Kidney International, Vol. 34 (1988), pp. 4–12

LABORATORY INVESTIGATION

Role of the urinary concentrating process in the renal effects of high protein intake

NADINE BOUBY, MARIE-MARCELLE TRINH-TRANG-TAN, DENISE LAOUARI, CLAIRE KLEINKNECHT, JEAN-PIERRE GRÜNFELD, WILHELM KRIZ, and LISE BANKIR, with technical assistance of MONIQUE DOUTÉ, BRUNI HÄHNEL, and CHRISTINE COUTAUD

INSERM Unité 90 and INSERM Unité 192, Hôpital Necker, 161 rue de Sèvres, 75743 Paris Cedex 15, France, and Anatomisches Institut der Universität Heidelberg, 6900 Heidelberg, Federal Republic of Germany

Role of the urinary concentrating process in the renal effects of high protein intake. High protein diet is known to increase glomerular filtration rate (GFR) and induce kidney hypertrophy. The mechanisms underlying these changes are not understood. Since the mammalian kidney comprises different nephron segments located in well-delineated zones, it is conceivable that the hypertrophy does not affect all kidney zones and all nephron segments uniformly. The present experiments were designed to study the chronic effects of high or low isocaloric protein diets (HP = 32% or LP = 10% casein, respectively) on kidney function and morphology in Sprague-Dawley rats. HP diet induced significant increases in kidney mass, GFR, free water clearance, and maximum urine concentrating ability. Kidney hypertrophy was characterized by: 1. a preferential increase in thickness of the inner stripe of the outer medulla (IS) (+ 54%, $P < 0.001$, while total kidney height, from cortex to papillary tip, increased only by 18%); 2. a marked hypertrophy of the thick ascending limbs (TAL) in the inner stripe (+40% epithelium volume/unit tubular length, $P < 0.05$) but not in the outer stripe nor in the cortex; 3. an increase in heterogeneity of glomeruli between superficial (S) and deep (D) nephrons (D/S = 1.47 in HP vs. 1.17 in LP, $P < 0.05$). In contrast, normal kidney growth with age and kidney hypertrophy induced by uninephrectomy were not accompanied by preferential enlargement of IS structures. The morphologic changes induced by high protein intake parallel those we previously reported in rats fed a normal diet (25% protein) but in which the operation of the urine concentrating mechanism was chronically stimulated by ADH infusion or by reduction in water intake. This similarity and the dramatic increase in free water reabsorption induced by HP diet suggest that high protein intake affects kidney function and morphology by increasing the level of operation of the urine concentrating process. The preferential increase in TAL epithelium disclosed in this study, and the recent demonstration by others of a decreased salt concentration in the early distal tubule of HP rats raises the possibility that the protein-induced increase in GFR is mediated by a depression of tubuloglomerular feedback resulting from an increased salt transport in the medullary TAL in relation with an increase in free water generation.

The possible role of protein intake on the progression of renal insufficiency has recently been re-emphasized by several experimental and clinical studies [1–3]. In order to understand why dietary proteins may have a deleterious effect on the function of the diseased kidney, it is useful to study how they influence the function of the normal kidney. It has long been known that a high protein intake induces kidney hypertrophy [4] and increases glomerular filtration rate (GFR) [5, 6]. Only rarely mentioned in these studies is the fact that a high protein diet also increases urine concentrating ability as observed in rats [7], sheep [8], dogs [5, 9], and humans [10].

It has been shown that sustained stimulation of urine concentrating process, obtained either by chronic infusion of antidiuretic hormone (ADH) or by chronic reduction in water intake, induced a marked kidney hypertrophy [11–13], an increase in GFR [14], and an enhanced response to subsequent ADH injections (M-M. Trinh-Trang-Tan and L. Bankir, unpublished observations). The kidney hypertrophy did not affect all kidney zones and all nephron segments uniformly. The inner stripe of the outer medulla and the thick ascending limbs of Henle's loops (TAL) in their early part were hypertrophied far more than the rest of the kidney. This marked increase in volume of TAL epithelium in the inner stripe [11, 13] and the accompanying increase in transepithelial potential difference [15] probably reflect an increased transport activity in the medullary TAL, the nephron segment responsible for the "single effect" enabling the building up and maintenance of the osmotic pressure gradient in the medulla. In addition, sustained urine concentration significantly accentuated the well known heterogeneity between superficial and deep nephrons, with regard to single nephron filtration rate, length of proximal tubule and volume of the glomeruli [13, 16, 17].

Since high protein intake induces an increase in urine concentrating ability [5, 7–10], we designed experiments aimed at determining the relationships between protein-induced changes in renal mass and renal function, and the operation of the concentrating system of the kidney. Glomerular filtration rate, free water reabsorption on ad libitum water intake, and maximum urine concentrating ability after water deprivation were compared in rats fed a low or a high protein diet. Another goal of this study was to determine if the protein-induced kidney hypertrophy exhibits the same specific morphologic pattern as that induced by ADH or water restriction in rats fed a standard diet. For comparison, the pattern of changes occurring in the enlarged kidney after high protein intake was also compared to that observed during normal kidney enlargement, that is, nor-

Received for publication July 1, 1986
and in revised form April 13 and November 17, 1987

mal growth with age, and in another form of kidney hypertrophy, namely compensatory hypertrophy after uninephrectomy.

Methods

High and low protein intake

Two series of rats were used to study the functional and morphologic effects of protein intake on the kidney. In the first series (experiment A) male Sprague-Dawley rats (Charles River Labs., France), body weight 120 to 140 g, were fed either a 10% casein diet (low protein, LP, $N = 6$) or a 32% casein diet (high protein, HP, $N = 6$) for six weeks. Starch and phosphate were added to the low protein diet to maintain a similar caloric and phosphate content per gram. Sodium content of both diets was 0.5% (CNRZ, Guyancourt, France). Rats were housed individually. LP rats were offered LP food ad libitum. Every day at about 10 a.m. each HP rat received the same weight of HP food as that consumed by LP rats (mean of the 6 rats) on the previous day. All rats had free access to tap water.

During the fourth and fifth weeks of treatment, rats were placed for three days in metabolic cages. On the third day, urine was collected for 24 hours and a blood sample was taken from the jugular vein under ether anesthesia at about 10 a.m. Creatinine concentration was measured with a Technicon SMA 6 Autoanalyzer for urine samples and with an ASTRA 8 Beckman analyzer for plasma samples. Urine osmolality was measured on a freezing point osmometer (Fiske, Uxbridge, Inc.). Creatinine, osmolar, and free water clearances were calculated and averaged for both weeks. During the sixth week, rats underwent a dehydration test. Water, but not food, was withdrawn for 24 hours. Urine from periods 0 to 18 hours, 18 to 22 hours, and 22 to 24 hours was collected under oil and its osmolality measured.

Two or three days after return to normal water supply, rats were anesthetized with Nembutal, and a 5% solution of Alcian blue 8GS in 1% acetic acid was infused into a jugular vein at a rate of 40 μl/min for 10 minutes, as previously described [18], in order to stain the kidneys. Kidneys were cut out, decapsulated, weighed, then dehydrated in graded ethanol and kept in methylsalicylate. When impregnated with methylsalicylate kidney tissue becomes relatively hard and can easily be cut free hand with a razor blade. Under transillumination, the different renal zones [cortex (C), outer stripe (OS) and inner stripe (IS) of the outer medulla, and inner medulla (IM)] can be clearly distinguished by their specific staining [18]. Coronal sections about 0.5 mm thick were photographed (Leitz, Focotar lens 1.9) and color pictures with a final magnification of 10 × for HP rats and of 12 × for LP rats were prepared. In these pictures, the total height of the kidney (from cortex surface to papillary tip) and the height of each renal zone along the cortico-papillary axis were measured to the nearest 0.1 mm. The choice of different magnifications for HP and LP kidneys was made to prevent immediate recognition of HP or LP status during the double blind measurements, the difference in kidney size being otherwise easily noticeable.

In the second series (experiment B), male Sprague-Dawley rats (Iffa Credo, France) were fed high ($N = 3$) or low ($N = 3$) protein diets as described in the preceding experiment. After six weeks, under Nembutal anesthesia, kidneys were washed and perfusion-fixed in situ with a flushing solution followed by a

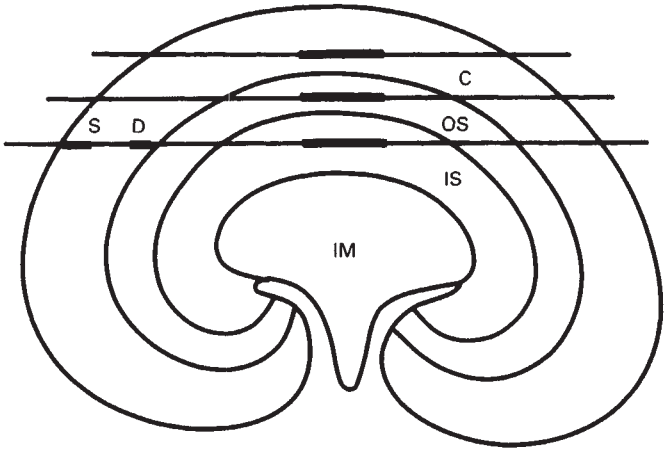

Fig. 1. *Sectioning of the kidney in experiment B.* Serial sections 7 μm thick were cut from paraffin-embedded kidneys. TAL were measured in the central part (heavier line) of sections from the mid-inner stripe (IS), mid-outer stripe (OS) and mid-cortex (C). Superficial (S) and deep (D) glomeruli were measured in the cortex of the mid-inner stripe section, as shown.

1.5% glutaraldehyde-1.5% formaldehyde fixative as described previously [11]. Kidneys were then processed for standard light microscopy. The entire right kidney of each rat was cut in serial paraffin sections 7 μm thick, oriented perpendicular to the cortico-papillary axis (Fig. 1). Sections from the six rats were coded so that subsequent measurements were performed in a double blind fashion. These measurements comprise height of the different renal zones, cross sectional surface area of TAL epithelium, and cross sectional area of superficial and deep glomeruli.

The height of the different renal zones along the cortico-papillary axis was determined from the number of sections in which the characteristic tubular components of each zone were present, multiplied by the section thickness [11]. Observation of the central part of successive sections (Fig. 1) enabled a clear distinction of the limits between OS and IS, and between IS and IM. The border between C and OS was, however, less clearly defined.

Measurements of epithelium cross-sectional surface area were performed in randomly selected medullary thick ascending limbs (MTAL) at the mid-inner stripe, mid-outer stripe and mid-cortical levels (Fig. 1) using a MOP digitizer [11]. The surface area of TAL epithelium was calculated from the difference between the total area covered by a tubule section and its luminal area. Ten thick ascending limbs per section were measured in four different sections per level in each rat. At the mid-inner stripe level, it is possible to distinguish thick ascending limbs from short and from long loops, due to their different locations with regard to vascular bundles and collecting ducts (Fig. 3 in [11]). Ten thick ascending limbs of both short- and long-looped nephrons were measured in each section at this level.

The cross sectional area of glomeruli was measured in superficial (S) and deep (D) cortex in sections of "mid-inner stripe" level (Fig. 1). Twelve superficial and twelve deep glomeruli per section were measured in two different sections per rat. These measurements represent an underestimation of

Table 1. Renal function in rats on low and high protein diet (Experiment A)

	LP 10% Casein $N = 6$	HP 32% Casein $N = 6$	*t*-test
Creatinine clearance *ml/day*	767 ± 54	1,523 ± 27	$P < 0.001$
ml/day · 100 g body wt	586 ± 19	730 ± 12	$P < 0.001$
Urine flow rate *ml/day*	5.33 ± 0.67	13.50 ± 1.33	$P < 0.001$
Urine osmolality *mOsm/kg H_2O*	1,530 ± 176	1,750 ± 156	NS
Osmolar excretion *mOsm/day*	7.52 ± 1.03	23.99 ± 1.24	$P < 0.001$
Osmolar clearance[a] *ml/day*	20.37 ± 2.79	83.29 ± 4.46	$P < 0.001$
Free water clearance *ml/day*	−19.04 ± 2.25	−67.79 ± 3.92	$P < 0.001$

Values are means ± SE.

[a] Osmolar clearance and free water clearance were calculated assuming plasma osmolality was 300 mOsm/kg H_2O in both groups.

the actual equatorial glomerular cross-sectional surface area since many glomeruli are not cut in their equatorial plane. However, this bias should affect the measurements equally in HP and LP rats and in S and D glomeruli.

Normal kidney growth and compensatory hypertrophy

Two groups of male Wistar rats (Iffa Credo, France) of widely different ages and body weights (6 rats ≈ 2 months old and 6 rats ≈ 6 months old) were studied. They had free access to regular pellet diet (containing 25% protein of vegetable, meat, and fish origin) and tap water ad libitum (experiment C). Under Nembutal anesthesia, they received an i.v. infusion of Alcian blue as described for rats of experiment A. Measurement of kidney zones on color prints was performed as described above for the first series of rats.

In addition, twelve male Wistar rats (Iffa Credo, France), 150 to 220 g body weight, were used to assess the characteristics of compensatory renal hypertrophy (experiment D). In six rats, the left kidney was removed under ether anesthesia by flank incision, whereas the six others underwent a sham operation. After six weeks on a regular pellet diet and tap water ad libitum, the 12 rats were sacrificed after in vivo Alcian blue staining, and kidney zones were measured as described in experiment A.

Statistics

Results are given as means ± 1 SE of each group. Group means were compared by Student's *t*-test or by paired *t*-test when appropriate.

Results

Results concerning the functional parameters studied in metabolic cages during the fourth and fifth weeks on the two different diets are given in Table 1. As already well known, creatinine clearance (C_{Cr}), an index of filtration rate, was higher in rats fed the high protein diet than in those fed the low protein diet. Even factored by body weight, C_{Cr} was still significantly higher in HP than in LP rats. Plasma creatinine was lower in HP than in LP rats (38.8 ± 1.1 μmol/liter in HP and 46.0 ± 1.5 μmol/liter in LP, $P < 0.001$).

Urine flow rate was much greater in HP than in LP rats (Table 1). Urine osmolality (UOsm) was not significantly different between the two groups, although it was slightly higher in HP rats. Osmolar excretion was more than threefold higher on HP than on LP feeding, a difference mainly accounted for by a much higher urea excretion in the latter group (urea excretion measured in 2 LP and 2 HP rats in another experiment was respectively 2.6 and 22.3 mmol/day [19]). As a consequence, osmolar clearance was significantly greater and free water clearance significantly smaller on HP than on LP diet. This difference in free water clearance reflects the fact that a much greater amount of solute-free water was reabsorbed by HP than by LP rats.

As seen above, the difference in urine osmolality between HP and LP rats on ad libitum water intake, was modest and non-significant. A significant difference appeared during the dehydration test as shown in Figure 2. During the first 18 hours of water deprivation, UOsm rose to 2.640 ± 230 mOsm/kg H_2O in HP versus 1.615 ± 85 mOsm/kg H_2O in LP ($P < 0.01$). From 18 to 22 and 22 to 24 hours of water deprivation, UOsm reached 2.890 ± 130 and 2.940 ± 160 mOsm/kg H_2O in HP versus 2.040 ± 35 and 1.840 ± 80 in LP ($P < 0.001$ between HP and LP for each period).

Table 2 shows the mean absolute and relative kidney weights in the two series of HP and LP rats and in the "normal growth" and "compensatory growth" series. As expected, HP diet induced a marked hypertrophy of the kidney, although the difference did not reach significance in experiment B, due to the small number of animals. During normal growth in mammals, kidney weight increases more slowly than body weight so that kidney weight becomes a smaller fraction of total body weight with increasing age and/or increasing body weight [20]. This was indeed observed in the "normal growth" experiment. In contrast, in both series of "protein experiments" the relative kidney weight was 17% higher in the HP rats although they were heavier. In the "compensatory growth" experiment the remaining kidney increased in weight by 47% in the absence of any difference in body weight. In Table 2, group means should be compared only for pairs belonging to the same experiment since kidneys of the different experiments were processed with different techniques and/or were not studied in parallel.

The height of the different kidney zones in rats of experiment A is shown in Figure 3. Each of the four zones was increased in HP compared to LP rats. However, all zones did not increase to the same extent. The inner stripe of the outer medulla grew out of proportion to the rest of the kidney. Table 3 shows the height of each kidney zone in percentage of the total kidney height for all experiments. A greater enlargement of the inner stripe was found by two different techniques of evaluation in the two series of experiments in which protein intake was varied, that is, direct measurements on pictures of kidney transverse sections (experiment A; + 31%), or calculation from number of serial sections along the cortico-papillary axis (experiment B; + 22%). The 13% increase for cortex and 8% decrease for OS in experiment B (Table 3) might result from slight inaccuracies in determining the exact limit between the two zones on histological sections. In the whole, in both protein experiments the sum of the relative height of C + OS (two zones which include the same nephron segments) was unchanged, and a significant increase in IS relative thickness appeared at the expense of IM, the relative height of which was significantly decreased. Such

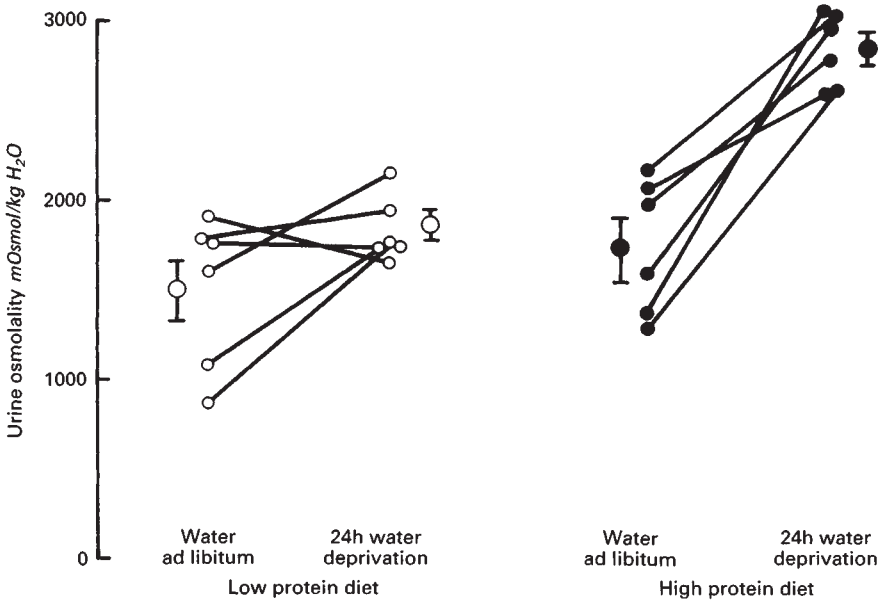

Fig. 2. *Urine osmolality on ad libitum water intake and after 24 hours of water deprivation in individual LP (open circles) and HP rats (closed circles).* Means ± SE are indicated. Urine osmolality was significantly greater in HP than in LP rats after water deprivation ($P < 0.001$) but not on ad libitum water intake.

Table 2. Influence of several factors on kidney weight

Experiment	Condition	N	Body weight g	Right kidney weight mg	Right kidney weight mg/100 g body wt
Protein intake					
A	Low protein	6	178 ± 8	753 ± 19	424 ± 13
	High protein	6	282 ± 5[c]	1401 ± 42[c]	497 ± 15[b]
B	Low protein	3	306 ± 2	1310 ± 59	429 ± 21
	High protein	3	341 ± 10[a]	1727 ± 193	505 ± 50
Normal growth					
C	2 month old	6	206 ± 9	821 ± 20	401 ± 13
	6 month old	6	484 ± 19[c]	1400 ± 99[c]	289 ± 15[c]
Compensatory growth					
D	Sham	6	350 ± 6	1149 ± 66	328 ± 16
	Uninephrectomy	8	349 ± 10	1691 ± 80[c]	482 ± 10[c]

Values are means ± SE. N = number of rats in each group. Student's t-test between two groups of the same experiment: [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$.

an unequal growth of the different kidney zones was not observed in kidneys enlarged by normal growth or by compensatory growth (experiments C and D). In these two cases, the increase in kidney weight did not include an unhomogenous increase of the different kidney zones.[1]

The nephron segment which makes up most of the tissue in the inner stripe is the thick ascending limb. In rats of experiment A, it was not possible to perform morphometric measurements of the tubules because kidneys had not been fixed. In experiment B, in which kidneys were perfusion-fixed, a marked difference in the development of TAL epithelium was observed in inner stripe between LP and HP rats (Fig. 4). Mean epithelium surface area per TAL, hence volume of epithelium per unit length of tubule, reached in the inner stripe of HP rats values higher than those which would have been expected from the overall difference in kidney weight between the two groups[2] (Fig. 5). Cross-sectional surface area of the TAL epithelium in HP rats was 1.46-fold higher in short-looped and 1.38-fold higher in long-looped nephrons than in LP rats, while the average difference expected from the difference in total renal mass (scaled to the two-thirds power of mass) is only 1.20

[1] The relative height of the IS in Wistar rats of the "normal growth" study (≈ 17%) is slightly less than that in sham-operated Wistar rats of the "compensatory growth" study (19%). This difference might result from the fact that the two studies were not carried out at the same time of the year and that rats of experiment C just stayed for 2 to 3 days in our laboratory before the study whereas those of experiment D stayed there for several weeks. Prior housing and feeding conditions, hygrometry, and other factors able to alter the degree of operation of the urine concentrating mechanism might affect kidney weight relative to body weight, and the relative development of the inner stripe and thick ascending limbs [11–13, 30, 31]. For this reason, in Table 3, as in Table 2, group means should be compared only for pairs belonging to the same experiment.

[2] If all structures in the kidney were to hypertrophy homogeneously, the length of any structure (first power dimension) would increase by a factor equal to the cube root of the weight (volume) increase. Surface areas (second power dimension) would increase by a factor equal to the square of this cube root. In the present case, kidney weight increased by a factor of 1.318. Surface areas would be expected to increase by $(\sqrt[3]{1.318})^2 = 1.20$.

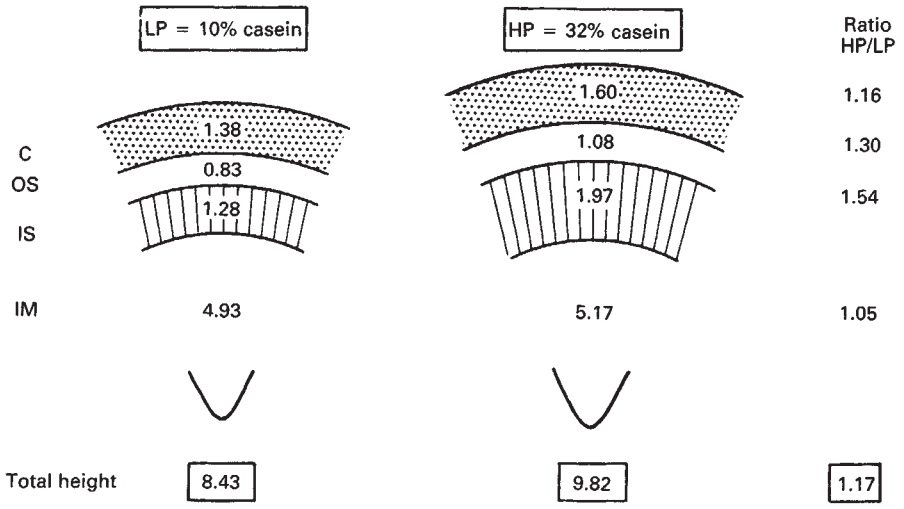

Fig. 3. *Mean height (in mm) of the renal zones along the cortico-papillary axis in LP and HP rats.* SE (not shown) never exceed 6% of the means ($N = 6$ in each group). Student's t-test between HP and LP rats: $P < 0.05$ for cortex, $P < 0.001$ for outer and inner stripe, NS for inner medulla, $P < 0.01$ for total kidney height.

Table 3. Relative height of the different kidney zones (in % of whole kidney height)

Experiment	Condition	Cortex	Outer stripe	Inner stripe	Inner medulla
Protein intake					
A	Low protein	16.5 ± 0.8	9.9 ± 0.4	15.3 ± 0.7	58.2 ± 1.7
	High protein	16.4 ± 0.8	11.1 ± 0.6	20.1 ± 0.5[b]	52.4 ± 1.3[a]
B	Low protein	16.6 ± 0.6	11.8 ± 0.6	15.5 ± 0.2	56.1 ± 1.2
	High protein	19.5 ± 0.7[a]	10.1 ± 0.7	18.9 ± 0.8[a]	51.5 ± 0.6[a]
Normal growth					
C	2 month old	18.3 ± 0.4	11.0 ± 0.6	16.5 ± 0.6	54.2 ± 1.0
	6 month old	18.2 ± 0.6	12.1 ± 0.4	17.6 ± 0.8	52.0 ± 1.0
Compensatory growth					
D	Sham	17.4 ± 0.4	12.4 ± 0.4	19.0 ± 0.6	51.2 ± 0.7
	Uninephrectomy	17.6 ± 0.4	10.7 ± 0.6[a]	18.7 ± 0.4	53.1 ± 0.8

Values are means ± SE. Number of rats as in Table 2. Student's t-test between two groups of the same experiment: [a] $P < 0.05$, [b] $P < 0.001$.

(dashed line in Fig. 5). This hypertrophy is comparable to that observed in another study on cryostat sections of unfixed kidneys of HP and LP rats [19]. This shows that kidney fixation did not induce artifacts. In contrast with what was observed in the inner stripe, the hypertrophy of the TAL in the outer stripe and in the cortex (× 1.11 and 1.23, respectively) was not greater than that expected from whole kidney enlargement, and the difference between the two groups did not reach significance (Fig. 5).

Figure 6 displays the results of the glomeruli measurements. In keeping with the well-known internephron heterogeneity, cross-sectional surface area of deep (D) glomeruli was significantly larger than that of superficial (S) glomeruli in both HP and LP rats ($P < 0.01$ in HP and $P < 0.02$ in LP by paired t-test). However, the difference was much greater in HP than in LP rats. The deep/superficial ratio was 1.47 ± 0.02 in HP, and 1.17 ± 0.02 in LP ($P < 0.001$, by t-test). Horizontal bars in Figure 6 indicate the cross sectional area for S and D glomeruli that would have been expected if all glomeruli had increased proportionately to the whole kidney (× 1.20). Superficial glomeruli increased much less and deep glomeruli more than expected. As a consequence, heterogeneity of glomerular size was much greater in rats fed a high than a low protein intake.

Discussion

Several studies have demonstrated that high protein intake increases GFR and kidney mass [4–6, 28, 29], and increases the kidney's ability to concentrate urine [5, 7–10]. Conversely, chronic feeding a low protein diet leads to a concentrating defect [8, 9, 21]. The results reported in the present experiments demonstrate again these well known effects of dietary proteins and disclose two additional features. 1) Measurements of urine osmolality and calculation of osmolar clearance and free water clearance when water was available ad libitum illustrate a large variation in free water reabsorption with protein content of the diet. 2) Morphometric measurements reveal that the protein-induced kidney hypertrophy affects the different kidney zones and nephron segments unequally.

Increase in free water reabsorption

As could be expected, the solute excretion was much higher on HP than on LP diet. It is well known that increasing solute excretion generally leads to a decrease in urine osmolality [22], except when urea contributes to the solute load to be excreted. Gamble et al in 1934 demonstrated that urea, but no other osmotically active solute, has the property to induce "an economy of water in renal function" [23]. In other words, urea

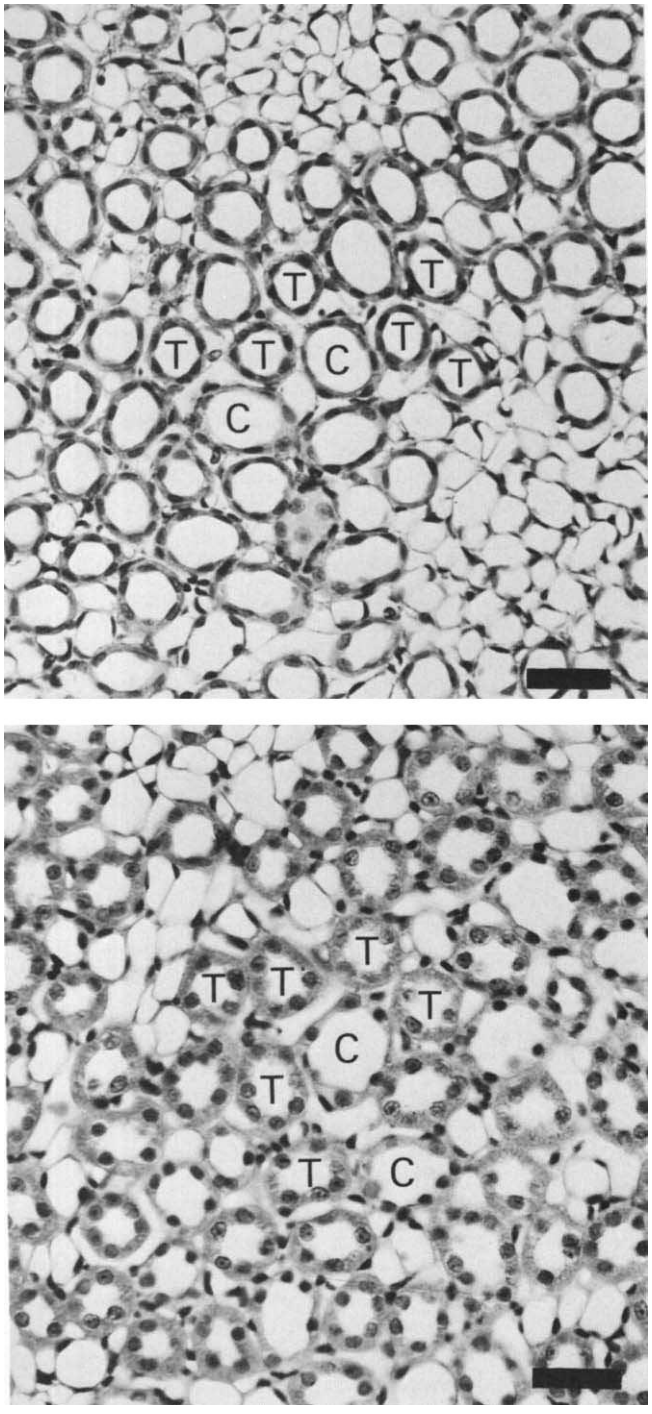

Fig. 4. *Cross sections through the inner stripe of a LP rat (top) and a HP rat (bottom).* T: thick ascending limbs, C: collecting ducts (Bar = 50 μm).

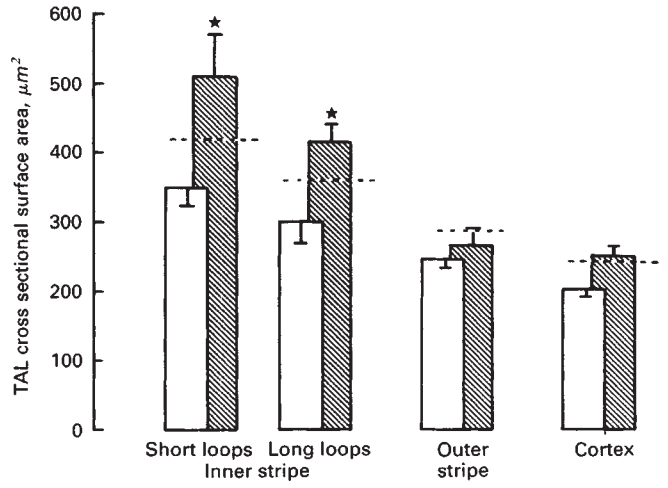

Fig. 5. *Thick ascending limb epithelial cross sectional surface area in LP (open bars), and in HP (hatched bars) rats in different kidney zones.* Dashed lines indicate values expected from the overall kidney increase ($N = 3$ in each group). Student's t-test between HP and LP, $^*P < 0.05$.

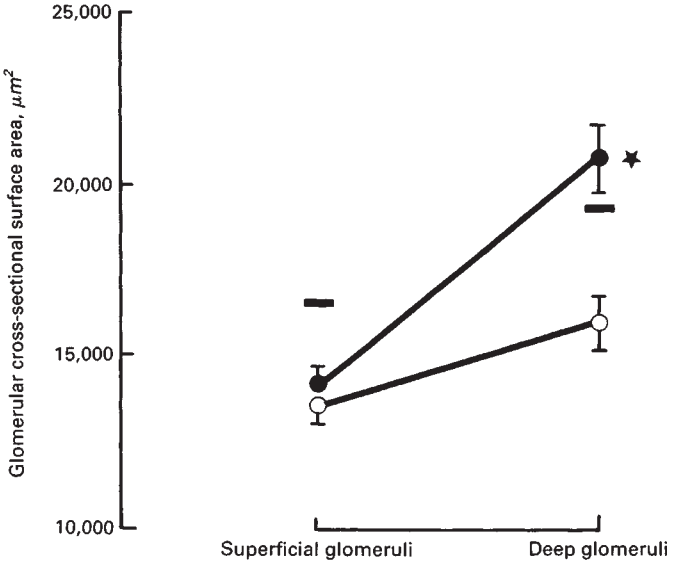

Fig. 6. *Cross sectional surface areas of superficial and deep glomeruli in LP (open circles) and in HP (closed circles) rats.* Values are means ± SE ($N = 3$ in each group). Horizontal bars indicate values expected from the overall kidney increase. $^*P < 0.001$ by Student's t-test between HP and LP.

reduces the volume of water required for the excretion of a given amount of osmotically active solutes. Later, Crawford, Doyle and Probst showed that this holds true only within certain limits of urea to non-urea solute ratio [24]. This effect is now thought to depend on the accumulation of urea in the cortico-medullary solute gradient, contributing to better extract water from the collecting ducts. However, in spite of many investigations [8, 9, 25] the exact mechanism responsible for this effect is not yet fully understood.

Results displayed in Table 1 show that the amount of solute-free water removed from the filtrate in HP rats was 3.5 times higher than that in LP rats (T^cH_2O = negative free water clearance = 67.8 ml/day in HP vs. 19.0 in LP). Had solute-free water reabsorption not been increased on HP diet, urine osmolality would have fallen and urine volume would have largely increased. Although urine osmolality was about the same in HP and LP rats (Table 1), it appears from the large difference in T^cH_2O that the urine concentrating process

worked more intensely in HP than in LP rats, reabsorbing more free water in face of a greater solute excretion.

Intrarenal morphologic changes

How reduction in protein intake affects intrarenal morphology was recently investigated by Schmidt-Nielsen et al [26]. They specificially analyzed vascular bundle anatomy and thin limb morphometry in the inner stripe of the outer medulla at the electron microscopic level. The only difference resulting from feeding rats 8 versus 24% protein (consisting of blood meal and soy bean) was a thinning of the wall of the thin descending limbs of long-looped nephrons [26]. In our experiment, thin descending limbs could not be validly measured on paraffin sections, but no obvious difference was apparent by light microscopy. Our measurements showed that the inner stripe of the outer medulla was thickened by high casein feeding and that, within this zone, the thick ascending limb grew out of proportion to the rest of the kidney, so that TAL epithelium volume per unit kidney mass in the inner stripe of the outer medulla was 50% higher in HP than in LP rats[3]. In addition, the internephron heterogeneity, as judged by glomerular size, was amplified.

This pattern of hypertrophy differs from that observed during normal kidney growth or compensatory hypertrophy. This may be due to the fact that the different situations leading to increase kidney weight induce different types of "loads" on the kidney and its successive nephron segments. During normal growth, all kidney functions are probably increased uniformly with the progressive increase in body mass. After uninephrectomy, all excretory and regulatory tasks the kidney assumes are suddenly doubled for the remaining nephrons, but the proportion between the different tasks is unchanged. The hypertrophy adapts each nephron to an increased filtration and reabsorption with unchanged tubuloglomerular balance [27]. In this situation, no change occurs in solute or water excretion, neither in urea to non-urea solute ratio. In contrast, after high protein feeding, excretion of urea is increased several-fold while other functions of the kidney are presumably not greatly modified and excretion of non-urea solutes remains unchanged. Thus, the urea to non-urea solute ratio is increased. As apparent in our results (Table 1), this condition leads the kidney to increase solute-free water reabsorption, that is, to improve its concentrating ability. It should be stressed, however, that the increase in urea excretion and the water economy that follows are not the only factors involved in the protein-related changes in kidney function and morphology, since feeding a high protein diet increases urinary concentrating ability and kidney weight more than does feeding urea in amounts leading to the same urea excretion [28, 29].

The pattern of kidney hypertrophy resulting from high protein feeding closely resembles that seen after sustained stimulation of the urinary concentrating process. It has been shown that chronic administration of ADH to homozygous Brattleboro rats (unable to synthesize this hormone) leads to increased GFR [14], and to hypertrophy of the kidney [11, 12], with a preferential increase of the inner stripe of outer medulla and of the medullary thick ascending limbs [11] and an increase in internephron heterogeneity [16, 17]. Very similar changes were observed in normal Wistar rats submitted to chronic stimulation of urine concentration (obtained by restricting water intake to one-third of spontaneous intake) when compared to Wistar rats undergoing chronic high diuresis (obtained by offering 5% glucose as drinking fluid) [13]. Adaptation of MTAL to alterations in status of urinary concentration, comprising anatomical, enzymatic, and functional changes has also been documented in rat and another rodent [15, 30, 31].

Possible relationship between MTAL hypertrophy and T^cH_2O

The similarity between intrarenal patterns of ADH- and protein-induced kidney hypertrophy may be related to the fact that in both cases a marked increase in solute-free water reabsorption (T^cH_2O) takes place. Solute-free water reabsorption depends both on the amount of solutes to be excreted and on the osmotic pressure to which these solutes are raised above that of plasma. With ADH-stimulation of urine concentration in Brattleboro rats, T^cH_2O increased by $\approx$ 70 ml/day (from -17 to + 54 ml/day, data from experiment reported in [11]). This increase was due to enhancement of urine osmolality with no change in amount of solutes excreted. In the case of high protein diet, solute-free water reabsorption was enhanced by $\approx$ 50 ml/day. This resulted from an increase in osmolar excretion with no significant change in urine osmolality (Table 1).

In these two situations in which T^cH_2O was enhanced and MTAL hypertrophied, an increased salt reabsorption has been demonstrated in this nephron segment. With respect to ADH, Besseghir, Trimble and Stoner showed by microperfusion of isolated tubule that transepithelial voltage (a reflection of active salt transport) was doubled in MTAL of Brattleboro rats chronically infused with AVP compared to that of untreated rats [15]. In several other studies, the acute effect of AVP or dDAVP to stimulate salt transport in this segment has been demonstrated [32–34]. With respect to protein intake, Seney, Persson and Wright showed by micropuncture and by in situ microperfusion that salt reabsorption in the loop was significantly increased (+ 40%) by prior feeding the rats for 10 days with a 40% versus 6% casein diet [35].

Several decades ago, Addis suggested that the "burden" imposed on the kidney by high protein intake was not that of excreting but that of *concentrating* the resulting urea [36]. Although ignoring the role of the thick ascending limb in the concentrating mechanism, Addis had conceived that "the special renal tissue directly concerned with this most specific renal work is only a small fraction of the whole kidney" (page 255 in [36]). We know now that most of, if not all (according to the "passive model") the energy necessary to concentrate urine (hence, to reabsorb solute-free water) originates from active salt reabsorption in medullary TAL [37, 38]. It may be assumed that the increase in T^cH_2O observed after high protein feeding is dependent upon a chronic increase in salt transport in the medullary TAL in this circumstance. This increase, observed by Seney et al [35], is accompanied by an hypertrophy of medullary TAL (present study) and by increases in Na-K-ATPase [19, 39] and AVP- and glucagon-dependent adenylate cyclase activities [40]. The enhanced transport activity in the TAL could contribute to increase GFR by decreasing salt

[3] A similar intrarenal pattern of hypertrophy of inner stripe and TAL was also observed by J. Schnermann, J. Briggs and W. Kriz in perfusion-fixed kidneys of Sprague-Dawley rats fed either a 50% or a 5% protein diet (personal communication).

concentration at the macula densa [35] and altering the tubuloglomerular feedback. Indeed, this feedback loop is depressed [35, 41, 42] and the renin-angiotensin system is stimulated [43, 44] in high protein fed rats.

From the present work and available data in the literature it is not possible to determine how high protein intake may stimulate salt reabsorption in MTAL. A role for glucagon, a hormone known to increase in plasma after protein ingestion and to induce a great stimulation of adenylate cyclase in the medullary TAL [45] may be assumed. Antidiuretic hormone also stimulates adenylate cyclase activity [46] and salt transport in the rat TAL [15, 32, 34]. Relatively high levels of ADH are required to induce these effects [47]. Whether this hormone increases on a high protein diet is not known. Nonetheless ADH is necessary for the manifestation of the effects of HP diet since this diet failed to induce a kidney hypertrophy in homozygous Brattleboro rats [48].

In conclusion, this study shows that the medullary TAL is hypertrophied after chronic high protein intake, a finding consistent with the demonstration of an increased active transport in this segment, and is most probably related to the increased solute-free water reabsorption observed in this circumstance. Unraveling the mechanism by which these changes are induced needs further investigation.

Acknowledgments

Part of this work was presented at the IXth International Congress of Nephrology, June 1984, Los Angeles, Abstract n° 405 A, and at the IVth International Congress on Nutrition and Metabolism in Renal Disease, October 1985, Williamsburg, Virginia USA. We thank Kevin Lemley (Stanford, California) for reading the manuscript and Francoise Buhot (Paris) for secretarial assistance.

Reprint requests to Lise Bankir, Ph.D., INSERM Unité 90, Hôpital Necker, 161 rue de Sèvres, 75743 Paris Cedex 15, France.

Note added in proof

Since completion of this study, it was shown that ADH plasma level is increased in man after a large protein meal [A] or after five days on a high protein diet (4.3 ± 0.9 vs. 1.6 ± 0.2 pg/ml on low protein diet) [B].

A. Chan AYM, Cheng ML, Myers BD: Functional response of diseased glomeruli to a large protein meal. (abstract) *Kidney Int* 31:381, 1987

B. Daniels BS, Hostetter TH: Effect of dietary protein intake on vasoactive hormones. (abstract) *Kidney Int* 33: 260, 1988

References

1. Kleinknecht C, Salusky I, Broyer M, Gubler MC: Effect of various protein diets on growth, renal function, and survival of uremic rats. *Kidney Int* 15:534–541, 1979
2. Hostetter TH, Olson JL, Rennke HG, Venkatachalam AM, Brenner BM: Hyperfiltration in remnant nephrons: A potentially adverse response to renal ablation. *Am J Physiol* 241 (Renal Fluid Electrol Physiol 10): F85–F93, 1981
3. Mitch WE: The influence of the diet on the progression of renal insufficiency. *Ann Rev Med* 35:249–264, 1984
4. MacKay EM, MacKay LL, Addis T: Factors which determine renal weight. V The protein intake. *Am J Physiol* 86:459–465, 1928
5. Pitts RF: The effect of infusing glycin and of varying the dietary protein intake on renal hemodynamics in the dog. *Am J Physiol* 142:355–365, 1944
6. Pullman NT, Alving AS, Dern RJ, Landowne M: The influence of dietary protein intake on specific renal functions in normal man. *J Lab Clin Med* 44:320–332, 1954
7. Hendrikx A, Epstein FH: Effect of feeding protein and urea on renal concentrating ability in the rat. *Am J Physiol* 195:539–542, 1958
8. Rabinowitz L, Gunther RA, Shoji ES, Freeland RA, Avery EH: Effects of high and low protein diets on sheep renal function and metabolism. *Kidney Int* 4:188–207, 1973
9. Schmidt-Nielsen B, Robinson RR: Contribution of urea to urinary concentrating ability in the dog. *Am J Physiol* 218:1363–1369, 1970
10. Epstein FH, Kleemlan CR, Pursel S, Hendrikx A: The effect of feeding protein and urea on the renal concentrating process. *J Clin Invest* 36:635–641, 1957
11. Bouby N, Bankir L, Trinh-Trang-Tan MM, Minuth WW, Kriz W: Selective ADH-induced hypertrophy of the medullary thick ascending limb in Brattleboro rats. *Kidney Int* 28:456–466, 1985
12. Bankir L, Bouby N, Trinh-Trang-Tan MM, Kriz W. Grünfeld JP: Effect of long term presence or absence of vasopressin on kidney function and morphology, in *Vasopressin*, edited by Schrier RW, Raven Press, 1985, pp. 497–506
13. Bankir L, Fischer C, Fischer S, Jukkala K, Specht HC, Kriz W: Adaptation of the rat kidney to altered water intake and urine concentration. *Pflügers Arch* (in press)
14. Gellai M, Silverstein JH, Hwang JC, La Rochelle FT Jr, Valtin H: Influence of vasopressin on renal hemodynamics in conscious Brattleboro rats. *Am J Physiol* 246 (Renal Fluid Electrol Physiol 15):F819–F827, 1984
15. Besseghir K, Trimble ME, Stoner L: Action of ADH on isolated medullary thick ascending limb of the Brattleboro rat. *Am J Physiol* 251 (Renal Fluid Electrol Physiol 20):F271–F277, 1986
16. Trinh-Trang-Tan MM, Diaz M, Grünfeld JP, Bankir L: ADH-dependent nephron heterogeneity in rats with hereditary hypothalamic diabetes insipidus. *Am J Physiol* 240 (Renal Fluid Electrol Physiol 9):F372–F380, 1981
17. Trinh-Trang-Tan MM, Bouby N, Doute M, Bankir L: Effects of long-term and short-term anditiuretic hormone availability on internephron heterogeneity in the adult rat. *Am J Physiol* 246 (Renal Fluid Electrol Physiol 15):F879–F888, 1984
18. Bankir L, Hollenberg NK: In vivo staining of the kidney with Alcian Blue: An adjunct to morphological and physiological studies. *Renal Physiol* 6:151–155, 1983
19. Bouby N, Bankir L: Effect of a high protein intake on the Na-K-ATPase activity in the thick ascending limb of Henle's loop in the rat. *Clin Sci* 74:319–329, 1988
20. Provoost AP, Keijzer de MH, Wolff ED, Molenaar JC: Development of renal function in the rat. The measurement of GFR and ERPF and correlation to body and kidney weight. *Renal Physiol Basel* 6:1–9, 1983
21. Klahr S, Tripathy K, Garcia FT, Mayoral LG, Ghitis J, Bolaños O: On the nature of the renal concentrating defect in malnutrition. *Am J Med* 43:84–96, 1967
22. Schrier RW, Berl T: Disorders of water metabolism, in *Renal and Electrolyte Disorders*, edited by Schrier RW, Boston, Little Brown and Company, 1980, pp. 1–64
23. Gamble JL, McKhann CF, Butler AM, Tuthill E: An economy of water in renal function referable to urea. *Am J Physiol* 109: 139–154, 1934
24. Crawford JD, Doyle AP, Probst JH: Service of urea in renal water conservation. *Am J Physiol* 196:545–548, 1959
25. Pennel JP, Sanjana V, Frey NR, Jamison RL: The effect of urea infusion on the urinary concentrating mechanism in protein-depleted rats. *J Clin Invest* 55:399–409, 1975
26. Schmidt-Nielsen B, Barrett JM, Graves B, Crossley B: Physiological and morphological responses of the rat kidney to reduced dietary protein. *Am J Physiol* 248 (Renal Fluid Electrol Physiol 17):F31–F42, 1985
27. Fine L: The biology of renal hypertrophy. *Kidney Int* 29:619–634, 1986
28. Mac Kay EM, Mac Kay LL, Addis T: Factors which determine renal weight. XII. The nitrogen intake as varied by addition of urea

to the diet. *J Nutr* 4:379–383, 1931
29. WILSON HEC: An investigation on the cause of renal hypertrophy in rats fed on a high protein diet. *Biochemical J* 27:1348–1356, 1933
30. TRINH-TRANG-TAN MM, BANKIR L, DOUCET A, EL MERNISSI G, IMBERT-TEBOUL M, MONTEGUT M, SIAUME S, MOREL F: Influence of chronic ADH treatment on adenylate cyclase and ATPase activity in distal nephron segments of diabetes insipidus Brattleboro rats. *Pflügers Arch* 405:216–222, 1985
31. DOUCET A, BARLET C, BADDOURI K: Effect of water intake on Na-K-ATPase in nephron segments of the desert rodent, *Jaculus orientalis*. *Pflügers Arch* 408:129–132, 1987
32. ROUFFIGNAC C DE, CORMAN B, ROINEL N: Stimulation by antidiuretic hormone of electrolyte tubular reabsorption in rat kidney. *Am J Physiol* 244 (Renal Fluid Electrol Physiol 13):F156–F164, 1983
33. WORK J, GALLA JH, BOOKER BB, SCHAFER JA, LUKE RG: Effect of ADH on chloride reabsorption in the loop of Henle of the Brattleboro rat. *Am J Physiol* 249(Renal Fluid Electrol Physiol 18):F698–F703, 1985
34. PETERSON L, ROUFFIGNAC C DE, SONNENBERG H, LEVINE DZ: Thick ascending limb response to dDAVP and atrial natriuretic factor in vivo. *Am J Physiol* 252 (Renal Fluid Electrol Physiol 21):F374–F381, 1987
35. SENEY FD JR, PERSSON AEG, WRIGHT FS: Modification of tubuloglomerular feedback signal by dietary protein. *Am J Physiol* 252 (Renal Fluid Electrol Physiol 21):F83–90, 1987
36. ADDIS T. *Glomerular Nephritis. Diagnosis and Treatment*. The Macmillan Company, New York, 1949
37. BERLINER RW: Mechanism of urine concentration. *Kidney Int* 22:202–211, 1982
38. JAMISON RL, KRIZ W: *Urinary Concentrating Mechanism, Structure and Function*. Oxford University Press, New York, Oxford, 1982
39. SENEY FD JR, MARVER D: Effect of dietary protein on medullary thick ascending limb Na-K-ATPase activity. (abstract) *Clin Res* 34:609A 1986
40. BOUBY N, TRINH MM, BANKIR L: Influence of dietary protein on adenylate cyclase activity of the medullary thick ascending limb. (abstract) *Kidney Int* 31:430, 1987
41. SENEY FD JR, WRIGHT FS: Dietary protein suppresses feedback control of glomerular filtration in rats. *J Clin Invest* 75:558–568, 1985
42. SCHNERMANN J, BRIGGS JP: The effect of variations in protein intake on tubuloglomerular feedback and single nephron filtration rate in rats with normal and elevated blood pressures. (abstract) *Renal Physiol* 7:258–259, 1984
43. FERNANDEZ-REPOLLET E, MARTINEZ-MALDONADO M: Influence of dietary protein intake on the renin-angiotensin-aldosterone system in the rat. *Proc 9th Int Congr Nephrol*, Los Angeles (abstract) 311 A, 1984
44. PALLER MS, HOSTETTER TS: Dietary protein increases plasma renin and reduces pressor reactivity to angiotensin II. *Am J Physiol* 251 (Renal Fluid Electrol Physiol 20):F34–F39, 1986
45. BAILLY C, IMBERT-TEBOUL M, CHABARDES D, HUS-CITHAREL A, MONTEGUT M, CLIQUE A, MOREL F: The distal nephron of rat kidney: A target site for glucagon. *Proc Natl Acad Sci USA* 77: 3422–3424, 1980
46. IMBERT-TEBOUL M, CHABARDES D, MONTEGUT M, CLIQUE A, MOREL F: Vasopressin-dependent adenylate cyclase activities in the rat kidney medulla: Evidence for two separate sites of action. *Endocrinology* 102:1254–1261, 1978
47. ELALOUF JM, DI STEFANO A, DE ROUFFIGNAC C: Sensitivities of rat kidney thick ascending limbs and collecting ducts to vasopressin in vivo. *Proc Natl Acad Sci USA* 83:2276–2280, 1986
48. BOUBY N, LAOUARI D, TRINH-TRANG-TAN MM, KLEINKNECHT C, KRIZ W, BANKIR L: Protein-induced kidney hypertrophy: Selective increase of the inner stripe of the outer medulla and possible involvement of ADH and/or urine concentrating mechanism. (abstract) *Proc IXth Int Cong Nephrol, Los Angeles* 1984, 405A