OBSERVATIONS ON PHOSPHATE TRANSPORT IN EXPERIMENTAL RENAL DISEASE *

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In the normal adult, the renal excretion of phosphate may be varied over a wide range depending on the level of phosphate intake. When the diet contains the usual amounts of phosphate (about 1 g of phosphate per day), only a small fraction of the filtered phosphate is excreted in the urine. This fraction is decreased when the dietary phosphorus is low and increased when it is high. Homeostasis is thus maintained with only slight fluctuations of the plasma phosphate concentration (2-6).

In the course of advancing renal failure in man, normal plasma phosphate concentrations typically prevail until the glomerular filtration rate (GFR) falls below 25 to 30 ml per minute (7). This occurs by virtue of the fact that, as GFR decreases, the fraction of the filtered phosphate excreted increases proportionately; as a result, phosphate balance may be maintained in the presence of a decrease in the filtered load. However, when the GFR decreases below 25 ml per minute, the filtered load of phosphate is decreased to such an extent that even the excretion of a large fraction of the filtered load fails to maintain phosphate homeostasis, and hyperphosphatemia supervenes. The serum phosphate concentration will then increase until the filtered load of phosphate is sufficiently high for the re-establishment of phosphate balance. Advanced bilateral renal disease in man

thus is characterized by hyperphosphatemia, but the degree of phosphate retention is minimized by the continuing excretion of a large fraction of the filtered phosphate.

The mechanism underlying the excretion of an increasing fraction of the filtered phosphate has been variously attributed to secondary hyperparathyroidism and to an inability of the diseased kidney to reabsorb phosphate (7, 8). Because renal insufficiency due to bilateral renal disease causes many alterations in the composition of extracellular fluid that could secondarily influence phosphate transport, it seemed desirable to study phosphate excretion by the diseased kidney under conditions in which the variables of extrarenal factors were minimized. The experimental model of permanent unilateral renal disease in dogs appeared to be well suited for such an investigation (9). This model makes possible the serial and simultaneous measurement of clearances in the diseased and normal kidneys of the same animal; owing to the presence of one intact kidney, the essentially normal composition of the extracellular fluid is maintained. Any consistent differences in the pattern of phosphate excretion between the diseased and contralateral control kidney might reflect alterations in phosphate transport induced by the renal disease per se.

METHODS

Thirty-four experiments were performed on 26 female mongrel dogs weighing 6 to 20 kg. In each animal, one of three types of unilateral renal disease was induced: 1) aminonucleoside-induced nephritis, 2) chronic pyelonephritis, 3) antikidney serum glomerulonephritis. In one dog, bilateral pyelonephritis was induced.

Techniques for induction of these diseases, their histologic features, and details of the clearance measurements have been described in previous publications (9-11).

Patterns of phosphate excretion were studied by simultaneous determination of the clearances of phosphate and

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TABLE I
Patterns of phosphate excretion of diseased and intact kidneys under baseline conditions *

Clearance period	Plasma P	C _{Cr}		V		UV _P		C _P		C _P /C _{Cr} ×100	
		Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.
	<i>μmoles/ml</i>	<i>ml/min</i>		<i>ml/min</i>		<i>μmoles/min</i>		<i>ml/min</i>			
Dog A	Antikidney serum nephritis, wt, 7.8 kg										
1	1.52	7.1	27.0	1.08	3.75	2.23	7.98	1.47	5.26	21.0	19.5
2	1.61	6.5	23.9	0.89	2.96	2.61	8.78	1.62	5.45	24.8	22.8
3	1.61	6.8	26.3	0.86	2.93	3.19	11.2	1.98	6.97	28.9	26.5
Dog B	Pyelonephritis, wt, 8.6 kg										
1	1.24	4.66	50.4	0.578	3.61	0.841	12.0	0.675	9.63	14.6	19.1
2	1.31	3.16	45.7	1.000	2.97	0.590	12.2	0.450	9.27	14.2	20.3
3	1.17	3.96	45.7	1.09	2.44	0.738	11.6	0.629	9.85	15.9	21.6
4	1.25	4.42	49.4	1.13	3.13	0.860	14.8	0.687	11.8	15.6	24.0
5	1.24	3.72	42.4	1.09	2.81	0.777	12.5	0.624	10.0	16.7	23.6
6	1.48	4.99	54.1	0.708	4.24	1.28	16.1	0.865	10.9	17.2	20.1
7	1.53	4.22	55.1	0.659	4.44	0.916	16.6	0.600	10.8	14.2	19.6
8	1.40	4.35	52.9	0.745	4.72	1.07	15.8	0.769	11.3	17.7	21.4

* Dogs were maintained on an *ad lib.* diet of Purina dog chow. The clearance periods were usually 10 to 20 minutes in duration. P = phosphate, C_{Cr} = creatinine clearance, V = urine volume, UV_P = phosphate excretion rate, C_P = phosphate clearance.

creatinine in the diseased and contralateral control kidneys. Creatinine was measured by the method of Bonsnes and Taussky (12) and phosphate by the method of Gomori (13).

RESULTS

Baseline pattern. Eighteen experiments were performed in 16 fasting dogs without phosphate loading. Each experiment consisted of three to ten clearance periods.

Two representative experiments are shown in Table I. In Dog A, the fraction of filtered phosphate excreted (clearance ratio C_P/C_{Cr}) was closely comparable in the experimental and control kidneys despite a fourfold difference in filtration rates. Similar results were obtained in five experiments in five dogs with pyelonephritis and in five experiments in four dogs with antikidney serum nephritis.

TABLE II
Pyelonephritic dogs with moderate phosphate loading *

Clearance period	Plasma P	C _{Cr}		V		UV _P		C _P		C _P /C _{Cr} X100		Reab. P	
		Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.
	<i>μmoles/ml</i>	<i>ml/min</i>		<i>ml/min</i>		<i>μmoles/min</i>		<i>ml/min</i>				<i>μmoles/min/ ml GF</i>	
Dog A	Wt, 15.9 kg												
1	3.21	26.4	42.1	1.20	1.64	45.3	75.1	14.1	23.4	53.4	55.6	1.49	1.42
2	3.42	26.1	42.2	1.34	1.87	51.9	82.0	15.2	24.0	58.2	56.9	1.43	1.48
3	3.23	26.4	41.8	1.53	2.09	56.8	87.6	17.6	27.2	66.7	65.1	1.08	1.13
4	3.42	25.9	41.2	1.65	2.29	57.5	91.6	16.8	26.8	64.9	65.0	1.20	1.20
Dog B	Wt, 18.2 kg												
1	4.35	20.3	59.9	1.92	3.88	58.8	146.4	13.5	33.6	66.5	56.1	1.45	1.91
2	4.58	24.5	44.7	2.22	3.53	76.6	129.8	16.7	28.3	68.2	63.3	1.45	1.68
3	4.68	21.1	45.9	2.04	3.78	67.8	143.9	14.5	30.8	68.7	67.1	1.46	1.54
4	4.84	23.8	42.6	2.34	3.63	80.8	141.7	16.7	29.3	70.2	68.8	1.44	1.51
Dog C	Wt, 15.4 kg												
1	4.16	31.7	52.4	1.53	1.88	71.1	117.6	17.1	28.3	53.9	54.0	1.92	1.92
2	4.48	29.5	47.1	1.62	1.90	78.4	122.6	17.5	27.3	59.3	58.0	1.82	1.88
3	4.68	27.9	49.6	1.62	2.18	78.4	139.2	16.8	29.8	60.2	60.1	1.87	1.87
4	4.68	25.7	48.6	1.62	2.30	76.8	142.4	16.4	30.4	63.8	62.5	1.69	1.75

* Dogs received 4 g of ammonium chloride daily for 1 to 3 days before the study. Buffered phosphate (pH 7.4) was infused at a rate of 0.15 mmole phosphate/min. Creatinine was infused at a rate of 7 mg/min and PAH at a rate of 2.5 mg/min. Reab. P = reabsorbed phosphate, C_{Cr} = creatinine clearance, V = urine volume, UV_P = excreted phosphate, C_P = phosphate clearance.

The data on Dog B are representative of experiments in which the difference in clearance ratios was somewhat more marked. In the experiment depicted, the diseased kidney excreted a smaller fraction of its filtered phosphate than did the contralateral kidney. Comparable results were obtained in one other dog with pyelonephritis, in two experiments on a dog with aminonucleoside nephritis, and in two experiments on two dogs with antikidney serum nephritis. In only one experiment (a dog with pyelonephritis) was the clearance ratio higher on the diseased side. These small differences in clearance ratios were

not related to 1) the difference of filtration rates, 2) the interval between induction of the experimental lesion and study, 3) the serum phosphate concentration, and 4) the absolute value of the clearance ratios. In any one experiment, the relationship between the clearance ratios was consistent in all clearance periods.

Phosphate loading. Thirteen experiments were performed in eight pyelonephritic dogs in which the serum phosphorus concentration was moderately elevated as a result of intravenous infusions of buffered phosphate; 1.5 to 4-fold differences in filtration rates were observed in this group of ani-

TABLE III
*T_{mp} in unilateral renal disease **

Time	Plasma P	C _{cr}		UV _P		Reab. P		T _{mp}		T _{mp} /C _{cr}	
		Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.
min	μmoles/ml	ml/min		μmoles/min		μmoles/min		μmoles/min		μmoles/min/ ml GF	
Dog A	Pyelonephritis, wt, 9.5 kg										
0-20	0.910	13.8	27.2	2.00	3.65	10.6	21.0				
20-36	0.906	11.7	28.0	1.57	2.99	9.0	22.4				
36-54	0.910	9.1	24.8	0.963	1.93	7.32	20.7				
55	Infusion of 0.16 mmole phosphate/min begun										
54-86.5	3.02	12.8	26.9	9.26	2.68	29.4	78.5				
86.5-100	3.60	11.5	27.0	20.5	58.2	21.0	39.0				
100-117.5	4.21	12.6	28.2	34.5	70.2	18.5	48.5				
117.5-138	4.74	11.1	29.7	35.3	92.2	17.3	48.6				
139	Infusion of 0.33 mmole phosphate/min begun										
138-171.5	7.04	13.6	28.0	64.1	145	31.6	52.0				
171.5-187.5	7.67	16.0	30.5	89.1	191	33.9	43.0				
187.5-204	8.20	16.0	33.6	102	216	29.0	60.0	31.5	51.7	2.06	1.68
Dog A'	Same dog studied 10 days later										
0-10	1.05	9.97	23.8	2.39	5.40	8.11	19.5				
11.5	Infusion of 0.16 mmole phosphate/min begun										
10-32.5	2.38	8.88	19.9	8.02	19.8	13.1	27.6				
32.5-46	3.02	9.29	20.7	14.7	31.8	13.3	30.8				
46-58.5	3.74	8.71	11.4	18.3	23.8	14.3	18.8				
60	Infusion of 0.33 mmole phosphate/min begun										
58.5-81	6.22	9.15	21.8	36.0	87.4	21.1	48.4				
81-98	7.58	10.72	22.5	59.5	122	21.8	49.0				
100	Infusion of 0.82 mmole phosphate/min begun										
98-129	13.7	9.84	23.7	109	264	26.0	62.0				
129-141	16.3	10.77	24.2	161	347	14.0	47.0	20.7	51.6	2.05	2.24
Dog B	Antikidney serum, wt, 12.3 kg										
0-21	1.37	1.55	53.2	0.259	9.21	1.86	63.5				
21-43.5	1.34	1.70	52.8	0.103	8.73	2.18	62.3				
50	Infusion of 0.16 mmole phosphate/min begun										
43.5-100	3.42	2.24	59.3	4.39	107	3.25	96.0				
100-115	3.71	2.38	53.5	7.42	129	1.42	69.0				
116.5	Infusion of 0.33 mmole phosphate/min begun										
115-138.5	5.00	2.20	59.0	7.58	193	3.42	102				
138.5-149	5.84	2.42	59.0	10.3	237	3.80	107				
149-158.5	6.32	2.78	60.4	13.8	275	3.80	107				
160	Infusion of 0.82 mmole phosphate/min begun										
158.5-180	10.1	2.81	53.9	22.4	451	6.00	93				
180-185	11.4	2.97	53.0	28.0	511	5.50	91	4.5	98	1.70	1.71

* Continuous infusions of creatinine (7 mg/min) and para-aminohippuric acid (2.5 mg/min) were maintained in all studies. P = phosphate, C_{cr} = creatinine clearance, UV_P = phosphate excretion rate.

mals. The pattern of phosphate excretion for three representative experiments is shown in Table II. The fractions of filtered phosphate excreted by diseased and control kidneys were in close agreement. There was no statistically significant difference ($p > 0.05$) in any one experiment or in group data when the results of all experiments were pooled (degrees of freedom = number of clearance periods minus 1 in a paired t test). In some experiments, the serum phosphate concentration was sufficiently high that saturation of the tubular capacity for phosphate reabsorption (Tm_P) might have occurred. For this reason, the values for net phosphate reabsorption per unit of GFR are also shown in Table II.

The plasma phosphate concentrations were elevated progressively to values well in excess of Tm_P in three experiments on two dogs (Table III). The value for Tm_P was calculated as the mean of the observed rate of phosphate reabsorption (micromoles per minute) of all clearance periods in which the plasma phosphate concentration was $5 \mu\text{moles per ml}$ or higher (3). Although the absolute value of the Tm_P (micromoles per minute) was always lower on the experimental side, the ratio Tm_P/GFR was closely comparable in the experimental and control kidneys. Furthermore, the differences of these ratios between kidneys were not consistent in direction; in the dog with pyelonephritis, the Tm_P/GFR ratio was higher in the experimental kidney in one study (A, Table III) and higher in the control kidney in a subsequent study (A', Table III). In Dog B, the ratio was nearly identical in the two kidneys. The comparability of the Tm_P/GFR in the two kidneys is particularly noteworthy in the latter dog, since filtration rates differed by a factor of 20.

DISCUSSION

In most of these experiments, the pattern of phosphate excretion was comparable in the diseased and contralateral control kidneys, regardless of the type of experimental renal disease studied or of the severity of the renal lesion. The similarity of the pattern in the two kidneys was particularly impressive in experiments with phosphate loading. Total excretion of phosphate and phosphate clearances were always reduced in the diseased organ, but this reduction was propor-

tional to the decrease of the GFR. Intrinsic failure of tubules to perform phosphate transport was not evident.

In several of the experiments performed without phosphate loading, a difference of moderate degree was observed in the pattern of phosphate excretion between diseased and normal kidneys. In these experiments the diseased kidney generally excreted a smaller fraction of the filtered phosphate than did the control organ. The explanation for this phenomenon is not apparent; however, if nephrons in the diseased kidney lost their capacity for phosphate reabsorption, the differences between the two kidneys would have been in the opposite direction.

The results of the present studies may be interpreted in terms of current concepts of renal phosphate transport. Serum inorganic phosphate is filtered at the glomerulus and reabsorbed by the tubules to a variable extent. A tubular maximum for phosphate transport can be demonstrated when the serum phosphate concentration is progressively increased. However, in contrast to the Tm for glucose, the Tm_P is highly variable, in both man and dog (3, 14). Thus at high filtered loads, the absolute rate of phosphate reabsorption often varies appreciably from period to period in individual studies, and the calculated value for Tm_P may vary over a wide range when repeated studies are performed on the same subject. The reasons for this variability are not completely clear, but two factors appear to be of major importance. 1) Parathyroid hormone is thought to decrease the maximal rate of net phosphate reabsorption, and the measurement of the Tm_P may require the induction of changes in parathyroid function. These changes may be brought about by hyperphosphatemia per se or by hyperphosphatemia-induced depression of the serum calcium concentration. 2) Phosphate may be secreted by the tubules as well as reabsorbed. Phosphate secretion is well established in some species but has not been unequivocally established in mammals (15, 16). It is possible, in the experiments in which the clearance ratio was lower in the diseased kidney, that less phosphate secretion was accomplished by the tubules of the diseased kidney.

Two experimental details require special comment. The variation of Tm_P noted in these experiments was of the same order of magnitude as

that observed by most other investigators (3, 14). In the experiments with moderate phosphate loading (Table II), the animals were acutely acidotic. It has been shown previously that acidosis does not influence the T_{mP} (3).

If experimental renal disease in the dog is functionally comparable with spontaneous renal diseases in man, the characteristically high clearance ratios of advanced bilateral human disease require explanation. Inability of the surviving tubules to transport phosphate effectively would appear to be unlikely on the basis of the dog experiment. Bilateral renal disease in the dog with moderate uremia is characterized by patterns of phosphate excretion very much like those of bilateral human disease. This is illustrated in Table IV, in which several experiments on a dog with bilateral pyelonephritis are shown. The combined GFR (i.e., two kidneys) was 11 to 13 ml per minute and the plasma urea nitrogen concentration was approximately 60 mg per 100 ml. From 37 to 59 per cent of the filtered phosphate was excreted (a range considerably higher than that noted in animals with unilateral renal disease). Despite the marked lowering of GFR and a normal dietary intake, hyperphosphatemia was prevented.

The excretion of an increasing fraction of filtered phosphate in bilateral chronically diseased kidney may therefore represent an adaptive change in phosphate transport which facilitates the maintenance of phosphate balance. Of the various factors responsible for the modifications in phosphate transport, small changes of the plasma phosphate concentration and secondary hyperparathyroidism must be considered. If total phosphate reabsorption is decreased in proportion to the reduction of the GFR, as the present data indicate, even a slight increase of the plasma phosphate concen-

tration, at a given level of GFR and phosphate reabsorption, would result in an increase of the clearance ratio. In the clinical situation, the change in plasma phosphate concentration may be so subtle as to escape detection, and the plasma phosphate concentration may "remain within normal limits" while the clearance ratio is increased. That minimal hyperphosphatemia is probably not the sole cause of the increased clearance ratio in bilateral human disease is suggested by the observation that the clearance ratio remains high after the serum phosphate concentration has been reduced by a low phosphate diet Basagel administration (17). In addition, preliminary experiments have shown that the very high clearance ratio in man with advanced renal insufficiency can be substantially lowered by an infusion of calcium salts, a procedure that probably decreases parathyroid hormone release (18).

SUMMARY

1. Phosphate transport was studied in dogs with unilateral renal disease. The experimental model made possible simultaneous clearance measurements in diseased and contralateral control kidneys in the absence of uremic changes in the extracellular fluid.

2. Under these conditions, phosphate transport, as measured by T_m and by moderate phosphate loading, was entirely comparable in the diseased and normal organs when transport was expressed per unit of glomerular filtrate. In the absence of phosphate loading, the fraction of filtered phosphate excreted by the diseased kidney generally approximated that of the contralateral control kidney.

3. It is concluded that the intrinsic capacity of the residual nephrons in the dog to transport phosphate remains essentially intact, and it is suggested that the excretion of an increasing fraction of filtered phosphate in advancing renal disease in man may relate principally to adaptive changes induced by the uremic state.

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TABLE IV
*Bilateral pyelonephritis **

Date	Serum P	C _{Cr}	C _P	C _P /C _{Cr} ×100
	<i>μmoles/ml</i>	<i>ml/min</i>	<i>ml/min</i>	
5/6	1.42	11.1	4.11	37.0
5/20	1.63	11.6	5.31	45.8
5/24	1.70	12.6	5.58	44.3
6/3	1.74	11.2	6.58	58.8

* Each value is the mean of at least 3 clearance periods. P = phosphate, C_{Cr} = creatinine clearance, C_P = phosphate clearance.

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