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QUANTITATIVE HISTOCHEMISTRY OF THE NEPHRON. IV.
ALKALINE PHOSPHATASE AND LACTIC DEHYDROGENASE
ACTIVITIES IN RENAL TUBULAR DISEASES *

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The present investigations were designed to study by a quantitative technique the distribution of enzyme activity in the various individual anatomical and functional units of the human nephron. Our first objective was to study the role played by enzymes in the formation of urine, particularly in the secretion and reabsorption of various substances in the renal tubule. The second objective was to discover enzymes in the kidney which may appear in the blood stream or in urine, and whose measurement in renal disease could be used as an index of damage to specific structures in the nephron.

In this paper investigations are reported on the relationship between isolated and combined defects in the function of the renal tubules and alkaline phosphatase and lactic dehydrogenase (LDH) activity in the nephron. Assays of enzyme activity were made on the individual anatomical units of the nephron of kidneys from patients with familial renal glycosuria, hypophosphatasia, phosphate-losing renal tubular disease, cystine storage disease, and the adult Fanconi syndrome. The results were compared with those from kidneys of persons free of renal disease.

MATERIAL AND METHODS

Healthy kidneys. In five instances specimens of healthy kidneys were taken by biopsy during the course of a laparotomy for disease unrelated to the kidney, e.g., during elective cholecystectomy or repair of a ventral hernia. Two specimens were taken at autopsy, 3 and 6 hours after

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death. All of the kidney specimens so obtained were from patients with no recent or past history of renal disease, and in whom examination of the urine and blood revealed no evidence of renal disease. In none was the primary disease one known to have secondary effects on the kidney.

Abnormal kidneys. The renal tissue was removed by percutaneous renal biopsy (1) from 5 patients (see below). Tissue was also obtained postmortem from a patient with the adult Fanconi syndrome (Case 1) and from an infant with the infantile form of hypophosphatasia. These specimens were obtained and frozen within 1 and 5 hours after death, respectively. Two specimens of kidney tissue were analyzed from the patient with the phosphate-losing renal tubular disease, who died suddenly from a subarachnoid hemorrhage. A specimen had been obtained by biopsy 11 weeks before death, the second was taken at autopsy 4 hours after she died.

Methods of dissection and analysis of tissue for enzyme activity. These have been described in detail in previous communications from this laboratory (2, 3). Optimal conditions for the assay of alkaline phosphatase and LDH activities in human kidneys were used for the assays (2, 3). The results were expressed in moles of substrate split or in moles of DPNH oxidized per kilogram dry weight of tissue per hour at 37° C.

RESULTS

Statistical handling of data (Tables I and II)

In the previous two papers the results of enzyme assays were expressed in terms of the total number of fragments of each structure studied (2, 3). A different form of analysis was necessary in the present study in order to compare data from single pathological cases with data from analyses of several normal kidneys. Therefore, in the case of healthy kidneys the results were expressed as means with standard deviations of the means of all the healthy kidneys grouped together. The results for the single pathological specimens were expressed differently—as means with standard deviations of the *individual* renal fragments analyzed.

TABLE I
Alkaline phosphatase activity in the anatomical units of nephrons from healthy kidneys and from the kidneys of patients with renal tubular dysfunction
(expressed in moles of substrate split per kilogram dry weight of tissue per hour at 37° C.) *

Structure analyzed	Hypophosphatasia						Adult Fanconi syndrome		
	Healthy adult kidneys	Familial renal glycosuria	Juvenile		Phosphate-losing renal tubular disease†	Cystine storage disease	Case 1		Case 2
			Case 1	Case 2			Case 1	Case 2	
Glomeruli	0.48±0.17 5 [36]	0.70±0.07 [5]	0.16±0.20 [5]	0.32±0.28 [6]	0.10±0.098 [6]	0.32±0.21 [14]	0.90±0.58 [7]	0.40±0.40 [7]	0.21±0.11 [7]
Proximal convolutions	5.70±0.90 5 [40]	2.60±0.81 [9]	0.54±0.40 [7]	0.49±0.12 [7]	0.20±0.20§ [8]	1.40±0.42 [18]	7.20±1.99 [5]	0.35±0.21 [7]	2.00±0.60 [9]
Distal convolutions	2.70±0.50 6 [40]	1.20±0.61 [7]	0.30±0.25 [5]	0.37±0.21 [6]		1.21±0.93 [12]		1.10±0.50 [4]	0.13±0.23 [2]
Degenerated convolutions						0.92±0.81 [11]	1.20±0.58 [7]	0.40±0.29 [6]	0.35±0.12 [7]
Medullary rays	2.84 1 [9]	2.70±1.42 [8]	0.79±0.21 [5]	0.34±0.20 [6]	0.30±0.29 [6]	1.10±0.45 [24]	2.00±0.40 [3]	0.60±0.59 [6]	2.06±0.18 [7]
Medulla (outer and inner medullary zones)	0.80±0.20 2 [11]				0.50±0.81 [5]	0.70±0.29 [12]	0.80±0.40 [3]		
Papilla	1.70±0.89 2 [15]					0.37±0.13 [6]	0.60±0.79 [13]	0.60±0.41 [14]	
Small artery	1.20±0.70 5 [25]	0.17±0.19 [3]	0.15±0.054 [3]	0.10±0.089 [5]	0.14±0.086 [7]	0.60±0.19 [7]	0.50±0.29 [6]	0.40±0.40 [7]	0.092±0.086 [11]

* For the healthy kidneys the mean value and standard deviation are given for the averages of each patient; for the pathologic kidneys the mean value and standard deviation are given for the fragments of tissue analyzed. The total number of tissue fragments analyzed is given in brackets.

† Number of kidneys analyzed in each group is shown in italic type.

§ Pooled data from one biopsy and one autopsy specimen from the same patient; no significant differences were found between these specimens.

|| Histologically normal medullary ray.

¶ Histologically atrophic and degenerated medullary ray.

Values removed more than 2 standard deviations from the mean values obtained for healthy kidneys were regarded as significantly different statistically ($p = < 0.05$). In evaluating the functional significance of changes in enzyme activity, however, it must be kept in mind that very considerable changes in enzyme activity may be necessary before functional effects can be expected.

Healthy kidneys

Alkaline phosphatase. The level of alkaline phosphatase activity was found, on a molar basis, to be low throughout the nephron, the lowest activity being in the glomeruli (Table I). The highest activity was found in the proximal convolutions, and this activity was significantly greater than that in the distal convolutions ($t = 6.65$; $p = < 0.001$). Comparatively low levels of activity were found in the medulla and papilla.

LDH. There was a high level of activity, on a molar basis, of this enzyme throughout the nephron (Table II). The enzymatic activity of the proximal and distal convolutions was higher than that of any other segment of the nephron, and as in the case of alkaline phosphatase, the lowest activity was found in the glomeruli. In the case of both enzymes there were no significant

differences in activity between specimens taken by biopsy and at autopsy.

Results in disease states

Renal glycosuria. A 31 year old bank clerk and many members of his family were known to have renal glycosuria. He was in excellent health and fasting blood glucose levels and oral glucose tolerance tests were normal. The renal functions measured were within normal limits, as was the histology of the renal biopsy.

Alkaline phosphatase activity was decreased in the proximal (> 3 SD) and in the distal convolutions ($= 3$ SD), but was not significantly altered in the glomeruli. The LDH activity was not significantly different from normal in any unit of the nephron analyzed.

Hypophosphatasia. Although hypophosphatasia is not usually considered to be a disease with clinically significant renal tubular dysfunction, it was chosen for study because of the possible relationship between alkaline phosphatase activity and the reabsorption of glucose by the proximal tubule (4, 5). Kidneys from three children with hypophosphatasia were studied. Full details of the three cases have been published elsewhere (6).

The first patient, a two and one-half year old

TABLE II

*Lactic dehydrogenase activity in the anatomical units of nephrons from healthy kidneys and from the kidneys of patients with renal tubular dysfunction (expressed in moles of DPNH oxidized per kilogram dry weight of tissue per hour at 37° C) **

Structure analyzed	Healthy adult kidneys 7†	Familial renal glycosuria	Hypophosphatasia		Phosphate-losing renal tubular disease‡§	Cystine storage disease	Adult Fanconi syndrome		
			Case 1‡	Case 2‡			Case 1	Case 2	Case 3‡
Glomeruli	108±22 6 [46]	116±21.5 [8]	122±32.4 [5]	142±49.9 [6]	213±44.1 [6]	146±15.2 [6]	39±7.06 [8]	58±23.0 [7]	50±0.99 [2]
Proximal convolutions	257±41 7 [55]	299±44.1 [6]	206±10.2 [6]	312±64.0 [7]	314±15.0 [7]	171±21.1 [21]	114±26.8 [7]	165±41.0 [8]	314±51.0 [7]
Distal convolutions	256±44 7 [49]	334±35.8 [5]	286±52.6 [8]	299±77.0 [6]	357±33.1	336±53.8 [7]	131±63.0 [7]	191±28.0 [7]	336±53.8 [7]
Medullary rays	231±25 3 [25]	249±34.4 [7]	234±64.9 [6]	260±45.6 [6]	375±27.0 [6]	271±69.0 [6]	134±29.1 [7]	99±23.0 [7]	137±31.1 [6]
Medulla (outer and inner medullary zones)	165±21 3 [22]				326±30.8 [5]	289±40.9 [12]		108±24.0 [6]	152±100.0 [10]
Papilla	175±82 3 [8]					152±30.3 [12]		89±18.1 [6]	
Small artery	131±61 6 [31]		151±24.8 [4]	196±14.7 [5]	203±39.2 [6]	77±16.0 [6]	30±13.1 [2]	53±23.0 [6]	26±7.08 [8]
									62±19.5 [6]

* For the healthy kidneys the mean value and standard deviation are given for the averages of each patient; for the pathologic kidneys the mean value and standard deviation are given for the fragments of tissue analyzed. The total number of tissue fragments analyzed is given in brackets.

† Number of kidneys analyzed in each group is shown in italic type.

‡ Multiplied with factor 3.45 to correct for inhibitor present in DPNH used in these experiments (3).

§ Autopsy specimen only.

|| Proximal and distal convolutions could not be dissected out separately in this patient.

white girl, presented because of loss of the deciduous teeth. There were no abnormal clinical findings but changes consistent with those seen in hypophosphatasia were found on X-ray of the long bones. The serum alkaline phosphatase level was 0.5 Bodansky unit.

The second patient, a three year old white girl, had widespread bony deformities, premature loss of deciduous teeth, and radiological changes consistent with hypophosphatasia. The serum alkaline phosphatase level was 0.6 Bodansky unit.

The third patient, a two month old white infant, had had gross skeletal defects from birth. Seizures characteristic of hypsarrhythmia appeared at eight days. The child died of pneumonia. Physical abnormalities included gross skeletal deformities, with wide flat fontanelles and widely separated sutures. Radiologically, there was delayed ossification of the skull, demineralization of the spine, marked flaring of costochondral junctions and an irregular appearance of the rib shaft. The metaphyses were demineralized and there were deformities of the diaphyses of the long bones. The serum alkaline phosphatase was 0.0 and 0.7 Bodansky unit; calcium was 11 mg per 100 ml and inorganic phosphate was 5.2 mg per 100 ml. Overgrowth of cartilage, failure of bone resorption, much osteoid, poor calcification and irregularity of bone trabeculae were noted postmortem. Calcium deposits were found in the interstitial tissue of the kidney among normal glomeruli and tubules. The tissue alkaline phosphatase activities were measured in homogenates of liver, spleen, kidney and bone, and were found to be from 0.7 to 15 per cent of levels in tissues from an infant of the same age who had died in an accident (6).

Serum alkaline phosphatase levels were abnormally low in members of the families of all three children. Phospho-ethanolamine was demonstrated by two-dimensional paper chromatography in the urine of all three patients. None of the three patients had glycosuria. The first patient, however, was found to have aminoaciduria.

Extremely low levels of alkaline phosphatase activity were found in nephrons from these three patients, but the levels of activity in the two juvenile cases of hypophosphatasia were approximately double that found in the infantile. All these results were significantly lower than measurements from two normal infant kidneys, ana-

lyzed as controls (7). The LDH activity in the juvenile cases did not differ significantly from that found in the healthy adult or infant kidneys, but there was a slight increase of LDH activity in the case of infantile hypophosphatasia.

Phosphate-losing renal tubular disease. The kidney from a 53 year old white advertising executive was studied on two occasions. She had enjoyed excellent health until the age of 50, when she had a sudden pain in the chest and was found to have several fractured ribs. Thenceforth she sustained numerous spontaneous fractures. The serum calcium was between 9.5 and 10.8 mg per 100 ml, inorganic phosphate between 1.8 and 2.5 mg per 100 ml, and alkaline phosphatase from 3 to 4 Bodansky units. The tubular reabsorption of phosphate was 47 per cent on a diet containing 1.5 g phosphate per day. It was not affected by treatment with 500,000 units of vitamin D daily for 6 days, but it increased from 47 to 59 per cent when calcium (1.5 mg per kg) was infused. The urine specific gravity varied from 1.002 to 1.018, and the serum nonprotein nitrogen was 12 mg per 100 ml. There was no aminoaciduria or glycosuria. There was no evidence of intestinal malabsorption. Several bone biopsies were characteristic of osteomalacia, and the parathyroid glands were found to be normal on exploration of the neck and mediastinum (8). The patient died from a subarachnoid hemorrhage. No morphologic abnormalities were found in the renal biopsy specimen or in the kidneys examined postmortem.

The alkaline phosphatase activity was low throughout the nephron, but least abnormal in the glomeruli. These differences were significant in the proximal convolutions (> 4 SD) and in the distal convolutions ($= 3$ SD). Even lower activities were found in histologically degenerated convoluted tubules. The LDH activity was slightly but not significantly increased throughout the nephron, except in the papilla.

Cystine storage disease with Fanconi syndrome. Nephrons from one patient, aged four and one-half years, with cystine storage disease, were studied. Failure to thrive had been noted from the age of 18 months, and polyuria and polydipsia from the age of three years. Cystine crystals were found in the corneas, and in the reticuloendothelial cells of the bone marrow. The urine volumes varied between 2 and 5 L daily, and the urine specific

gravity did not exceed 1.005 during dehydration or after injection of Pitressin (vasopressin). There was no glycosuria but there was a pronounced aminoaciduria (total α -amino-nitrogen excretion 1,545 mg per 24 hours). The serum potassium was consistently low. On histological examination of the renal biopsy, slight degenerative changes were found in the convoluted tubules, and there was a slight increase of fuchsin-positive and periodic acid-Schiff (PAS)-positive granules in the cytoplasm. No other abnormality was found. The father, paternal grandfather and one sister were found to have a heavy aminoaciduria. The second sister was shown to have cystine storage disease; cystine crystals were seen in corneas and bone marrow, and rickets, aminoaciduria and glycosuria were noted.

The alkaline phosphatase activity was significantly higher than normal in the glomeruli (> 2 SD) and was slightly elevated in the histologically normal proximal convolutions (< 2 SD). However, the alkaline phosphatase activity was significantly decreased in the histologically degenerated convolutions. By contrast, LDH activity was significantly decreased in glomeruli (> 3 SD), proximal convolutions ($= 2$ SD) and medullary rays (> 3 SD).

Adult Fanconi syndrome. The kidneys from three patients with the adult Fanconi syndrome were studied. Full details of the clinical histories and metabolic studies on Patients 1 and 2 will be published elsewhere (9). Although there were considerable pathophysiological similarities in these three patients, we report them below individually because of the different underlying renal pathologies.

Patient 1, a 51 year old man, died in cardiac and hepatic failure with postnecrotic cirrhosis, osteomalacia and atypical multiple myeloma. During life the urine volume varied from 2 to 5 L, and the urine specific gravity from 1.012 to 1.018. The creatinine clearance was 55 ml per minute, the urea clearance was 60 per cent of average normal renal function, and the excretion of phenolsulfonphthalein was 15 per cent in 2 hours. The serum nonprotein nitrogen was 27 mg per 100 ml. The following substances were excreted in excess in the urine: glucose, glycine, α -amino-nitrogen, inorganic phosphate, calcium, uric acid, and potassium. Tubular reabsorption of phosphate was 21

to 38 per cent. On microscopic examination of the kidney, moderately severe degenerative changes were seen in the tubular cells, and there was patchy tubular atrophy. Several tubules contained hyaline or pigmented casts, and some of the casts contained bilirubin. In some tubules there was syncytial formation such as occurs in multiple myeloma. Cuboidal crystals of unknown composition were seen in proximal convoluted tubules and interstitial tissue. The glomeruli were normal but there was diffuse fibrosis of the interstitial tissue.

Patient 2, a 52 year old housewife, was ill with osteomalacia and multiple pseudofractures. Her urine volume varied from 1.5 to 2 L per day, the specific gravity from 1.012 to 1.017, and the pH from 6.0 to 7.5. The endogenous creatinine clearance was 21.4 ml per minute, the urea clearance was 22 per cent of average normal renal function, and the excretion of phenolsulfonphthalein was 12 per cent in 2 hours. The serum nonprotein nitrogen was 50 mg per 100 ml. She excreted the following substances in excess in her urine: glucose, glycine, α -amino-nitrogen, uric acid, sodium, chloride, potassium, and phosphate. Pyelonephritis and arterial and arteriolar nephrosclerosis were diagnosed by means of a renal biopsy. Several glomeruli were hyalinized and there was a moderate degree of tubular atrophy and degeneration.

Patient 3, a 38 year old Negro housewife, was also ill with osteomalacia and multiple pseudofractures, and in addition had metastases from an osteogenic sarcoma. The serum calcium ranged from 9.4 to 10.3 mg per 100 ml, and the inorganic phosphate from 1.1 to 1.8 mg per 100 ml. The serum alkaline phosphate level was normal. The urine volume varied from 1.4 to 2.0 L daily, and the specific gravity from 1.022 to 1.031. The endogenous creatinine clearance was 119 ml per minute, and the serum urea nitrogen was 8 mg per 100 ml. She excreted the following substances in excess in her urine: glucose, phosphate, glycine, and other amino acids. No abnormality was found on histological examination of the renal biopsy specimen.

Biochemical analyses of the kidneys from these three patients revealed a marked decrease of alkaline phosphatase activity throughout the nephrons, except for the glomeruli, where it was normal. Particularly low levels were found in the proximal and distal convolutions (> 3 SD). LDH activity

was also significantly decreased in the glomeruli, proximal convolutions and medullary rays of Patients 1 and 2, and in the distal convolutions of Patient 1. However, there was a slight increase of LDH activity throughout the nephrons of the third patient, but this was significant only in the medullary ray (> 3 SD).

DISCUSSION

Very little is known currently of the distribution and functions of enzymes in the individual anatomical and functional units of the nephron. The limited knowledge we possess of the distribution of enzyme activity in the nephron has been gleaned by histological staining techniques. Because these techniques yield only qualitative information of limited value, the quantitative techniques reported in the previous two papers (2, 3) were applied to the study of human renal tissue.

Method of dissection of specimens. The justification for and validity of the microdissection technique were discussed in a previous paper (2), in which it was shown that individual units of the healthy nephron could be dissected out accurately and assayed with precision for enzyme activity. Several new problems arose when nephrons from diseased kidneys were studied. In particular, there were considerable variations in the histological appearance both of glomeruli and of the individual anatomical units of the tubules. For example, the appearance of the proximal convolutions was more heterogeneous than that in healthy kidneys. Some appeared normal with brush borders, whereas others showed either degenerative changes or atrophy, or both. Thus, histologically normal proximal convolutions were more easily identified than were histologically abnormal proximal convolutions; this might lead the dissector, consciously or unconsciously, to dissect out the histologically normal tubules rather than the damaged tubules. In order to have a representative sample, every effort was made to dissect out for analysis both histologically normal and histologically abnormal tubules whenever there were distinct variations in the histological appearance of the tubules. Those tubules which were so abnormal that they could not be identified were analyzed as a separate group—"degenerated convolutions."

Levels of enzyme activity in renal tubular dysfunction. The results suggest that there are sig-

nificant differences in enzyme activity between healthy kidneys and the nephrons in various disease states. Alkaline phosphatase activity was found to be decreased significantly in the convoluted tubules in all diseases studied, with the exception of cystinosis.

LDH activity was decreased significantly only in two of the three cases of adult Fanconi syndrome and also in the child with cystine storage disease and Fanconi syndrome. All three were excreting very large amounts of α -amino-nitrogen in the urine. The third adult with the Fanconi syndrome was excreting only a slight excess of α -amino-nitrogen; the LDH activity of the tubules was not decreased. These findings might suggest that the long continued massive amino acid depletion resulted in a decreased synthesis of enzyme protein in these patients. However, the finding of normal alkaline phosphatase activity in the tubules of the child with cystinosis makes this explanation unlikely. Therefore, alternative explanations must be sought.

In lupus glomerulonephritis, for example, where the tubular degeneration was of a comparatively mild degree, *increased* LDH activity was found in the convoluted tubules (10). There was no evidence of severe impairment of renal function in this disease, and glucose, inorganic phosphate, and amino acids appeared to be reabsorbed normally by the tubules. Thus far, *decreased* LDH activity has been found only in nephrons from the three kidneys with histologically severe tubular disorganization, and with severe derangements of tubular function; the lowest activity was observed in nephrons of the most severely damaged kidney. Thus the decreased LDH activity of these severely damaged nephrons may reflect or be a consequence of the severe damage sustained by the tubular cells in the basic disease processes of severe adult Fanconi syndrome and of cystine storage disease.

The *coefficient of variation* within individual kidneys was invariably larger than the coefficient of variation between individual kidneys (Table III), suggesting that the variation from nephron to nephron within a single healthy kidney was as great as, or greater than, the variation from healthy kidney to healthy kidney. There was little difference in the coefficient of variation for LDH activity in individual healthy and abnormal kidneys, suggesting that the nephron population of abnormal

TABLE III

Coefficients of variation for alkaline phosphatase and lactic dehydrogenase activities in healthy kidneys and in kidneys from patients with renal tubular dysfunction

Structure analyzed	Alkaline phosphatase			Lactic dehydrogenase		
	Healthy kidneys		Renal tubular dysfunction	Healthy kidneys		Renal tubular dysfunction
	Coefficient of variation within individuals*	Coefficient of variation between individuals	Coefficient of variation within individuals	Coefficient of variation within individuals	Coefficient of variation between individuals	Coefficient of variation within individuals
Glomeruli	65.3 (42.9-100)	35.4	75.4 (10.0-125.0)	31.9 (17.9-53.6)	20.4	22.4 (9.9-39.6)
Proximal convolutions	28.1 (16.4-36.0)	15.8	36.2 (11.6-74.1)	20.4 (8.4-34.4)	15.9	15.2 (4.8-24.8)
Distal convolutions	48.8 (28.1-65.2)	18.5	75.3 (34.3-179.0)	21.9 (12.0-36.9)	17.4	21.4 (10.7-48.0)

* Figures in parentheses indicate the range.

kidneys was as homogenous in this respect as was that of healthy kidneys. There was greater variation of alkaline phosphatase activity in individual abnormal than in individual healthy kidneys. This was due, at least in part, to the fact that the alkaline phosphatase activities were low in the abnormal kidneys, and the readings obtained were very close to those of the blanks (2).

Alkaline phosphatase and tubular reabsorption of glucose. It has been suggested by some investigators that alkaline phosphatase plays a significant role in the reabsorption of glucose from the glomerular filtrate by the proximal convolution (4, 5). If this were so, the alkaline phosphatase activity of the proximal convolution might be expected to be low in renal glycosuria. In the single patient with familial renal glycosuria studied, the alkaline phosphatase activity in the proximal convolution was decreased to approximately 50 per cent of that found in proximal convolutions from healthy kidneys. The decrease of alkaline phos-

phatase activity was of a similar magnitude in patients with lupus nephritis (10), none of whom had glycosuria. Moreover, in three patients with hypophosphatasia there was no glycosuria and the alkaline phosphatase activity was only 3.5 to 9.5 per cent of that found in healthy proximal convolutions. Unless an alternate pathway for glucose reabsorption can be invoked for patients with hypophosphatasia, these facts exclude the possibility that alkaline phosphatase plays a key role in the tubular reabsorption of glucose. To obtain further confirmation, glycosuria was produced in the dog by injection of phlorhizin. Table IV shows that there was no significant difference in the activity of this enzyme before and after injection of phlorhizin, so that phlorhizin-induced glycosuria is not mediated by way of inhibition of alkaline phosphatase.

SUMMARY

1. Alkaline phosphatase and lactic dehydrogenase (LDH) activities were measured quantitatively in the various anatomical and functional parts of the nephrons from healthy kidneys and from the kidneys of six patients with diseases affecting the renal tubules: familial renal glycosuria, phosphate-losing renal tubular disease, cystine storage disease, and adult Fanconi syndrome.

2. Alkaline phosphatase activity was decreased in the convoluted tubules in all diseases studied except in cystine storage disease. LDH activity was decreased only in two of the three cases of

TABLE IV

*The effect of phlorhizin on the alkaline phosphatase and lactic dehydrogenase activity of the proximal convolutions in the dog**

Enzyme	Before phlorhizin	After phlorhizin	p
Alkaline phosphatase	20.6 ± 3.1 [9]	26.9 ± 16.3 [11]	0.29
Lactic dehydrogenase	215 ± 38 [10]	192 ± 28 [11]	0.67

* Total number of tissue fragments analyzed is given in brackets.

adult Fanconi syndrome and in a child with cystinosis and the Fanconi syndrome. A parallel decrease of both enzymes was observed in two of the patients with adult Fanconi syndrome.

3. The kidneys of three patients with hypophosphatasia were also studied. None had glycosuria. The extremely low level of alkaline phosphatase activity found in the proximal convolutions indicates that this enzyme does not play a key role in glucose reabsorption. This was confirmed by the observation that prolonged phlorhizin-induced glycosuria did not affect the level of alkaline phosphatase activity in the proximal convolution of the dog.

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