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Mechanism of Inhibition of the Proximal Tubular Isotonic Fluid Absorption by Polylysine and Other Cationic Polyamino Acids*

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Summary. The present study was initiated with the hope of clarifying the role of negative charges in the luminal brush border membrane in the overall process of transepithelial isotonic sodium and water absorption. Using micropuncture techniques, cationic polyamino acids such as polylysine (mol wt 100,000, 17,000 and 1,500–5,000, 1 mg/ml), tetralysine, polyornithine (mol wt 100,000, 1 mg/ml), polyethyleneimine (2 mg/ml), polymyxin B (2 mg/ml), protamine sulfate (25 mg/ml) and histone (0.5 mg/ml) were perfused through the segments of rat kidney proximal tubule for 30 sec to 2 min. The rate of isotonic fluid absorption was measured before and after each perfusion with the Gertz's split drop method using Ringer's solution as a shrinking drop. Polylysine 100,000 and 17,000 and polyornithine were the most potent, inhibiting isotonic reabsorption by 93%. The sequence of inhibitory effect was: polylysine 100,000 $\simeq$ polyornithine 100,000 $\simeq$ polylysine 17,000 > polyethyleneimine > polylysine 1,500–5,000 $\simeq$ polymyxin B > protamine sulfate $\simeq$ histone. In contrast, tetralysine (2 mg/ml) showed no inhibitory effect. Electrical potential difference (p.d.) of the proximal tubular cells was destroyed within 10 sec of luminal perfusion with polylysine 100,000 (1 mg/ml). Simultaneously with the drop in p.d., electrical resistance of the luminal brush border membrane was nearly totally eliminated, whereas transepithelial input resistance remained unaltered. Furthermore, trypan blue dye was taken up by polylysine 100,000-perfused tubular cells but not by normal cells. Expanding drop analysis (mannitol solution as a split drop) was performed as a screening test to examine if the permeability for water and sodium in the lateral paracellular pathway is altered by polylysine 100,000. No significant difference was observed in the velocity of split drop expansion between untreated and polylysine-perfused tubules. A lower concentration of polylysine 100,000 (0.1 mg/ml) showed a much less inhibitory effect on fluid absorption and on cell p.d. These observations indicate that the strong inhibition on proximal tubular fluid absorption exerted by polylysine and perhaps also by other cationic polyamino acids is due not to modification of membrane negative charges but to the lysis of tubular cells by these polycations.

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Despite numerous studies, the detailed mechanisms of isotonic Na^+ reabsorption across the mammalian kidney proximal tubule is still far from clear. A recent study from this laboratory using thermodynamic analysis has indicated, however, that at least part of the net Na^+ transport across this epithelium is active [10].[1] Stated in another way, at least part of the net Na^+ transport is basically ascribable to the pattern of transport originally proposed by Koefoed-Johnsen and Ussing [22] and Ussing and Zerahn [45], namely that passive influx of Na^+ occurs across the brush border membrane of tubular cells and active efflux from the cell of Na^+ occurs at the basal and/or lateral cell membrane. It was also inferred that Na^+, actively pumped out into the basal infoldings and lateral intercellular space, further drags water, Cl^- and more Na^+ from the tubular lumen through the tight junction [10]. The electrophysiological basis for such a paracellular pathway for the isotonic transport has recently been elaborated by Frömter [6]. However, the mechanism whereby Na^+ crosses the luminal brush border membrane of the mammalian kidney proximal tubule is still poorly understood except that Na^+ influx is coupled, at least partially, with H^+ secretion into the lumen [44] and partially with influx into the cell of glucose and amino acids [8]. The brush border membrane of the kidney proximal tubule carries a net negative charge at a neutral pH [17, 21]. In epithelial membranes [47] and phospholipid bilayers [1] attempts have been made to explain ionic selectivity and permeability by the fixed charges of the membrane. If the negative charges in the proximal brush border membrane can be modified artificially then the resultant alteration in the tubular reabsorptive function would allow insight into the role of negative membrane charges in the overall process of Na^+ movement across this membrane. Although H^+ and Ca^{++} are known to modify the membrane charges and reverse the permselectivity of Na^+ and Cl^- in the passive route [47], they do not appear to have profound effects on the overall reabsorption rate [42]. Furthermore, the use of highly permeable small molecular substances may pose a difficulty in interpreting the sites of action even if they modify the overall reabsorption rate. For this reason, we have employed several positively charged macromolecules, cationic polyamino acids (also referred to as polycation or basic polyelectrolyte), such as poly-L-lysine (polylysine, mol wt 100,000, 17,000 and 1,500–5,000), tetralysine, poly-L-ornithine (polyornithine), polyethyleneimine, polymyxin B, protamine sulfate and histone with the hope that we can specifically modify the surface charges of the

1 In that study, active transport of Na^+ and other ions has been defined as those portions of the net transport unaccounted for by solvent drag, diffusion and electrical transference.

luminal membrane by applying them directly into the proximal tubular lumen for a short period of time using micropuncture techniques. The effects of cationic polyamino acids on the transepithelial Na^+ transport have already been studied in the rat salivary duct by Mangos and McSherry [24] and in the toad bladder by Mamelak, Wissig, Bogoroch and Edelman [23], the former showing a marked inhibition of Na^+ reabsorption and the latter an increase in the membrane permeability to Na^+ and the damage of the superficial epithelial cells. The present study was also intended to fill some discrepancies between the above two studies and to further elucidate the effects and the mechanisms of action of these cationic macromolecules on the isotonic fluid and sodium absorption of the rat kidney proximal tubule. The results show, as in the rat salivary duct, that cationic polyamino acids expecially polylysine and polyornithine exert a strong and prompt inhibitory effect on the proximal tubular isotonic fluid absorption. However, evidence has been obtained to indicate that the inhibition of membrane transport in the proximal tubule may not be due to modification of membrane charges but rather due to the lysis of tubular cells by these polycationic compounds.

Materials and Methods

Micropuncture Experiments

Male Wistar rats, weighing 180–240 g, were anesthetized with Inactin (promonta-80 mg/kg body weight) and their left kidneys were prepared for micropuncture as prey viously described [43]. The kidney capsule was completely stripped off and the kidney surface covered with paraffin oil. Cotton wool was used to immobilize the kidner instead of agar. The ureter was identified and canulated with a polyethylene tubing o, simply cut and urine allowed to drain into the abdominal cavity. Fluid loss, if any, during the experiments was not replaced. The rats had free access to usual food and tap water until the time of experiments. The micropuncture technique used was the split drop method [11] in combination with the luminal perfusion of test substances [37]. Split oil droplets were made with either double-barrelled pipettes with approximate outside diameter of 12 μm, or two single-barrelled pipettes of from 4 to 7 μm outside diameter. Ringer's solution splitting the colored castor oil always contained, in mEquiv/liter: $Na^+=150$, $Cl^-=128$, $Ca^{++}=3$, $HCO_3^-=25$ with measured osmolarity of 285 mOsm/kg water. This Ringer's solution was also used as a medium for dissolving cationic polyamino acids or other test compounds throughout the present study. Surface proximal tubular segments, irrespective of their distance from glomerulus were identified for suitability with split droplet formation, which was always performed toward the glomerulus. Care was taken to use only straight or nearly straight tubular segments. The change in the volume of the split droplet was photographed at 5-sec intervals with a Robot motor driven camera and a Robot timer. The camera was attached to one ocular of a Wild (Switzerland) trinocular stereo microscope at a magnification of 100. The illumination used for micropuncture was a Monla low-voltage lamp while the photographs were taken with a flash from a Zeiss Ukatron flash generator at 60 W synchronized with the timer and led onto the kidney surface beneath the oil through a Zeiss dia-

cleral lamp cone. Absorptive half time ($t_{1/2}$) was evaluated according to Gertz [11] except that one tubular diameter was used as a correction factor for estimating the length of split drop as described by Györy [14]. Absorption rate was expressed as volume flow (Jv) per unit tubular length

$$Jv = \frac{\ln 2 \cdot \pi r^2}{t_{1/2}} \tag{1}$$

where r is the radius of the oil column in each individual tubule [14]. Thus, the dimension of Jv is 10^{-7}cm^3/cm sec. In some Figures, absorption rate is presented only as $t_{1/2}$, which is an inverse function of Jv. The solution containing basic polyamino acids or other test compounds was then perfused downstream from a point proximal to the test site. Immediately after the perfusion of test solution, the rate of isotonic reabsorption was measured again at the same tubular site by splitting the oil column with Ringer's solution.

Preparation of Perfusates

All the substances tested, namely, cationic polyamino acids, acid mucopolysaccharide, anionic polyamino acids and enzymes, were dissolved in Ringer's solution immediately before use. Cationic polyamino acids include polylysine (mol wt 100,000 and 17,000, Serva, Heidelberg; mol wt 1,500–5,000, Miles-Seravac, Lausanne, Switzerland), tetralysine (Miles-Yeda, Rehovot), polyornithine (Serva), histone (Serva), polyethyleneimine (Serva), polymyxin B (Sigma), protamine sulfate (Sigma); acid mucopolysaccharides include heparin (Roche or Nutritional Biochemical, Cleveland), chondroitin sulfate (Serva) and hyaluronic acid (Schuchardt). Trypsin was obtained from Serva, neuraminidase (from *Cl. perfringens*) from both Serva and Sigma. Whenever molecular weight is not specified "polylysine" refers to polylysine 100,000.

Measurement of Static Head Concentration

Using the split drop recollection technique [18], the static head concentration (also referred to as steady-state concentration) was measured for Na^+ and Cl^- before and after the perfusion of tubules with polylysine 100,000 (1 mg/ml). In order to shorten the time needed for equilibration an approximately equilibrated Ringer's solution (in mM: raffinose = 50, $Na^+ = 125$, $HCO_3^- = 25$, $Ca^{++} = 1$, $Cl^- = 102$) was injected into the lumen as a split drop.

Volume Expansion of Intraluminally Injected Isotonic Mannitol

A long loop of proximal tubule was identified and filled with a length (6 tubular diameters) of colored castor oil. A constant volume (0.045 nl) of isosmotic mannitol solution was injected into the oil column (expanding drop). Velocity of drop volume expansion was measured by taking serial photographs at 5-sec intervals. Calibration of a constant volume of mannitol solution (0.045 nl) injected was accomplished by transferring mannitol solution from a precalibrated constant bore capillary pipette to the tip of an oilfilled micropuncture pipette. The transferred mannitol solution in the puncture pipette was again sealed with oil. The moment that the expanding drop was injected into the oil column, the first photograph was taken (time zero). Since the initial length of the expanding drop was only two or three tubular diameters, the correction for oil meniscus and thus the volume of hemispherical head of the oil column was essential in calculating the volume of the expanding drop (V). V was thus calculated according to

$$V = \pi \left(\frac{D}{2}\right)^2 L - \frac{4}{3}\pi \left(\frac{D}{2}\right)^3 = \pi \left(\frac{D^2 L}{4} - \frac{D^3}{6}\right) \tag{2}$$

where D is the diameter of the expanding drop (assuming that it is equal to the diameter of oil column during droplet expansion); L is the intermenisci length + D. The agreement between the calculated initial ($t=0$) volume of the expanding drop and that of actually injected volume indicates the adequacy of the procedure (*see* Fig. 6). In four tubules, the expanding drops were formed immediately after the tubular lumen was perfused with polylysine 100,000 (1 mg/ml) for 30 sec.

Trypan Blue Dye Incorporation Test

3% trypan blue in Ringer's solution was first perfused intraluminally with a micropipette for 2 min. The dye was not taken up by the cells by this procedure. Then polylysine 100,000 (1 mg/ml) was infused into the lumen for 30 sec and immediately afterwards trypan blue solution was perfused for 2 min.

Electrophysiological Study

The preparation of kidney was similar to that of the micropuncture study except that the kidney was immobilized with Ringer-agar in a double kidney cup. Tail skin was stripped off and the tail was grounded via an agar bridge and a calomel electrode. The electronic set-up used was essentially the same as described previously [16] except that a semifloating electrode [33] was used to obtain a stable cell p.d. This electrode was constructed immediately before use by simply replacing the shaft of the conventional Ling-Gerard glass microelectrode with a length (6 cm or longer) of KCl-filled flexible silicon-rubber tubing (Leitz). With this improved electrode, it was possible to obtain stable cell p.d.'s in the proximal tubular cells lasting for as long as 40 min [33]. The connection to the electrometer (Keithley 604 differential electrometer amplifier) was achieved by a pair of saturated calomel half-cells. Only those electrodes with tip resistance $\geqq 50$ MΩ and tip potential $\leqq 5$ mV were selected for constructing the cell-impaling semifloating electrodes. Either a Ling-Gerard electrode or a semifloating electrode was used as a reference electrode, the tip of which was placed on the kidney surface in the vicinity of the cell-impaling semifloating electrode. When the cell electrode was introduced into a tubular cell, an electrical potential difference (p.d.) across the contraluminal cell membrane of approximately -70 mV, cell interior negative, was recorded. If the cell p.d. remained stable for longer than 2 min, polylysine 100,000 either 10^{-5}M (1 mg/ml) or 10^{-6}M (0.1 mg/ml) in Ringer's solution was gently infused into the tubular lumen so that the tubular diameter was not visibly changed by infusion. In half the experiments, twice concentrated polylysine was infused so that the final concentration of polylysine in the lumen after probable dilution with luminal fluid may not be less than 10^{-5} or 10^{-6}M; however, we have not observed a consistent difference of effect in so doubling the concentrations. Thus, for the sake of simplicity, these data will be grouped under 10^{-5} or 10^{-6}M, respectively. The electrical resistance of the brush border membrane was estimated as illustrated in Fig. 1. Square pulses of 5×10^{-7} amp and 0.3- to 0.5-sec duration were injected intraluminally via a 100-MΩ resistor connected between a Tektronix pulse generator (Type 161 and 162) and a low tip resistance ($\leqq 5$ MΩ) microelectrode of Ling-Gerard type. A reference electrode (electrode R in Fig. 1), a Ling-Gerard type electrode with relatively low tip resistance ($\leqq 10$ MΩ), was placed in the lumen in the vicinity (less than 20 μm distant) of the pulse-injection electrode. When the cell-impaling semifloating electrode is located in the immediate peritubular space (Fig. 1 *A*) above the reference electrode, the current-induced voltage pulse thus obtained represents a qualitative measure of transepithelial input resistance (input resistance = pulse height in volt divided by the injected current, 5×10^{-7} amp). Then the cell electrode was carefully introduced into the tubular cells. The height of voltage under these conditions was

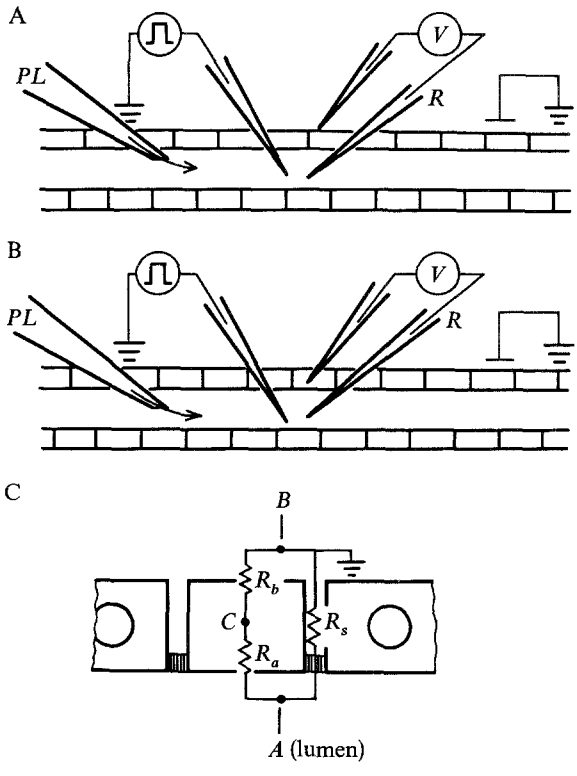

Fig. 1. Schematic drawing of the methods for measuring transepithelial (*A*) and luminal brush border membrane (*B*) resistance of the rat kidney proximal tubule. *V*, Keithley differential electrometer. *R*, reference electrode. *PL*, pipette for perfusion of polylysine and other cationic polyamino acids. (*C*) Shows a simplified circuit diagram, where R_a, R_b and R_s represent resistances of brush border membrane, basal or contraluminal cell membrane and paracellular shunt pathway. Current pulse-induced voltage deflection indicates only a qualitative measure of the electrical resistance of the luminal cell membranes and the tubular wall

usually 3/4 that of the transepithelial voltage pulse and represents a qualitative measure of the electrical resistance of the luminal brush border membrane. Polylysine 100,000 was subsequently perfused intraluminally from a pipette inserted proximally to the impaled cell.

Electron-Microscopic Study

A total of 12 proximal tubules were subjected to the electron-microscopic study. In two tubules, two times more concentrated polylysine 100,000 (2 mg/ml) than that used for transport study (1 mg/ml) was continuously infused into the lumen for 2 min and in the other two tubules for 6 min. Fixation was performed *in situ* by perfusing 1% glutaraldehyde in cacodylate buffer (pH 7.4) into the peritubular blood capillary until the blood capillary was blocked by tissue hardening, which usually occurred within 20 sec. The perfused tubules were marked by injecting colored oil into the adjacent tubules. The entire kidney was immediately removed and dipped in several changes of cold 1% glutaraldehyde solution. The surface layer of the kidney was sliced free hand

with a razor blade and transferred to the glutaraldehyde solution. The perfused tubules were identified under a stereoscopic microscope and isolated with a pair of Dumont No. 5 tweezers and a sharp steel needle. One control tubule from each experiment was treated in the same way except that Ringer's solution was perfused intraluminally for 2 and 6 min, respectively. The isolated tubules were postfixed in 1% OsO_4 buffered with veronal acetate (pH 7.4). In the second set of experiments, two tubules from each experiment were first perfused intraluminally with polylysine 100,000 (2 mg/ml) for 2 and 6 min, respectively, followed by the luminal perfusion for 2 min of buffered 1% OsO_4 solution. The perfused tubules were stained dark purple and thus easily identified during the subsequent isolation procedure in cacodylate buffer solution. Appropriate controls were taken. Following fixation all the tissues were dehydrated in ethanol and embedded in Epon 812. For the electron-microscopic examination, sections 600 to 800 Å thick were cut and stained sequentially in uranyl acetate and in lead citrate.

Results

Micropuncture Experiments

Table 1 lists the effects of cationic polyamino acids on the isotonic fluid absorption by the rat proximal tubule. Polylysine, both mol wt 100,000 and 17,000 at 1 mg/ml, inhibited fluid absorption by 92% after 30-sec intraluminal perfusion. The smaller molecular polylysine 1,500–5,000 with the same dosage and perfusion time showed 84.1% inhibition. In contrast, tetralysine exerted no significant inhibitory effect despite the fact that the doubled concentration (2 mg/ml) was perfused for 2 min or longer and that split drop itself contained the same concentration of tetralysine. Thus

Table 1. Effects of basic polyamino acids on the isotonic fluid absorption by the rat kidney proximal tubule

Basic polyamino acid	mol wt	Concentration (mg/ml)	Perfusion time (min)	*n*	*Jv* (experimental)	*Jv* (control)	% Inhibition
polylysine	100,000	1 (10^{-5} M)[a]	0.5	27	0.38±0.04	4.88±0.25	92.3
polylysine	100,000	0.1 (10^{-6} M)	0.5	8	4.07±0.28	5.39±0.40	24.5
polylysine	100,000	0.1 (10^{-6} M)	2.0	5	3.16±0.41	5.03±0.47	37.2
polylysine	1,500–5,000	1	0.5	10	0.92±0.21	5.80±0.20	84.1
polylysine	17,000	1 (6×10^{-5} M)	0.5	7	0.36±0.04	4.95±0.34	92.8
etralysine	724	2 (2.7×10^{-3} M)	2.0[b]	6	4.25±0.29	4.84±0.50	12.0
polyornithine	100,000	1 (10^{-5} M)	0.5	15	0.35±0.04	5.07±0.32	93.1
polyethyleneimine	>2,000	2	0.5	7	0.52±0.10	5.99±0.70	91.3
polymyxin B	?	2	1.0	5	0.82±0.15	4.94±0.69	83.4
protamine sulfate	(≃4,000)	25	2.0	7	1.07±0.17	5.32±0.49	80.0
istone	?	0.5	2.0	4	2.56±0.61	7.85±1.00	67.4

= number of experiments; *Jv* = volume flow expressed in 10^{-7}.

10^{-5} M polylysine 100,000 corresponds to 5.5 mM lysine residues.

The same concentration of tetralysine was also included in the split drop.

it should be noted that under this latter condition, unlike the case of the higher molecular polylysines, the luminal brush border membrane has been continuously exposed to the solution containing a high positive charge density even during the period of split drop test. Table 1 also shows that the inhibitory effect of polylysine 100,000 was drastically reduced when one-tenth dose (0.1 mg/ml) was perfused, the inhibition being only 24 and 37% after 0.5- and 2-min perfusions, respectively. Among the other cationic polyamino acids, polyornithine and polyethyleneimine exhibited a comparably strong inhibitory effect as polylysine 100,000, which was followed by polymyxin B, protamine sulfate and histone in decreasing order of inhibitory action. For the sake of comparison with these cationic polyamino acids, we have also tested the effect of anionic polyelectrolytes such as heparin (1,250 U/ml, mol wt 10,000–26,000), poly-L-glutamic acid (5 mg/ml, mol wt 40,000–100,000) and poly-L-aspartic acid (5 mg/ml, mol wt 20,000 to 30,000). Although these compounds were perfused as long as 2 min, they showed no inhibitory effect (not included in Table 1). Hence, in order to rule out the possibility that the inhibitory effect of polycations is due to the effect of possible contaminants in the preparations but not due to the cationic nature of these macromolecules, we mixed polylysine and various anionic polyelectrolytes, namely polyaspartic acid, polyglutamic acid, heparin, chonroitin sulfate and hyaluronic acid in the test tubes. In all cases, white precipitates were formed and the supernatants thus obtained were without any inhibitory effect on the proximal tubular fluid absorption (Fig. 2). This is consistent with the observation by Katchalsky [19] that acidic and basic polyelectrolytes interact electrostatically with precipitation at the point of polymeric neutrality. The findings obtained hitherto agree well with the studies by others [25] that the biological effects of cationic polyamino acids are dependent on molecular size, concentration and the charge density of positive charges. We then proceeded to estimate how much of the luminal brush border membrane must be covered by polylysine 100,000 to reach a maximum inhibitory effect. Such a study was also intended to exclude the possibility that the inhibitory effect of cationic polyamino acids is simply due to the precipitation of polycations to the negatively charged brush border membrane and thus the formation of a new diffusive barrier. The amount of polylysine to effect a maximal inhibition on isotonic reabsorption was estimated by injecting polylysine 100,000 (1 mg/ml) in Ringer's solution into the tubular lumen as shrinking droplet (Fig. 3). The shrinking of such a droplet was photographed at 5-sec intervals for 20 sec. The shrinkage was fast in the beginning but increasingly slowed down with time. Hence the Jv value of the first split drop should be re-

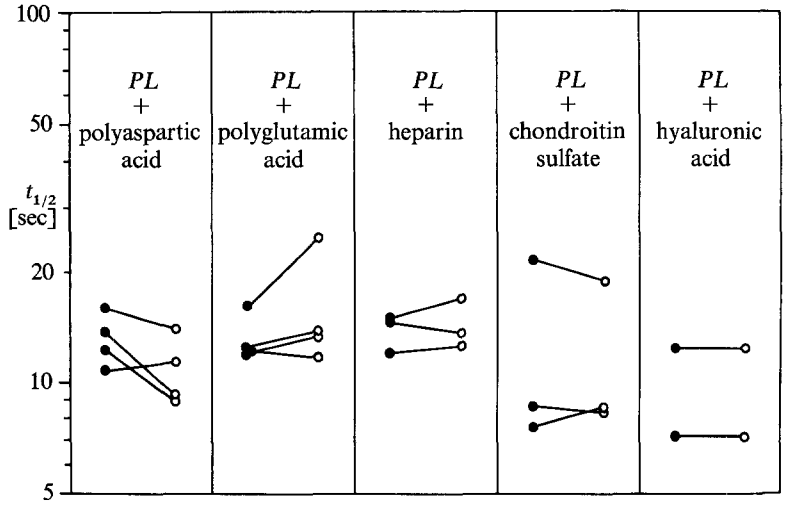

Fig. 2. Effect on proximal tubular fluid absorption of polylysine-perfusate pretreated with acid mucopolysaccharides (polyacids). To polylysine-containing Ringer's solution (1.0 mg/ml), 2.0 mg/ml mucopolysaccharides were added and the precipitate was centrifuged out. Supernatants thus obtained were perfused downstream intraluminally. Filled symbols represent control absorption rates and empty circles those after perfusion of perfusates. The rate of fluid absorption was given as absorption half time ($t_{1/2}$). Each line indicates a single observation. *PL*, polylysine 100,000

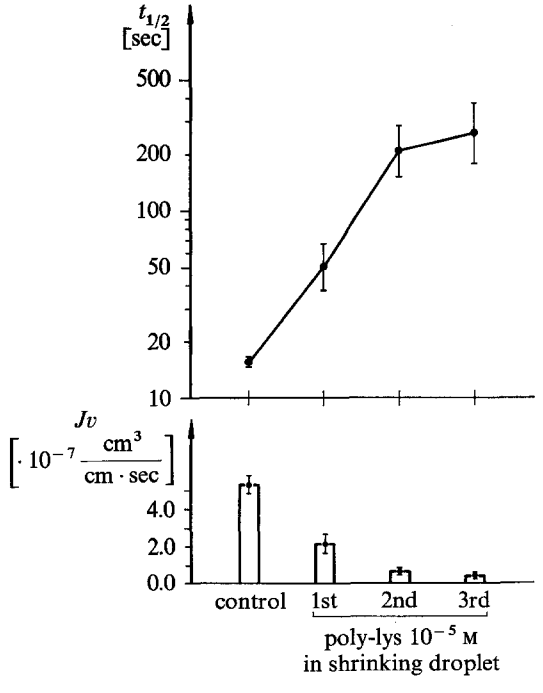

Fig. 3. Absorption of polylysine 100,000 (1 mg/ml or 10^{-5} M) containing solution injected as a shrinking drop. The data are given as both $t_1/_2$ and *Jv*. Each plot or column is the mean $\pm$ SEM of five observations. For more detail, *see text*

garded as only the rough estimate of mean absorption rate during this period of time. After the 20 sec the remaining droplet containing polylysine was moved slowly back and forth several times along the entire length of the tubule which had been initially exposed to the injected shrinking droplet until nearly all the droplet was absorbed. The second droplet containing polylysine, however, shrank extremely slowly from the beginning. Based on these observations, we estimated that the amount of polylysine required to effect a maximal inhibition of the isotonic reabsorption is less than that contained in two droplets. The polylysine molecule to brush border surface ratio (SR) was calculated by assuming the following two boundary configurations of polylysine molecules: (a) when polylysine is in a globular form and (b) when the polylysine molecule is stretched out and all the lysine residues are available for the interaction with the negative charge of the brush border membrane.

(a) $$\mathrm{SR}\left(\frac{\text{polylysine}}{\text{brush border}}\right)=\frac{2\times10^{-5}\cdot\pi r_t^2\, l\cdot No\cdot R_a^2}{2\pi r l\cdot 60}=0.08$$

(b) $$\mathrm{SR}\left(\frac{\text{polylysine}}{\text{brush border}}\right)=\frac{2\times10^{-5}\cdot 685\cdot\pi r_t^2\, l\cdot No\cdot R_b^2}{2\pi r l\cdot 60}=0.26$$

where

10^{-5} = concentration of polylysine, 10^{-5} moles/liter
r_t = radius of the tubule, 16 μm
l = length of the tubular segment tested
No = Avogadro's number, 6×10^{23}
R_a = radius of polylysine in globular from, 40 Å
R_b = radius of each lysine residue, 3 Å
685 = number of lysine residues in polylysine
60 = surface correction factor for the brush border [29].

The surface ratios of polylysine molecules to brush border membrane thus estimated for the two boundary conditions (a) and (b), were only 8 and 26%, respectively. These values are not in favor of the possibility that the precipitated polylysine forms a continuous coat, which functions as a physical diffusive barrier for Na^+ and water at the surface of brush border membrane and thus inhibited isotonic fluid absorption.

Many mammalian as well as amphibian cells are covered with a layer of negatively charged surface mucous coat which consists predominantly of sialoglycoprotein, glycolipid and free polysaccharide [28, 32]. In human erythrocyte membrane the release of sialic acid by neuraminidase causes

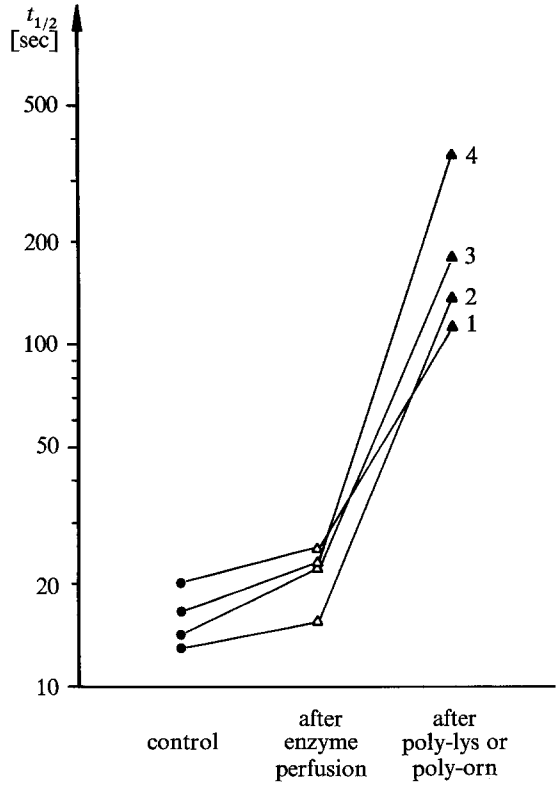

Fig. 4. Effect of neuraminidase- and trypsin-treatment of the tubules on the effect of subsequently perfused polylysine 100,000 or polyornithine. (1) Neuraminidase (Serva, cat. No 30294, in 0.05 M acetate-buffer, pH 5.5. Original 1-ml enzyme solution which has a capacity to hydrolyze 500 µg sialic acid from human glycoprotein in 15 min at 39 °C was diluted 2× and its electrolytes adjusted to isotonicity) was perfused intraluminally for 2 min and thereafter polylysine (1 mg/ml) was perfused for 1 min. All the plots represent the mean of two experiments. (2) Trypsin in Ringer's (0.1 mg/ml) was perfused for 4 min and then polyornithine (not hydrolyzable by trypsin, 1 mg/ml) for 1 min. (3) Neuraminidase, the same dose as above, was perfused for 15 min and subsequently polylysine (2 mg/ml) was perfused for 1 min. (4) Trypsin (0.2 mg/ml) was perfused first for 5 min and then neuraminidase, the same dose as above, for 10 min. Polylysine (2 mg/ml) was subsequently perfused for 1 min

a drastic reduction in the electrophoretic mobility [5, 41]. If the sialic acid contributes predominantly to the negative charges of the brush border membrane and offers binding sites for basic polyamino acids, then the enzymatic removal of sialic acid residues in vivo might alter the interaction with the membrane of those basic macromolecules subsequently administered. Fig. 4 shows the effects on the fluid absorption of enzymatic treatments of the luminal wall of proximal tubules *in situ* and that of the subsequent luminal perfusion of polylysine or polyornithine. In some tubules, perfusion

of trypsin was done either singly or preceeding the perfusion of neuraminidase with the hope of exposing any masked or neuraminidase-inaccessible glycoprotein or glycolipid. The treatment of the tubules with enzymes did not by itself cause a significant reduction in the rate of fluid absorption, nor did it significantly change the effect of subsequently perfused basic polyamino acids. Although there is no evidence that this enzymatic treatment significantly altered the membrane charge or removed the surface coat, more harsh enzymatic treatment of the tubule was not practical since it very frequently destroyed the tubular wall.

Effect of Polylysine on the Static Head Concentration Difference of Na^+ and Cl^-

When an isotonic solution containing an impermeable solute is introduced into the lumen, the volume continues to expand until passive influx of Na^+ is balanced by active outflux [18]. Thus the concentration difference under this condition (static head concentration difference) is dependent on both passive and active (used here in a broad sense) components of membrane transport. As shown in Table 2, polylysine totally eliminated the static head concentration difference for Na^+, indicating either passive back flux of Na^+ is increased or active outflux of this cation is inhibited, or both. The concentration gradient of Cl^- between split drop and peritubular fluid which is normally generated by active HCO_3^- reabsorption also disappeared after polylysine treatment.

Expanding Drop Analysis

As shown in Fig. 5 isotonic mannitol solution expanded almost linearly with time during the first 15 sec. No significant difference was observed between control and polylysine-treated tubules whereas a chloroform-treated tubule did not exhibit such a drop expansion. Since the initial net influx into the lumen of both Na^+ and water proceeds almost exclusively through intercellular channels and tight junctions [3, 46] the data can be interpreted to indicate that the paracellular shunt pathway was not damaged by polylysine. This is further supported by the observation that such a drop expansion of mannitol solution did not occur when the tubular wall was destroyed by flushing the kidney surface with chloroform.

Trypan Blue Dye Incorporation Test

As can be seen in Fig. 6 trypan blue dye is incorporated into the tubular cells pretreated with luminal perfusion of polylysine, indicating that the tubular cells are damaged by this cationic polyamino acid.

Table 2. Effect of polylysine (mol wt 100,000) on Na, K and Cl concentration of the stop-flow luminal perfusate at the steady-state zero net flux (static head) condition

Sample	Rat No.	Sample No.	Na (mM)	Cl (mM)	K (mM)
serum[a]	2	1	153	109	—
	4	1	150	101	—
		2	153	110	—
	mean		152	107	
glom. ultrafiltrate[b]	2	1	155	109	—
		2	156	110	—
		3	151	113	—
	3	1	139	116	—
	4	1	155	110	—
	mean		151	112	
split drop[c]	1	1	113	—	—
(static head conc.,		2	121	—	—
control)	5	1	132	—	—
	mean		122		
split drop[d]	1	1	156	—	5.0
(static head conc.,		2	165	—	5.5
after PLY)	3	1[e]	138	108	—
	4	1[e]	—	113	—
		2[e]	—	110	—
		3[e]	140	117	—
		4	137	119	—
		5	166	109	—
	mean		150	113	5.3
tubular fluid	4	1	156	131	—
		2	153	129	—
		3	149	131	—
	mean		153	130	

[a] Na^+ and Cl^- in serum (collected from femoral vein) are not corrected for Donnan distribution ratio and protein space. Invalidity of the theoretical Donnan ratio for correction has been reported by Terepka, Chen and Toribara [40]. Using their new correction factors of 0.93 for Na^+, 0.99 for Cl^- and protein-bound Na^+ of 0.25 mM/g [40] and the protein space of 5%, the equivalent concentrations are calculated to be 150 mM for Na^+ and 113 mM for Cl^-, which correspond well to those of glomerular ultrafiltrate.

[b] Glomerular ultrafiltrate was sampled directly from the Bowman's capsule.

[c] Luminal stop-flow perfusate at the steady-state zero net volume flux condition. Equilibrated Ringer's solution containing 50 mM raffinose, was injected into the lumen as a split drop and recollected after 30 sec.

[d] Luminal stop-flow perfusate at the stationary state condition after the lumen had been perfused with polylysine (mol wt 100,000, 1 mg/ml) for 1 min. Equilibrated solution described above was injected as a split drop and recollected after 60 sec by which time volume expansion had stopped completely. The mean for Na^+ concentration was calculated for those samples obtained after injection of raffinose-Ringer's.

[e] The experiment was the same as in d except that normal Ringer's solution (150 mM Na^+, 25 mM HCO_3^-, 1.5 mM Ca^{++}, 128 mM Cl^-, no raffinose) was used for measuring the change in the concentration difference of HCO_3^- in normal split drops.

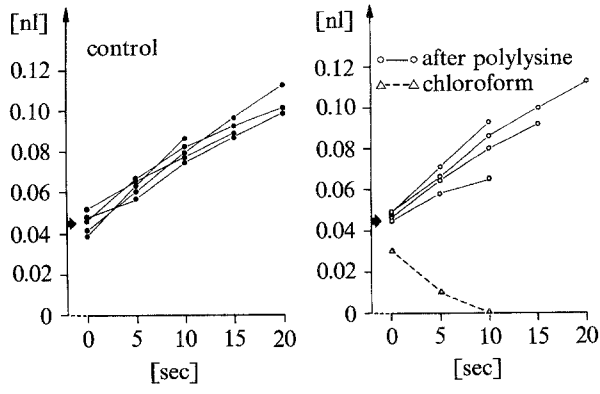

Fig. 5. Drop expansion of luminally injected isotonic mannitol solution. Isotonic mannitol was injected as a split drop and its rate of expansion was followed for as long as 20 sec in both control and polylysine-treated (mol wt 100,000) tubule. One tubule was flushed with chloroform since this procedure is known to completely destroy the permselectivity of the tubular wall [7]. Each line represents an experiment. Arrows indicate the volume of mannitol solution initially injected

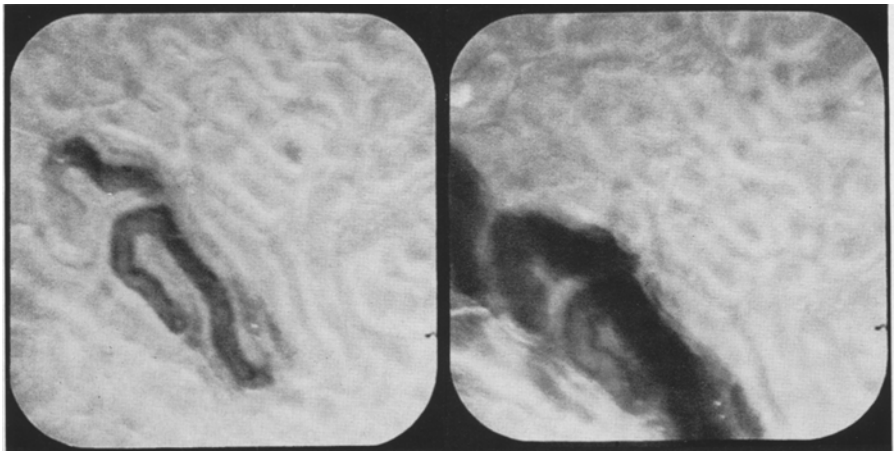

Fig. 6. Trypan blue dye incorporation test. *Left:* 3% trypan blue in Ringer's solution was perfused for 2 min intraluminally, which did not stain the tubular cells. In order to show the contour of the lumen, this picture was taken while the dye was still remaining in the lumen. *Right:* The same tubule after 30-sec luminal perfusion with polylysine followed by 2-min luminal perfusion of trypan blue dye. The tubular cells are now stained intensely. Magnification, ×100

Electrophysiological Study

Fig. 7 demonstrates the effect of intraluminally perfused polylysine 100,000 (1 mg/ml or 10^{-5} M) on the cell p.d. of tubular cells. When the electrode is introduced into a tubular cell, a sharp deflection of negative

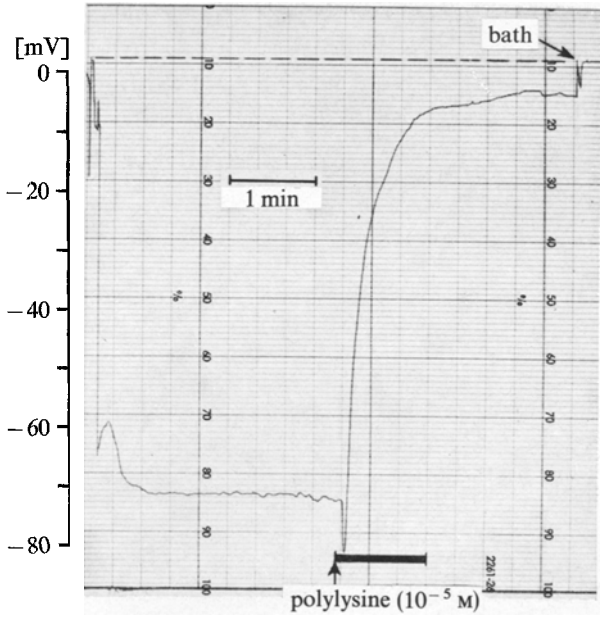

Fig. 7. Effect of polylysine on the cell p.d. of tubular cells; a typical example. Polylysine 100,000 (1 mg/ml or 10^{-5} M) was continuously perfused intraluminally during the period as marked by an arrow and a thick bar. Bath; the electrode was placed in the medium on the surface of the kidney

cell p.d. is recorded. Usually, after a brief period of decline in p.d., the p.d. increases (becomes more negative) and becomes stable at around −70 mV, which is presumably due to sealing of the membrane around the electrode. In all 10 tubules successfully impaled the cell p.d.'s started to decay within 10 sec of luminal perfusion with polylysine. In eight of these experiments, a sharp spike of hyperpolarization preceded the onset of rapid decay in cell p.d. This pattern of p.d. decay is so typical that it is readily distinguishable from those of natural decay in cell p.d. usually seen when the electrode is dislocated. In contrast, the response of the cell p.d. to the 1/10 concentration of polylysine (0.1 mg/ml or 10^{-6} M) varied widely from cell to cell. Of the 10 cells successfully impaled, three cells did not respond at all for as long as 5 to 10 min perfusion with this lower concentration of polylysine, while the other cells showed a decay in cell p.d. in from 1 to 5 min. Some cells in the latter group showed a rather rapid decay once the p.d. started to break down while the others showed a gradual decrease in p.d. until the p.d. reached −20 or −30 mV, where it stayed for several minutes. One example demonstrated in Fig. 8 showed the onset of a rapid drop in cell p.d. after 2 min of continuous luminal perfusion with polylysine, at which

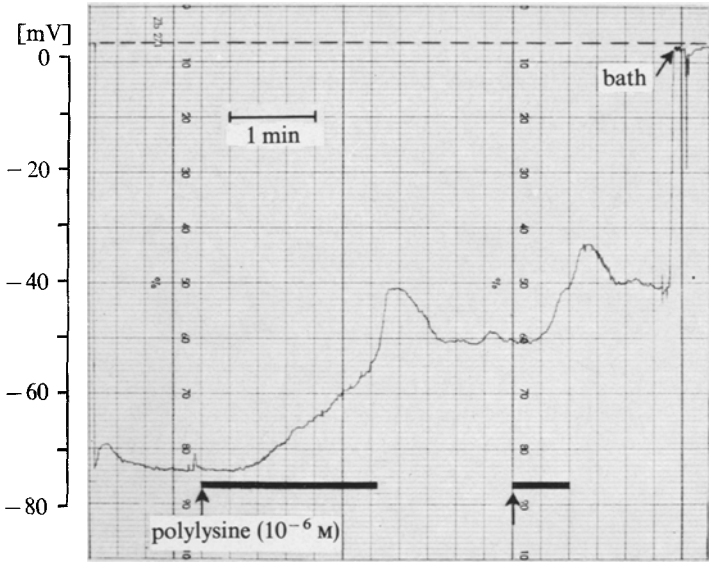

Fig. 8. Effect of polylysine 100,000 (0.1 mg/ml or 10^{-6} M) on p.d. of a tubular cell; one example. In this case polylysine was perfused for two intermittent periods as indicated by thick bars

point the perfusion was discontinued. Twenty seconds later, the p.d. showed a tendency to recover, however only partially, which suggests the occurrence in the cell of a repair process of the damaged membrane. Fig. 9 illustrates a typical response of voltage pulse to luminal perfusion of polylysine (1 mg/ml or 10^{-5} M). In all six cells successfully impaled, polylysine totally eliminated the voltage-pulses generated across the brush border membrane, indicating a drastic decrease in the electrical resistance of the membrane, whereas the transepithelial input resistance was not altered (Fig. 10). The significance of the decrease in voltage-pulse across the luminal membrane is indicated by the fact that in cases where natural decay of p.d.'s occurred due to dislocation of the electrode or incomplete sealing of the membrane around the electrode, the height of voltage pulses remained unchanged or even increased. The possibility of impaling the luminal membrane with the electrode during the luminal perfusion with polylysine appears unlikely since the electrode was always introduced into the lateral tubular wall in a direction parallel to the tubular loop. The analysis of the simplified circuit illustrated in Fig. 1C gives

$$R_t = \frac{R_s(R_a + R_b)}{R_a + R_b + R_s} \tag{3}$$

where R_t is the transepithelial resistance.

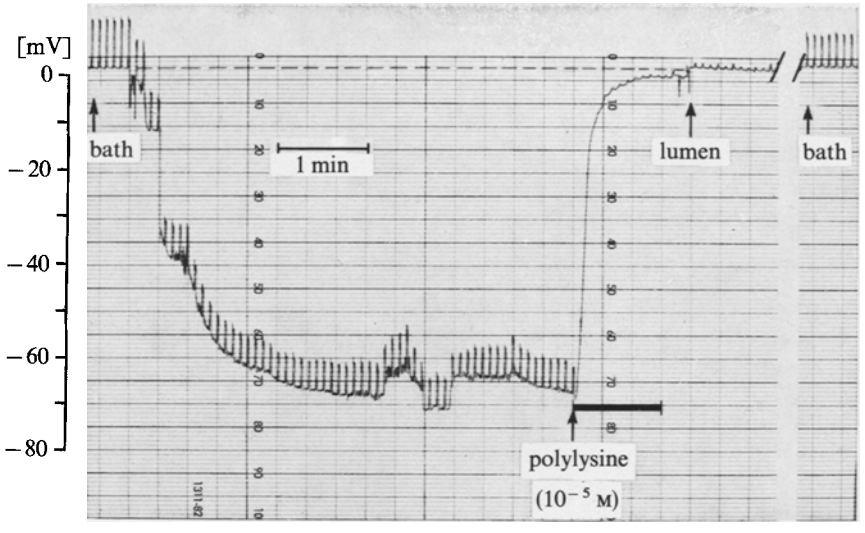

Fig. 9. Effect of luminally perfused polylysine 100,000 (1 mg/ml) on the brush border membrane resistance. Voltage trace from left to right: Transepithelial p.d. (near baseline) with superimposed voltage pulse (a measure of transepithelial input resistance; *see* Fig. 1 *A*). In this particular case, it required nearly 1 min from cell impalement with an electrode until the impaled membrane sealed around the electrode. An irregular shift in resting p.d. (4 min from the start) was caused by tissue distortion when inserting the polylysine pipette near the electrode. The height of voltage pulses now indicates a measure of the electrical resistance of the luminal brush border membrane, provided transepithelial input resistance remains constant, which is the case in the present study. Perfusion of polylysine as indicated by an arrow and a thick bar totally eliminated the voltage pulse. "Lumen" and "bath" indicate the position of electrode tip

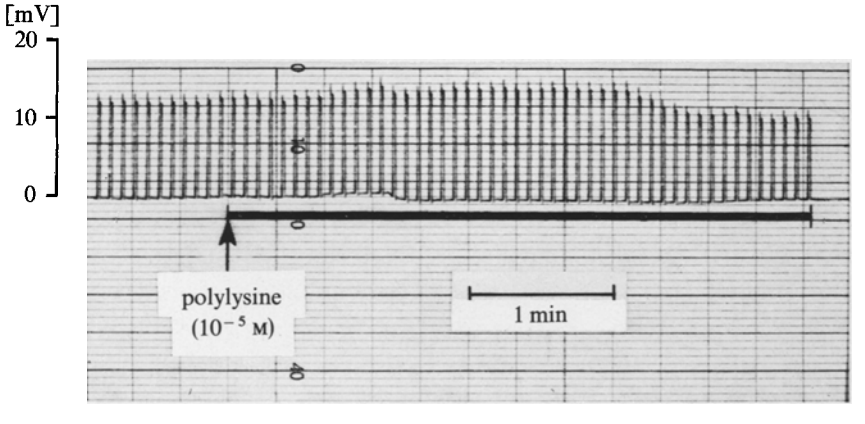

Fig. 10. Effect of luminally perfused polylysine 100,000 (1 mg/ml or 10^{-5} M) on transepithelial input resistance. The height of the voltage-pulse represents a measure of input resistance. Perfusion of polylysine is indicated by an arrow and a thick horizontal bar. Seven other tubules studied showed the same pattern

The rat kidney proximal tubule is classified as the leaky membrane [9]. Thus it can be inferred that R_s is much smaller than R_a and R_b (R_a, $R_b \gg R_s$) as in the *Necturus* proximal tubule [2]. Hence to the first order of approximation, we can probably write Eq. (3) as

$$R_t \simeq R_s.$$

The disappearance of voltage pulses (Fig. 9) after the perfusion of polylysine indicates that R_a is reduced to near zero. Our data that the decrease in R_a did not accompany any change in R_t can probably be interpreted as indicating that the above assumption on the membrane resistance profile in the rat proximal tubule is correct and also that polylysine affected only the resistance of the brush border membrane.

Electronmicroscopic Study

Irrespective of the differences in the method of fixation, all the isolated tubules appeared to be free of fixation artifacts. In light of the detailed description on the ultrastructural changes of the polylysine-treated toad bladder epithelium by Mamelak *et al.* [23], special attention was paid to the appearance of the mucous coat, plasma membrane, brush border microvilli, tight junction, cytoplasmic organelles and nucleus. Within the resolution of ultrathin section, we have been unable to detect any alterations in the membrane surface such as clumping, thickening or disappearance of the mucous coat. There was no clumping of microvilli or other signs of cytological alteration in any tubules treated with polylysine for either 2 or 6 min, except that pinocytotic cytoplasmic inclusion bodies were more numerous in the treated tubular cells. Thus, in the light of other data in the present study, the negative ultrastructural finding should be interpreted with extreme caution especially in terms of the correlation between acute structural and functional changes of the cell. It should also be noted that the previous studies by others on the toad bladder [23] and the rabbit gallbladder [31] which reported ultrastructural changes after polylysine treatment used longer exposure (incubation) time, i.e. from 10 to 30 min.

Discussion

At a preliminary stage of the present study, we reported [35] that the polylysine-induced inhibition of proximal tubular fluid absorption can be reversed by the subsequent luminal perfusion with heparin. Although the

observation itself is reproducible, we have since then dismissed the significance of such an observation since we soon found that the tubular fluid absorption seemingly restored by heparin is not inhibited by the local capillary perfusion with 1 mM cyanide. Nor have we been able to reverse the polylysine-induced depolarization of tubular cells by the subsequent luminal perfusion of heparin *(unpublished observation)*. We currently suspect that the apparent restoration of tubular absorption might have been due to leakage of the split drop presumably through the damaged tight junction. Recapitulating the main observations in the present study: (1) Cationic polyamino acids strongly inhibit the proximal tubular isotonic fluid absorption. The sequence of their potency is polylysine 100,000 $\simeq$ polyornithine 100,000 $\simeq$ polylysine 17,000 > polyethyleneimine > polylysine 1,500–5,000 $\simeq$ polymyxin B > protamine sulfate $\simeq$ histone. (2) Tetralysine (mol wt 720) has no inhibitory effect. (3) Polylysine eliminates the static head concentration differences for Na^+ and Cl^- across the tubular wall. (4) Polylysine-treated tubular cells incorporate trypan blue dye. (5) Polylysine destroys cell p.d. and simultaneously eliminates the electrical resistance of the brush border membrane. (6) The lower concentration of polylysine 100,000 (0.1 mg/ml or 10^{-6} M) has a much less inhibitory effect on fluid absorption and also exhibits varyingly lesser effects on the cell p.d. (7) The paracellular pathway does not seem to be affected by polylysine since drop expansion of luminally injected mannitol occurred normally and the transepithelial input resistance was unchanged after the luminal perfusion with polylysine. Thus, the data obtained strongly indicate that polylysine caused a drastic increase in permeability of the luminal cell membrane and that the resultant cell lysis is the mechanism for inhibition of fluid absorption by polylysine and possibly also by other polycations. The possibility of charge modification or neutralization as an alternative mechanism of their inhibitory actions seems unlikely since tetralysine (Table 1) and other small molecular size cationic polyelectrolytes such as spermine spermidine, methyllysine, amiloride and di-lysine are also without any significant inhibitory effect even at high concentrations *(unpublished observation)*. Furthermore, the molecular weight dependency of polylysine action is also against the possibility of charge neutralization as its mechanism. The cytotoxic effect of polylysine has recently been reported in a number of tissues, such as toad urinary bladder [23], rabbit gallbladder [31], Ehrlich ascites cells [25] and mouse peritoneal macrophages [38], to name just a few. Interestingly, however, red cells are not hemolyzed by polylysine [25] except at high concentrations of the polycation [27]. Similar diversity of response to polycations also occurs in artificial lipid bilayers. For example, Montal [26]

reported that in asymmetrical lipid bilayers the addition of Ca^{++} or polylysine to the compartment limited by a monolayer of the neutral lipids results in no modification of the resistance and stability of the membrane, whereas a drastic decrease in both parameters was observed by the addition of those cations on the opposite compartment containing a monolayer of the negatively charged lipids. In consonance with the above report, polylysine increased ^{22}Na permeability only in liposomes made of phosphatidylserine (negatively charged) but not in those made of phosphatidylcholine (neutral) [20]. Thus, such a difference of response among cells or among different lipids itself offers interesting problems for further studies. Aside from the mechanism of action of polycations on the proximal tubular fluid absorption, our present results shed light on the following two relevant problems, namely, (1) relationship between cystic fibrosis (CF) factor (so-called Mangos factor) in the exocrine secretory fluid and exocrine ductal dysfunction, and (2) the role of luminal hydrostatic pressure in the passive fluid efflux through the paracellular pathway. (1) In CF, the inability of the exocrine gland duct, especially the eccrine sweat duct to reabsorb Na^+ is one of the basic defects of the disease [4]. Mangos and McSherry [24] have observed that Na^+ reabsorption by the salivary duct is inhibited when sweat or saliva from CF patients are inversely perfused through the duct. Since Na^+ reabsorption is also inhibited by inverse perfusion with polylysine and other polycations [24], they assumed that polycationic factors in CF sweat or saliva inhibit Na^+ reabsorption by modifying the surface charge of luminal membrane of exocrine ducts. In our preliminary study, we could not inhibit proximal tubular fluid absorption by luminal perfusion with CF saliva *(unpublished)* although a direct comparison of the epithelia is not possible at present. In the exocrine gland ducts of patients with CF no ultrastructural abnormality has ever been detected [4] despite the fact that the ductal system of the patient is continuously exposed to the CF factors in the secretory fluid. Thus, careful future studies are obviously indicated to clarify whether polycations (including CF factors) can inhibit or modify membrane transport by means of membrane charge modification alone under some special conditions. (2) The second relevant problem concerns the contribution of luminal hydrostatic pressure as a driving force for the overall transtubular net fluid transport in the proximal tubule. In normally hydrated rats, luminal hydrostatic pressure is approximately 20 cm H_2O under free-flow condition and 40 cm H_2O under stop-flow condition [34] whereas that of subcapsular interstitial space is only 3.8 cm H_2O [48]. Opinions are divided among nephrologists as to whether the transtubular hydrostatic pressure difference (ΔP) contributes significantly to the net

fluid absorption (Jv) across the proximal tubular wall. For example, Grantham, Qualiza and Welling [13] in isolated single rabbit proximal tubules and we [34] in rat proximal tubule in vivo could not enhance Jv significantly by increasing luminal hydrostatic pressure. However, the study by Persson, Agerup and Schnermann in the rat kidney proximal tubule in vivo [30] and that by Grandchamp and Boulpaep in the proximal tubule of *Necturus* kidney [12] indicate that the tubular walls have relatively high values of apparent hydraulic conductivity due to hydrostatic pressure (L_{p_h}). L_{p_h} value as calculated from the data of Persson *et al.* [30] of 0.11×10^{-7} cm^3/cm sec · cm H_2O and the approximate transtubular ΔP of 20 cm H_2O give water flux (Jv) due to ΔP of 2.2×10^{-7} cm^3/cm sec indicating that approximately half of the transtubular net Jv is ascribable to ΔP. Since the accumulated evidence suggests that the paracellular pathway offers a main route for the passive permeation of electrolytes and water in the so-called "leaky epithelia" [6], it may be reasonable to assume that the hydrostatic pressure-induced volume flux also takes this paracellular route. The present study has shown that polylysine inhibited Jv by 92% without interfering with the function of paracellular route. In addition to polylysine, three other inhibitors occur in the rat proximal tubule, which inhibit Jv as much as 95%; namely, serum complement [36, 37, and *unpublished*], phospholipase C [39, and *unpublished*] and cyanide [15, 34]. Our most recent data suggest that serum complement inhibits Jv in the same manner as polylysine, i.e. lysis of the tubular cells [36, 37, and *unpublished*]. It should be noted that the measurement of Jv was made under the stop-flow condition (split drop method) in the present study and the luminal hydrostatic pressure is 40 cm H_2O. Thus, if the L_{p_h} value is as high as that calculated from the data of Persson *et al.* [30], the inhibition of Jv as high as 95% by polylysine and other inhibitors can hardly be explained.

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References

1. Bangham, A. D., Standish, M. M., Watkins, J. C. 1965. Diffusion of univalent ions across the lamellae of swollen phospholipids. *J. Mol. Biol.* **13**:238
2. Boulpaep, E. L. 1970. Electrophysiological properties of proximal tubules: Importance of cellular and intercellular transport pathway. *In:* Electrophysiology of Epithelial Cells. G. Giebisch, editor. p. 91. Schattauer Verlag, Stuttgart-New York
3. Boulpaep, E. L. 1972. Permeability changes of the proximal tubule of *Necturus* during saline loading. *Amer. J. Physiol.* **222**:517

4. DiSant'Agnese, P. A., Talamo, R. C. 1967. Pathogenesis and physiopathology of cystic fibrosis of the pancreas. *New Engl. J. Med.* **277**:1287, 1343, 1399
5. Eylar, E. H., Madoff, M. A., Brody, O. V., Oncley, J. L. 1962. The contribution of sialic acid to the surface charge of the erythrocyte. *J. Biol. Chem.* **237**:1992
6. Frömter, E. 1972. The route of passive ion movement through the epithelium of *Necturus* gallbladder. *J. Membrane Biol.* **8**:259
7. Frömter, E. 1972. Progress in microelectrode techniques for kidney tubules. *Yale J. Biol. Med.* **45**:414
8. Frömter, E. 1973. Electrical studies on sugar kinetics of rat proximal tubule. *Pflüg. Arch.* **343**:R47
9. Frömter, E., Diamond, J. 1972. Route of passive ion permeation in epithelia. *Nature, New Biol.* **235**:9
10. Frömter, E., Rumrich, G., Ullrich, K. J. 1973. Phenomenologic description of Na^+, Cl^- and HCO_3^- absorption from proximal tubules of the rat kidney. *Pflüg. Arch.* **343**:189
11. Gertz, K. H. 1963. Transtubuläre Natriumchloridflüsse und Permeabilität für Nichtelektrolyte im proximalen und distalen Konvolut der Rattenniere. *Pflüg. Arch.* **276**: 336
12. Grandchamp, A., Boulpaep, E. L. 1972. Effect of intraluminal pressure on proximal tubular sodium reabsorption. A shrinking drop micropuncture study. *Yale J. Biol. Med.* **45**:275
13. Grantham, J. J., Qualiza, P. B., Welling, L. W. 1972. Influence of serum protein on net fluid reabsorption of isolated proximal tubules. *Kidney Int.* **2**:66
14. Györy, A. Z. 1971. Reexamination of the split oil droplet method as applied to kidney tubules. *Pflüg. Arch.* **324**:328
15. Györy, A. Z., Kinne, R. 1971. Energy source for transepithelial sodium transport in rat renal proximal tubules. *Pflüg. Arch.* **327**:234
16. Hegel, V., Frömter, E. 1966. Erfahrung mit der Öltropfenmethode zur Lokalisation der Mikroelektrodenspitze bei transtubulären Potentialmessungen an der Rattenniere. *Pflüg. Arch.* **291**:121
17. Heidrich, H. G., Kinne, R., Kinne-Saffran, E., Hanning, K. 1972. The polarity of the proximal tubule cell in rat kidney. Different surface charge for the brush border microvilli and plasma membranes from the basal infoldings. *J. Cell Biol.* **54**:232
18. Kashgarian, M., Stöckle, H., Gottschalk, C. W., Ullrich, K. J. 1963. Transtubular electrochemical potential of sodium and chloride in proximal and distal renal tubules of rats during antidiuresis and water diuresis (Diabetes insipidus). *Pflüg. Arch.* **277**:89
19. Katchalsky, A. 1964. Polyelectrolytes and their biological interactions. *Biophys. J.* **4**:9
20. Kimelberg, H. K., Papahadjopoulos, D. 1971. Interactions of basic proteins with phospholipid membranes. Binding and change in the sodium permeability of phosphatidyl serine vesicles. *J. Biol. Chem.* **246**:1142
21. Kinne, R., Schmitz, J. E., Kinne-Saffran, E. 1971. The localization of the Na^+-K^+-ATPase in the cells of rat kidney cortex. A study on isolated plasma membranes. *Pflüg. Arch.* **329**:191
22. Koefoed-Johnsen, V., Ussing, H. H. 1958. The nature of the frog skin potential. *Acta Physiol. Scand.* **42**:298
23. Mamelak, M., Wissig, S. L., Bogoroch, R., Edelman, I. S. 1969. Physiological and morphological effects of poly-L-lysine on the toad bladder. *J. Membrane Biol.* **1**:144
24. Mangos, J. A., McSherry, N. R. 1968. Studies on the mechanism of inhibition of sodium transport in cystic fibrosis of the pancreas. *Pediat. Res.* **2**:378

25. Mayhew, E., Harlos, J. P., Juliano, R. L. 1973. The effect of polycations on cell membrane stability and transport processes. *J. Membrane Biol.* **14**:213
26. Montal, M. 1973. Asymmetric lipid bilayers. Response to multivalent ions. *Biochim. Biophys. Acta* **298**:750
27. Nevo, A., DeVries, A., Katchalsky, A. 1955. Interaction of basic polyamino acids with red blood cell. 1. Combination of polylysine with single cells. *Biochim. Biophys. Acta* **17**:536
28. Parsons, D. F., Subjecl, J. R. 1972. The morphology of the polysaccharide coat of mammalian cells. *Biochim. Biophys. Acta* **265**:85
29. Passow, H. 1967. Steady state diffusion of non-electrolytes through epithelial brush borders. *J. Theoret. Biol.* **17**:383
30. Persson, E., Agerup, B., Schnermann, J. 1972. The effect of transtubular hydrostatic and oncotic pressure differences on reabsorption net flux. *In:* Recent Advances in Renal Physiology. *Int. Symp. on Renal Handling of Sodium*, Brestenberg. H. Wirz and F. Spinelli, editors. p. 51. Kargir, Basel
31. Quinton, P. M., Philpott, C. W. 1973. A role for anionic sites in epithelial architecture. *J. Cell Biol.* **56**:787
32. Rambourg, A. 1971. Morphological and histological aspects of glycoproteins at the surface of animal cells. *Int. Rev. Cytol.* **31**:57
33. Sato, K. 1974. The application of semi-floating electrode to the study of cell p.d.'s of the rat proximal tubules. *Pflüg. Arch.* **347**:R 32
34. Sato, K. 1974. Reevaluation of some micropuncture techniques: Some of the factors which affect the rate of fluid absorption by the proximal tubule. *In:* Symposium on Biochemical Aspects on Renal Function. S. Angielski, editor. *(In press)*
35. Sato, K., Ullrich, K. J. 1972. Effects of acid and basic polyelectrolytes on the rat proximal tubular isotonic reabsorption. *Pflüg. Arch.* **335**:R 30
36. Sato, K., Ullrich, K. J. 1974. Serum-induced inhibition of proximal tubular isotonic fluid absorption. I. Mechanism of inhibition. *Biochim. Biophys. Acta* **343**:609
37. Sato, K., Ullrich, K. J. 1974. Serum-induced inhibition of proximal tubular isotonic fluid absorption. II. Evidence that complement is involved. *Biochim. Biophys. Acta* **354**:182
38. Selielid, R., Selverstein, S. C., Cohen, Z. A. 1973. The effect of poly-L-lysine on the uptake of Resvirus double-stranded RNA in macrophages in vitro. *J. Cell Biol.* **57**:484
39. Solomon, S., Vanatta, J. C. 1966. Implication of phospholipids in rat tubular reabsorption. *Proc. Soc. Exp. Biol.*, N.Y. **122**:1040
40. Terepka, A. R., Chen, P. S., Toribara, T. Y. 1970. Ultrafiltration: A conceptual model and a study of sodium, potassium, chloride and water distribution in normal human sera. *Physiol. Chem. Phys.* **2**:59
41. Uhlenbruck, G., Wintzer, G., Voigtmann, R., Salfner, B., Cohen, E. 1971. Surface topography of glycoproteins in blood cell membranes. *In:* Glycoproteins of Blood Cells and Plasma. G. A. Jamielson and T. J. Greenwalt, editors. p. 74. Lippincott, Philadelphia-Toronto
42. Ullrich, K. J., Baldamus, C. A., Uhrich, E., Rumrich, G. 1969. Einfluß von Calciumionen und antidiuretischer Hormone auf den transtubulären Natrium-Transport in der Rattenniere. *Pflüg. Arch.* **310**:369
43. Ullrich, K. J., Frömter, E., Baumann, K. 1969. Micropuncture and microanalysis in kidney physiology. *In:* Laboratory Techniques in Membrane Biophysics. H. Passow and R. Stampfli, editors. p. 106. Springer-Verlag, Berlin-Heidelberg-New York
44. Ullrich, K. J., Radtke, H. W., Rumrich, G. 1971. The role of bicarbonate and other buffers on isotonic fluid absorption in the proximal convolution of the rat kidney. *Pflüg. Arch.* **330**:149

45. Ussing, H. H., Zerahn, K. 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiol. Scand.* **23**:110
46. Whittemburg, G., Sugino, N., Solomon, A. K. 1961. Ionic permeability and electrical potential differences in *Necturus* kidney cells. *J. Gen. Physiol.* **44**:689
47. Wright, E. M., Diamond, J. M. 1968. Effect of pH and polyvalent cations on the selective permeability of gallbladder epithelium to monovalent ions. *Biochim. Biophys. Acta* **163**:57
48. Wunderlich, P., Persson, E., Schnermann, J., Ulfendahl, H. 1971. Hydrostatic pressure in the subcapsular interstitial space of rat and dog kidneys. *Pflüg. Arch.* **328**:307