but the frog and the toadfish, were composed of the straight

[1] The terminology used in this paper to describe the various zones of the medulla compares as follows with that of Oliver and associates (11):

Present authors		Oliver and associates (11)	
Medulla	Outer medullary zone	Outer stripe	Outer zone → Medulla
	Inner medullary zone	Inner stripe	
	Base of papilla	Inner zone	→ Medulla
	Apex of papilla		

part of the proximal tubules (pars recta), descending and ascending loops of Henle, and collecting tubules. In the stained sections the morphology of the pars recta was similar to that of the convoluted portion of the proximal tubules. The ascending limbs of the loops of Henle resembled the distal convoluted tubules. The collecting tubules were broad and contained many nuclei which appeared in two layers. Their cells were columnar, stained lightly with PAS, and the lumen contained many granules stained with PAS. The descending limbs of the loops of Henle were small round tubules with flat cuboidal cells.

In the unstained lyophilized sections the medullary ray contained two types of tubules, gray and dark tubules. The dark tubules were identified as the ascending limb of the loop of Henle. Two types of gray tubules were distinguished in the rabbit—one a dark gray, the other a light gray. It was difficult to identify them with certainty.

The *outer medullary zone* [outer stripe of outer medullary zone of Oliver and colleagues (11)], was composed mainly of pars recta, loop of Henle, and collecting tubules. The brush border of the pars recta of the rat stained more densely with PAS than did the convoluted portions of the proximal tubules.

The *inner medullary zone* (inner stripe of the outer medullary zone of Oliver and associates), contained collecting tubules and loops of Henle. The tubules were straight and converging toward the papilla. The loop of Henle appeared to curve around at the base of the papilla and return toward the outer medullary zone. In the dry section the collecting tubules were light and broad, whereas the loops of Henle were dark and narrow.

The *papilla* (inner medullary zone of Oliver and associates) was divided into a base and an apex, both consisting of collecting tubules, which were broad and composed of tall cuboidal cells which contained many nuclei. Many granules stained with PAS were seen in their lumina. In the dry section the papilla appeared uniformly light gray from base to apex. In the base there was a preponderance of longitudinal sections, while in the apex there was a considerable number of cross sections of tubules.

Convoluted tubules and collecting ducts were the only tubular structures recognizable in the kidney of the aglomerular toadfish, and there were large amounts of lymphoid tissue between the tubules. Proximal and distal convolutions could not be distinguished one from the other.

B. Alkaline phosphatase activity. The levels of alkaline phosphatase activity in the individual units of the nephron of healthy man, monkey (*Macacca mulatta*), dog (mongrel), rat (Sprague-Dawley), rabbit (New Zealand white), frog (*Rana pipiens*), and aglomerular toadfish (*Opsanus tau*) are given in Table II. This table also gives the number of individuals or animals studied and the number of fragments analyzed, together with the mean values obtained and the standard error of the means. The quantitative distributions are shown diagramatically in Figure 2. It should be noted that the alkaline phosphatase activity in the kidney of those species studied (man and dog) was inhibited completely by cyanide, whereas in human serum, liver and bone, appreciable amounts of noncyanide-sensitive alkaline phosphatase activity were found.

There were striking differences in the distribution and activity of alkaline phosphatase from one species to another. The highest levels of activity were found in the rat. The levels of activity were low in the nephrons of man and monkey compared with those in the other species.

The alkaline phosphatase activity of the glomeruli was particularly low in man and monkey; slightly higher levels were found in the dog and rabbit, whereas considerable activity was found in the rat glomeruli. Particularly high levels of activity were observed in the glomeruli of *Rana pipiens,* in which species there was higher activity in the glomeruli than in any other part of the nephron. This observation is similar to that reported by Pillai and Iyengar, who found, by histochemical staining techniques, that alkaline phosphatase activity was higher in the glomeruli than in the proximal tubules of *Rana hexadactylia* (12).

The alkaline phosphatase activity of proximal convolutions was significantly greater than that of distal convolutions in all species studied (Table II). Although this confirms in general the results obtained by histochemical staining techniques, the differences observed were less striking. For example, alkaline phosphatase activity is not usually detected in distal convolutions by histochemical staining techniques, whereas the ultra-microchemical technique revealed low but definite activity in this part of the nephron. Significant activity was

TABLE II

Alkaline phosphatase activity in the anatomical units of nephrons from healthy kidneys in man and in various animal species (expressed in moles of substrate split per kilogram dry weight of tissue per hour at 37° C) *

Structure analyzed	Man 6‡	Monkey 2‡	Dog 3‡	Rat 2‡	Rabbit 2‡	Frog 2‡	Toadfish† 5‡
Cortex:							
Glomeruli	0.56±0.072 [36]	0.60±0.081 [18]	1.90±0.15 [19]	9.70±0.88 [10]	1.03±0.25 [11]	29.8 ±3.80 [13]	Absent
Proximal convolutions	5.48±0.28 [40]	2.32±0.21 [24]	10.3 ±1.16 [27]	30.9 ±1.59 [21]	10.4 ±1.41 [13]	14.4 ±1.39 [16]	4.92±0.295 [37]
Distal convolutions	2.67±0.20 [41]	1.10±0.34 [3]	0.85±0.27 [17]	7.43±1.60 [9]	1.46±0.30 [9]	10.3 ±1.44 [12]	
Medullary ray							
(Superficial cortex)	2.55±0.15 [5]	4.88±0.43 [27]	0.66±0.13 [21]	39.7 ±2.88 [16]	5.85±0.81 [3]	Absent	Absent
(Deep cortex)	3.08±0.53 [5]	1.44±0.39 [11]			12.5 ±1.68 [10]		
Medulla:							
Outer medullary zone	0.81±0.13 [11]	0.36±0.13 [16]	2.25±0.65 [29]	50.1 ±4.50 [9]	13.8 ±1.32§ [9] 1.40±0.40‖ [12]	6.40±1.53 [10]	Absent
Inner medullary zone	1.27±0.11 [8]	0.25±0.11 [6]	0.70±0.18 [18]	3.65±0.68¶ [10] 3.20±0.25** [10]	0.10±0.037 [9]	8.70±1.73 [10]	Absent
Base of papilla	1.15±0.48 [6]	0.72±0.27 [5]	0.63±0.27 [3]	3.50±0.27 [9]	0.11±0.038 [10]	Absent	Absent
Apex of papilla	1.61±0.49 [9]	0.27±0.078 [11]	0.38±0.11 [6]	3.88±0.40 [10]	0.15±0.027 [5]	Absent	Absent
Artery	1.12±0.19 [25]	0.51±0.097 [19]	0.76±0.20 [12]	15.3 ±0.69 [13]	2.72±0.85 [5]	3.40±0.60 [16]	0.25±0.042 [7]

* Results are expressed as mean values with the standard error of the means. The total number of specimens of each structure analyzed is given in brackets.

† Toadfish, convoluted tubules only; lymphoid tissue = 30.9 ± 1.28, [19]; Wolff canal = 3.23 ± 0.15 MKH units, [27].

‡ Total number of kidneys analyzed.

§ Light tubules: descending limb of Henle's loop, collecting ducts and pars recta of proximal tubule.

‖ Dark tubules: ascending limb of Henle's loop.

¶ Dark tubules: Henle's loop.

** Light tubules: collecting ducts.

also found in the convoluted tubules of the aglomerular toadfish. Wilmer was unable to demonstrate any stainable alkaline phosphatase except in the interstitial lymphoid tissue of this species (13), but later work by Longley has demonstrated stainable alkaline phosphatase in the convoluted tubules of *Opsanus tau* (14).

Particularly high levels of activity were found in the glomerulus, in the proximal convolutions, the medullary ray, and outer medullary zone of the rat. The activity of the vessels in the rat was also high, and this was not due to contamination from blood within the vessels.

In the monkey higher levels of alkaline phosphatase were found in the medullary ray near the capsule than in the deeper regions of the cortex. The reverse was true in the rabbit, but in other species no such differences were observed.

In the rat we were able to dissect the loop of Henle from the collecting tubules in the inner medullary zone (dark bar and light bar, respectively, in Figure 2). No significant difference was found in their alkaline phosphatase activity.

In the outer medullary zone of the rabbit kidney, the ascending loop of Henle (dark bar, Figure 2) was dissected from the descending loop of Henle and from the collecting tubules (light bar, Figure 2). In this species the mixture of collecting tu-

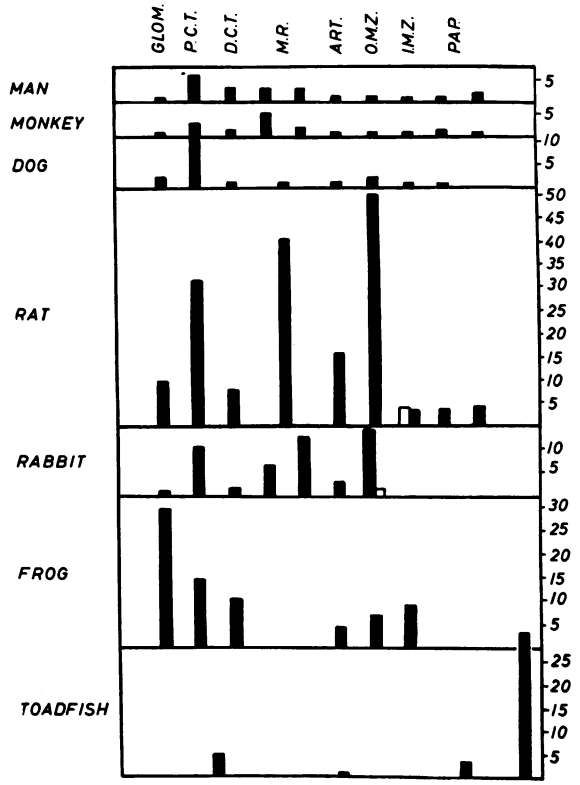

FIG. 2. THE QUANTITATIVE DISTRIBUTION OF ALKALINE PHOSPHATASE ACTIVITY IN THE NEPHRON. The bars represent mean alkaline phosphatase activities expressed in moles of substrate split per kilogram dry weight of tissue per hour at 37° C. Note that the same scale is used for all animal species studied. (See text and Table II for details.)

bules and descending limb of Henle's loop was found to have much greater alkaline phosphatase activity than the ascending limb of Henle's loop.

DISCUSSION

Techniques. When Lowry (4) developed his microtechniques for the study of brain cells, he first froze and then mounted the tissue specimens in two separate operations. In the case of the small fragments of tissue obtained by needle biopsy, it was not possible to mount the frozen specimen on a microtome sample holder without allowing the specimen to warm up appreciably. After trying several techniques unsuccessfully, we found that the two operations of freezing and mounting could be combined successfully into one. The tissue was placed directly onto a sample holder covered with a layer of 5 per cent tragacanth gel and the sample holder with the tissue already mounted was then immediately immersed in liquid nitrogen. Using this technique there was no visible evidence of ice crystal formation in biopsy material because the diameter of the sample was so small that rapid freezing could take place. When surgical biopsies and postmortem material were used, it was necessary to use slices of tissue less than 2 mm thick, to avoid harmful ice crystal formation.

Initially we employed sections cut at 30 μ, but these were too thick to allow satisfactory recognition of individual tubules. Sections cut at 6 to 10 μ were difficult to handle and provided too small a piece of tissue for analysis. These disadvantages did not occur with sections cut at 16 μ.

Most workers freeze-dry specimens at or below $-40°$ C (15, 16), because a uniform quality of preservation of tissue is thereby obtained. We found that kidney tissue could be frozen-dried at $-20°$ C without noticeable shrinking of the tissue. This had the advantage of allowing the freeze-drying process to be carried out in the cryostat in which the tissue was sectioned. Moreover, the drying process took place more rapidly at $-20°$ C, being complete in 4 to 5 hours.

Identification of individual parts of the nephron, particularly the proximal and distal convolutions, proved to be difficult in unstained frozen-dried tissue. McCann approached this problem by injecting trypan blue *in vivo,* and dissecting out separately the areas of proximal convolutions stained with trypan blue and unstained distal convolutions (8). As it was necessary to develop a technique applicable to human tissue, and as the dosage of trypan blue needed to outline the proximal convolutions would have resulted in a blue patient, McCann's technique was unsuitable for our needs. Moreover, renal biopsies in man could not necessarily be obtained at the time when there was optimal concentration of trypan blue in the proximal convolutions. For these reason we tried fluorescence, phase contrast, and polarization microscopy of the frozen-dried sections. With all these techniques no clear-cut differences were observed between proximal and distal convolutions at the low magnifications necessary for dissection. Thus far the only technique which allowed us to distinguish clearly between proximal and distal convolutions was the alternate section technique we used in this study. In addition, this technique allowed us to recognize and dissect separately

tubules in pathological human material, which showed various degrees of histological changes (17, 18).

It has been shown previously by conventional histochemical staining techniques in many species (19, 20) and with the trypan blue technique used by McCann in dogs (8) that the alkaline phosphatase activity of proximal convolutions was greater than that of distal convolutions. Our results confirm these findings since they demonstrate a consistently and significantly higher alkaline phosphatase activity in proximal than in distal convolutions. This suggests that our technique for identification and dissection of the individual parts of the nephron was accurate.

The validity and reproducibility of the quantitative histochemical technique used in these experiments has been demonstrated by Lowry and associates (6, 21). The sample size used in our studies was of the order of 15 to 100 mμg dry weight, which is slightly larger than that used by Lowry, Roberts and Chang (21) in their study of single brain cells (8 to 25 mμg). The average relative standard error of the mean for three replicate analyses of each of eight homogenates of human kidney was 1.49 per cent; for lactic dehydrogenase activity, the average relative standard error of the mean for four replicate analyses of each of eight homogenates was 2.31 per cent. These values are comparable to the average relative standard error of the means (10.5 per cent) for three different enzymes studied by Lowry and co-workers. In our experiments on dissected portions of the nephron the average relative standard error of the means for alkaline phosphatase was 15.4 per cent (Table III). For lactic dehydrogenase (22), the average relative standard error of the means was considerably smaller (8.3 per cent) (Table III). This difference between the standard errors of the means for alkaline phosphatase and lactic dehydrogenase can be accounted for partly by the fact that the readings for alkaline phosphatase were comparatively close to the blank values, whereas those for lactic dehydrogenase were well above the blank values. In this respect, it is significant that the average relative standard error of the means for alkaline phosphatase was only 8.8 per cent in the proximal convolutions, which had the highest activity. An alternate explanation is that the variation of enzyme activity from nephron to nephron is greater for alkaline phosphatase than for lactic dehydrogenase.

TABLE III

Average relative standard errors of the means for alkaline phosphatase and lactic dehydrogenase activities (all species combined) *

Structure analyzed	Alkaline phosphatase	Lactic dehydrogenase
	%	%
Glomerulus	13.5	6.9
Proximal convolutions	8.8	6.3
Distal convolutions	19.2	6.2
Medullary ray	14.4	12.3
Outer medullary zone	17.4	9.9
Inner medullary zone	13.8	8.5
Papilla	28.4	8.0
Over-all average	15.4	8.3

* Values for the arteries are not included in this table because the arterial tissue examined was not homogenous and consisted of arteries of different dimensions, varying from interlobular arteries to terminal branches.

Many authors, using histochemical staining techniques, have found no evidence of stainable alkaline phosphatase activity in the distal convolutions of many species (23). Our data indicate that there is a small but definite alkaline phosphatase activity in the distal convolution. The difference in enzyme activity demonstrated by these two techniques could be the result of inaccuracies in the dissection technique, resulting in intermixing of proximal and distal convolutions. However, this is unlikely for the following reasons: *1*) Significant alkaline phosphatase activity was found in the glomeruli, loops of Henle, collecting tubules and vessels—parts of the nephron in which no stainable alkaline phosphatase activity has been found (24, 25). In dissecting the glomeruli, papillary collecting tubules and vessels, there was virtually no opportunity for obtaining an admixture of structures with high activity. *2*) Our data are comparable to those obtained by McCann for the dog (8)—the only other quantitative study of alkaline phosphatase distribution in the nephron. McCann found that the ratio of activity in the distal to that of the proximal convolutions in the dog was 0.19. He considered that his samples of proximal convolutions contained about 10 per cent of distal convolutions and vice versa. When the ratio of activity is corrected on the basis of complete homogeneity of samples, a ratio of 0.081 is obtained, which is

virtually identical with the ratio of 0.083 found in our experiments.

The inherent limitations of the histochemical staining technique offer an alternative and more likely explanation for the discrepancies observed between the staining technique and the quantitative method. Visualization of stainable alkaline phosphatase depends on the product of activity and incubation time exceeding an indefinable threshold value for recognition by light microscopy. If the incubation time is so chosen as to obtain suitable staining of structures with high activity, such as the proximal convolutions, the threshold value may not be reached in structures with lower activity, such as the distal convolutions. The fact that measurable alkaline phosphatase activity was found in all parts of the nephron, including those in which stainable alkaline phosphatase has not been demonstrated, suggests that the differences between the two techniques are due to the inherent limitations of the histochemical staining technique. This view gains further support from a comparative study of the alkaline phosphatase distribution in the developing hamster tooth (*26*). When the alkaline phosphatase activity was measured quantitatively by the ultra-microtechniques used in this paper, the range of activity found in the various structures taken from the same dried section was from 0.85 ± 0.20 to 298 ± 26 moles of *p*-nitrophenylphosphate split per kg dry weight per hour (MKH units) at 37° C. If the adjacent section was stained for alkaline phosphatase by the Gomori technique under conditions optimal for structures having an activity of 298 MKH units, structures with an activity of 140 MKH units exhibited unequivocal staining for alkaline phosphatase, but an adjacent structure with an activity of 85 MKH units showed minimal stainable alkaline phosphatase even when the tissue was incubated for periods varying from 1 to 10 minutes. Structures with activities of 21 MKH units or less always gave a negative reaction under these conditions.

These experiments demonstrate that the quantitative measurement of enzyme activity provides accurate data on a wide range of activities in all structures of tissue, provided they can be dissected out accurately. By contrast, the standard histochemical staining techniques demonstrate qualitatively a comparatively narrow range of activities, and may give negative results for structures with appreciable levels of enzyme activity.

SUMMARY

1. A quantitative technique for enzyme assay of individual functional units of the nephron is described. This technique depends on: *a*) exact identification of individual parts of the nephron, using stained frozen sections; *b*) microdissection of the individual parts of the nephron in adjacent unstained frozen-dried sections; *c*) weighing and subsequent enzyme assay.

2. The optimal assay conditions for the alkaline phosphatase activity of the human kidney were determined. It was found that the enzyme was of the magnesium-activated, cyanide-inhibited type.

3. The morphology of frozen-dried unstained kidney sections of man, monkey, dog, rat, rabbit, frog and toadfish is described.

4. The quantitative distribution of alkaline phosphatase activity in the nephron of these species is reported. In general, alkaline phosphatase activity was found in all parts of the nephron analyzed, although there was considerable species variation. The highest activities were found consistently in the proximal convolutions, and the activities in the proximal convolutions were consistently significantly higher than those in the distal convolutions.

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