Effect of Thyroparathyroidectomy and Parathyroidectomy on Renal Function and the Nephrotic Syndrome in Rat Nephrotoxic Serum Nephritis

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ABSTRACT Dietary phosphorus restriction (PR) prevents uremia in rats with nephrotoxic serum nephritis (NSN). One possible mechanism by which PR could be protective would be through the suppression of parathyroid hormone. To evaluate this possibility two separate protocols were designed. In the first rats were thyroparathyroidectomized (TPTX) before ($n = 11$) or 5 wk after ($n = 7$) NSN induction and compared to sham-operated parathyroid intact rats with NSN ($n = 12$). At the end of the 23-wk study, intact rats were azotemic, plasma creatinine 3.80±0.81 mg/100 ml vs. 0.65±0.07 for TPTX rats ($P < 0.001$). During the study 75% of intact rats died of uremia in contrast to none of the TPTX rats ($P < 0.001$). Renal histological damage was greatly diminished and calcification prevented in TPTX rats. The proteinuria of the heterologous phase was unaffected, but the protein excretion and hypertriglyceridemia (HTG) of the autologous phase were markedly decreased in the TPTX rats. The degree of HTG and proteinuria had a high positive correlation ($P < 0.001$). Late TPTX also produced significant decreases in proteinuria and HTG regardless of the degree of azotemia, and prevented azotemia if the plasma creatinine at the time of TPTX was ≤0.85 mg/100 ml.

In additional studies selective parathyroidectomy (PTX) was performed. The adequacy of this procedure was documented by showing a similar fall in plasma Ca and urinary cyclic AMP in PTX animals as found in TPTX animals. However, selective PTX had no effect on proteinuria, histologic damage, or functional deterioration. These studies further showed that early, histologic damage and functional deterioration preceeded renal parenchymal calcification. Because animals were pair fed and both groups were given 1,25-dihydroxycholecalciferol to normalize serum Ca and P levels these studies exclude alterations in plasma Ca and P levels, dietary intake, urinary P excretion, and vitamin D administration in promoting the protective effect of TPTX on renal function.

We conclude that TPTX is equally effective in preventing functional deterioration and more effective in reducing proteinuria in NSN than PR. The mechanism of this protective effect remains to be elucidated, since it does not primarily involve either the elimination of parathyroid hormone or the prevention of renal parenchymal calcification.

INTRODUCTION

Recent studies have demonstrated that dietary restriction of phosphorus will decrease histological damage and prevent renal functional deterioration in immunological and nonimmunological forms of experimental renal disease (1, 2). It is important to note that the rate of functional deterioration in patients with chronic renal disease also appears to be decreased by phosphorus restriction (PR)[1] (3). Since PR completely prevented

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[1] *Abbreviations used in this paper*: Cr, creatinine; 1,25-$(OH)_2D_3$, 1,25-dihydroxycholecalciferol; GBM, glomerular basement membrane; GFR, glomerular filtration rate; HTG, hypertriglyceridemia; NSN, nephrotoxic serum nephritis; NTS, nephrotoxic serum; PR, phosphate restriction; PTH, parathyroid hormone; PTX, selective parathyroidectomy; TG, triglycerides; TPTX, thyroparathyroidectomy.

the elevated tissue Ca levels observed in end-stage kidneys, it has been suggested that this protective effect may be mediated through the prevention of renal parenchymal calcification, possibly by reducing the Ca × P product. Another mechanism for reduction of renal damage and calcification, however, is suppression of parathyroid hormone (PTH) secretion secondary to hypercalcemia produced by PR (2). Experimentally, parathyroid extract causes Ca deposition primarily in renal tubular epithelial cells and basement membrane, a pattern similar to that described in human and experimental end-stage kidneys (4–9).

Mechanisms other than prevention of renal calcification could also be involved in a protective effect of PTH suppression, since PTH-specific receptors have been detected in rat glomeruli (10). Of interest, PTH-induced glomerular cyclic AMP production is apparently confined largely to glomerular epithelial cells (11), the barrier to albumin passage in the nephron. PTH and dibutyryl cyclic AMP, in addition, acutely reduce the glomerular capillary ultrafiltration coefficient (K_f) and the glomerular filtration rate (GFR) in rats (12, 13). Thus, it seems reasonable to hypothesize that PTH could alter renal function, calcification, and proteinuria in chronic renal failure.

The purpose of the present study was to determine the role of PTH in PR protective effect by analyzing the effect of thyroparathyroidectomy (TPTX) and selective parathyroidectomy (PTX) on renal function and the nephrotic syndrome in nephrotoxic serum nephritis (NSN).

The results of the studies indicate that whereas TPTX is completely protective of renal function, markedly decreases proteinuria and renal calcification, PTX has no effect on the nephrotic syndrome, histologic damage, or development of chronic renal insufficiency. From these studies we conclude that the protective mechanism(s) of PR and TPTX in NSN are not mediated primarily through PTH suppression or prevention of early renal parenchymal calcification.

METHODS

Nephrotoxic serum. Nephrotoxic serum (NTS) was obtained from rabbits immunized bimonthly repeatedly with 10–12 mg purified rat glomerular basement membrane (GBM) in complete Freund's adjuvant prepared according to Krakower's and Greenspon's method (14). Biological activity of the NTS was assessed by double layer immunofluorescence of rat kidney slices. All rabbit NTS was complement inactivated at 56°C for 30 min and absorbed with normal rat erythrocytes for 60 min before injection.

Protocols. Three separate studies were done. For studies I and II male Sprague-Dawley rats weighing 250–300 g (Simonsen Mill Rendering Plant, Quimby, Iowa) were uninephrectomized to decrease the quantity of NTS required to induce disease and to shorten the time required for development of renal insufficiency. Following uninephrectomy the rats were either TPTX or selectively PTX by thermocautery. Adequacy of TPTX and PTX was determined one to three weeks postsurgery by a serum Ca consistently <7.0 mg/100 ml following overnight fasting. Mean serum Ca was 6.3±0.8 mg/100 ml in TPTX animals vs. 4.8±0.4 in the PTX group (P = NS). PTX animals were demonstrated to have urinary cyclic-AMP excretions in the same range as TPTX rats for as long as five months (TPTX, 5.4±1.04 nmol/100 ml GFR; PTX 4.9±0.4 nmol/100 ml GFR; non-TPTX 13.5±2.9 nmol/100 ml GFR). Non-TPTX control rats were sham operated.

2 wk following their surgical procedure and 5 d before receiving NTS all animals were preimmunized with 10–12 mg of ammonium sulfate fractionated rabbit gamma globulin in incomplete Freund's adjuvant intramuscularly and subcutaneously. All rats were fed Purina normal rat chow diet (Ralston Purina Co., St. Louis, Mo.) and plasma Ca normalized with thrice weekly injections of 0.015 μg/kg body wt 1,25-dihydroxycholecalciferol (1,25-$[OH]_2D_3$) i.p., in order to eliminate the possible effects of hypocalcemia on renal calcification and triglyceride (TG) metabolism.

Study 1. The effect of TPTX on renal insufficiency and the nephrotic syndrome was examined over a 23-wk period at which time all rats that had survived were killed. Rats were either TPTX before induction or 5 wk after induction (late TPTX group) of NSN. Non-TPTX rats were sham operated before induction of disease. These rats were fed ad lib., and the final weights for the TPTX and non-TPTX groups were 357±12 and 348±26 g, respectively (P = NS). The average plasma Ca of TPTX rats was increased from 6.3±0.8 to 9.0±0.14 mg/100 ml in the TPTX group and 9.6±0.38 mg/100 ml in the late TPTX group by vitamin D replacement. Although the TPTX group's plasma Ca was significantly less than the non-TPTX group, it was within the normal range found in our laboratory, and the late TPTX group's plasma Ca was virtually identical to non-TPTX rats (Table I). The average plasma P was similar in all groups as was the Ca × P product (Table I). These values represent animals still alive at the time of plasma analysis. In rats progressing to end-stage renal disease, the plasma Ca and P rose rapidly during the final 1–2 wk reaching values of 10–12.5 and 10–24 mg/100 ml, respectively. Synthroid was given to TPTX rats sporadically but never more than weekly at a dose of 10 μg i.p.

Blood was obtained by tail artery cannulation 1 wk before and 1 wk after NTS injection and every 2 wk thereafter for analysis of plasma Ca, P, and creatinine (Cr). Plasma TG, cholesterol, and albumin were determined 30 and 60 d after NTS injection in the TPTX and non-TPTX groups. The subgroup of non-TPTX animals receiving a TPTX at 35 d had

TABLE I
Study I. Effect of 1,25-$(OH)_2D_3$ Therapy on Plasma Ca, P, and Ca × P Product

Group	Plasma Ca	Plasma P	Ca × P
	mg/100 ml		*mg/100 ml²*
Non-TPTX	9.7±0.14	6.71±0.35	64.4±3.41
TPTX	9.0±0.27*	6.77±0.20	58.8±1.64
TPTX at 35 d	9.6±0.38	6.90±0.48	69.9±5.15

Values are average plasma Ca and P over the course of the experiment demonstrating normalization of serum Ca in TPTX animals treated with 1,25-$(OH)_2D_3$.
* TPTX vs. non-TPTX P value < 0.05. All other comparisons did not yield significant differences.

their plasma TG, cholesterol, and albumin levels determined immediately before and 30 d post-TPTX (65 d into the study).

24-h urinary protein excretion was measured before and 1 wk after NTS injection and at monthly intervals thereafter.

Study II. This study was designed to compare the effect of PTX and TPTX on the development of early renal insufficiency and the nephrotic syndrome. Rats were TPTX, PTX, or sham operated before induction of disease. The experiment was designed to end when one group's Cr became significantly elevated. Therefore, all rats were killed at 5 wk for histologic study and kidney Ca and P content determination.

All rats were placed in individual metabolic cages 1 wk before induction of NSN, and pair-fed throughout the entire study. All rats in the TPTX and PTX groups had their plasma Ca normalized with 1,25-$(OH)_2D_3$ (as above) and the TPTX group received Synthroid 10 μg i.p. weekly. Plasma analyses were done weekly for Ca, P, Cr, albumin, cholesterol, and TG.

Study III. This protocol was carried out to determine the effect of PTX on the development of chronic renal insufficiency, and designed to end when one group developed advanced renal disease. Nine sham operated rats comprised the non-TPTX group, whereas the PTX and TPTX groups consisted of 10 and 11 animals, respectively. Adequacy of PTX and TPTX was assessed as in studies I and II. Plasma Ca before induction of disease was 6.9±0.5 mg/100 ml for TPTX rats and 6.8±0.3 mg/100 ml for the PTX groups. All rats were uninephrectomized at least 4 wk before induction of nephritis and were preimmunized with 8–10 mg of DEAE-cellulose fractionated rabbit gamma globulin in incomplete Freund's adjuvant 5 d before the intravenous injection of 1.0 ml NTS. PTX and TPTX rats received 1,25-$(OH)_2D_3$ as in the above protocols. No thyroid replacement was given to the TPTX group. The groups were placed in cages with two to three rats per cage and given 20 g of ICN-purified rat diet (ICN Pharmaceuticals, Inc., Plainview, N. Y.) per rat per day.

Histology. Kidneys from all animals who died or were killed were examined after hematoxylin and eosin staining and after Alizarin red-S staining to demonstrate divalent cations. Immunofluorescence studies were performed only on kidneys from killed rats. Kidney tissue was embedded in O.C.T. compound (Lab-Tek Products Div. Miles Laboratories Inc., Naberville Ill.) and snap-frozen immediately in dry ice-ethanol. Tissue sections were reacted with fluoresceinated rabbit anti-rat IgG, or goat anti-rat C3 (third component of complement) (Microbiological Associates Walkersville Md.).

Chemical analysis. Plasma was analyzed for Cr (15) and P (16) on a Technicon II AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.). Serum Ca was determined by atomic absorption spectrophotometry (Perkin Elmer model 290B Perkin Elmer Corp. Instrument Div., Norwalk, Conn.) Urinary protein was determined by a modified Lowry method (17) or a trichloracetic acid precipitation method (18). Plasma TG were measured by a modified colorimetric technique (19) using a Sigma kit No. 405 (Sigma Chemical Co., St. Louis, Mo.) Plasma cholesterol was determined by an enzymatic degradation method (20) using Calbiochem-Behring kit reagents (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) Plasma albumin was measured with the bromcrescol green method at 600 nm wavelength (21). Kidney mineral content was determined by methods reported previously (22). Urinary cyclic-AMP was measured by a radioimmunoassay using Becton Dickinson Kit No. 201618 (Becton, Dickinson & Co., Rutherford, N. J.) (23).

Statistical methods. Statistical comparisons were performed using the paired or unpaired Student's t test, analysis of variance with Duncan's test of significance, or χ^2 as appropriate. All data are expressed as the mean±SEM.

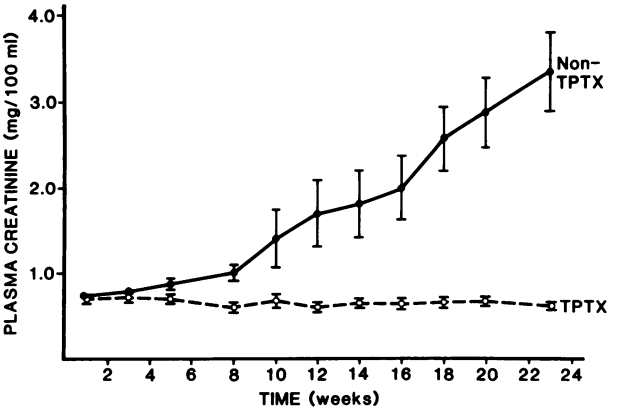

FIGURE 1 Plasma Cr vs. time after induction of NSN in TPTX (n = 11) and non-TPTX (n = 12) rats. $P < 0.05$ at 5 wk. $P < 0.001$ at 18–24 wk.

RESULTS

Effect of TPTX on renal function (study I). TPTX had a profound effect in preventing the development of uremia. Fig. 1 demonstrates that the non-TPTX animals had significantly higher mean plasma Cr than TPTX rats by 5 wk ($P < 0.005$). The non-TPTX rats' mean Cr progressively rose throughout the study while the TPTX group's mean Cr remained constant at 0.65 mg/100 ml. Survival was 100% in the TPTX group as depicted in Fig. 2. In contrast, only 25% of the non-TPTX rats survived through the 23-wk study. All deaths were related to renal failure with a mean Cr of 3.8±0.81 mg/100 ml before death. Final weights between the two groups were not significantly different.

TPTX performed after induction of NSN also provided some protection against the development of renal insufficiency (Fig. 3). Only one of seven rats TPTX 5 wk

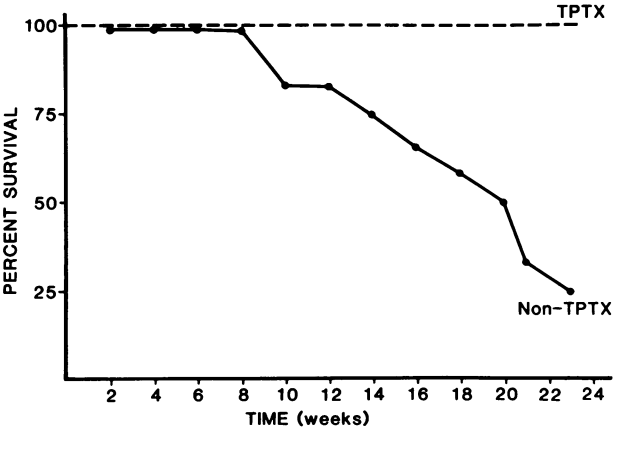

FIGURE 2 Percentage of animals with NSN surviving throughout the study. At the conclusion of the experiment 3 of 12 non-TPTX survived but all were uremic with lowest plasma Cr 1.65 mg/100 ml. n = 11 for TPTX group.

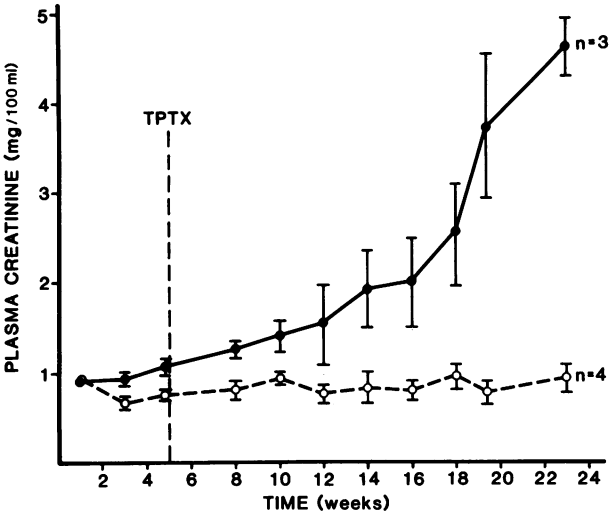

FIGURE 3 Plasma Cr vs. time after NSN induction in rats thyroparathyroidectomized 35 d after NTS injection. Animals were separated on the basis of plasma Cr ≥ 1.0 mg/100 ml (solid line) or <1.0 mg/100 ml (dashed line) at time of TPTX. $P < 0.02$ before TPTX. $P < 0.001$ at 23 wk.

after induction of nephritis died of uremia by the end of the 23-wk study period ($P < 0.01$; χ^2) despite disease as manifested by proteinuria in all rats at the time of surgery. Analysis of these rats revealed that animals with Cr ≥ 1.0 mg/100 ml at the time of TPTX surgery developed progressive uremia whereas those with Cr ≤ 0.85 mg/100 ml had a more stable course without developing uremia (Fig. 3).

Effect of TPTX on the nephrotic syndrome. NTS induced high grade proteinuria (262±14 mg/24 h) within 4 d after injection (Table II). TPTX had no significant effect on the degree of protein excretion during this early phase of NSN, but prevented the twofold increase observed in non-TPTX rats 30 d following nephritis induction when the autologous phase was well established (298±24 vs. 601±44 mg/24 h; $P < 0.001$). Proteinuria in the TPTX group decreased further without returning to base line over the ensuing 12 wk whereas it remained essentially unchanged in the non-TPTX group.

Even more striking was the complete absence of hypertriglyceridemia (HTG) in the TPTX rats in contrast to the intact group (Fig. 4). At 4 wk the mean plasma TG level of TPTX rats was not significantly different from control animals, whereas the non-TPTX rats experienced a five- to sixfold increase. Hypercholesterolemia was only partially prevented (134±12 in TPTX rats vs. 205±17 mg/100 ml in non-TPTX rats; $P < 0.005$) and hypoalbuminemia only partially corrected (2.80±0.06 vs. 2.43±0.07 g/100 ml; $P < 0.001$) (Fig. 4) in the TPTX animals. There was no significant correlation between plasma cholesterol and either plasma albumin or total protein excretion. However, there was a strong positive correlation between the plasma TG level and proteinuria ($r = 0.711$; $P < 0.001$) and a borderline negative correlation between plasma TG concentrations and plasma albumin ($r = 0.437$; $P < 0.05$).

Late TPTX demonstrated that the autologous phase proteinuria and HTG are reversible as well as preventable. As shown in Table II, proteinuria decreased in all rats from a mean of 570±59 just before TPTX to 315±48 mg/24 h 4 wk after TPTX ($P < 0.001$) and the decrease was maintained for the duration of the study. In association with the fall in proteinuria there was a highly significant decrease in plasma TG (Fig. 5). All rats showed a marked decrease in plasma TG concentrations although not necessarily to control values. Plasma cholesterol was less uniformly affected and the small decrease was not statistically significant.

Effect of TPTX on kidney Ca and P content. NSN induced marked renal calcification which was prevented totally by TPTX (Table III). Non-TPTX kidneys had 619±170 mmol Ca/kg dry kidney wt vs. 14.8±2.8 mmol Ca/kg dry kidney wt for TPTX kidneys.

TABLE II
Effect of TPTX on Urinary Protein Excretion

Group	Time after nephritis induction				
	4 d	4 wk	9 wk	13 wk	21 wk
Non-TPTX	267±17*	601±44	506±55	406±31	489±60
TPTX	225±47	298±24*	154±24*	90±15	107±25
TPTX at 35 d		579±59*	315±48	278±45	265±62

Urinary protein excretion was measured at various time periods after NTS injection and is expressed as milligrams excreted per 24 h. P values between groups at time periods: 4 wk, non-TPTX vs. TPTX < 0.01; 9 wk, non-TPTX vs. TPTX or TPTX at 35 d < 0.01; TPTX vs. TPTX at 35 d < 0.05; 13 wk and 21 wk, non-TPTX vs. TPTX or TPTX at 35 d < 0.01.

* $P < 0.01$ for adjacent values in rows.

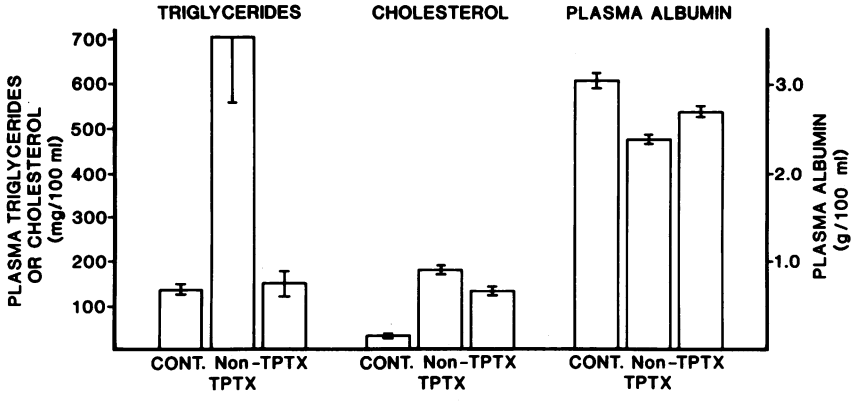

FIGURE 4 Effect of TPTX on plasma TG, cholesterol, and albumin. TG and cholesterol were measured in the nonfasting state at 4 wk after nephritis induction. Controls (Cont.) are normal rats without nephritis (n = 5). Data are expressed as mean±SEM. P values for TG: TPTX vs. non-TPTX < 0.001; TPTX vs. Cont. NS; for cholesterol: TPTX vs. non-TPTX < 0.005; TPTX vs. Cont. < 0.01; for albumin: TPTX vs. non-TPTX < 0.005; TPTX vs. Cont. < 0.001.

Non-TPTX kidneys had 796±110 mmol P/kg dry kidney wt. TPTX also limited the increase in kidney P content to 430±11 mmol P/kg dry kidney wt but there remained a small but statistically higher P content in the TPTX group than in the control animals (359±7 mmol P/kg dry kidney weight, $P < 0.001$).

Effect of TPTX on immunofluorescence and histology. Both TPTX and non-TPTX rats displayed intense linear staining of the glomerular basement membrane with fluoresceine-labeled anti-rat IgG. When stained with anti-C3 antibody, the non-TPTX group kidneys displayed glomerular fluorescence in a granular pattern which was undetectable in the TPTX animals. Tubules failed to stain with either conjugate in either group.

The histologic pattern was markedly altered by TPTX. Fig. 6 shows the typical appearance of NSN end-stage kidneys, manifesting a diffuse interstitial inflammatory reaction, interstitial fibrosis, tubular dilatation, and atrophy, with marked cellular proliferation and severe crescent formation in all glomeruli. In contrast the histologic appearance of TPTX kidneys at 23 wk showed little or no interstitial inflammatory reaction, tubular dilatation, or atrophy (Fig. 7). The majority of glomeruli showed mild increase in cellularity but only an occasional glomerulus developed a small crescent.

Effect of PTX (study II). In this protocol the rats were pair-fed during the study with no group showing a

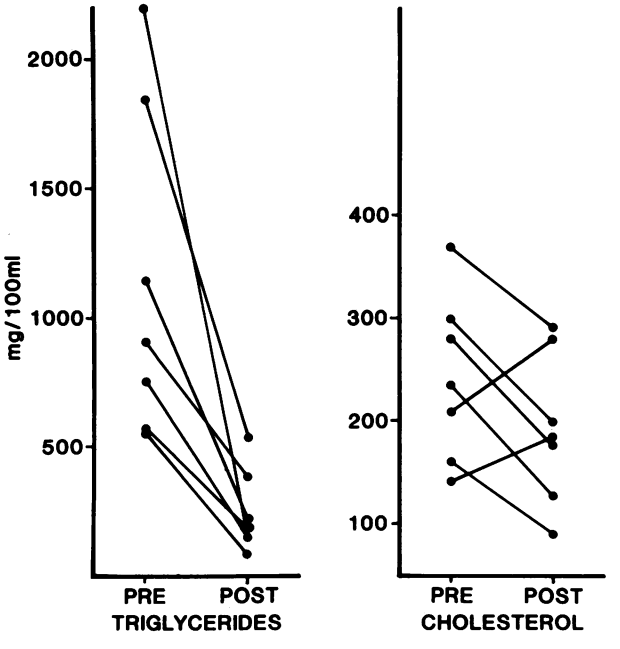

FIGURE 5 Effect of TPTX on established HTG and hypercholesterolemia. Sham-operated rats had a TPTX performed 35 d after injection of NTS. Lipids were measured 30 d after TPTX in the nonfasting state. Data are for each individual rat. n = 7. P value for TG < 0.001. P value for cholesterol < 0.05.

TABLE III
Kidney Ca and P Content

Group	Ca	P
	mmol/kg dry wt	
Control	13.1±1.0	359±7.1
Non-TPTX	619±170	796±110
TPTX	14.8±2.8	430±11.2

Control values were determined for normal Sprague-Dawley rats (n = 10). P values for Ca content: TPTX vs. control, NS; TPTX vs. non-TPTX, < 0.001; P values for P content: TPTX vs. control, < 0.001; TPTX vs. non-TPTX < 0.001.

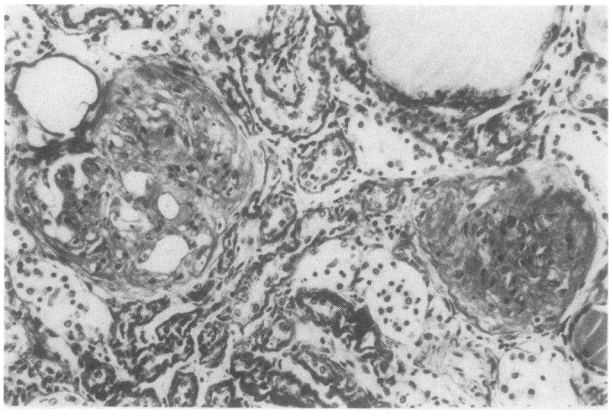

FIGURE 6 Light micrograph of renal cortex from a non-TPTX rat with NSN demonstrating crescenteric glomerulonephritis, severe tubular dilatation and atrophy, and interstitial inflammation. Hematoxylin and eosin stain. (×140.)

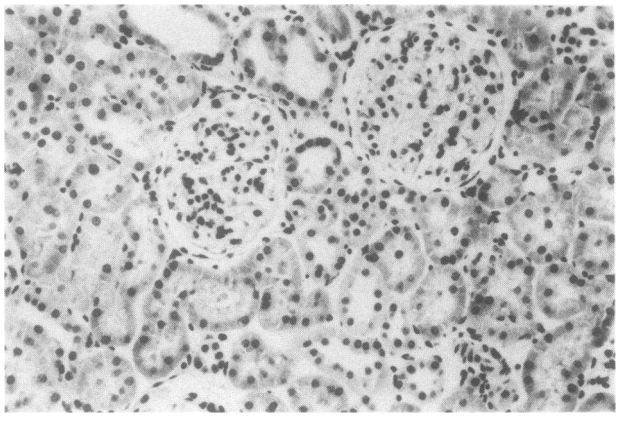

FIGURE 7 Light micrograph of renal cortex from a TPTX rat with NSN illustrating absence of crescents, inflammatory infiltrates, and intact tubules. A mild proliferative glomerulonephritis persists. Hematoxylin and eosin stain. (×140.)

significant weight gain during the 5-wk period (PTX, 342±28 to 345±35 g; TPTX, 308±16 to 303±24 g; non-TPTX 365±46 to 371±30 g; mean±SD). Similarly, neither the urinary P excretions (Table IV) nor the average fractional excretions of phosphate (PTX, 0.16±0.04, TPTX, 0.18±0.03, non-TPTX, 0.21±0.02% P = NS) were significantly different between the groups.

At 5 wk the mean Cr of the PTX group was not significantly different from the TPTX rats, while in non-TPTX rats Cr was 1.02±0.19 mg/100 ml ($P < 0.01$) with six of the seven rats having Cr ≥ 0.9 mg/100 ml. Plasma Ca was normalized in PTX and TPTX animals with 1,25-$(OH)_2D_3$ (Table IV). Histologically, in six of seven rats, the non-TPTX group revealed significant interstitial disease, and tubular dilatation with atrophy. Their glomeruli had increased cellularity and mesangial matrix, minimal thickening of the GBM, and 0–5% mild cresent formation. The PTX rats separated themselves into two distinct groups on the basis of histology. Three of six rats were indistinguishable from non-TPTX rats (Fig. 8a and b) while the others had minimal if any histologic alterations. The degree of histologic damage correlated with the plasma Cr. The PTX rats showing the highest Cr had the greatest histologic damage (Cr 0.65, 0.75, 1.0 mg/100 ml). In contrast, the TPTX group again had essentially complete absence of the interstitial reaction, tubular dilatation, and atrophy. Glomerular cellularity was slightly less than the non-TPTX rats. These anatomical differences were exemplified by the finding that kidney weight was significantly greater in non-TPTX (3.19±0.35, $P < 0.01$) and PTX (2.83±0.40 g, $P < 0.05$) groups as compared with TPTX (1.76±0.22 g) animals. Kidney weight in this latter group was not different from that found in uninephrectomized control animals (1.81±0.07 g).

The effect of PTX on proteinuria was markedly different from that of TPTX. By the 3rd day proteinuria was significantly increased over the TPTX group but

TABLE IV
Study II. Plasma and Urinary Indices in Short-term Protocol

Group	Average urinary phosphate excretion	Plasma Cr at 5 wk	Mean plasma Ca	Plasma TG	
				3 wk	5 wk
	mg/d		*mg/100 ml*		
PTX	21.1±1.9	0.67±0.07*	10.6±0.2	481±121‡	419±129‡
TPTX	17.9±1.4	0.65±0.04§	10.4±0.2	74±36	108±99
Non-TPTX	20.0±1.4	1.02±0.07	10.4±0.2	488±82‡	389±91‡

The average urinary phosphate excretion is the average of seven 24 h urinary collections from the 2nd to 13th d of the study (P NS for all group comparisons). The mean plasma Ca for each group was the result of the weekly plasma analysis (n = 6) (P NS for all groups).
* $P < 0.01$ vs. non-TPTX group.
‡ $P < 0.05$ vs. TPTX group.
§ $P < 0.001$ vs. non-TPTX group.

less than non-TPTX animals albeit not significant (Fig. 9). At 4 wk the PTX and non-TPTX proteinuria were identical remaining significantly greater than TPTX animals. The large standard deviation was caused by individual variations in the rate of development of high grade proteinuria.

Table IV shows that PTX failed to prevent HTG at 3 and 5 wk. No significant difference between the non-TPTX and PTX groups was detected whereas both were significantly greater than the TPTX animals.

In contrast to the extensive calcification observed in non-TPTX end-stage kidneys, the kidney Ca content of the non-TPTX rats at 5 wk in the short-term protocol was only 12.9±0.4 mmol/kg dry wt, a value essentially identical to normal rat kidneys (Table III). Thus, despite the fact that these non-TPTX rats were developing progressive renal insufficiency and histologic damage their kidneys were essentially devoid of calcification.

Effect of PTX on functional deterioration (study III). The disease in this study was more intense than in previous studies as evidenced by a mean Cr of 4.8±0.6 mg/100 ml in the non-TPTX rats by 7 wk. At this time five of nine animals in this group had died of uremia. The PTX rats had a mean Cr 4.3±0.5 mg/100 ml with 6 of 10 rats dying of renal failure. In contrast none of the TPTX rats died during the study, and their mean Cr of 0.9±0.3 mg/100 ml was significantly less than the TPTX and PTX groups ($P < 0.01$). Histologically the non-TPTX and PTX rats were indistinguishable, both manifesting severe interstitial disease, tubular dilitation, and glomerular disease similar to that observed in study 1.

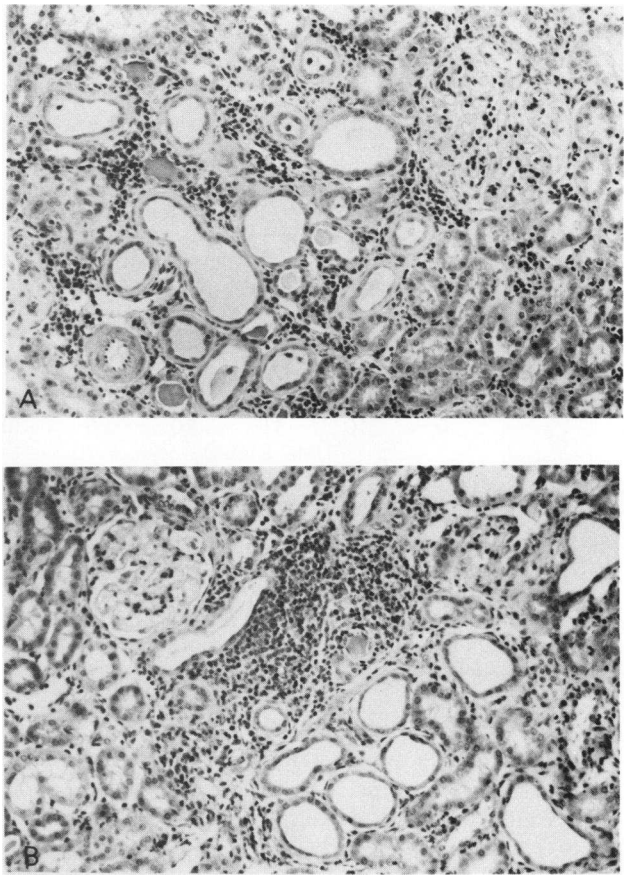

FIGURE 8 Light micrograph of hematoxylin and eosin stained renal cortex section from a PTX rat with NSN of 5 wk duration in study II (a). This illustrates the diffuse interstitial inflammatory reaction and tubular dilatation with atrophy observed in PTX rats, which was indistinguishable from that noted in the non-TPTX group (b). (×160.)

DISCUSSION

NSN is an experimental immunologic model of glomerulonephritis with two distinct clinical and pathogenetic phases. The initial, or heterologous phase, results from the immediate binding of infused rabbit anti-GBM antibody to the rat GBM, which is characterized clinically by the development of proteinuria within hours. The later, or autologous phase, occurs when an immune response to the injected rabbit gamma globulin develops at varying periods of time following NTS injection. The renal response, which begins when rat anti-rabbit gamma globulin antibodies attach to the GBM, is characterized clinically by increasing proteinuria and the progressive development of uremia. In the present studies the autologous phase was potentiated by preimmunization of the rats with rabbit gamma globulin (24).

Although the time required for the Cr to become elevated varied considerably among individual rats and studies NSN invariably progressed to end-stage renal disease in the non-TPTX animals. In contrast, all of the TPTX rats retained near-normal plasma Cr during the experimental periods.

In addition, rats TPTX 5 wk after NSN induction followed two distinct courses. If their Cr was ≤0.85 mg/100 ml before TPTX, they maintained a stable GFR, while those with Cr ≥1.0 mg/100 ml at the time of TPTX developed uremia. These two observations suggest that TPTX prevents an early event in the pathogenesis of uremia, and once that critical event occurs, TPTX can no longer alter the process of progressive damage.

In contrast to TPTX, selective PTX had little effect on functional deterioration or histologic damage. Although histologic changes were not as consistent as found in non-TPTX animals at 35 d, when the Cr was ≤1.0 mg/100 ml, one-half of the PTX rats did have comparable renal involvement to the non-TPTX group whereas only one TPTX rat had any interstitial reaction, and this was extremely mild. Furthermore, when studied at more advanced stages of renal failure

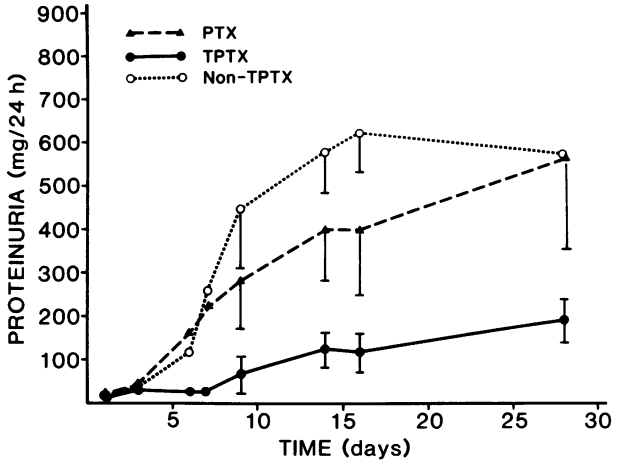

FIGURE 9 Proteinuria vs. time after induction of nephritis in study II. This illustrates that PTX had no significant effect on the proteinuria induced by NTS. Data are expressed here as mean±SD. Proteinuria was determined by the trichloroacetic acid precipitation method. The PTX group's proteinuria was significantly greater than the TPTX group's ($P < 0.05$) from day 3 to the end of the study. The PTX proteinuria was never significantly less than the non-TPTX group.

histologic damage was equally severe in both the PTX and non-TPTX groups. This is in marked contrast to TPTX rats studied at similar time points who demonstrated only minimal glomerular and interstitial disease. These differences between the TPTX and PTX groups cannot be ascribed to ineffective PTX as the completeness of selective PTX was documented by showing a similar fall in plasma Ca and urinary cyclic AMP as found in TPTX animals. Thus the protective effect of TPTX is not mediated through the removal of PTH.

The nephrotic syndrome was significantly altered by TPTX as evidenced by the prevention of the marked increase in autologous phase proteinuria and a 50% reduction of urinary protein excretion when TPTX was done as late as 35 d after establishment of disease (Table II). In a manner similar to protein excretion, HTG was reversible as well as preventable by TPTX. The effect on the nephrotic syndrome appears to be independent of renal functional deterioration and histologic changes since the rats TPTX at 35 d had a halving of their proteinuria regardless of their renal function at the time of TPTX or of the subsequent development of progressive renal insufficiency. This implies that TPTX has separate effects on glomerular selective permeability, GFR and histologic damage.

There was a strong positive correlation between the TG level and proteinuria ($r = 0.711$; $P < 0.001$). Recent studies suggest that lipoprotein lipase activators may be lost with other proteins in the nephrotic syndrome (25, 26). If this is true, then HTG would be expected to be modified by changes in protein excretion. Thus TPTX could affect plasma TG levels indirectly by amelioration of proteinuria. Plasma albumin and plasma TG levels correlated marginally or not at all in this and other studies (27) suggesting that hypoalbuminemia is not an important variable.

While it has been demonstrated that PTH has a direct effect on TG metabolism producing an increase in TG in normal rats (28–30) and acute (31) and chronically uremic rats (32), the animals in our studies were not adequately replaced with Synthroid so that an effect of hypothyroidism on TG metabolism complicated the TPTX studies. In contrast to TPTX, proteinuria and plasma TG in PTX rats were not significantly different from the non-TPTX group (Fig. 9, Table IV). Thus, removal of PTH had no direct effect on TG metabolism and further supports the hypothesis that the alterations in plasma TG in the nephrotic syndrome is a function of proteinuria. However, a direct effect of thyroxine or calcitonin has not been eliminated.

Several possible mechanisms for the protective effect of TPTX exist. The protective effect does not appear to be a consequence of plasma chemistry alterations. Although the plasma Ca was slightly less in the TPTX group, this was not the case for the TPTX rats at 35 d or the TPTX rats in the short-term protocol who derived a similar benefit from the procedure. Furthermore, plasma P levels and Ca × P products were identical in all groups when progressive renal insufficiency initially developed in the non-TPTX animals. Plasma Ca and P levels did not become elevated in the uremic animals until one to two weeks before death. Vitamin D therapy does not play an important role since non-TPTX rats treated with $1,25\text{-}(OH)_2D_3$ in a separate group of experiments developed renal insufficiency at a rate equivalent to non-TPTX rats without vitamin D supplementation,[2] and $1,25\text{-}(OH)_2D_3$ administered to the selectively PTX rats failed to alter proteinuria, histological damage, or functional deterioration.

Since phosphate load per nephron has recently been demonstrated to have renal toxicity (33), and dietary PR is protective (2), a decrease in phosphate intake or excretion in TPTX animals may be the mechanism for this beneficial effect. Although the rats in protocol I were fed ad lib., we feel this is unlikely, because the weights of the TPTX and non-TPTX rats at the termination of the study were essentially identical (TPTX, 357±12 g vs. non-TPTX, 348±26 g). In addition, in protocol II the TPTX, PTX, and non-TPTX groups were pair fed and had similar urinary phosphate excretions (Table IV) and fractional excretions of phosphate. Under these conditions, the TPTX animals had the same protection afforded as in the previous protocol.

Prevention of renal parenchymal calcification, which is thought to produce renal insufficiency by inciting an

[2] Tomford, R. C., and A. C. Alfrey. Unpublished observations.

inflammatory response in the diseased kidney is another mechanism for the protective effect. Consistent with this hypothesis, in this study the calcification in end-stage kidneys in non-TPTX rats was extensive and predominently located in renal tubular cells and basement membrane, a pattern invariably found in human and experimental end-stage kidneys. TPTX rats had essentially complete prevention of this calcification and renal failure. Against calcification as a mediator of renal damage, however, is the fact that non-TPTX animals at 35 d had reduced renal function, marked proteinuria, and extensive histologic damage, whereas kidney Ca content was normal. This would suggest that renal damage precedes calcification and implies that the early protective effect of TPTX is not mediated through prevention of calcification. Although it seems unlikely that calcification induces early progressive damage, the possibility remains for it to accelerate functional deterioration in late renal failure.

PR and TPTX both prevent renal functional deterioration, parenchymal calcification, and histologic damage associated with NSN. In contrast to TPTX however, PR failed to prevent or reduce proteinuria (2). Combining this with the fact that proteinuria but not necessarily functional deterioration was affected by TPTX performed 35 d after induction of disease suggests that these two processes are mediated by different mechanisms. A unifying concept explaining the protective mechanism(s) of PR and TPTX must involve modes of action in addition to the removal or suppression of PTH. It is possible that PR suppresses thyroxine and/or calcitonin secretion, but this has not been studied. Both thyroid ablation and PR could affect ATP metabolism (34, 35) and could damage cell membrane phospholipids (36, 37) that alter cell viability. Because NSN is an immunologic disease involving both humoral and cell mediated mechanisms (38, 39), PR and thyroid ablation could also blunt the intensity of the immunologic response although the effects of thyroid hormones on the immune response have yet to be investigated. Hypophosphatemia, however, has been demonstrated to depress chemotactic, phagocytic, and bactericidal activity of granulocytes (40).

Finally it is possible that PR and TPTX have independent mechanisms of protection. This might be suggested by the above differences on the autologous phase proteinuria.

It can only be concluded at this time that PR and TPTX have a consistent and reproducible effect on NSN, and that the specific mechanism(s) of this protective effect remains to be elucidated.

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