Kidney International, Vol. 36 (1989), pp. 918–937

NEPHROLOGY FORUM

Phosphate excess and progressive renal failure: The precipitation-calcification hypothesis

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Case presentation

A 44-year-old black woman with incomplete exstrophy of the urinary bladder has been followed for renal insufficiency and radiopaque calculi over the last 5 years. Twenty-two years ago, the right kidney was removed and the left ureter implanted into an ileal conduit because of severe infection and obstruction; the serum creatinine then was 1.3 mg/dl. During the ensuing 8 years, serum creatinine fluctuated between 1.4 mg/dl and 1.8 mg/dl, serum calcium between 8.9 and 10.2 mg/dl, and serum phosphorus between 3.3 and 4.5 mg/dl. The 24-hour urine calcium and phosphorus levels ranged between 30 and 150 mg/dl and 350 and 680 mg/day respectively. Fourteen years ago, a left nephrolithotomy was performed; postoperative serum creatinine levels were 1.6 and 1.7 mg/dl. Bacteriuria has been present consistently and, a couple of times yearly, she has required systemic antibiotics for overt urinary tract infections. No urosepsis has been documented.

The serum creatinine concentration averaged 1.8 to 1.9 ten years ago. An ileal "loopogram" at that time revealed three 2.5 cm opaque calculi in the left lower pole calyces, with a dilated middle calyx and reflux of contrast material. Serum chloride (108 to 111 mEq/liter), carbon dioxide (18 mEq/liter), and blood pH (7.34) 7 years ago were consistent with mild hyperchloremic metabolic acidosis. The 3 calculi appeared denser 6 years ago, although their size was unchanged. The serum creatinine gradually rose to 2.2 mg/dl; the serum calcium (9.6 to 10.0 mg/dl) and phosphorus (3.2 mg/dl) remained within normal limits. In a typical 24-hour urine sample, volume was 2000 ml; creatinine, 1.37 g; oxalate, 40 mg; phosphorus, 560 mg; sodium, 178 mEq; uric acid, 512 mg; calcium, 102 mg; and citrate, 373 mg. No gross hematuria was noted, and no gravel was passed. She took trimethoprim-sulfamethoxazole between 5 and 8 years ago.

Five years ago, her blood pressure was 132/72 mm Hg. She weighed 80.5 kg and had a normal cardiopulmonary examination. The ileal cutaneous fistula was clean and healthy. There was no tenderness in the costovertebral angles or in her abdomen. The extremities were free of edema. The urinary specific gravity was 1.009. Dipstick testing of the urine revealed: pH, 6; no glucose or ketone; 1+ proteinuria, and a trace of blood. Occasional bacteria, 2 red blood cells, and 10 to 15 white blood cells/high-power field were apparent on microscopic examination of the urine sediment. Crystals and casts were absent. The hemoglobin was 14.1 g/dl and the hematocrit was 40.8%.

At that time, the working diagnosis was mild renal insufficiency due to reflux nephropathy. The problem was complicated by chronic bacteriuria and pelvocalyceal calculi, presumably containing phosphate. Lithotripsy, attempted twice, was only partially successful in breaking up the calyceal stones. On analysis, one calculus consisted of 58% struvite ($Mg NH_4 PO_4$), 32% carbonate apatite, 2% NH_4 acid urate, and 8% blood. She was treated with calcium carbonate, and small doses of aluminum hydroxide if she became hypercalciuric, to prevent phosphate retention. Fluids were prescribed to increase urine volume to greater than 2.5 liters/day; this goal was easily achieved. Initially her diet was not modified. Two years ago, sodium bicarbonate was given to maintain the serum bicarbonate over 16 mEq/liter. Furosemide soon was added to sustain a normal blood pressure. Between one and five years ago, her urine phosphorus ranged between 101 and 412 mg/day (mean ± SE, 233 ± 32 mg); the mean ± SE for urine calcium was 116 ± 16 mg/day. Urine phosphorus-to-creatinine ratio fell from 0.41 to 0.19 ± 0.030. The serum phosphorus ranged from 2.7 to 4.3 mg/dl (mean ± SE, 3.6 ± 0.2). Serum creatinine averaged 2.2 ± 0.1 mg/dl. Serum calcium averaged 9.8 ± 0.4 mg/dl. The patient has remained normotensive and asymptomatic from overt infection, renal colic, or signs of complications from azotemia.

Discussion

DR. KAI LAU (*Director of Nephrology, Michael Reese Hospital, and Associate Professor of Medicine, University of Chicago, Chicago, Illinois*): After the process of renal disease is initiated, limited therapeutic modalities are available to nephrologists for reversing and/or stabilizing the disease course. For this reason we have learned to be vigorous and aggressive in controlling hypertension. We deliberately avoid nephrotoxic insults like unnecessary exposure to contrast materials, aminoglycosides, phenacetin-containing analgesics and, lately, nonsteroidal antiinflammatory agents. We attempt to correct reversible factors such as infection, obstruction, and renal hemodynamic compromise, and we counsel against the continued use of heroin or sensitizing antibiotics. Unfortunately, the clinical course of an established disease is relentlessly downhill. Therefore the concept that manipulation of dietary factors like

Presentation of the Forum is made possible by grants from Pfizer, Incorporated; Merck Sharp & Dohme; and Sandoz, Incorporated.

sodium, protein, calories, and lipids might halt progressive renal disease is exciting.

In the last 8 years, three Forum discussants have reviewed the role of dietary protein [1–3]. Although my focus is on phosphate, some reference to protein becomes inevitable and perhaps even useful. I would like to raise a testable hypothesis that I hope will stimulate more research on this clinical problem. This hypothesis, known as the precipitation-calcification hypothesis, proposes that phosphate absorbed in excess of residual nephron excretory capacity produces precipitation and deposition of calcium phosphate microcrystals in the tubular lumen, peritubular space, capillaries, and interstitium and is thus responsible for progressive functional deterioration in chronic renal failure. In other words, an undue phosphate burden is deleterious to the anatomic and functional integrity of residual nephrons. Pathophysiologic alterations and eventually pathologic destructions ensue, further compromising renal function and setting up a vicious cycle by precipitating more phosphate and calcium in the renal parenchyma.

Despite a number of studies on the presumed benefits of a low-phosphate diet on renal function (Table 1), it is still unclear whether reduced dietary phosphorus per se is *solely* responsible.

To assess the separate roles of phosphate and protein in the progession of renal disease, it is logical to turn first to studies in humans with renal disease. Unfortunately, none of the published studies provides such insights. Each published study contains the following confounding variables that make it impossible to identify the unique effects of protein and phosphate restriction. (*1*) Protein restriction and phosphate restriction were simultaneous. (*2*) Study periods were too short to observe an effect. (*3*) The number of patients studied was small. (*4*) Glomerular filtration rate (GFR) was rarely measured. Instead, serum creatinine was usually employed to evaluate renal function. Given that creatinine production fell in many such patients, the stability in serum creatinine over time might not have been a reflection of stable glomerular function. (*5*) In some studies, patients included in one study might have been included in a later study. (*6*) Contemporaneous, well-matched controls were rarely used; historic controls were more commonly employed. (*7*) The possible role of more intensive medical care related to more frequent visits to physicians was not excluded. Although the results were negative, one of the earliest studies [4] amply illustrates the problems created by these confounding variables. Given these serious flaws in experimental design, we are unable to draw any conclusion about the independent effects of phosphate restriction on renal function from clinical trials.

To validate this opinion, I shall first review the principal studies in this area. Maschio and coworkers examined the effects of a 650 mg/day phosphorus diet in 3 groups of patients with mild-to-moderate renal insufficiency, and compared these effects with those of a 900 mg intake before the diet and with those of 30 different patients on an ad-libitum phosphorus diet in the same patients [5, 6]. The reciprocal of serum creatinine against time was stable in the patients with an initial serum creatinine of 2.2 mg/dl, it progressed minimally in those with a creatinine of 4.2 mg/dl, and it declined significantly less in those with a creatinine of 6.1 mg/dl relative to the control. There are several problems in their study design, however.

First, their controls were derived from a different population, and the subjects were followed according to a different protocol. Second, the study was brief, especially for those with a starting serum creatinine of less than 2.5 mg/dl. In the group with a serum creatinine of 6.1 mg/dl, for example, 40% experienced a deterioration in the reciprocal of serum creatinine against time in 2 years [6]. Third, they failed to measure GFR. Diets similar to the ones they used are known to produce muscle wasting. Without serial body weights or 24-hour urine creatinine measurements, it is unclear whether the minimally elevated or unchanged serum creatinine values truly reflected a stable GFR. Fourth, the dietary protein was also reduced from 70 to 42 g/day; simultaneously, dietary calcium was increased to 1.25 g/day. Two years later [7], this same group reported similar improvements in 78 patients treated for an average of 42 months with a phosphate-restricted *and* protein-restricted diet compared with 22 retrospective controls on a free diet followed for 24 months. Although the hypertensive and nephrotic patients experienced faster renal functional decline than did patients with either polycystic kidney disease or chronic pyelonephritis, these concerns still apply.

About the same time, Barsotti et al tried to retard progressive renal failure by using a diet low in phosphorus (475 versus 850 mg/day) and nitrogen (39 versus 56 g protein/day). Over the 11 and 21 months of studies respectively in one group of 20 and another group of 29 patients with moderate renal insufficiency [8, 9], urinary phosphorus was reduced (324 versus 650 mg/day) in the first group. Endogenous creatinine clearance showed either no change in the shorter study (versus 5 ml/min fall per year in the pre-experimental-diet control period) [8] or a small decline in the longer study (1.7 versus 6.6 ml/min per month in the pre-diet control period) [9]. The fall in creatinine clearance directly correlated with 24-hour urinary phosphorus but not with urinary urea, implicating a closer relationship with dietary phosphorus (Fig. 1). In 1986, the same authors reported that a similar dietary approach, that is, a 500 mg phosphorus and 35 g protein diet supplemented by 1.2 g calcium, yielded stable creatinine clearance and body weight for 12 months in 85 patients with a mean creatinine clearance of 9.5 ml/min [10]. Besides being brief in duration, it is impossible to know how many of the original 49 patients, if any, were included in this later study. The major problem with these studies is the lack of prospectively randomized contemporaneous controls with identical characteristics and followup protocol.

Three other studies, involving 7, 10, and 4 patients and lasting only 3, 4, and 6 months respectively [11–13], also suggested that a diet reduced to 35% to 50% of "normal" phosphorus and protein intake stabilizes serum creatinine [12] and creatinine clearance [11, 13] in patients with moderate-to-advanced renal insufficiency. These studies suffer from the same problems, however: inappropriate controls, brevity, lack of GFR data, and failure to exclude other nutrients as confounding variables. For example, 5 years after the original publication, Tessitore et al reported a decline in iodothalamate clearance from 44 to 12 ml/min in 6 other patients treated with a similarly low (700 mg) phosphorus diet for 29 months [14].

Could we tease out the role of a low phosphorus intake by examining the effects of a low-protein diet per se? Unfortunately, similar reservations also apply to studies on protein restriction (Table 2). For example, although Barsotti et al

Table 1. Beneficial effects of low-phosphorus diet in clinical renal diseases

Ref.	Diet P	Rx Time	No. of Patients	Basal serum creat	Control data	Parameters compared: BUN	Serum creat	Serum creat^{-1}	Urine protein	C_{creat}	Serum Ca	Serum P	Other variables: Dietary protein	BW data	Comments
4	"Low P" + $Al(OH)_3$	10 mon	10	5.3 mg/dl	Historic data in same + 10 others	NA[a]	1.9 mg% rise vs 1.9 mg% in control	Variable Δ	NA	NA	↑	↓	?↓	Not given	↓ [Ca × P] from 48 to 34; no concurrent control
5			25	2.2 mg/dl	Historic data in same pts. + 30 others (S_{creat} = 2.3 mg/dl)		↑ in only 12% or 3 pts.	Stable with time							↑ diet Ca to 1.25 g/day; ↓ urine P (377 vs 558 mg/day); controls different in source and followup only
		3–4 yrs	20	4.2 mg/dl		↓	↑ in only 20% or 4 pts.	Little decline							
6	650 vs 900 mg/day	2 yrs	8	6.1 mg/dl		↓	↑ in 38% or 3 pts.	Decline in 3 pts.	NA	NA	↑	↓	↓ to 42 vs 70 g/day	Not given	
7	700 vs 900 mg/day	44 mon	33 GN[a]	2.2 mg/dl	22 retrospective controls on free diet	NA	Doubling time = 8.7 yr.	Fell at 9% of control	NA	NA	NA	NA	↓ to 42 vs 70 g/day	Not given	↑ diet Ca to 1.25 g/day; controls followed only 24 mon; functional decline faster in hypertensives and nephrotics
		42 mon	17 PKD[a]	2.4 mg/dl			= 8.5 yr	Fell at 13% of control							
		41 mon	28 CP[a]	2.6 mg/dl			= 14.9 yr	Fell at 5% of control							
8	500 vs 850 mg/day	11 mon	20	3.85 mg/dl	Historic free diet in same + 19 others	↓	No Δ vs a rise in control	NA	NA	No fall (vs 5 ml/min fall in control)	↑ (↓ PTH)	↓	↓ to 35 vs 56 g/day	Not given	↑ diet Ca by 1 g/day; ↓ urine P (325 vs 650 mg/dl)
9	455 vs 850 mg/day	21 mon	29	C_{creat} = 22 ml/min	Same as above + 26 others on low-protein diet alone	↓	No Δ	Not given	NA	↓ by 1.7 (vs 6.6 ml/min in control)	↑	↓	↓ to 42 vs 56 g/day	Not given	↑ diet Ca by 1 g/day; urine P (362 vs 629 mg/day); followup 1 vs 4 mon
10	500 mg/day	12 mon	85	C_{creat} = 9.5 ml/min	In same pts. fed only low-protein diet	NA	Not given	NA	NA	No Δ	NA	NA	↓ to 35 g/day	No Δ	↑ diet Ca by 1.2 g; ↓ diet Na; diuretics
11	500 mg/day	3 mon	7	C_{creat} = 6.1 ml/min	Historic control in same pts.	NA	Not given	NA	NA	No Δ	↑ (↓ PTH)	↓	↓ to 14 g/day	Not given	EAA/KAs added (8.4 mg N_2/kg/day)
12	350 mg/day	4 mon	10	5.7 mg/dl	Historic control in same pts.	↓	No Δ	NA	NA	No Δ	No Δ	No Δ	↓ to 21 vs 70 g/day	No Δ	EAA added
13	21 vs 46 mg/kg BW/day	6 mon	4	C_{creat} = 16.4 ml/min/1.73 m^2	Historic control in same pts.	No Δ	0.2 ↑ vs 0.4 mg/dl in control	NA	NA	NA	No Δ (↓ PTH)	No Δ	↓ from 2.5 to 1.7 g/kg/day	Not given	↓ urine P by 60%; ↓ diet Ca by 30%; all on calcitriol

[a] BW, body weight; GN, glomerulonephritis; PKD, polycystic kidney disease; CP, chronic pyelonephritis; C_{creat}, creatinine clearance; EAA, essential amino acid; KAs, keto acid analogues; NA, not applicable.

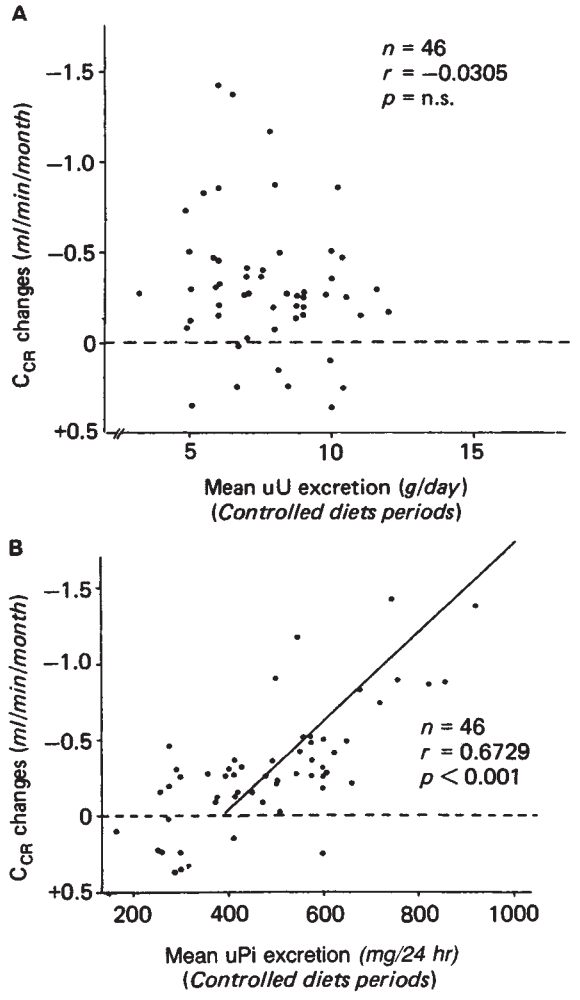

Fig. 1. *The change in creatinine clearance (negative signs indicating a fall) was not correlated with 24-hour mean urine urea (uU) excretion (A), but with mean urine phosphorus (uPi) excretion during periods of control diets (B).* (From Ref. 9.)

attributed the slower decline in creatinine clearance over 10 months in 12 patients to a low-protein diet, we face the same problem in assessing this result. In particular, the simultaneous reduction in dietary phosphorus (from 600 to 300 mg/day) and increase in dietary calcium confounds interpretation of the effect of a single variable [15].

This is also true in another study that compared a low-protein diet in 31 patients with a mean serum creatinine over 6 mg/dl to another 10 patients on an ad-libitum diet [16]. Although the rate of increase in serum creatinine was slower over 2 to 3 years in the treated patients, GFR was not measured. Such a study leaves open the possibility that decreased creatinine production, rather than a change in GFR, is responsible for the stability in serum creatinine over time [12]. In addition, an independent effect of dietary phosphorus restriction was not excluded.

A Swedish study also showed that in 17 patients with a serum creatinine of 10 mg/dl, treatment with an 18 g/day protein diet for 1.5 years reduced the slope of the reciprocal of serum creatinine against time [17]. Such studies in a smaller group and lasting even a shorter time are open to identical criticisms: without serial body weights and GFR data [17], changes in serum creatinine might merely reflect muscle wasting and falling creatinine production [18, 19]. Furthermore, the role of reduced phosphorus intake (to 350 mg/day), enteric phosphate binders, and more frequent physician visits (every 33 versus every 55 days), which could affect blood presure control, could not be dissociated.

Mitch and coworkers studied a 25 g/day protein diet supplemented with essential amino acids (EAA) and ketoacid analogues (KA) [20]. Although an improvement was evident in 60% of the 17 patients who had a defined rate of decline during the pre-diet period, serum creatinine deteriorated in 6 of the 7 patients without a defined rate of decline in the pre-diet period. Again, the use of historic controls, the short duration of the study, the limited creatinine clearance data (measured only in one-third of the subjects), and the simultaneous reduction in dietary phosphorus (600 mg/day) preclude conclusions about the separate effects of the individual dietary manipulations.

A British group treated 39 patients with a 35 g/day protein diet for 6 months and found an improvement in the slope of the serum creatinine in 93% of the patients, with the best response in those with tubulointerstitial diseases [21]. Unfortunately, this study is vulnerable to the same four criticisms.

Finally, 3 other studies that report improvement in the reciprocal of serum creatinine plotted against time were similarly flawed by their failure to specifically eliminate the confounding variable of phosphate restriction [18, 19, 22]. Two of these also employed historic data as the control [19, 22], had a small sample size, and provided no [22] or uninterpretable data on creatinine clearance (24-hour urine creatinine excretion fell during treatment because of diminished muscle mass) [19]. One study provided prospective, appropriately randomized controls and compared 100 subjects on protein restriction with a similar number on an ad-libitum diet [18]. Again, however, GFR was not measured and the possible role of phosphorus restriction is uncertain [18].

Can we learn more about the single role of protein restriction from animal models of renal disease [23–28]? Several dozen studies demonstrate and confirm a beneficial effect of dietary *protein restriction* on renal pathology [23, 28], proteinuria [23, 25, 27, 28], BUN [18, 21, 23], serum creatinine [18, 22, 26, 27], and survival [23, 24, 26, 27] (Table 3).

In one study of rats with nephrotoxic serum glomerulonephritis, for example, a low-protein diet for 10.5 months improved renal histology, decreased proteinuria, decreased BUN, and decreased the number of deaths due to renal disease [23]. In a model of immune glomerulonephritis, a low-protein diet for 6 months was also found to ameliorate the severity of the renal lesion [24]. In rats subjected to eight-tenths nephrectomy, Ritz et al found that the mortality rate due to uremia was decreased by a lower protein intake [29]. In another study, the histopathology, proteinuria, and number of renal-related deaths was decreased by a protein-restricted diet in rats with five-sixths nephrectomy [26]. In all these studies, the presence of other nutrients cannot be factored out as confounding variables, and the lack of data on GFR leaves the conclusions questionable.

Estimates of GFR were attempted in 3 experimental studies that suggest a salutary effect of a low-protein diet on renal disease [25, 27, 28]. Two showed no changes in GFR [25, 28]. In

Table 2. Beneficial effects of low-protein/low-nitrogen diets in clinical renal diseases[a]

Ref.	Dietary protein	Rx Time	No. of Patients	Baseline	Control data	Parameters compared							Other variables		Comments
						BUN	Serum creat	Serum creat^{-1}	Urine protein	C_{creat}	Serum Ca	Serum P	Dietary protein	BW data	
15	Daily 0.2 vs 0.5 g/kg BW	10 mon	12	C_{creat} = 7.2 ml/min	Historic data in same pts + 30 others	NA	NA	NA	NA	↓ monthly decline from 0.5 to 0.003	NA	↓ by 1.3 mg/dl	300 vs 600 mg/day	None	↑ diet Ca to 1.53 g/day; EAA and KAs added
16	30 g/day vs ad lib	2–3 yrs	31	Serum creat ≥6 mg/dl	Another & larger group (N = 107)	NA	Slowed the ↑ to 10 mg/dl by > twofold	NA	NA	NA	NA	NA	?	None	KAs used
17	18 vs 50 g/day	1.5 yrs	17	Serum creat = 9.8 mg/dl	Same pts checked q 33 vs 55 day	NA	Done, but not given	Better	NA	NA	↑	↓	↓ to 350 mg/day	None	↑ diet Ca to 0.85 g/day; EAA or KAs, PO_4 binders used
18	0.6 g/day/kg BW 0.4 g/day/kg BW vs ad lib	18 mon	65 vs 66 45 vs 29	C_{creat} 31–60 10–30 ml/min/1.73^2	Prospective randomized control	↓	Slowed the rate of increase	Better (but 24-hr urine ↓ 10%)	↓	NA	No Δ	No Δ	?	No Δ	Measured urine PO_4 not given
19	0.8 g/day/kg BW	6–12 mon	12	Serum creat = 10 mg/dl	Historic control in same pts	↓	↓ in 10/12 pts	Better (24-hr U. creat fell)	↓	No Δ	↑	↓ by 1.7	?	Fell by 3.3 kg	EAA & KAs added; muscle mass fell; 7 pts withdrew
20	25 g/day vs ad lib	20 mon	24	C_{creat} = 7.6 ml/min	Historic control in same pts	↓	Done, but not given	Better in 60% of 17 pts	NA	No Δ in 8 pts studied	No Δ	No Δ	↓ to ≤600 mg/day	No Δ	EAA or KAs added; PO_4 binders, $CaCO_3$ & $NaHCO_3$ used
21	35 g vs ad lib	6 mon	39	Serum creat = 2.7 mg/dl	Historic control in same pts	↓	Done, but not given	93% better		No Δ after 2 wks.	NA	No Δ	↓ to 700 mg/day	No Δ	Best response in tubular diseases
22	0.6 g/day/kg BW 5 day/wk	2–20 yr	20	Serum creat = 2 mg/dl	Historic control in same pts	NA	Slowed the doubling time	Better	NA	NA	NA	NA	?	None	Pre-Rx little decline & ? protein take

[a] Abbreviations are the same as those in Table 1.

Table 3. Beneficial effects of low-protein diets in experimental renal diseases[a]

Ref.	Rat model	Dietary protein	Rx time	Renal lesion	Parameters compared					Exclusion of other variables restriction of diet						
					Urinary protein	Serum creat	GFR	Deaths	"Renal" survival	Na	Ca	PO_4	Calorie	BW	BP	Food data
23	Nephrotoxic serum GN	5 vs 18%	10.5 mon	GN better	↓	(BUN: better	(C_{urea}: better	↓	↑	ND	ND	ND	ND	Yes	ND	None
		40 vs 18%		Worse	↑	Worse)	Worse)	↑	↓							
24	NZB × NZW mice	6 vs 20% (10 vs 20 cal/day too)	6 mon	Reduced immune GN	NA	NA	NA	NA	NA	No	No	No	No	No	ND	None
25	Nephrotoxic serum GN	4 vs 23%	2 mon	GN better	↓	No Δ	No Δ	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes	None
		58 vs 23%		Worse	↑											
26	5/6 Nx	8 vs 16%	5 mon	Better	NA	"Better"	ND	"↓"	↑	Yes	Yes	Yes	Yes	No	No	None after 2 mon
		32 vs 16%		Worse		"Worse"		"↑"	↓							
27	5/6 Nx	8 vs 18%	5.5 mon	Better	↓	Better	Better	↓	↑	No	Yes	Yes	Yes	Yes	Yes	None
28	Adriam-GN	6 vs 20%	1 mon	Better	↓	No Δ	No Δ	NA	NA	No	No	No	No	ND	ND	Qualitative
29	8 × 10 Nx	50 vs 15%	25 days	NA	NA	NA	NA	NA	↓	Yes	No	No	No	Yes	ND	None

[a] ND, not done; NA, not analyzed; No Δ, not different from normal protein diet group; GN, glomerulonephritis; BP, blood pressure; Nx, nephrectomy; Adriam, adriamycin.

one, all the other nutrients were kept identical between the low- and normal-protein diets, but the authors failed to quantify food intake, leaving open the possibility that the improved renal lesion and proteinuria were due to variations in intake of other nutrients [25]. In the other study, treatment with a low-protein diet for one month in rats with adriamycin-induced glomerulonephritis was found to improve the renal lesion and proteinuria, but not the inulin clearance [28].

To the best of my knowledge, only in the study by El-Nahas et al was endogenous 24-hour creatinine clearance found to be improved by 5.5 months of a low-protein diet in rats with five-sixths nephrectomy [27]. Unfortunately, absolute food intake, and therefore phosphorus intake, were not quantified despite identical phosphorus content; thus one cannot exclude the possibility that protein-restricted rats consumed less phosphorus because of anorexia. In addition, measurements as long as 129 days after protein restriction revealed no differences in serial serum creatinine. Although not specified in the text, I surmise (from the data points on the graph depicting creatinine clearances) that the creatinine clearance measurements were made at 129 days [27]. To me, however, it is not clear how a better creatinine clearance result could be obtained in rats fed a low-protein diet when their serum creatinine (at 129 days) was numerically higher and body weight numerically lower.

Such skepticism about the efficacy of protein restriction in preserving renal function is warranted in view of evidence suggesting that a chronic high-protein diet has a *beneficial* effect on glomerular filtration rate [30–39] (Table 4). In sheep with intact kidneys, a low-protein diet decreased GFR [30]. In rats with one-half or three-fourths nephrectomy, Schoolwerth et al showed that one week of a high-protein (40%) diet increased inulin clearance by 29% and 38% [31]. In rats with more severe renal ablation, Hostetter et al also found that a normal-protein diet (24%) sustained a higher GFR than did a low-protein (6%) diet [32]; this finding is one of the bases for the hyperfiltration hypothesis [40–43]. Single nephron GFR in residual nephrons of a remnant kidney model is two- to threefold of the normal unless dietary protein is reduced [32].

After 5 weeks, the increase in GFR produced by a high-protein diet was sustained in normal animals and in animals treated with adriamycin [34]. Even into the eighth week, the beneficial effects of a higher protein diet on renal plasma flow and GFR persisted in these animals [35]. Does the same hemodynamic response occur in humans? One study, which evaluated the effect of a 90 g protein diet for one week, answered affirmatively [34]. In another study, 24 patients with renal insufficiency responded to 4 weeks of protein supplementation with similar increments in GFR (58 ml/min versus 48 ml/min) and effective renal plasma flow (240 ml/min versus 205 ml/min) [37].

It is appropriate, of course, to raise concern about the possible adverse effects of hyperfiltration and glomerular hypertension. These physiologic adjustments to a high protein intake presumably lead to glomerular sclerosis and impaired glomerular function. In adult dogs fed a high- or normal-protein diet, the clearances of both paraaminohippuric acid (PAH) and inulin were significantly higher than those in animals fed a low-protein diet [38]. Even 4 years after three-fourths nephrectomy, the effects of high-protein diets were sustained. In these adult animals, neither histology on light microscopy nor ultrastructure on electron microscopy differed among the 3 different protein diets [38]. In the rat with either uninephrectomy or five-sixths nephrectomy, Hostetter et al also observed sustained benefits on GFR after 8 months of treatment with a high- versus a low-protein diet, despite heavier proteinuria and increased glomerular sclerosis [39]. Considering the average longevity of dogs and rats, these results cast doubt on the prediction or implication that chronic glomerular hypertension and hyperfiltration will eventually cause a progressive decline in GFR.

Given the paucity of evidence from animal studies that protein restriction per se improves GFR, and the substantial data suggesting a salutary influence of a high-protein diet on GFR, we can reasonably conclude that the effects of a low-phosphorus diet on GFR are not mediated by the associated protein restriction. Do we have more concrete evidence that supports a role of phosphorus from animal studies? If excess phosphorus is indeed nephrotoxic, a low-phosphorus diet should ameliorate, and a high-phosphorus diet should aggravate, the impairment of GFR. In addition, at a given dietary phosphorus content, agents that reduce phosphorus absorption or inhibit calcium phosphate precipitation should result in a higher GFR.

Let us examine the evidence (Table 5). In 1978, Ibels et al found that a low-phosphorus diet (0.04 g% versus 0.5 g%), initiated 6 weeks before and continued for 24 weeks after seven-eighths nephrectomy in adult rats, sustains a better serum creatinine with or without enteric phosphate binders [$Al(OH_3)$] [44]. This diet also reduces the serum calcium × phosphorus product, ameliorates the histologic lesions, and increases survival.

Two years later, using a model of antiglomerular-basement-membrane glomerulonephritis, Karlinsky and colleagues not only confirmed these results with a phosphorus-restricted diet but also found that the calcium content in the kidney was reduced compared with that from rats fed a normal-phosphorus diet [45]. The reduced body weight in phosphorus-restricted rats left uncertain whether the serum creatinine truly reflected GFR. These two 6-month-long studies [44, 45] were corroborated, however, and extended by a similar experiment in rats with nine-tenths nephrectomy [46]. In the latter study, a 0.03 g% phosphorus diet decreased the serum calcium × phosphorus product, serum creatinine, incidence of end-stage renal disease (9% versus 70%), tubular lesions, and deaths attributed to uremia when compared with a normal-phosphorus diet [46].

Two studies in the rat subjected to five-sixths nephrectomy failed to detect differences in serum creatinine [47, 48] or creatinine clearance [47], but the negative results are likely due to less stringent phosphorus restriction (20% to 24% of normal) and a shorter evaluation period (3 to 4 weeks [47, 48]). These studies, however, did confirm the attenuation of calcium and phosphorus in renal tissue [48] and prolonged survival with a lower phosphorus intake [47]. Thus, calcium and phosphorus deposits might precede functional deterioration. In one of the studies, survival rates improved with a low-protein diet, and the beneficial effects of low-protein and low-phosphorus diets were additive [47], although the number of animals studied was small.

Thus, increasing evidence suggests that phosphorus restriction exerts a salutary effect on renal damage and GFR. The next question is whether dietary phosphorus loading potentiates

Table 4. Improvement of glomerular filtration rate by chronic increase in dietary protein[a]

Ref.	Model	Dietary protein	Rx time	GFR[b,c]	Urinary protein	Glomerular histology	Food intake	Comments
30	Normal sheep (N = 19 vs 13)	14 vs 5 g%	2 wks	per kg BW, ↑ 31%	NA	NA	? Same	No BW data
31	Rats with 3/4 Nx (N = 6 vs 6)	40 vs 6 g%	1 wk	↑ 29%	NA	NA	? Same	No BW data
	1/2 Nx (N = 6 vs 6)			↑ 38%				
32	Rats with 11/12 Nx (N = 9 vs 11)	24 vs 6 g%	2.5 wks	↑ 24%	NA	Worse	? Same	—
33	Adult dogs with 7/8 Nx (N = 4)	54 & 26 vs 18 & 8 g%	2 wks	21% (C_{PAH} 25%)	No Δ	NA	? Same	6% BW Δ between diets
34	Rats with adriamycin-GN (N = 10 vs 15)	35 vs 20 g%	5 wks	↑ 75%	↑	NA	? Same	Slight ↑ in BW
	Normal rats (N = 15 vs 15)			↑ 45%	↑			
35	Rats with 5/6 Nx (N = 9 vs 8)	25 vs 20 g%	8 wks	↑ 37%	↑	Worse	? Same	BP & BW no Δ
36	Normal (N = 5)	90 vs 70 g/day	1 wk	↑ 18%	NA	NA	? Same	? Same diet
37	30 men with chronic renal disease	85 vs 35 g/day	4 wks	↑ 16% (C_{PAH} ↑ 9% in 24 compliant pts)	↑	NA	? Same	BP no Δ; BW ↑ by 1 kg
38	Adult dogs with 3/4 Nx (N = 10 and 5 vs 6)	56 27 vs 19 g%	4 yrs	↑ 29% & 33%; (C_{PAH} ↑ 32% & 51%)	No Δ	No Δ	? Same	Uremic deaths not correlated with diet; BP, no Δ
39	Intact rat kidneys (N = 9 vs 12)			(4 mon) (8 mon) ↑ 26% ↑ 30%	↑	No Δ or ↑	? Same for 8 mon	Diet P (0.86 vs 0.63 g%) & BW were ↑ by high-protein diet
	with 1/2 Nx (N = 9 vs 9)	40 vs 6 g%	8 mon	↑ 35% ↑ 27%	↑	No Δ or ↑		
	with 5/6 Nx (N = 11 vs 11)			↑ 33% ↑ 22%	↑	↑		

[a] Nx, nephrectomy; GN, glomerulonephritis; PAH, para-aminohippurate; NA, not analyzed; BW, body weight.
[b] GFR measured by clearance of inulin or iodothalamate;
[c] Changes expressed as % of value for normal-protein diet, either measured or interpolated.

renal injury. At least 3 groups have examined this issue; all yielded a positive answer [49–52]. In 1979, Kleinknecht et al reported a deterioration in serum creatinine at 4 weeks and in survival of weanling rats at 20 weeks after seven-eighths nephrectomy, if they were fed a diet high in protein, sodium, and phosphorus (1.58 g% in the experimental rats versus 0.98

Table 5. Effects of low-P diet ion chemistry, histology, and survival in experimental renal diseases[a]

Ref.	Rat (*model*)	Diet P (*g%*)	Rx time	Serum [Ca] × [P]	Serum creat	Urinary protein	Renal lesions	Survival	Comments
44	Adults with 7/8 Nx (N = 12 vs 26)	0.04 ± $Al(OH)_3$ vs 0.5	6 pre- & 24 wks post-Nx	39 vs 105	Better 7 to 24 wks	↓ to 33%	Better	75% vs zero at 24 wks	No BW; no food data
49	Weanlings with 7/8 Nx (N = 10 vs 9)	0.98 vs 1.58	20 wks post-Nx	NA	Better at 4 wks	NA	NA	70% vs 22% at 2 wks	Diet NA & protein also ↓; ↑ food intake
45	Anti-GBM GN (N = 11 vs 13)	0.14 vs 0.05	23 wks	NA	Better from 5 to 13	No Δ	Better	70% vs 10% at 23 wks	↓ kidney Ca; ↓ BW; no food data
50	1/3 and 1/2 Nx (N = 4 per group per diet)	0.5 vs 1 and vs 2	18 wks	NA	↓ at 18 wk; [↑ C_{creat} in 1/3 Nx at 7 wk]	NA	Better; (↑ kidney Ca & PO_4 with Nx & with ↑ diet P)	NA	Fed only 10 g/ day; ↓ BW on 2 g% P diet; no Δ in BP
	Intact on 3 g% P	EHDP vs none	5 wks	NA (↓ s_p)	No Δ	NA	Better	NA	No Δ in C_{creat}
51	Weanlings with 7/8 Nx (N = 11 vs 13)	0.75 vs 0.98	30 wks	NA	Better from the 4th wk on	NA	Better	90% vs 10% at 30 wks	No food data; ↓ BW; ↓ BP; ↓ diet protein, Ca, & Na
52	7/10 Nx (N = 5 vs 12 vs 15)	0.58 vs 2.2 ± phosphocitrate	4 wks	75 vs 102 vs 95	0.58 vs 1.90 vs 0.77	NA	Tubular lesions & calcinosis; only 2.2% P diet	NA	↑ Tissue CA & ↓ BW on 2.2% P diet alone; food data spotty
46	9/10 Nx (N = 11 vs 10 vs 10)	0.03 vs 0.2 vs 0.5	36 wks	↓ with low-P diet	Lower with 0.03% P diet	NA	Fewer tubular lesions	80% vs 10% at 36 wks	After 26 wks, no food data; ↓ BW & ↓ ESRD (9 vs 70%) with LPD; benefits partly mimicked by ↓ food intake
47	5/6 Nx (N = 10 vs 11)	0.12 vs 0.5 g	3 wks	Na (↓ s_p)	No Δ (C_{creat} also similar)	NA	NA	↑ by LPD only in very uremic rats	No food data; low (6%) protein diet ↑ survival & ↓ s_p regardless and synergistic with ↓ P diet
48	5/6 Nx (N =?)	12 vs 63 mg P/ day	3 wks	NA	No Δ	NA	NA	NA	↓ kidney Ca & P; no food or BW data; no Δ in BP
53	5/6 Nx (N = 9 vs 9)	Normal P chow ± DHAAA	14 wks	43 vs 127	↓ at 12th and 14th wks (↑ C_{creat})	↓ 80% by DHAAA	↓ sclerosis 8 vs 67%; ↓ tubulointerstitial lesions	↑ by DHAAA to 67% vs 22%	BP no Δ; ↓ kidney Ca; inulin clearance in only 3 rats; no food data

[a] Nx, nephrectomy; NA, not analyzed or not assessed; DHAAA, dihydroxaluminum aminoaceate; GBM, glomerular basement membrane; EHDP, disodium ethane-l-hydroxy-l-l-diphosphonate; s_p, serum phosphorus; LPD, low-protein diet.

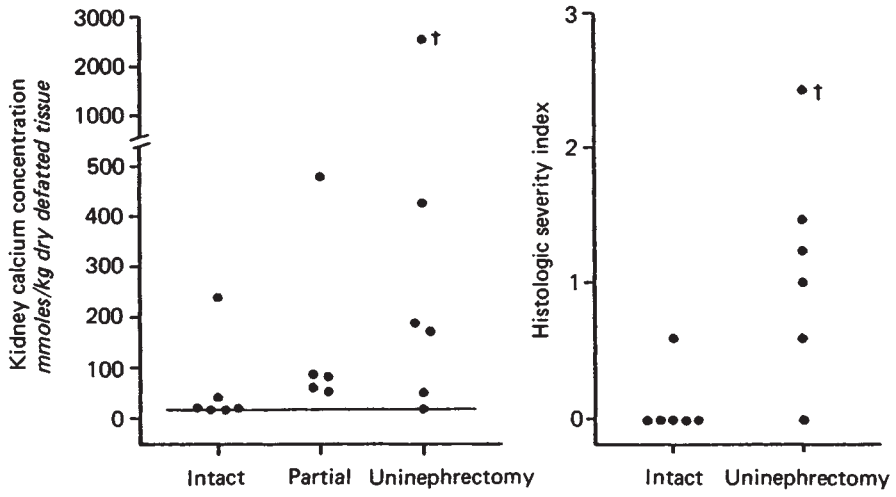

Fig. 2. *Increasing nephron losses were associated with increasing Ca concentration and worsening histologic abnormalities in the remnant kidneys harvested from rats subjected to partial (1/3) nephrectomy and to uninephrectomy and raised on 1% phosphorus diet.* (From Ref. 50.)

g% in the control rats) [49]. The followup study, in which the authors used a smaller load of phosphorus (0.98 g% versus 0.75 g%), protein, and sodium, confirmed the adverse effect on serum creatinine 4 weeks into the dietary treatment, on mortality at 30 weeks, on renal pathology, and on hypertension [51]. Although no data were given on body weight and GFR, this study illustrated the deleterious potential of excessive phosphorus and/or protein intake in renal failure.

At about the same time, Haut et al substantiated the nephrotoxicity of a high-phosphorus diet (2.0 g% versus 0.5 g%) in rats with prior one-third or one-half nephrectomy [50]. The endogenous creatinine clearance was lower in the one-third nephrectomy model after 7 weeks of a high-phosphorus when compared with the normal phosphorus diet. Both partial and complete unilateral nephrectomies were associated with increased renal calcium and phosphate deposits (Fig. 2), which were aggravated by doubling or quadrupling dietary phosphorus in rats after uninephrectomy [50] (Fig. 3). Calcification was most prominent in tubular lumens near the corticomedullary junction, and some calcium in the interstitium was thought to have been derived from degenerated tubules. Severely affected tubules appeared to have calcification of the epithelial cells [50]. The importance of excessive dietary phosphorus alone (for example, 2.0 or 3.0 g% versus 0.5 g%, the level generally considered normal) was underscored by the demonstration by Alfrey and coworkers that after 5 weeks of such a diet, tubulointerstitial abnormalities, renal calcification, and increased calcium and phosphorus content developed even in rats without prior renal ablation [50]. Although creatinine clearance was not detectably worsened at 5 weeks, these authors suggested that functional deterioration in previously healthy kidneys can be produced by dietary phosphorus loading, and that such deterioration is preceded by renal parenchymal calcification [50]. The severity of the histologic lesion and net kidney weight correlated positively with kidney calcium concentrations. Renal phosphorus content rose in association with renal calcium content; this finding suggests the deposition of compounds composed of both calcium and phosphate [50]. Finally, these investigators found a direct correlation between the severity of the histologic lesions for the intact and uninephrectomized rats and the daily excreted load of phosphate per nephron (assuming 30,000 nephrons per rat

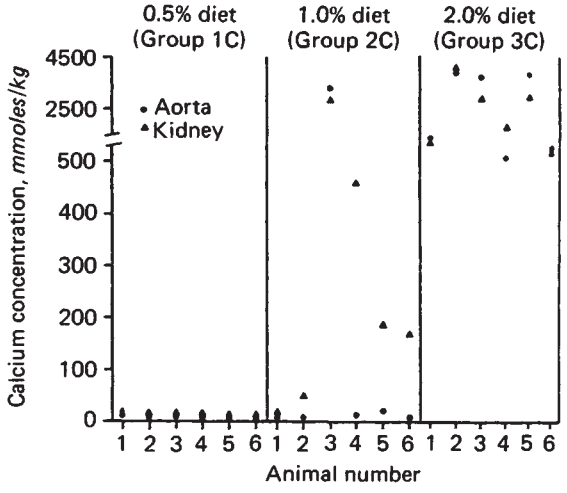

Fig. 3. *Increasing dietary phosphorus from normal (0.5%) to 1% and 2% was associated with increased Ca concentration in the aorta* (solid circles) *and in the kidney* (open triangles) *harvested from rats previously subjected to uninephrectomy.* (From Ref. 20.)

kidney) at 3 levels of dietary phosphorus (0.5, 1.0, and 2.0 g%) [50] (Fig. 4).

The third line of evidence implicating phosphate nephrotoxicity comes from four studies. First, enteric phosphate binders are known to sustain a lower serum creatinine and to ameliorate the renal lesions in rats with seven-eighths nephrectomy when used with a low-phosphorus diet [44]. Concerns can be raised regarding the anorexic effect of a low phosphorus diet, but a followup study by Alfrey and colleagues partially resolved this and the GFR issues [53]. Rats with five-sixths nephrectomy fed chow containing the usual amount of phosphorus (normal-phosphorus diet) received either dihydroxaluminum-aminoacetate (DHAAA) as a phosphate binder for 14 weeks (experimental group) or an equivalent quantity of glycine mixed with the normal-phosphorus diet (control group) [53]. The DHAAA raised serum calcium and lowered serum phosphorus, as expected, and led to a lower serum creatinine and higher endogenous creatinine clearance at 12 and 14 weeks of treatment. This effect was assumed to reflect GFR because they found few

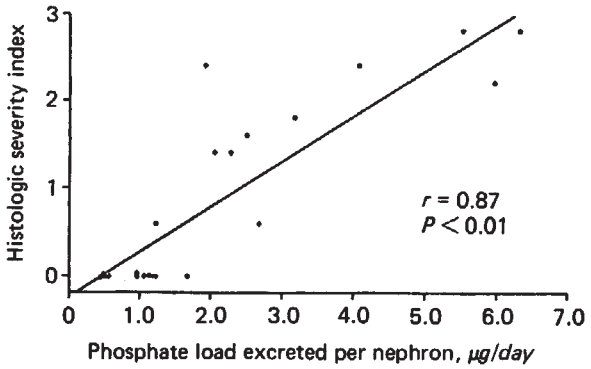

Fig. 4. *Correlation between estimated phosphate load excreted per nephron based on a 24-hour urine collection at week 7 and the degree of renal histologic abnormalities determined at the conclusion of the study at week 18.* Data points were derived from rats with intact kidneys and with uninephrectomy that were fed a 0.5%, 1%, and 2% phosphorus diet, assuming 30,000 nephrons per kidney. Rats with 1/3 nephrectomy were excluded from this analysis because the exact number of residual nephrons could not be estimated. (From Ref. 50.)

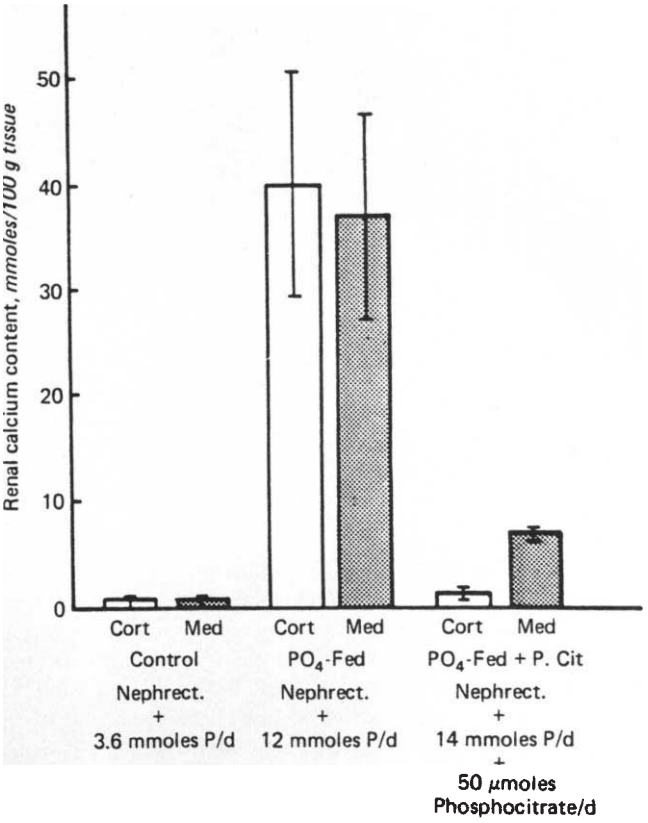

Fig. 5. *Renal Ca content (mmoles/100 g wet weight of renal tissue) as a function of dietary phosphorus and origin of the renal tissue.* Cort. and med. refer to cortical and medullary locations. Control diet contained 3.6 mmoles P/day as opposed to PO_4-fed rats that received 12 to 14 mmoles P/day. The latter exhibited a marked and highly significant increase in both cortical and medullary Ca content in the kidney remnant. In contrast, rats receiving increased phosphate intake plus phosphocitrate (P. Cit) showed no significant increase in cortical Ca and only a modest rise in medullary Ca. (From Ref. 52.)

discrepancies between inulin clearance and creatinine clearance measured concomitantly in 3 rats [53]. The incidence of glomerulosclerosis and tubulointerstitial lesions and renal-tissue calcium content were markedly reduced by DHAAA, as was the mortality rate (33% versus 78%). As with their original study [44], interpretation of this study remains ambiguous because without data on food and protein intake, the reduction in proteinuria by $Al(OH)_3$ [44] or DHAAA [53] cannot be definitively interpreted.

Gimenez et al evaluated the effect of 3-phosphocitrate, an inhibitor of calcium phosphate precipitation and tissue calcification, in a rat model with seven-tenths nephrectomy fed a high phosphorus (2.2 g%) diet [52]. Compared with a normal-phosphorus (0.58 g%) diet, rats fed the high-phosphorus diet experienced a fourfold increase in serum creatinine levels after 4 weeks. These animals developed tubulointerstitial disease and nephrocalcinosis, and more calcium accumulated in their renal tissue compared with rats fed a normal-phosphorus diet (Fig. 5) [52]. The concomitant administration of 3-phosphocitrate prevented these abnormalities despite a comparably high phosphorus diet and a similar degree of initial ablation (Fig. 5).

Contrary to the studies in which enteric phosphate binders were used [44, 53], the calcium × phosphorus product was not significantly reduced by 3-phosphocitrate. Thus, the beneficial effects probably are mediated at the tissue level. A definitive statement on improvement of GFR by this agent cannot be made because the non-treated, phosphate-loaded rats might have been volume contracted, as suggested by their decreased body weight [52]. The emerging evidence argues strongly, however, that in the remnant kidney model, a low-phosphorus diet per se [44, 46, 49, 51] or agents that reduce phosphorus absorption [44, 53] preserve renal function, as assessed by serum creatinine [44–46, 51] or creatinine clearance [53], independent of other nutrients [44, 53]. The obverse, namely, that excess dietary phosphorus is detrimental to residual renal function, also is reasonably well supported [49–51]. Finally, the adverse consequence of phosphorus loading on histologic abnormalities [50] and on serum creatinine [52] can be largely blocked by diphosphonate [50] and by 3-phosphocitrate [52]. Collectively, these 3 lines of data support a pathogenetic role of phosphorus in progressive renal insufficiency.

How might excess phosphorus exert its deleterious effect? The animal studies I have cited suggest that it does so by precipitation in the kidney as calcium salts. From a pathophysiologic point of view, this hypothesis considers excess phosphate deleterious because: (*1*) Via a fall in ionized calcium, high plasma phosphate levels raise parathyroid hormone (PTH), thus increasing tubular fluid phosphorus (TF[P]) (Fig. 6) [57] and elevating cytosolic-free calcium [54–56]. (*2*) Hyperphosphatemia (coupled with increased single-nephron GFR) increases the single-nephron filtered load of phosphorus and increases the absolute flux of phosphorus. (*3*) Hyperphosphatemia and a high PTH level potentiate the effects of each other in raising TF[P] and in predisposing to tubular $CaPO_4$ precipitation.

Let us examine the scientific basis for the hypothesis that excess phosphate is deleterious. In the remnant kidney, the SNGFR is increased by 66% [57]. Although in this study serum phosphorus was not increased, single-nephron filtered load of phosphorus was more than double that of normal [57]. Frac-

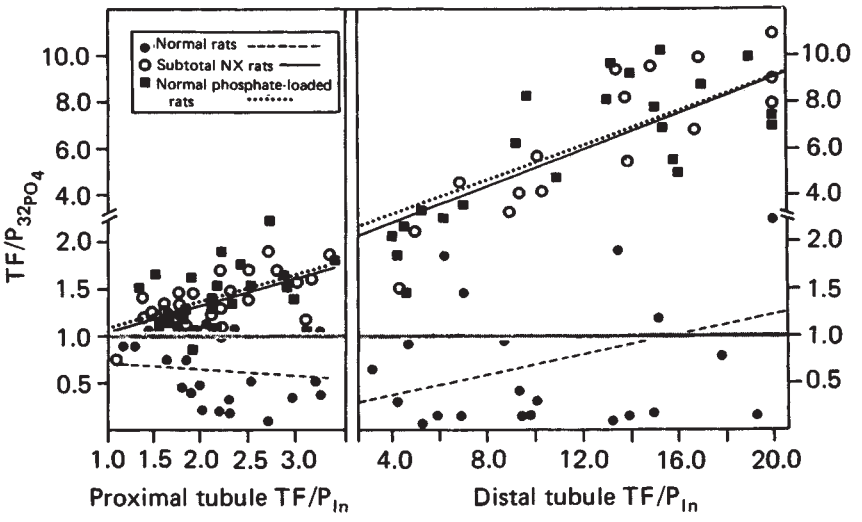

Fig. 6. *Tubular fluid-plasma $^{32}PO_4$ ratios ($TF/^{32}PO_4$) related to corresponding tubular fluid/plasma inulin ratios (TF/P_{In}) obtained from micropuncture studies in 3 groups of rats (normal rats with intact kidneys, rats with 2/3 nephrectomy, and normal rats infused with inorganic PO_4).* (From Ref. 57.)

tional reabsorption of phosphorus (FRP) fell, as confirmed in uremic dogs by Wong et al [58] and by Wen and Stoll [59], because of non-PTH-mediated [60, 61] and PTH-mediated inhibition of transport [62, 63]. The fall in FRP was slightly less than one-half, so that absolute reabsorption of phosphorus (ARP) by the proximal convoluted tubule up to the latest accessible micropuncture sites (TF/plasma inulin of 2.5) still increased. Clearly this increase in ARP is a function of the magnitude of rise in SNGFR, which depends in part on dietary protein intake [39] among other factors. In this scenario, transepithelial flux of phosphorus across the proximal convoluted tubule increases. Similar reasoning probably holds true for transepithelial flux of calcium and traffic through the renal tubular cytosol. Cytosolic free calcium in proximal tubule [54, 56] as well as in distal nephron segments, like that in the connecting tubule [55], is increased by PTH both acutely [54] and chronically [56]. The TF[P] also increases markedly in the late proximal convoluted tubule [57] (Fig. 6). The increased TF [Ca] × [P] product leads to lumen calcification. The increased transepithelial calcium and phosphorus fluxes increase the risk of the renal tubules to calcium intoxication. The peritubular capillary and the interstitium in turn are rendered more vulnerable to the deposition of calcium and phosphorus. In essence, the entire nephron and its immediate surrounding structures become more susceptible to calcification.

Phosphate handling by the pars recta and the very early distal tubule is altered similarly and adversely. Increased SNGFR, via increased single-nephron filtered load, increases ARP several-fold and raises TF[P] in the early distal tubule (Fig. 6) [57] because of the inhibitory action of PTH and the intrinsic diminution in tubular phosphate transport [63]. Phosphate in the tubular fluid coursing through the distal tubule undergoes a further increase; the ARP continues to be higher than normal. In brief, both the tubular lumen phosphorus and the transepithelial phosphate traffic increase. Using calcium-sensitive fluorescent dye, we obtained preliminary results suggesting that in the proximal tubule, cytosolic free calcium increases after uninephrectomy [64]. Further, this abnormality can be partially corrected by a low-phosphorus diet.

The TF[P] level at which calcium phosphate crystals are likely to precipitate is unknown because many other factors, including tubular fluid concentrations of other ions and crystal growth inhibitors, levels of sulfate and oxalate [65], flow rate, and pH also must be involved. Despite the paucity of in-vivo information, we can estimate the critical concentration range. In normal blood, one can derive a crude [Ca] × [P] product as 38 mg/dl^2, or 3.1 mM^2. Knowing that plasma ultrafilterable calcium is about 60% of the total, we can approximate better by using the product 1.9 mM^2.

As an example, let us consider a patient with chronic renal failure who does not take phosphate binders. Let us assume a serum phosphorus of 12 mg/dl and a serum ultrafilterable calcium concentration of 8 mg/dl. The calculated [Ca] × [P] product is thus 96 mg/dl^2, or 5 mM^2 using the ultrafilterable calcium. This figure would persuade most nephrologists to start aggressive treatment. Micropuncture studies indicate a value of 1.5 mM for the TF[Ca] in the surface proximal tubule [66–69], 3.1 mM in the hairpin turn of the juxtamedullary nephron [66, 67], and 0.4 to 0.8 mM in the surface distal tubule [68–70]. Division of 5 mM^2 by these TF[Ca] data yields an estimate for the critical TF[P] at risk for precipitation: 3.3 mM for the proximal convoluted tubule (a concentration attained in the uremic rats studied by Bank et al [57]), 1.6 mM in the hairpin turn; and between 6 and 17 mM respectively in the early and late portions of the distal tubule. Micropuncture studies in Munich-Wistar rats from portions of the accessible loop of Henle [66, 71–75] suggest that the critical phosphorus concentration of 1.6 mM is reached during infusion with phosphate, PTH, or both [57, 68, 69, 71, 73, 75–77]. It is reasonable to expect that in renal insufficiency this threshold can be exceeded because serum PTH and phosphorus are only expected to increase. Dietary phosphate restriction predictably reduces TF[P] [74], theoretically offsetting these effects of renal failure.

The possible pathophysiology of phosphate nephrotoxicity is schematically depicted in Figure 7. Besides other changes not relevant to our discussion today, renal failure results in hydrogen and phosphate retention and in an adaptive increase in SNGFR. Metabolic acidosis reduces citrate production and increases citrate reabsorption [78, 79], reducing TF[citrate] in the proximal tubule. Simultaneously, hyperphosphatemia and

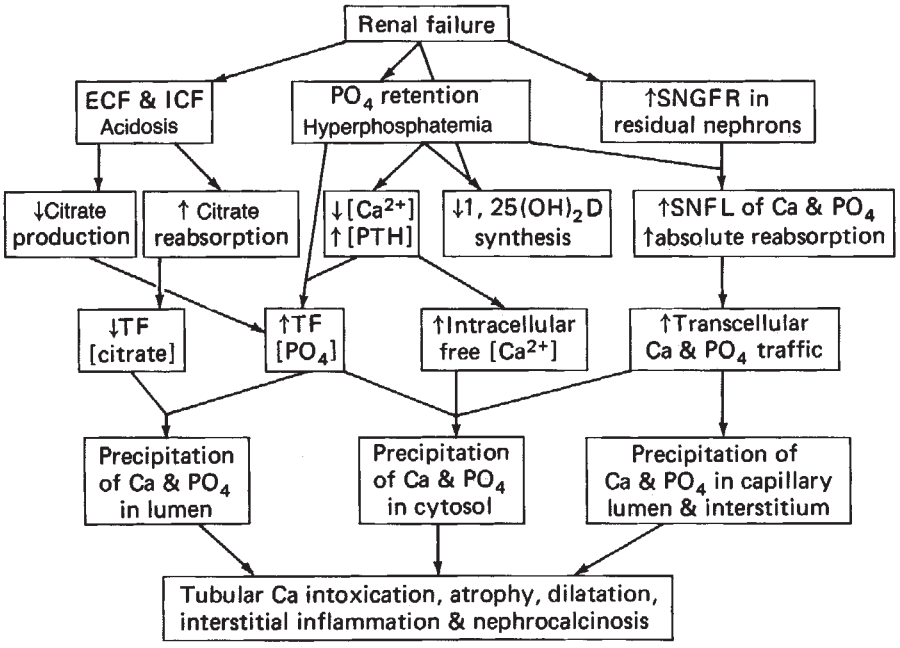

Fig. 7. *Theoretical schema of the calcification-precipitation hypothesis, outlining the pathophysiology of phosphate nephrotoxicity.* ECF and ICF refer to extracellular and intracellular fluids respectively. SNGFR, single-nephron glomerular filtration rate; SNFL, single-nephron filtered load; *vertical arrows* within boxes indicate increase or decrease as directed; *solid lines* ending in an arrow connecting boxes indicate a process or change leading to another alteration.

hyperparathyroidism raise TF[P], as I explained earlier [57, 70, 77]. The decreased TF[citrate] and increased TF[P] favor precipitation of calcium phosphate in the lumen [78]. The medullary location of Henle's loop is particularly vulnerable, given the higher TF[pH], the normally rising TF[Ca] [66, 67], and TF[P] [66] as tubular fluid descends from the cortex toward the papillary tip. By reducing ionized calcium [79] and $1,25(OH)_2D$ synthesis, phosphate retention indirectly increases PTH synthesis [80] and secretion, which in turn elevate cytosolic free calcium [54–56]. Coupled with the increased transcellular calcium and phosphorus traffic and decreased citrate availability, these changes increase the risk of cellular calcium intoxication.

As I mentioned, the increased SNGFR raises the mass fluxes of calcium and phosphorus through the cytosol and into the interstitial space and capillary lumen. Exposure to increased loads and/or concentrations of calcium and phosphorus in the tubular lumen, cytosol, and the interstitium leads to tubular calcification, atrophy, dilation, fibrosis, interstitial inflammation, and nephrocalcinosis as were found in experimental renal diseases [44, 45, 50, 52, 53].

If this pathophysiologic schema is correct, a low protein intake might exert its benefits by attenuating the rise in SNGFR. Judicious alkali therapy might sustain a better TF[citrate], as it presumably does as the basis for the effective management of nephrolithiasis and nephrocalcinosis of familial distal renal tubular acidosis. Finally, the restriction of phosphate intake or phosphate absorption is key to interrupting the course and multitude of adverse metabolic consequences outlined here (Fig. 7).

Vitamin D metabolites also play a role. Serum $1,25(OH)_2D$ not only is directly related to GFR but also inversely related to 24-hour urinary phosphorus excretion, which reflects phosphorus intake [14]. In children with moderate renal insufficiency, Portale et al found that 5 days of phosphate restriction plus administration of $Al(OH)_3$ or supplementation of dietary phosphorus produced the expected rise and fall, respectively, in serum $1,25(OH)_2D$. Serum PTH changed inversely as expected [81]. These findings are consistent with the concept that increases in $1,25(OH)_2D_3$ within the physiologic range downregulate the synthesis and release of PTH.

Is it feasible to reduce the phosphate burden without resorting to aluminum-containing enteric binders? I believe so. The steps worthy of trying are: (*1*) Repeated patient education and reinforcement. (*2*) Modest, not extreme, dietary phosphate restriction (for example, allowing 70% to 80% of whatever one was consuming before). (*3*) Administration of calcium-containing enteric phosphate binders until serum calcium exceeds 11.5 mg/dl and/or urinary calcium exceeds 275 mg/day. Whenever possible, one should encourage a copious fluid intake compatible with the patient's ability to excrete water. Water diuresis increases tubular fluid flow rate, decreases TF[Ca] and TF[P] in the medulla, offsets the known hypercalciuric side effect of phosphate deprivation [69], and ameliorates the constipating effect of most phosphate binders. (*4*) Addition of small doses of $Mg(OH)_2$ as adjuncts and/or laxatives. Of course, patients should be instructed to withhold these agents if oliguria ensues.

The end point should be the lowest possible 24-hour urinary phosphorus achievable; a rough rule of thumb is to achieve a phosphate excretion 7 times the clearance of creatinine, with the former expressed in milligrams and the latter expressed in milliliters per minute. Since our demonstration in rats that pharmacologic amounts of calcium carbonate virtually eliminate intestinal phosphorus absorption [82], a number of clinical trials [83–87] have emerged confirming the efficacy of calcium carbonate in suppressing PTH [85] and in lowering serum phosphate [83]. Recently, Sheikh et al reported that phosphate-binding potencies are dependent on pH [87]. Calcium carbonate is most effective at a pH of 5; the percentage of binding is time dependent, whereas aluminum carbonate $[Al_2(CO_3)_3]$ is relatively insensitive to pH and to contact time [87]. Calcium citrate

is not as effective as calcium carbonate. Interestingly, calcium acetate is more effective in the alkaline pH range, and its phosphate binding potency is almost as great after 1 hour as after 4 or 10 hours [87]. In 10 normal humans given a meal containing 345 mg of phosphorus, calcium carbonate and calcium citrate reduced net phosphorus absorption from 76% to 44% and 50%, respectively. Calcium acetate, however, was found to produce the efficacy approaching that of $Al_2(CO_3)_3$ (26% versus 18% of net phosphorus absorption). Net calcium absorption was also lowest with calcium acetate. These results with calcium acetate are encouraging, because the avoidance of aluminum toxicity by using calcium-containing binders depends on an effective substitute that doesn't carry the risk of producing significant hypercalcemia.

Guillot et al reported a modest reduction in serum phosphorus with $Mg(OH)_2$ (0.75 to 3 g/day) in uremic patients on maintenance hemodialysis [84]. The serum phosphorus fell by approximately 1 mg/dl compared with 2 mg/dl by $Al(OH)_3$ alone (in doses of 1.5 to 3.8 g), whereas serum magnesium rose by approximately 1.4 mg/dl. Had they used a dialysate with a magnesium level less than or equal to 1 mEq/liter, it might have been possible to administer a larger amount of $Mg(OH)_2$ without producing significant hypermagnesemia [84].

In summary, I have proposed the precipitation-calcification hypothesis of phosphate nephrotoxicity and reviewed the available but inconclusive evidence. I also have outlined the rationale and feasibility of reducing the total-body and renal phosphate burden by shunting the excretion of phosphorus from the kidney to the lower intestine. Finally, I have raised the notion that phosphate and protein are nephrotoxic via different mechanisms; hence they carry the potential for different but synergistic therapeutic interventions [47]. Many issues remain. When is the optimal time to begin reducing phosphate stores? How severely should dietary phosphate be restricted? What is the utility of hypophosphaturia as an end point? What is the role of phosphate overload in initiating or sustaining various "idiopathic" tubulointerstitial disorders, and what is the precise interrelationship, if any, between nephrotoxicity of phosphate and protein? Clinical nephrology has entered a new and exciting era of interventional therapy, and these questions should provide additional impetus for further research.

Questions and answers

DR. NICOLAOS E. MADIAS (*Chief, Division of Nephrology, New England Medical Center, Boston, Massachusetts*): It is my impression that the diffuse renal calcification observed in uremic rats fed a normal diet in the study by Ibels and colleagues [44] is at substantial disparity with the reported histology in the rat remnant kidney model using normal diet. The variance raises some concern about the applicability if the dietary phosphorus hypothesis is accounting for the progressive nature of renal disease.

DR. LAU: I am not sure whether there is really any disparity in renal histopathology between what Ibels and colleagues observed with a normal-phosphorus (0.5 g%), normal-protein (24 g%) diet on the one hand [44], and what Hostetter, Brenner, and coworkers observed with a normal-phosphorus (0.63 to 0.86 g%) [39], normal-protein (24 g%) diet [32] on the other. The apparent disparity merely might reflect a difference in emphasis between studies directed at different objectives. For example, Ibels et al did describe "glomerular fibrosis," although those two words were "buried" by the detailed description of the tubulointerstitial abnormalities, in particular renal calcification [44]. As best can be surmised from the methods section and supported by the results section, they did not plan to dwell on the glomerular pathology [44]. In contrast, Brenner, Hostetter, et al designed their experiments to concentrate heavily on the glomeruli. They examined 200 to 600 glomeruli per rat, but they made no mention of using special stains such as Von Kossa stain, Alizarin-red stain, or Bunting stain for calcium and phosphate minerals on light microscopy [32, 39]. Less than 5% of their pathologic description was spent on the tubules and interstitium. Rare "Ca deposits," "tubular atrophy and interstitial fibrosis" were found, especially in areas where "glomerular abnormalities were most prominent." Had these two groups devoted equal effort and time on the other structures not evaluated or elaborated, they might have documented similar findings. Such an explanation is supported in part by the description in a later study of glomerular sclerosis in 57% of rats subjected to five-sixths nephrectomy and fed rat chow containing a normal protein and phosphorus content [53].

The applicability of the hypothesis of phosphate nephrotoxicity in progressive renal disease still holds, in my opinion, not only because of the functional evidence, but also because of pathologic findings elicited by a high-phosphorus diet in normal rats with intact kidneys [50, 88, 89]. Although no renal function data were obtained, the serial pathologic studies by MacKay and Oliver clearly demonstrated that a 2 g% phosphorus diet for 15 to 44 days in weanling rats without prior nephron losses produced predominantly a tubulointerstitial lesion, localized primarily in the outer stripe of the outer medullary zone and characterized by necrosis of the terminal portion of proximal convoluted tubules, regeneration of atypical epithelium, and calcification of the necrotic debris that filled the tubules [89]. The glomeruli on light microscopy were well preserved [89]. In a shorter-term study in 115 g rats, Craig reported similar tubular pathology but also documented intraluminal calcium deposits in the terminal proximal tubules and in the ascending thick limbs of Henle's loop, as early as 3 to 6 days of exposure to a 2.2 g% phosphorus diet [88]. These lesions were confirmed by Haut et al, who fed normal intact rats a 3 g% phosphorus diet and produced increased calcium accumulation in the renal tissue [50]. Quantitatively, renal tissue calcium increased 10- to 50-fold after feeding normal rats with intact kidneys a 3.2% phosphorus diet respectively for 3 to 6 days; the effect was sustained 6 weeks later [90]. Lumen calcification in the corticomedullary junction was similar to that found in nephrectomized rats studied by Haut et al, who substantiated the associated drop in creatinine clearance with a high-phosphorus diet [50]. As I mentioned earlier, several groups have substantiated a deterioration in serum creatinine with a high-phosphorus diet or improvement with a low-phosphorus diet [46, 49, 50–52]; both changes were associated with corresponding changes in tubulointerstitial pathology. Thus, I believe the literature is persuasive regarding the phosphate nephrotoxicity hypothesis and indeed supportive of its relevance in progressive renal failure.

DR. JEROME P. KASSIRER (*Associate Physician-in-Chief, New England Medical Center*): The idea that a single element was responsible for progressive damage to the kidney is intrigu-

ing, but one of our responsibilities is to be skeptical. How do we know that the calcium deposition in the kidney is not simply an epiphenomenon totally unrelated to the actual cause of the declining renal function?

DR. LAU: Several lines of evidence argue against the notion that the calcium deposition is a mere epiphenomenon. First, the studies by Gimenez, Walker, et al showed that they could revert or prevent the rise in serum creatinine in rats with seven-tenths nephrectomy fed a 2.2 g% phosphorus diet by the concurrent administration of 3-phosphocitrate, a known inhibitor of calcium phosphorus crystallization [52] (Table 5). They also showed parallel improvement in calcinosis and tubular abnormalities and an attenuation in calcium content in renal tissues in the rats treated with 3-phosphocitrate [52]. These data suggest that one can interrupt the pathophysiology, that is, the functional deterioration, by preventing calcium phosphate deposition in the kidney in spite of feeding a high-phosphorus diet.

The second line of evidence comes from the observation that structural abnormalities (necrosis of proximal S_3 [89], increased calcium and phosphorus deposits in renal tissues [50], and intraluminal mineral deposits [88]) can develop in intact kidneys [50, 88] during consumption of a diet containing 2 to 3 g% of phosphate [50, 88, 89]. More significantly, these tubulointerstitial lesions (evident within days of a high-phosphorus diet [88, 89]), preceded demonstrable deterioration in serum creatinine or creatinine clearance [50]; this finding is consistent with, although not necessarily supportive of, the notion that these calcium-phosphate-induced lesions play a pathogenetic role in the subsequent functional decline.

The third line of evidence can be drawn from the studies of Haut et al, who used disphosphonate, a nonspecific crystal growth inhibitor, admittedly with many other actions on mineral metabolism [50]. Diphosphonate administration prevented the deposition of calcium-phosphate in renal tissue. In these short-term (5-week) studies, the phosphate-loaded animals with intact kidneys probably had not been exposed long enough to raise serum creatinine. Thus, it was not possible to determine whether diphosphonate exerts a salutary effect on creatinine clearance.

DR. KASSIRER: I might have missed a point about the patients on dialysis. You indicated that it would have been better to eliminate magnesium from the dialysate, but shouldn't we be paying attention to patients before they require dialysis? It is these patients who are at risk of developing progressive renal failure.

DR. LAU: I appreciate your question, which allows me to clarify my comments. Of course we should focus more on patients with mild to moderate renal insufficiency and explore therapeutic measures to halt the progression to end-stage renal disease. The ultimate objective of our present discussion is precisely this. I proposed the phosphate nephrotoxicity hypothesis to stimulate more research in pursuit of this objective. I mentioned the clinical trial by Gennari et al in uremic patients [84] because of the applicability of magnesium salts as adjunct enteric phosphate binders (to the calcium salts) in pre-uremic patients. If used judiciously, a bedtime dose of magnesium hydroxide not only minimizes the constipating side effect of calcium carbonate without incurring undue risk of hypermagnesemia in a nonoliguric patient, but it also adds to the effects of the calcium salts. I didn't mean to suggest that management of phosphorus overload in patients with end-stage renal disease takes precedence over dealing with the same problem in patients with mild to moderate and potentially arrestable progressive renal disease. The top priority is to prevent end-stage renal disease.

Let me make two other points: First, the same propensity to accumulate excess phosphorus exists in pre-uremic and uremic stages, hence the need to restrict phosphate in both periods. More significantly, our current treatment in both instances still largely depends on enteric phosphate binders [91], but compliance unfortunately remains a common problem. Second, in the case of dialysis patients, my earlier suggestion was that more magnesium hydroxide (that is, more than the dose of 0.75 to 3.0 g/day used by Gennari et al) could be prescribed if a lower bath magnesium concentration were used.

DR. PAUL KURTIN (*Chief, Division of Pediatric Nephrology, New England Medical Center*): Is it possible to separate out the effects of phosphorus from those of hyperparathyroidism? For example, has a study been done comparing a low-phosphorus diet with a normal-phosphorus diet in parathyroidectomized animals?

DR. LAU: I am not aware of any study comparing a low- to a normal-phosphorus diet in parathyroidectomized animals, in exactly the way you suggested. It is an important issue because some of the pathophysiologic consequences outlined in my hypothesis (Fig. 7) are mediated by increased PTH (for example, increased TF[P]), whereas others depend directly or indirectly on phosphorus retention (for example, increased PTH, increased TF[P], and increased single-nephron filtered load of phosphorus). One important theoretical difference, however, should be emphasized. Phosphorus restriction is known to suppress PTH, thus ameliorating some of the adverse effects of both hyperphosphatemia and secondary hyperparathyroidism. In contrast, parathyroidectomy can be expected to aggravate phosphorus retention.

I do know of two slightly different versions of the experiment you mentioned. First, Borle and Clark measured renal calcium metabolism by microfluorometric ^{40}Ca and ^{45}Ca kinetic analysis in rats fed 6 to 8 times the normal dietary phosphorus (3.2 g%) [90]. They found that a high-phosphorus diet for as short as 3 to 6 days or as long as 6 weeks increased renal and mitrochondrial calcium, cytosolic and mitochondrial exchangeable pools, and all the calcium fluxes. Their quantitative data extended previous histochemical staining studies [88, 89], although none of these 3 groups evaluated renal function to establish the adverse impacts of a high-phosphorus diet, as some subsequent investigators did [49, 50, 52] (Table 5).

Borle and Clark next addressed the PTH question by feeding a high-phosphorus diet to parathyroidectomized rats. Because the changes in renal calcium metabolism appeared to be abolished by parathyroidectomy, these authors suggested that "nephrocalcinosis was caused by elevated PTH levels and not by elevated plasma PO_4." But their failure to prevent profound hypocalcemia (1.0 versus 2.0 to 2.5 mM) in phosphate-loaded, parathyroidectomized rats left open the possibility that an effect on tissue calcium, exerted by a high-phosphorus diet, might have been obscured by the associated hypocalcemia. Accordingly, these experiments didn't really differentiate the effects of phosphate from those of hyperparathyroidism.

The other study of relevance was done by Alfrey et al [92], who found that in uninephrectomized rats with nephrotoxic serum nephritis, thyroparathyroidectomy (TPTX) prevented the progressive increase in serum creatinine observed in non-TPTX rats (0.65 versus 3.8 mg/dl at 23 weeks). Thyroparathyroidectomy also markedly attenuated the proteinuria, uremic deaths, renal histologic damage, and calcification [92]. Mean serum calcium in TPTX rats was kept normal at 9 mg/dl by thrice-weekly calcitriol injections. Since dietary phosphorus was *not* reduced in either group and serum phosphorus levels were similar (6.77 versus 6.71 mg/dl in non-TPTX animals), these authors concluded that "TPTX is equally effective in preventing functional deterioration as PO_4 restriction" [92]. Unfortunately, these experiments were inconclusive. First, without GFR measurement and details on weight gain and food intake in both the 23-week and 7-week protocols, it is difficult to be sure that the differences in creatinine in these two protocols truly reflected changes in renal function. The second reservation arises from studies in which a weekly thyroxine replacement dose was given to the TPTX rats; theoretically, the differences between the TPTX and non-TPTX rats are simply the absence of PTH and calcitonin. A third group of rats with selective parathyroidectomy, studied by a pair-feeding schedule in a 5-week protocol, actually had similarly low plasma creatinine (0.67 mg/dl) and creatinine clearance (0.397 ml/min/100 g body weight) as did the TPTX rats given levothyroxine [92]; these data support a pathogenetic role of PTH. On the other hand, in a protocol lasting 7 weeks, but conducted without pair-feeding or monitoring of food intake, these authors found a lower plasma creatinine in TPTX rats given levothyroxine only (0.9 mg/dl), but not in rats that had had parathyroidectomy (4.3 mg/dl), when compared with non-TPTX controls (4.8 mg/dl). Again, without GFR and/or serial body weights, these plasma values might not be accurate indices of renal function. The internal inconsistencies and these two reservations are reflected by the authors' acknowledgment that the "mechanism of TPTX's protective effect remains to be elucidated" [92].

DR. MADIAS: Vigorous phosphate depletion has the theoretical potential for encouraging bone resorption. In fact, through PTH-independent mechanisms, phosphate depletion can pose limitations on titrable acid excretion that can, in turn, lead to acid retention and further mobilization of bone buffer. Should we be concerned about the potential for accentuation of renal osteodystrophy by vigorous phosphate restriction?

DR. LAU: I think we should. But we also should recognize two inherent features of phosphate restriction in renal failure, especially in adults. First, in contrast to growing children or young, growing rats, an adult not recovering from malnutrition, starvation, or bone disease should be in zero phosphorus balance. From day to day, an adult has the "problem" of excreting between 700 and 1000 mg of phosphorus daily in the urine. To maintain zero balance and avoid phosphorus intoxication, 60% [14] to 70% of the phosphorus intake, the amount absorbed by the gut, must be eliminated. In renal insufficiency, there is only a mild diminution in phosphorus absorption. The residual nephrons must continue to eliminate almost the same daily load of absorbed phosphorus, often at the expense of producing secondary hyperparathyroidism and hyperphosphatemia. The problem therefore is the tendency toward excessive retention. Patients with renal failure do not begin with a normal phosphorus balance and hence are susceptible to phosphate depletion if they are phosphate restricted. Phosphate restriction and/or enteric phosphate binders are measures that only diminish the phosphorus burden and prevent increased positive balance, not produce negative phosphorus balance.

Second, we must appreciate the fact that studies showing increased bone resorption or osteomalacia by phosphate depletion were by and large performed in young animals, which require positive phosphorus balance for healthy growth. In adult humans, osteomalacia develops because these patients lose more phosphorus in either the urine or stool than they absorb. Except for renal phosphate wastage seen in vitamin D-resistant rickets, renal adaptation defends against negative phosphorus balance produced by gastrointestinal losses. Their problem is not excessive phosphate retention or increased phosphorus balance, as is almost always inevitable in renal insufficiency, but rather inadequate retention, or negative phosphorus balance.

DR. KASSIRER: Let me ask you another question. There is a phenomenon in animals called nutritional hyperparathyroidism. If horses, for example, are given feed that is extremely high in phosphate, they develop hyperphosphatemia, hypocalcemia, and secondary hyperparathyroidism sufficiently severe to decalcify and deform their bones. Do such animals with severe, sustained hyperphosphatemia and secondary hyperparathryoidism develop progressive renal failure? If not, why not?

DR. LAU: The answer is probably yes, even though I can't cite you any studies on horses. The rat experiments by MacKay and Oliver showed that after 44 days of a high phosphorus intake (2 g%), previously untouched normal kidneys in these young rats were severely and irreparably damaged [89]. They did not measure GFR, but I suspect that if measured, it would be abnormal. Similar morphologic studies by Craig [88] and renal tissue calcium analyses by Borle and Clark [90] reinforced these findings.

DR. KASSIRER: Do you think that the acute renal failure that occurs during rapid phosphate infusion is the consequence of calcium phosphate deposition?

DR. LAU: My bias is yes, but in the few cases reported [93, 94], serum calcium fell from hypercalcemic to hypocalcemic levels and was associated with variable degrees of hypotension. Thus the acute renal failure might be hemodynamically mediated as well. At postmortem examination, interstitial calcification was found in the kidneys of these patients, but again, it could not be unequivocally established that the ectopic calcification was due to phosphate infusion rather than to pre-existing hypercalcemia [93, 94].

DR. MADAIO: At what level of renal function do you initiate therapy to lower serum phosphate? What precisely is the therapeutic approach, and how do you monitor the patient's response?

DR. LAU: I think the time to initiate therapy is a function of two equally important variables, the residual GFR and the amount of dietary phosphorus. The latter can be estimated by the 24-hour urinary phosphorus level at a relatively steady state on a self-selected diet. Under this condition, phosphate intake is roughly 1.5 times the 24-hour urinary phosphorus excretion [14]. If the 24-hour urinary phosphorus exceeds 800 or 900 mg, I would contemplate enteric phosphate binders and/or advise a modest reduction in dietary phosphorus, even with mild renal

insufficiency, for instance, a serum creatinine of 1.3 mg/dl. My prejudice is to prophylactically relieve the body—and the kidney—of any phosphate burden at the first sign that the intake is disproportionately high for the residual GFR. My rule of thumb is to limit 24-hour urinary phosphate excretion (in mg) to less than 7 times the creatinine clearance (in ml/min). Even when the GFR falls only slightly (for example, to 80 ml/min or 80% of the maximum), I believe that it behooves us to institute measures to proportionately ease the phosphate burden on the remaining nephron mass, say, by restricting urinary phosphorus excretion to 80% of the normal, or 560 mg/day. Analogous to the reversal of hyperparathyroidism by dietary phosphorus restriction in uremic dogs [95], I believe the regulation of urinary phosphorus should be achieved at each and every decrement in GFR. In patients with serum creatinines over 2.0 to 2.5 mg/dl, we should aim to reduce the urinary phosphorus to below 200 mg/day; the rationale for this is derived from the animal studies by Ibels et al [44]. Extrapolating from the relatively long duration of stabilized serum creatinine levels produced by phosphate binders and phosphate restriction (168 days in a rat's life), I believe that we should strive towards the lowest attainable urinary phosphorus excretion rate, as long as significant hypercalcemia ($\geq$ 11.5 mg/dl) and/or hypercalciuria ($\geq$ 275 mg/day) are absent.

In brief, if GFR is 100 ml/min, it is desirable to reduce urinary phosphorus to 700 mg/day. If GFR falls to 50 ml/min, urinary phosphorus should be lowered to less than 350 mg/day. I hope these guidelines are practical enough to be followed easily.

DR. MADIAS: Harris et al have reported that chronic administration of the calcium-channel blocker verapamil provides substantial protection from progression of chronic renal failure in the rat remnant kidney model independent of any effect on systemic blood pressure [96]. Do you have any insight about how this benefit might obtain, especially given the current view that the dominant hemodynamic effect of calcium blockers on the kidney is to dilate the afferent arteriole?

DR. LAU: Thank you for bringing to our attention this interesting study [96]. In this report, Harris and colleagues extended an early investigation on the chronic effects of verapamil from 3 [97] to 15 weeks in rats with five-sixths nephrectomy and fed a normal-phosphorus diet [96]. Not only did these authors confirm the significant amelioration in nephrocalcinosis and tubular damage first reported by Goligorsky et al with chronic verapamil therapy [97], but they also showed that at sacrifice, 12 weeks into treatment, serum creatinine (2.29 mg/dl versus 2.99 mg/dl), creatinine clearance (318 μl/min versus 164 μl/min) and mortality rates also were improved by the calcium-channel blocker [96]. These renal functional and morphologic benefits could not be attributed to mean arterial blood pressure, which was not measurably altered by verapamil, as assessed from 10 to 330 minutes after the previous dose [96].

I can't offer any insights on the mechanism of protection other than emphasizing some of the comments by these investigators. The role of increased cellular calcium uptake is attractive, because it can cause cellular damage by overloading the mitochondria with calcium, induce plasma membrane injury by activating phospholipases, and/or increase oxygen radical formation. Although experiments in cultured rabbit proximal and collecting tubules were cited as evidence for protection against anoxic cell injury [96], alterations in intrarenal hemodynamics (not detectable by systemic arterial pressure monitoring), as you suggested, are equally reasonable mediators of the protective action. The existence of calcium channels in vascular smooth muscle of renal microcirculation is better established than renal epithelial cells per se. Presently, the specific target element and the cellular mechanism of action are unclear to me. I would, however, caution against optimism in this as a mode of therapy, in view of the very modest benefit in serum creatinine, the meager inulin clearance data, and the failure to detect differences in survival after 12 weeks of verapamil treatment in one protocol, despite demonstration of benefit after 7 weeks in another protocol [96]. The results of both studies [95, 96] are consistent with the hypothesis that calcium accumulation in renal epithelium (reducible by calcium-channel blockers) and nephrocalcinosis (preventable by 3-phosphocitrate [52] or phosphate restriction [44–46, 49–53]) play an important role in the progression of renal failure.

DR. KURTIN: Children are known to have a higher range of normal serum phosphorus compared with adults. What do you think is going on in their tubules?

DR. LAU: Growth hormone has been postulated as the mechanism because growth hormone appears to increase the reabsorption of phosphorus [98] and contribute to the normally higher serum phosphorus level in children. There are probably other mechanisms or factors, but the complete picture is not understood, at least not by me.

DR. KURTIN: Are children not at risk for this calcium-phosphate precipitation?

DR. LAU: I don't think so. If the hypothesis I submitted to you is correct, one would predict that the major risk factor is increased TF[P] (Fig. 7). Growth hormone is expected to lower proximal (and perhaps distal) tubule [P] and by increasing reabsorption ultimately increase serum phosphate levels. Viewed from this framework, the lower TF[P] in children, as in young rats, should not pose increased risks to luminal calcium-phosphate precipitation. Consider that children have to be constantly laying down bone and constantly depending on supersaturated concentrations of calcium and phosphorus in the immediate microenvironment of the bone cell. It is teleologic, if not physiologic, for children to have higher normal serum phosphorus concentrations. If renal failure develops in a child, however, the physician should manage the child using as a guide the range appropriate for the age and gender of that child. Please bear in mind the studies by Portale et al [81] showing that serum phosphorus could actually be lower in children with moderate renal failure than normal, despite hyperparathyroidism and suppressed serum calcitriol levels, both of which are biochemical evidence of excessive phosphate retention.

DR. KURTIN: The lower GFR of infants, corrected for surface area, probably contributes to their slightly higher serum phosphorus concentration. Because of renal osteodystrophy, I think it is important to keep the serum phosphorus concentration normal in growing children. This is especially true now that we and several other centers are using growth hormone in growth-retarded children with renal disease. It is critical to keep the milieu normal for bone formation. We would be concerned about putting infants and young children on very low phosphorus diets because of the effects of that diet on bone formation and growth.

DR. LAU: I completely agree with that. As in adults, in

children with renal disease who are chronically receiving growth hormone, I would definitely encourage the use of 24-hour urinary phosphorus levels, not only as a therapeutic end point, but also as a monitor against excessive restriction of dietary phosphorus or overdose with enteric phosphate binders. Except for the disorders of vitamin D-resistant rickets characterized by renal phosphorus wastage, phosphate depletion resulting from dietary restriction (plus enteric phosphate binders) inevitably results in avid renal phosphate conservation. If phosphate deficiency begins to develop, urinary phosphate will become virtually zero [98]. This is true if demand of a growing child for phosphate exceeds the limited supply by the intestinal tract. Let me reiterate some of the key features of the studies by Portale et al in normophosphatemic children despite moderate renal insufficiency [81]. During periods of phosphate restriction, as reflected by reduced urinary phosphorus excretion, serum $1,25(OH)_2D_3$ levels responded by rising; conversely, during dietary phosphate supplementation (as reflected by increased urinary phosphorus excretion) serum $1,25(OH)_2D_3$ levels fell. The secondary hyperparathyroidism also responded appropriately in a reciprocal fashion. Because the phosphorus content in the extracellular fluid, of which plasma is a fraction, is less than 0.1% of the total body phosphorus stores [99], physicians are well advised to utilize and rely more on urinary phosphorus excretion to evaluate whether a growing child, with or without renal insufficiency, is getting too much or too little exogenous phosphorus.

DR. KLEMENS MEYER (*Fellow in Nephrology, New England Medical Center*): Apart from precipitation, how do increases in cytosolic calcium or in calcium and phosphorus transit damage tubular cells?

DR. LAU: Increases in cytosolic free-calcium concentration to the micromolar range adversely affect cellular process such as mitochondrial respiration and cause injury to plasma membrane and other membrane intracellular organelles. For example, the studies by Borle and Clark demonstrated increased mitochondrial calcium and increased calcium fluxes across all exchangeable pools of the kidney cells derived from phosphate-loaded rats [90]. The studies by Goligorsky et al [97] and by Harris and coworkers [96] emphasized structural damage and calcium deposition as well as increased cellular calcium uptake, which, earlier on, could be dissociable from tissue calcification [48, 50]. Although controversial in terms of the precise relationship, cell injury and death are closely linked to calcium overload. Very high intracellular calcium concentrations probably exert multiple deleterious effects on the tubules as well. I apologize if I gave the impression that calcium-phosphate could actually deposit in the cytosol in the same manner that it crystallizes in the tubular lumen. What I tried to suggest is that the increased cytosolic "traffic" of calcium and phosphorus (Fig. 7) perhaps is harmful to the cell. For instance, calcium entry from across the basolateral membrane and from across the apical membrane is stimulated by PTH [55], raising the cytosolic free calcium in cells that have reduced citrate production.

Acknowledgment

The author thanks Phyllis Lucente for secretarial support. The work was supported in part by an NIH grant (R01-DK33507).

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References

1. GIORDANO C: Nephrology Forum: Protein restriction in chronic renal failure. *Kidney Int* 22:401–408, 1982
2. FINE LG: Nephrology Forum: Preventing the progression of human renal disease: Have rational therapeutic principles emerged? *Kidney Int* 33:116–128, 1988
3. BRENNER BM: Nephrology Forum: Hemodynamically mediated glomerular injury and the progressive nature of kidney disease. *Kidney Int* 23:647–655, 1983
4. BARRIENTOS A, ARTEAGA J, RODICIO JL, ALVAREZ UDE F, ALCAZAR JM, RUILOPE LM: Role of the control of phosphate in the progression of chronic renal failure. *Miner Electrolyte Metab* 7:127–133, 1982
5. MASCHIO G, OLDRIZZI L, TESSITORE N, D'ANGELO A, VALVO E, LUPO A, LOSCHIAVO C, FABRIS A, GAMMARO L, RUGIU C, PANZETTA G: Effects of dietary protein and phosphorus restriction on the progression of early renal failure. *Kidney Int* 22:371–376, 1982
6. MASCHIO G, OLDRIZZI L, TESSITORE N, D'ANGELO A, VALVO E, LUPO A, LOSCHIAVO C, FABRIS A, GAMMARO L, RUGIU C, PANZETTA G: Early dietary protein and phosphorus restriction is effective in delaying progression of chronic renal failure. *Kidney Int* 24:273–277, 1983
7. OLDRIZZI L, RUGIU C, VALVO E, LUPO A, LOSCHIAVO C, GAMMARO L, TESSITORE N, FABRIS A, PANZETTA G, MASCHIO G: Progression of renal failure in patients with renal disease of diverse etiology on protein-restricted diet. *Kidney Int* 27:553–557, 1985
8. BARSOTTI G, MORELLI E, GIANNONI A, GUIDUCCI A, LUPETTI S, GIOVANNETTI S: Restricted phosphorus and nitrogen intake to slow the progression of chronic renal failure: A controlled trial. *Kidney Int* 24:278–284, 1983
9. BARSOTTI G, GIANNONI A, MORELLI E, LAZZERI M, VLAMIS I, BALDI R, GIOVANNETTI S: The decline of renal function slowed by very low phosphorus intake in chronic renal patients following a low nitrogen diet. *Clin Nephrol* 21:54–59, 1984
10. CIARDELLA F, MORELLI E, NIOSI F, CAPRIOLI R, BALDI R, CUPISTI A, PETRONIO G, CARBONE C, BARSOTTI G: Effects of low phosphorus, low nitrogen diet supplemented with essential amino acids and ketoanalogues on serum trigylcerides of chronic uremic patients. *Nephron* 42:196–199, 1986
11. LUCAS PA, BROWN RC, WOODHEAD JS, COLES GA: 1,25-dihydroxycholecalciferol and parathyroid hormone in advanced chronic renal failure: effects of simultaneous protein and phosphorus restriction. *Clin Nephrol* 25:7–10, 1986
12. GIN H, APARICIO M, POTAUX L, DE PRECIGOUT V, BOUCHET JL, AUBERTIN J: Low protein and low phosphorus diet in patients with chronic renal failure: influence on glucose tolerance and tissue insulin sensitivity. *Metabolism* 36:1080–1085, 1987
13. MCCRORY WW, GERTNER JM, BURKE FM, PIMENTAL CT, NEMERY RL: Effects of dietary phosphate restriction in children with chronic renal failure. *J Pediatr* 111:410–412, 1987
14. TESSITORE N, VENTURI A, ADAMI S, RONCARI C, RUGIU C, CORGNATI A, BONUCCI E, GIUSEPPE M: Relationship between serum vitamin D metabolites and dietary intake of phosphate in patients with early renal failure. *Miner Electrolyte Metab* 13:38–44, 1987
15. BARSOTTI G, GUIDUCCI A, CIARDELLA F, GIOVANNETTI S: Effects on renal function of a low-nitrogen diet supplemented with essential amino acids and ketoanalogues and of hemodialysis and free protein supply in patients with chronic renal failure. *Nephron* 27:113–117, 1981
16. GRETZ N, KORB E, STRAUCH M: Low-protein diet supplemented by keto acids in chronic renal failure: A prospective controlled study. *Kidney Int* 24:S263–S267, 1983
17. ALVESTRAND A, AHLBERG M, BERGSTROM J: Retardation of the progression of renal insufficiency in patients treated with low-protein diets. *Kidney Int* 24:S268–S272, 1983

18. Rosman JB, Meijer S, Sluiter WJ, Ter Wee PM, Piers-Becht T, Donker AJM: Prospective randomised trial of early dietary protein restriction in chronic renal failure. *Lancet* 2:1291–1296, 1984
19. Lucas PA, Meadows JH, Roberts DE, Coles GA: The risks and benefits of a low protein-essential amino acid-keto diet. *Kidney Int* 29:995–1003, 1986
20. Mitch WE, Walser M, Steinmen TE, Hill S, Zeger S, Tungsanga K: The effects of a keto acid-amino supplement to a restricted diet on the progression of chronic renal failure. *N Engl J Med* 311:623–629, 1984
21. El Nahas AM, Thomas AM, Brady SA, Farrington K, Wilkinson V, Hilson AJW, Varghese Z, Moorehead JF: Selective effect of low protein diets in chronic renal disease. *Br Med J* 289:1337–1341, 1984
22. Giordano C: Early dietary protein restriction protects the failing kidney. *Kidney Int* (suppl 17) 28:S66–S70, 1985
23. Farr LE, Smadel JE: The effect of dietary protein on the course of nephrotoxic nephritis in rats. *J Exp Med* 70:615–627, 1939
24. Friend PS, Fernandes G, Good RA, Michael AF, Yunis EJ: Dietary restrictions early and late: Effects on the nephropathy of the NZB × NZW mouse. *Lab Invest* 38:629–632, 1978
25. Neugarten J, Feiner HD, Schacht RG, Baldwin DS: Amelioration of experimental glomerulonephritis by dietary protein restriction. *Kidney Int* 24:595–601, 1983
26. Laouari D, Kleinknecht C, Gubler MC, Broyer M: Adverse effect of proteins on remnant kidney: Dissociation from that of other nutrients. *Kidney Int* 24:S248–S253, 1983
27. El-Nahas AM, Paraskevakou H, Zoob S, Rees AJ, Evans DJ: Effect of dietary protein restriction on the development of renal failure after subtotal nephrectomy in rats. *Clin Sci* 65:339–406, 1983
28. Remuzzi G, Zoja C, Remuzzi A, Rossini M, Battaglia C, Broggini M, Bertani T: Low-protein diet prevents glomerular damage in adriamycin-treated rats. *Kidney Int* 28:21–27, 1985
29. Ritz E, Mehls O, Gilli G, Heuck CC: Protein restriction in the conservative management of uremia. *Am J Clin Nutr* 31:1703–1711, 1978
30. Rabinowitz L, Gunther RA, Shouji ES, Freedland RA, Avery EH: Effects of high and low protein diets on sheep renal function and metabolism. *Kidney Int* 4:188–207, 1973
31. Schoolwerth AC, Sandler RS, Hoffman PM, Klahr S: Effects of nephron reduction and dietary protein content on renal ammoniagenesis in the rat. *Kidney Int* 7:397–404, 1975
32. Hostetter TH, Olsen JL, Rennke HG, Venkatachalam MA: Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. *Am J Physiol* 241:F85–F93, 1981
33. Bovee KC, Kronfeld DS: Reduction of renal hemodynamics in uremic dogs fed reduced protein diets. *J Am Anim Hosp Assoc* 17:277–285, 1981
34. Benigni A, Zoja C, Remuzzi A, Orisio S, Piccinelli A, Remuzzi G: Role of renal prostaglandins in normal and nephrotic rats with diet-induced hyperfiltration. *J Lab Clin Med* 108:230–240, 1986
35. Bidani AK, Schwartz MM, Lewis EJ: Renal autoregulation and vulnerability to hypertensive injury in remnant kidney. *Am J Physiol* 252:F1003–F1010, 1987
36. Bosch JP, Saccaggi A, Ronco C, Belledonne M, Glabman S: Renal functional reserve in humans: effect of protein intake on glomerular filtration rate. *Am J Med* 75:943–950, 1983
37. Schaap GH, Bilo HJG, Alferink THR, Oe Pl, Donker AJM: The effect of a high protein intake on renal function of patients with chronic renal insufficiency. *Nephron* 47:1–6, 1987
38. Robertson JL, Goldschmidt M, Kronfeld DS, Tomaszewski JE, Hill GS, Bovee KC: Long-term renal responses to high dietary protein in dogs with 75% nephrectomy. *Kidney Int* 29: 511–519, 1986
39. Hostetter TH, Meyer TW, Rennke HG, Brenner BM: Chronic effects of dietary protein in rat with intact and reduced renal mass. *Kidney Int* 30:509–517, 1986
40. Brenner BM: Nephron adaptation to renal injury or ablation. *Am J Physiol* 249:F324–F337, 1985
41. Anderson SM, Meyer TW, Rennke HG, Brenner BM: Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. *J Clin Invest* 76:612–619, 1985
42. Yoshida Y, Fogo A, Shiraga H, Glick AD, Ichikawa I: Serial micropuncture analysis of single nephron function in subtotal renal ablation. *Kidney Int* 33:855–867, 1988
43. Anderson S, Diamond JR, Karnovsky MJ, Brenner BM: Mechanisms underlying transition from acute glomerular injury to late glomerular sclerosis in a rat model of nephrotic syndrome. *J Clin Invest* 82:1757–1768, 1988
44. Ibels LS, Alfrey AC, Haut L, Huffer W: Preservation of function in experimental renal disease by dietary restriction of phosphate. *N Engl J Med* 298:122–126, 1978
45. Karlinsky ML, Haut L, Buddington B, Schrier NA, Alfrey AC: Preservation of renal function in experimental glomerulonephritis. *Kidney Int* 17:293–302, 1980
46. Laouari D, Kleinknecht C, Witmer GC, Habib R, Mounier F, Broyer M: Beneficial effect of low phosphorus diet in uraemic rats: a reappraisal. *Clin Sci* 63:539–548, 1982
47. Kikuchi H, Matsushita T, Hirata K: Improved dietary treatment with low protein and phosphorus restriction in uremic rats. *Kidney Int* 24:S254–S258, 1983
48. Klahr S, Buerkart J, Purkerson ML: Role of dietary factors in the progression of chronic renal disease. *Kidney Int* 24:579–587, 1983
49. Kleinknecht C, Salusky I, Broyer M, Gubler MC: Effects of various protein diets on growth, renal function, and survival of uremic rats. *Kidney Int* 5:534–541, 1979
50. Haut L, Alfrey AC, Guggenheim S, Buddington B, Schrier N: Renal toxicity of phosphate in rats. *Kidney Int* 17:722–731, 1980
51. Salusky I, Kleinknecht C, Broyer M, Gubler MC: Prolonged renal survival and stunting with protein-deficient diets in experimental uremia. *J Lab Clin Med* 97:21–29, 1981
52. Gimenez L, Walker WG, Tew WP, Hermann JA: Prevention of phosphate-induced progression of uremia in rats by 3-phosphocitric acid. *Kidney Int* 22:36–41, 1982
53. Lumlertgul G, Burke TJ, Gillum DM, Alfrey AC, Harris DC, Hammond WS, Schrier RW: Phosphate depletion arrests progression of chronic renal failure independent of protein intake. *Kidney Int* 29:658–666, 1986
54. Hruska KA, Goligorsky M, Scoble J, Tsutsumi M, Westbrook S, Moskowitz D: Effects of parathyroid hormone on cytosolic calcium in renal proximal tubular primary cultures. *Am J Physiol* 251:F188–F198, 1986
55. Bourdeau JE, Lau K: Effects of parathyroid hormone on cytosolic free Ca concentration in individual rabbit connecting tubules. *J Clin Invest* 83:373–379, 1989
56. Solanki J, Lau K, Eby B, Tan S, Rodrigues P, Lau K: Chronic effects of parathyroid hormone (PTH) on renal tubule cytosolic free Ca levels ($[Ca^{2+}]_i$): dependency on retained Ca but not on serum Ca. *Kidney Int* 35:387A, 1989
57. Bank N, Su WS, Aynedjian S: A micropuncture study of renal phosphate transport in rats with chronic renal failure and secondary hyperparathyroidism. *J Clin Invest* 61:884–894, 1978
58. Wong NLM, Quamme GA, Dirks JH, Sutton RAL: Phosphate handling by the remnant dog kidney in the presence and absence of the contralateral normal kidney. *Adv Exp Med Biol* 128:117–124, 1980
59. Wen SF, Stoll RW: Renal phosphate adaptation in uraemic dogs with a remnant kidney. *Clin Sci* 60:273–282, 1981
60. Fotino S: Phosphate excretion in chronic renal failure: evidence for a mechanism other than circulating parathyroid hormone. *Clin Nephrol* 8:499–503, 1977
61. Caverzasio J, Gloor HJ, Fleisch H, Bonjour JP: Parathyroid hormone-independent adaptation of the renal handling of phosphate in response to renal mass reduction. *Kidney Int* 21:471–476, 1982
62. Hruska K, Klahr S, Hammerman M: Decreased luminal membrane transport phosphate in chronic renal failure. *Am J Physiol* 242:17–26, 1982
63. Yanagawa N, Nissenson RA, Edward B, Yeung P, Trizna W, Fine LG: Functional profile of the isolated uremic nephron: Intrinsic adaptation of phosphate transport in the rabbit proximal tubule. *Kidney Int* 23:674–683, 1983
64. Tan S, Pesigan M, Loor E, Crockett R, Ondrizek T, Lau K: Pathogenetic role of increased tubule cytosolic free Ca concentra-

tion ($[Ca^{2+}]_i$) in progressive renal insufficiency: Amelioration by a low PO_4 diet. *Kidney Int* 35:438A, 1989
65. Worchester EM, Nakagawa Y, Bushinsky DA, Coe FL: Evidence that serum calcium oxalate supersaturation is a consequence of oxalate retention in patients with chronic renal failure. *J Clin Invest* 77:1888–1896, 1986
66. De Rouffignac C, Morel F, Moss N, Roinel N: Micropuncture study of water and electrolyte movements along the loop of Henle in psammomy with special reference to magnesium, calcium and phosphorus. *Pflugers Arch* 344:300–326, 1973
67. Jamison RL, Frey NR, Lacey FB: Calcium reabsorption in the thin loop of Henle. *Am J Physiol* 277:745–751, 1974
68. Lau K, Goldfarb S, Goldberg GM, Agus ZA: Effects of phosphate administration on tubular calcium transport. *J Lab Clin Med* 99:317–324, 1982
69. Lau K, Agus ZS, Goldberg M, Goldfarb S: Renal tubular sites of altered calcium transport in phosphate-depleted rats. *J Clin Invest* 64:1681–1687, 1979
70. Harris CA, Burnatowska MA, Seely JF, Sutton RAL, Quamme GA, Dirks JH: Effects of parathyroid hormone on electrolyte transport in the hamster nephron. *Am J Physiol* 236: F342–F348, 1979
71. Knox FG, Haas JA, Brendt T, Marchand GR, Youngberg SP: Phosphate transport in superficial and deep nephrons in phosphate-loaded rats. *Am J Physiol* 233:F150–F153, 1977
72. Haas JA, Berndt T, Knox FG: Nephron heterogeneity of phosphate reabsorption. *Am J Physiol* 234:F287–F290, 1978
73. Haramati A: Effects of phosphate infusions on phosphate transport in superficial and deep nephrons (*abstract*). *Kidney Int* 25:144, 1984
74. Haramati A, Haas JA, Knox FG: Adaptation of deep and superficial nephrons to changes in dietary phosphate intake. *Am J Physiol* 244:F265–F269, 1983
75. Haramati A, Haas JA, Knox FG: Nephron heterogeneity of phosphate reabsorption: effect of parathyroid hormone. *Am J Physiol* 246:F155–F158, 1984
76. Lau K, Guntupalli J, Eby B: Effects of somatostatin on phosphate transport: Evidence for the role of basal insulin. *Kidney Int* 24:10–15, 1983
77. Pastoriza-Munoz E, Colindres RE, Lassiter WE, Lechene C: Effects of parathyroid hormone on phosphate reabsorption in rat distal convolution. *Am J Physiol* 235:F321–F330, 1978
78. Brecevic L, Furedi-Milhofer H: Precipitation of calcium phosphates from electrolyte solutions. V: the influence of citrate ions. *Calcif Tissue Int* 28:131–136, 1979
79. Slatopolsky E, Caglar S, Pennel JP, Taggart DD, Canterbury JM, Reiss E, Bricker NS: On the pathogenesis of hyperparathyroidism in chronic experimental renal insufficiency in the dog. *J Clin Invest* 50:492–499, 1971
80. Finfeld DA, Sherwood LM: Parathyroid hormone and $1,25(OH)_2D_3$ in chronic renal failure. *Kidney Int* 33:1049–1058, 1988
81. Portale AA, Booth B, Balloran BP Jr, Morris RC: Effect of dietary phosphorus on circulating concentrations of 1,25-dihydroxyvitamin D and immunoreactive parathyroid hormone in children with moderate renal insufficiency. *J Clin Invest* 73:1580–1589, 1984
82. Lau K, Chen S, Eby B: Evidence for the role of PO_4 deficiency in antihypertensive action of a high-Ca diet. *Am J Physiol* 246: H324–H331, 1984
83. Slatopolsky E, Weerts C, Lopez-Hilker S, Norwood K, Zink M, Windus D, Delmez J: Calcium carbonate as a phosphate binder in patients with chronic renal failure undergoing dialysis. *N Engl J Med* 315:157–161, 1986
84. Guillot AP, Hood VL, Runge CF, Gennari FJ: The use of magnesium-containing phosphate binders in patients with end-stage renal disease on maintenance hemodialysis. *Nephron* 30:114–117, 1982
85. Mak RHK, Turner C, Thompson T, Powell H, Haycock GB, Chantler C: Suppression of secondary hyperparathyroidism in children with chronic renal failure by high dose phosphate binders: calcium carbonate versus aluminum hydroxide. *Br Med J* 291: 623–627, 1985
86. Ramirez JA, Emmett M, White MG, Fathi N, Santa Ana CA, Morawski, SG, Fordtran JS: The absorption of dietary phosphorus and calcium in hemodialysis patients. *Kidney Int* 30:753–759, 1986
87. Sheikh MS, Maguire JA, Emmett M, Santa Ana CA, Nicar MJ, Schiller LA, Fordtran JS: Reduction of dietary phosphorus absorption by phosphorus binders. *J Clin Invest* 83:66–73, 1989
88. Craig JM: Observations on the kidney after phosphate loading in the rat. *AMA Arch Pathol* 68:74–83, 1959
89. MacKay EM, Oliver J: Renal damage following the ingestion of a diet containing an excess of inorganic phosphate. *J Exp Med* 61:319–333, 1935
90. Borle AB, Clark I: Effects of phosphate-induced hyperparathyroidism and parathyroidectomy on rat kidney calcium in vivo. *Am J Physiol* 241:E136–E141, 1981
91. Coburn JW, Salusky IB: Control of serum phosphorus in uremia. *N Engl J Med* 320:1140–1142, 1989
92. Tomford RC, Karlinsky ML, Buddington B, Alfrey AC: Effect of thyroparathyroidectomy and parathyroidectomy on renal function and the nephrotic syndrome in rat nephrotoxic serum nephritis. *J Clin Invest* 68:655–664, 1981
93. Goldsmith RS, Ingbar SH: Inorganic phosphate treatment of hypercalcemia of diverse etiologies. *N Engl J Med* 274:1–7, 1966
94. Shackney S, Hasson J: Precipitous fall in serum calcium, hypotension, and acute renal failure after intravenous phosphate therapy for hypercalcemia. *Ann Intern Med* 65:906–916, 1967
95. Kaplan MA, Canterbury JM, Bourgoignie JJ, Veliz G, Gavellas G, Reiss E, Bricker NS: Reversal of hyperparathyroidism in response to dietary phosphorus restriction in the uremic dog. *Kidney Int* 15:43–48, 1979
96. Harris DCH, Hammond WS, Burke TJ, Schrier RW: Verapamil protects against progression of experimental chronic renal failure. *Kidney Int* 31:41–46, 1987
97. Goligorsky MS, Chaimovitz C, Rapaport J, Goldstein J, Kol R: Calcium metabolism in uremic nephrocalcinosis: Preventive effect of verapamil. *Biochim Biophys Acta* 27:774–779, 1985
98. Hammerman MR, Hruska A: Regulation of canine renal vesicle Pi transport by growth hormone and parathyroid hormone. *Biochem Acta* 603:322–25, 1980
99. Lau K: Phosphate disorders, in *Fluid and Electrolytes,* edited by Kokko JP, Tannen RL, Saunders, Philadelphia, 1986, pp 398–453