Renal Clearance of Calcium and Phosphorus in Experimental Urolithiasis

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THE occurrence of urolithiasis has long been a clinical problem in man as well as almost every other species in which it has been studied. The frequent presence of stones and/or calcification in kidneys of ulcer patients maintained on milk and alkali diets led to the description of a "milk-alkali syndrome" by Burnett et al.[1] in 1949. Since then, many references to specific cases, such as those included in a review by Kushner,[2] testify to the concern about urolithiasis in man.

So many factors have been implicated in urolithiasis that the etiology remains obscure. Several of the factors which have been suggested are mineral imbalance, avitaminosis A, hypercalcemia, hypercalciuria, hyper- and hypophosphatemia, hypervitaminosis D, hyperparathyroidism, infection, low pH, lack of colloids in urine, alkalosis, endocrine disturbances, skeletal neoplasms, dehydration, dietary deficiencies and excesses. It is clear that many of these are interrelated so that the over-all picture is complex.

For that reason several approaches to studying urolithiasis under experimental conditions have been initiated in recent years. Yarbro[3–7] in a series of studies on human beings investigated the physiochemical relationships of calcium in the urine of habitual calculus formers. Sager and Spargo[8] produced urinary calculi in protein-depleted rats on a low phosphorus diet, and by means of balance studies found that renal excretion of calcium and citrate were increased, precipitating as calcium citrate calculi. In their study on guinea pigs, when Maynard et al.[9] produced calcification in the kidney by creating a mineral imbalance (lowered magnesium in the diet), they found serum magnesium was lowered and serum phosphorus was increased. No report was found in the literature in which an attempt was made to study the functional activities of calcified kidneys as far as filtering and reabsorbing certain blood constituents such as calcium and phosphorus were concerned. That renal function is altered in the presence of urolithiasis was shown by Bjorneboe et al.,[10] who found that it was only 50 per cent of normal in two patients with calcinosis renis.

It appeared that a study of renal clearances in the presence of urolithiasis would be valuable in the understanding of basic processes leading to calcification. Since the rat has been firmly established as a valuable experimental animal in which to study human afflictions and since methods of making renal clearance studies in this animal were available in the literature, the rat was chosen for the present study. Urolithiasis was known to be producible by the feeding of a milk-alkali diet devised by Sambhavapol et al.[11] and modified by Empey.[12] Knowing that it could be produced by this dietary means, it was possible to study renal functions in both rats with urolithiasis and healthy ones by measuring the filtration and reabsorption of calcium and phosphorus in the two groups of animals.

METHODS AND PROCEDURES

Fifty albino female weanling rats were used. Thirty were of the Yale strain which has

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been maintained as a stock colony at the Cornell small animal laboratory for about thirty years; the others were of the Wistar strain. Ten of each strain were given a "non-calculus-producing" diet of ground Big Red Dog Food and ten of each were fed the following "calculus-producing" diet used by Empey.[12]

Parlac (dry whole milk)	1,000 gm.
$NaHCO_3$	30 gm.
Solka floc (cellulose)	20 gm.
Delsterol (vitamin D_3)	0.66 gm.
$MnCl_2.4H_2O$	115 mg.
$CuSO_4.5H_2O$	62 mg.
$FeCl_2.6H_2O$	420 mg.
KI	160 mg.

The ten remaining Yale strain rats were given the same milk-alkali diet plus 10 mg. per cent of magnesium. The trace minerals were added at levels to prevent known mineral deficiencies. All diets were fed *ad libitum* and distilled water was freely accessible.

The animals were maintained on the respective diets from the time of weaning until nine months of age, at which time each animal was subjected to a renal clearance test. Empey[12] had found that kidney calcification was detectable at seven months in female rats on the milk-alkali diet.

Within ten days of the time each renal clearance study was performed, each animal was unilaterally nephrectomized so that a roentgenogram of one kidney could be taken. The degree of calcification was determined by an arbitrary rating of the roentgenogram, on a scale from 1 to 6, with 1 representing no calcification and 6 representing massive calcification.

The animals were then allowed to recover approximately one week, after which time they were given a diet they had not received previously. Those which had been receiving the two milk-alkali diets were given laboratory chow and those which had been receiving chow were given the milk-alkali diet without the magnesium supplement. This time the laboratory chow was supplemented with 1 per cent ammonium chloride in an attempt to reverse calcification. After periods of from thirty-five to sixty-nine days on these diets, the rats were sacrificed, the remaining kidney was removed, a roentgenogram was taken and rated as the previously excised organ had been.

Modified methods of Friedman et al.[13] and Corcoran et al.[14] for renal clearance procedures, using *p*-aminohippurate to measure renal plasma flow, were used. The filtration and reabsorption rates of calcium and phosphorus were determined by measuring the respective elements in plasma and urine. Calcium was analyzed by the method of Bachra et al.[15] and inorganic phosphate was determined by modifications of the method of Shinowara et al.[16] *P*-aminohippurate concentrations were measured by the method described by Friedman et al.[13] The necessary modifications of these methods were set forth in detail by Payne.[17]

Statistical interpretation of data incorporated the methods of Snedecor.[18]

RESULTS AND COMMENTS

In all animals on the milk-alkali diets, an 85 per cent incidence of calcification occurred in comparison with an incidence of 20 per cent among animals on the control diet of ground laboratory chow. The Wistar strain rats showed a little more susceptibility to urolithiasis on the control diet and a greater degree of calcification on the milk-alkali diets.

The means of degree of calcification of the two groups of Yale strain rats receiving the milk-alkali diet and the milk-alkali diet plus magnesium were 2.3 ± 0.9 and 2.3 ± 1, respectively, as compared to 1.1 ± 0.4 in control animals. Calculation of the analysis of variance between each group and the control group resulted in "P" values of 0.14 and 0.20, respectively. In the rats of Wistar strain, the mean degree of calcification was 2.9 ± 1 in the group receiving the milk-alkali diet and 1.3 ± 0.5 in the control group. The "P" value in this case was 0.20. Although the analyses of variance did not show a statistically significant difference at the 5 per cent level between control animals and afflicted animals, the "P" values did indicate there was a biologic

TABLE I

Filtration and Reabsorption of Calcium and Phosphorus in the Kidneys of Yale Strain rats With and Without Renal Calcification

Rat No.	Effective Renal Plasma Flow (ml./min.)	Glomerular Filtration Rate (ml./min.)	Urine Flow (ml./min.)	Calcium				Phosphorus			
				Filtered (μg./min.)	Excreted (μg./min.)	Reabsorbed (μg./min.)	Per Cent Reabsorbed	Filtered (μg./min.)	Excreted (μg./min.)	Reabsorbed (μg./min.)	Per Cent Reabsorbed
Diet I											
1	2.73	0.428	0.0289	27.0	0.6	26.4	97.8	30.4	4.7	25.7	84.5
3	3.63	0.571	0.0267	38.4	0.0	38.4	100.0	43.4	4.8	38.6	88.9
4	2.84	0.445	0.0311	28.0	6.1	21.9	78.2	91.7	2.4	89.3	97.4
5	0.96	0.149	0.0133	9.0	0.4	8.6	95.6	6.7	0.5	6.2	92.5
6	1.25	0.198	0.0200	5.3	0.4	4.9	92.5	12.7	0.5	12.2	96.1
7	2.00	0.315	0.0200	22.9	0.0	22.9	100.0	15.1	0.8	14.3	94.7
8	2.41	0.378	0.0211	17.5	1.1	16.4	93.7	30.6	0.6	30.0	98.0
9	1.00	0.157	0.0178	4.3	0.0	4.3	100.0	11.5	0.3	11.2	97.4
10	2.04	0.320	0.0244	20.2	0.0	20.2	100.0	17.9	0.6	17.3	96.6
Diet II											
12	0.96	0.150	0.0133	10.7	1.7	9.0	84.1	13.5	0.9	12.6	93.3
13	2.51	0.394	0.0233	22.7	2.0	20.7	91.2	68.2	3.6	64.6	94.7
14	3.82	0.600	0.0411	33.8	0.9	32.9	97.3	58.2	4.8	53.4	91.7
15	2.30	0.361	0.0367	21.4	2.2	19.2	89.7	21.3	0.8	20.5	96.2
16	2.92	0.457	0.0244	27.1	6.1	21.0	77.5	26.5	0.7	25.8	97.4
17	1.66	0.261	0.0156	14.1	4.5	9.6	68.1	20.4	0.7	19.7	96.6
18	2.03	0.317	0.0300	11.2	1.4	9.8	87.5	28.5	2.3	26.2	91.9
Diet III											
21	1.45	0.225	0.0200	8.1	0.0	8.1	100.0	32.9	3.3	29.6	90.0
23	2.35	0.367	0.0289	8.1	0.0	8.1	100.0	34.9	1.4	33.5	96.0
24	0.80	0.125	0.0133	5.6	0.0	5.6	100.0	19.1	2.1	17.0	89.0
25	4.04	0.634	0.0289	31.6	3.4	28.2	89.2	54.4	1.3	53.2	97.6
26	1.81	0.282	0.0289	14.2	0.4	13.8	97.2	30.2	0.6	29.6	98.0
27	1.58	0.248	0.0167	12.9	3.0	9.9	76.7	22.1	1.4	20.7	93.7
28	2.06	0.326	0.0267	23.3	5.9	17.4	74.7	28.0	0.9	27.1	96.8
29	0.78	0.123	0.0133	6.9	0.7	6.2	89.9	15.3	0.6	14.7	96.1
30	2.95	0.461	0.0289	31.0	2.9	28.1	90.6	88.5	7.8	80.7	91.2

significance in the occurrence of urolithiasis in animals which received the milk-alkali diets. The significance of 5 per cent usually sought in biologic data might have been obtained had the experimental period been longer. The present experiment was not designed to test the idea.

The difference in mean values for calcium in plasma as between strains and between healthy and afflicted animals was not significant. The average values were in a normal range from 7.9 to 9.5 mg. per 100 ml. Thus, in the present study, the hypercalcemia implicated by other investigators in urolithiasis did not appear to play a part.

The mean level of phosphorus in plasma was significantly higher in the Yale strain rats receiving the milk-alkali diet plus magnesium (12.0 ± 4.4 mg. per 100 ml.) than in control animals (8 ± 3.3 mg. per 100 ml.). The "P" value was 0.04. Although the level of phosphorus in the plasma of the group receiving the milk-alkali diet without magnesium was not significantly higher (9.2 ± 3.7 mg. per 100 ml.), it was higher than in the control animals. In the Wistar strain rats, the mean level of phosphorus was 7.9 ± 2.8 mg. per 100 ml. plasma in the group receiving the milk-alkali diet and 13.9 ± 5.0 mg. per 100 ml. in the control group. The "P" value in this case was 0.005.

Hyperphosphatemia occurred in Yale rats only when magnesium was added to the milk-alkali diet and in Wistar rats when no supplement of magnesium was given. This seeming

TABLE II

Filtration and Reabsorption of Calcium and Phosphorus in the Kidneys of Wistar Strain Rats With and Without Renal Calcification

Rat No.	Effective Renal Plasma Flow (ml./min.)	Glomerular Filtration Rate (ml./min.)	Urine Flow (ml./min.)	Calcium				Phosphorus			
				Filtered (μg./min.)	Excreted (μg./min.)	Reabsorbed (μg./min.)	Per Cent Reabsorbed	Filtered (μg./min.)	Excreted (μg./min.)	Reabsorbed (μg./min.)	Per Cent Reabsorbed
Diet I											
31	1.63	0.245	0.0158	14.0	0.0	14.0	100.0	20.6	0.4	20.2	98.1
32	2.83	0.444	0.0289	26.4	0.0	26.4	100.0	28.9	1.4	27.5	95.2
33	1.31	0.204	0.0156	12.6	0.0	12.6	100.0	15.8	0.8	15.0	94.9
34	3.98	0.625	0.0195	37.9	5.4	32.5	85.8	31.9	0.6	31.3	98.1
35	3.05	0.480	0.0317	13.2	0.6	12.6	95.5	46.1	1.3	44.8	97.2
36	1.96	0.306	0.0156	15.1	0.3	14.8	98.0	24.5	1.7	22.8	93.1
38	3.96	0.620	0.0267	16.0	5.0	11.0	68 8	67.0	0.9	66.1	98.7
39	1.39	0.219	0.0089	6.3	0.0	6.3	100.0	15.8	1.6	14.2	89.9
40	2.17	0.339	0.0359	20.1	3.3	16.8	83.6	26.1	1.5	24.6	94.3
Diet II											
41	2.48	0.387	0.0222	22.5	12.4	10.1	44.9	42.6	3.7	38.9	91.3
42	3.58	0.564	0.0289	32.5	11.5	21.0	64.6	34.4	4.9	29.5	85.8
43	3.49	0.550	0.0452	18.5	16.1	2.4	13.0	64.4	1 8	62.6	97.2
44	2.19	0.346	0.6267	22.4	9.8	12.6	56.3	65.4	4.5	60.9	93.1
45	2.00	0.314	0.0178	24.5	7.7	16.8	68.6	35.2	3.5	31.7	90.0
46	2.24	0.351	0.0244	18.7	1.0	17.7	94.7	35.5	1.2	34.3	96.6
47	1.73	0.271	0.0200	17.6	1.5	16.1	91.5	58.5	3.3	55.2	94.4
48	1.66	0.260	0.0167	10.3	3.7	6.6	64.1	29.6	0.5	29.1	98.3
49	1.00	0.157	0.0144	9.1	2.5	6.6	72.5	37.7	0.5	37.2	98.7
50	2.00	0.314	0.0211	17.3	0.0	17.3	100.0	40.5	3.2	37.3	92.1

contradiction in regard to magnesium might indicate that the levels of calcium and magnesium in plasma are additive and that phosphorus exists in proportion to the total of the two elements rather than just calcium as expressed in the following relationship mentioned by Kushner:[2]

$$\frac{Ca^{++} \times HPO_4^{=} \times HCO_3^{-}}{pH} = K$$

There was no excess of phosphorus or deficiency of calcium in the milk-alkali diet, so that hyperphosphatemia mediated through hyperfunction of the thyroid, caused by hypocalcemia, did not appear to offer an explanation. The ratio of calcium to phosphorus was 1.3:1 in the milk diet, which lay within the range of ratios of 2:1 to 1:1 considered to be favorable for growth and bone formation. Only the fact that phosphorus was completely reabsorbed after filtration in the kidney, whereas calcium was not completely reabsorbed as in normal kidneys, seemed to explain the hyperphosphatemia in the present study.

The results of the renal filtration and reabsorption of calcium and phosphorus are shown in Tables I and II. The effective renal plasma flow values, as determined by measuring renal clearances of *p*-aminohippurate, were lower than values summarized by Smith.[19] However, our values were for female rats which, of necessity, were lightly anesthetized immediately before the collection period and analyses were made on blood taken by heart puncture. The fact that both control animals and afflicted animals were subjected to a highly standardized procedure appeared to validate the results for the comparative purposes we were interested in.

The glomerular filtration rate was calculated by multiplying the effective renal plasma flow per 100 gm. body weight by a factor of 0.16 on the basis of Smith's[19] figure of 0.157 ± 0.023 calculated from Friedman's[13] data. It was realized that it would be more satisfactory

to actually measure glomerular filtration rate in each individual animal, but so many analyses were being made on approximately 1 cc. plasma and 1 cc. urine that another analysis seemed prohibitive. Since the literature indicated the 0.157 was fairly constant, even among investigators, it was considered valid to apply the factor as long as interpretation of results were made in light of that application.

The quantity of calcium filtered was calculated by multiplying the calcium in the plasma by a factor of 0.6, a compromise figure between the 50 per cent and 75 per cent figures found in the literature for the diffusible fraction of calcium. Phosphorus was considered to be 100 per cent diffusible.

Data in Tables I and II show that calcium reabsorption was inhibited. The possibility existed that the actual glomerular filtration rate was impaired by calcification. However, the greater quantities of calcium excreted by the afflicted rats contraindicated the impairment of filtration, but indicated rather the inhibition of reabsorption. In only one instance was the rate of calcium reabsorption above 21 μg per minute in an animal (No. 25) with definitely calcified kidneys in contrast to rates up to 38.4 μg. per minute in healthy animals. The filtration and reabsorption of phosphorus, on the other hand, remained the same in healthy and unhealthy animals. It would appear, therefore, that the tubular reabsorption of calcium was independent of phosphorus, as stated by Fanconi,[20] and that the apparent imbalance of the two elements in plasma was a reflection of the kidney's handling of them.

Another question arises as to the role that sodium bicarbonate plays in the process of urolithiasis. Although calcification of the kidney occurs frequently in aged animals without sodium bicarbonate appearing in the diet as such, the presence of kidney stones in the older animal may reflect an accumulative effect of this or any other ion. The bicarbonate ion was included in the formula presented by Kushner[2] for the relationships between calcium and phosphorus in plasma. If the bicarbonate was being filtered out at the kidney with no reabsorption, then the presence of calcium (not being reabsorbed) and the absence of the phosphate (due to complete reabsorption) would be conducive to precipitation in the urinary tract. Unfortunately, the calculus material was not analyzed, nor were renal clearances of bicarbonate obtained. Future work on both these phases is anticipated.

Comparisons of the degree of calcification between the right kidney (removed surgically at time of renal clearance tests) and the left kidney (removed at death thirty-five to sixty-nine days later) showed no definite change. The animals which had been changed from the milk alkali diet to laboratory chow plus 1 per cent ammonium chloride in the interim did not show any evidence of reversal of calcification. Nor was there any indication of the development of calcification in those animals changed from laboratory chow to the milk-alkali diet during the intervening period. Unfortunately, only an average of five of every group of ten survived to be changed from one dietary regimen to another. The group III Yale strain rats were sacrificed immediately after renal clearance tests were performed.

It would appear that more studies of this type would not only unravel some of the complexities of urolithiasis, but also shed light on some basic facts about metabolism in general as it is reflected in blood levels of certain constituents which are normally filtered by the kidney. Whether calcification is the cause or the effect of changes in renal function could possibly be answered by such technics. It seems possible that urinary calculi are of different compositions depending upon the particular imbalance that exists in the tubules and urinary tract as a result of the filtration and reabsorption of imbalanced proportions of ions from the blood. If reversal of urolithiasis is to be accomplished, an understanding of such processes would be invaluable.

SUMMARY AND CONCLUSIONS

In a study of the renal filtration and reabsorption of calcium and phosphorus in healthy albino female rats and ones with urolithiasis, it was found that with calculus-producing diets calcium reabsorption was

inhibited, while phosphorus reabsorption was not. Hyperphosphatemia was found in Wistar strain rats receiving a milk-alkali diet and in Yale strain rats receiving the same milk-alkali diet with 10 mg. per cent of magnesium added. Wistar strain rats showed a slightly greater tendency toward urolithiasis than Yale strain rats, but not significantly so. No indication of reversal of calcification was found in animals given laboratory chow plus 1 per cent ammonium chloride, nor was urolithiasis observed in animals given a milk-alkali diet, after the nine-month experimental period.

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