Effect of Mannitol on Glomerular Ultrafiltration in the Hydropenic Rat

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ABSTRACT The effect of mannitol upon glomerular ultrafiltration was examined in hydropenic Munich-Wistar rats. Superficial nephron filtration rate (sngfr) rose from 32.0±0.9 nl/min/g kidney wt to 42.0±1.6 ($P < 0.001$) in eight rats. Hydrostatic pressure gradients acting across the glomerular capillary (ΔP) were measured in glomerular capillaries and Bowman's space with a servo-nulling device, systemic (π_A) and efferent arteriolar oncotic pressures (π_E) were determined by microprotein analysis. These data were applied to a computer-based mathematical model of glomerular ultrafiltration to determine the profile of effective filtration pressure ($EFP = \Delta P - \pi$) and total glomerular permeability (L_pA) in both states. Filtration equilibrium obtained in hydropenia ($L_pA \geqslant 0.099 \pm 0.006$ nl/s/g kidney wt/mm Hg) and sngfr rose because $\overline{EFP}$ increased from a maximum value of 4.2±1.1 to 12.8±0.5 mm Hg after mannitol ($P < 0.01$). This increase was due to both increased nephron plasma flow and decreased π_A. Computer analysis of these data revealed that more than half (>58%) of this increase was due to decreased π_A, consequent to dilution of protein. Since EFP was disequilibrated after mannitol, L_pA could be calculated accurately (0.065 ± 0.003 nl/s/g kidney wt/mm Hg) and was significantly lower than the minimum estimate in hydropenia.

Therefore, sngfr does increase with mannitol and this increase is not wholly dependent upon an increase in nephron plasma flow since the major factor increasing EFP was decreased π_A.

INTRODUCTION

There is evidence from both the renal physiology laboratory (1–4) and the clinical experience (5, 6) that osmotically active agents such as mannitol can augment or maintain the rate of glomerular filtration in both normal and hypotensive animals. Therefore, these agents may promote or restore urinary excretion of water and electrolytes through activities both as an osmotic diuretic within the tubules (7, 8) and by increasing or maintaining the load of filtrate to each nephron.

Recent studies which directly evaluate the factors governing filtration (9, 10) suggest that glomerular filtration rate may be increased by four potential mechanisms: (*a*) an increase in plasma flow, (*b*) an increase in the hydrostatic pressure gradient (ΔP)[1] acting across the glomerular capillary membrane, (*c*) and increase in total glomerular permeability (L_pA) (effective only if filtration equilibrium is not attained), and (*d*) a decrease in the systemic oncotic pressure (π_A). Previous studies examining the mechanism of action of osmotically active agents favor the first two explanations, increased plasma flow and increased ΔP, both of which could be mediated by a reduction in afferent arteriolar resistance (AR) (3, 11–14). However, mannitol appears more effective in restoring glomerular filtration than other agents which possess the capacity to vasodilate the renal vasculature (13), and this

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[1] *Abbreviations used in this paper:* AR, afferent arteriolar resistance (mm Hg/nl/min/g kidney wt); C, protein concentration; C_A, systemic protein concentration; C_E, efferent pertibular capillary protein concentration; ΔP, hydrostatic pressure gradient across glomerular capillary ($\Delta P = P_G - P_t$) (mm Hg); EFP, effective filtration pressure ($EFP = \Delta P - \pi$) (mm Hg); $\overline{EFP}$, mean EFP; EFP_A, afferent EFP; EFP_E, efferent EFP; L_pA, total glomerular permeability (60 s/min · L_pA = sngfr/EFP) (nl/s/g kidney wt/mm Hg); P_G, glomerular capillary hydrostatic pressure (mm Hg); P_t, Bowman's space or proximal tubular hydrostatic pressure (mm Hg); π, oncotic pressure (mm Hg); π_E, efferent arteriolar pressure; π_A, systemic oncotic pressure; Q_o, rpf, nephron plasma flow (nl/min/g kidney wt); snbf, single nephron blood flow; snff, superficial nephron filtration fraction; sngfr, nephron filtration rate (nl/min/g kidney wt); x^*, normalized glomerular capillary length (dimensionless parameter).

restoration can occur in clinical situations in which increases in renal blood flow are severely limited.

Utilizing recently developed microtechniques for direct analysis of both the hydrostatic and oncotic forces which determine the rate of glomerular ultrafiltration (15, 16), we have examined the specific mechanisms whereby mannitol exerts its influence upon the kidney (17). When data was obtained and analyzed by a computer-based mathematical model of glomerular ultrafiltration (16), a reduction in π_A attendant to the infusion of mannitol, was the major and most consistent factor causing a rise in filtration rate in superficial nephrons of the rat.

METHODS

Micropuncture studies were performed upon male rats of the Munich-Wistar strain which were grown in a colony at the San Diego Veterans Administration Hospital in La Jolla, Calif. Original stock for this strain were obtained from Dr. Klaus Thurau of the Physiologic Institute of Munich. The weight of the rats ranged between 150 and 225 g at the time of micropuncture and were maintained upon standard Purina Rat Chow diets (Ralston Purina Co., St. Louis, Mo.) and ad lib water intake until the time of micropuncture. The unique value of this strain of rats is that they possess several glomeruli directly on the surface of the kidney, immediately under the capsule, which are accessible to micropuncture techniques and measurement of pressure within the glomerular capillaries.

The animals were anesthetized with Inactin (100 mg/kg i.p., Promonta, Hamburg, West Germany). A tracheostomy tube (PE 240) was inserted. Polyethylene catheters were then placed in the left jugular vein and left femoral artery for both the infusion of solutions and the monitoring of blood pressure. Blood pressure was monitored with a Statham P 23dB pressure transducer and recorded on a Statham four-channel recorder (Statham Instruments, Inc., Oxnard, Calif.). A bladder catheter was placed (PE 50) to collect urine from the right kidney. The animal was then turned onto the right side and a left subcostal flank incision was made. The animal's temperature was constantly controlled on a heated micropuncture board by a servo-controlled thermoregulator at approximately 37.5°–38°C utilizing a rectal thermister probe. After the incision the perirenal fat and connective tissue were carefully dissected from the left kidney and the adrenal gland dissected free. The kidney was then placed in a Lucite cup with care such that the pedicle was not stretched. A PE-50 catheter was inserted into the left ureter near the pelvis of the kidney to collect urine from the micropuncture kidney. The Lucite cup was then lined and the kidney surrounded loosely with cotton. Clear agar at 37° to 39°C was then placed around the kidney leaving the dorsal surface exposed. The kidney was then covered with heated 37°C isotonic NaCl-$NaHCO_3$ solution. At the completion of surgery, [^{14}C]inulin (New England Nuclear, Boston, Mass.) was infused at a rate of 40–50 μCi/h for the measurement of glomerular filtration rate. There was no attempt to replace surgical losses, but the infusion of isotonic NaCl-$NaHCO_3$ was maintained at approximately 0.5% body wt/h.

In the control situation after equilibration of the inulin marker, glomerular capillary pressures, pressures in Bowman's space and proximal tubules were measured with the servo-nulling pressure sensor device (Instrumentation for Physiology and Medicine, San Diego, Calif.). The specific operation of the servo-nulling device has been described in detail in a previous publication (9). The basic principle is that of an electrical Wheatstone bridge, one arm of which is a micropipette with a 1-μm (OD) tip. It is filled with 1 M NaCl. Both during the control hydropenic periods and after the institution of the mannitol infusion, pressures were measured in all glomerular capillaries accessible on the surface of the kidney. Bowman's space pressures (P_t) were measured in the same nephron as the glomerular capillary pressure (P_G), and these values utilized to calculate the ΔP in each state. The validity of this method has been discussed in detail in a previous publication (9), but it should be restated that if evidence of disruption of the glomerular capillary was noted as evidenced by bleeding either into Bowman's space or onto the surface, the glomerulus was rejected and the pressure measurement not utilized.

After measurement of P_G and P_t at least three efferent arterioles were localized and punctured with 14–16-μm (OD) coated pipettes and samples of blood were obtained. At least six sngfr were obtained in six separate nephrons utilizing techniques previously described (9, 16). Total glomerular filtration rate was estimated from the count rate of [^{14}C]-inulin from both left and right kidneys in at least two periods in both the control and experimental states. After the measurement of sngfr and conclusion of clearance periods, a sample of left renal venous blood was obtained with a heparinized micropipette (35-μm OD tip). This blood sample was utilized to determine the extraction of inulin across the kidney and thereby kidney filtration fraction.

During hydropenia, it has been demonstrated by several authors that superficial nephron filtration fraction (snff) is equal to total kidney filtration fraction (18–20). Also in a recent study from this laboratory, snff was nearly identical to the total kidney filtration fraction in hydropenic rats (16). We have therefore elected to calculate efferent protein concentration (C_E) from the total kidney filtration fraction during hydropenia. To determine whether filtration equilibrium occurs, it is imperative to define the filtration fraction and the C_E by the method with the smallest possible error.

After completion of the control hydropenic periods, a solution of 10 g/100 ml mannitol in 0.45% NaCl was infused at a rate sufficient to deliver a volume equal to 1.5% body wt over a period of 20 min. A stable maximum urine flow was obtained usually within 10 min such that the net addition of fluid to the animal was usually less than 1 ml total. At the end of the 20-min period, the infusion rate was readjusted (Sage Instruments Div., Orion Research, Inc., Cambridge, Mass.) to equal the rate of urine output throughout the remainder of the experimental period. All measurements that were obtained in the control periods were then repeated during the continuous infusion of mannitol. Total glomerular filtration rates and total renal extraction of inulin were also evaluated during the infusion of mannitol.

Analytical methods. Urine was collected in preweighed containers under oil. Arterial plasma samples were collected in heparinized capillary tubes. Arterial blood samples for protein determinations were collected in presiliconized glass capillary tubes. ^{14}C counts in plasma, urine, venous, and tubular fluid samples were monitored on a Packard liquid scintillation counter (model 2425, Packard Instrument Co., Inc., Downers Grove, Ill.) and total glomerular filtration rate, renal plasma flow, and total filtration fraction calculated as previously described (16, 21). Nephron filtration rate (sngfr = UV/P) was calculated as the total count rate collected per minute (UV) divided by the plasma count rate (P) (corrected for plasma water).

The microprotein determinations were performed by modification of the Lowry protein method (22) similar to that

described by Brenner, Falchuk, Keimowitz, and Berliner (23) and as previously described in modified form from this laboratory (16). Microcapillary pipettes were specially coated to prevent clotting of blood (Dow Corning 1107 [Dow Corning Corp., Midland, Mich.], 2.5% in trichloroethylene and then baked at 200°C for 3 h). The peritubular capillary blood sample was sealed at the tip with Eastman 910 (Eastman Chemical Products, Inc., New York) and red cells separated from the plasma by centrifugation. At least three 7-nl plasma samples were obtained from each of three collections and each sample run in triplicate. Three 7-nl samples were taken from the aortic blood collection obtained concurrently with the peritubular capillary samples and also each sample read in triplicate. Concentrations from these samples were estimated by comparison with a standard curve of known protein concentrations handled in identical fashion with 7-nl plasma protein samples. The standard curve was determined by linear regression analysis to best fit for a curve utilizing all standard readings. Correlation coefficient for the standard curves was usually greater than $r = 0.99$ and always greater than 0.98. As a further check on protein values, another modification of Lowry protein method was performed on all aortic samples which utilized 2-μl plasma samples. Samples were rejected if obvious hemolysis occurred. Concentrations of mannitol up to 12.5 g/100 ml had no effect upon the protein determination by this method.

Urine and plasma sodium concentrations were determined on an Instrumentation Laboratory flame photometer (Instrumentation Laboratory, Inc., Lexington, Mass.). Urine osmolalities were measured on an Advanced Instrument osmometer (Advanced Instruments, Inc., Needham Heights, Mass.)

Calculations. snff was calculated as follows:

$$\text{snff} = 1 - C_A/C_E,$$

where C_A = systemic protein concentration and C_E equals efferent peritubular capillary protein concentration. Single nephron plasma flow (rpf) is calculated as follows:

$$\text{rpf} = \text{sngfr}/\text{snff}.$$

Single nephron blood flow (snbf) is calculated as follows:

$$\text{snbf} = \text{rpf}/(1 - \text{hct}),$$

where hct equals hematocrit as a fraction of 1. AR is calculated as follows:

$$\text{AR} = \text{MAP} - P_G/\text{snbf},$$

where MAP = mean arterial blood pressure in millimeters of Hg and P_G equals glomerular capillary hydrostatic pressure.

The relationship between protein concentration (C) and oncotic pressure (π) was described by Landis and Pappenheimer (24), where:

$$\pi = 2.1C + 0.16C^2 + 0.009C^3,$$

(π) is expressed in millimeters (Hg). We have simplified this expression for use in the mathematical calculations by a best fit analysis to the Landis-Pappenheimer equation by least squares using well-established computer methods. (Programs on file at the University of California at San Diego Computer Facility.) where:

$$\pi = 1.736C + 0.281C^2 \text{ (reference 16).}$$

The above coefficients for C and C^2 are utilized in all calculations in this study during hydropenia and mannitol. We have clearly demonstrated that total protein is approximately 50% albumin during hydropenia and it is assumed that the infusion of mannitol does not alter this distribution of total protein between globulin and albumin (16).

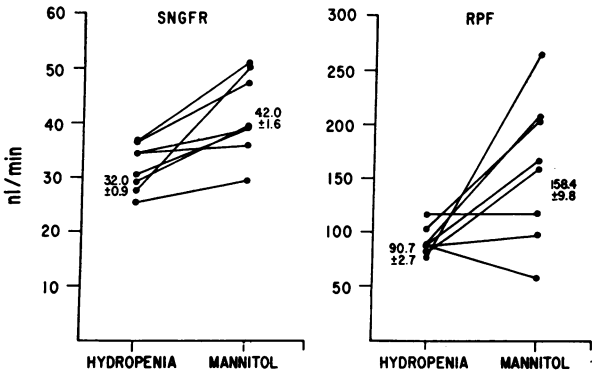

FIGURE 1 On the left is demonstrated as the mean sngfr in each of the 11 studies in hydropenia and during mannitol infusion. The values are expressed as the mean of each animal mean value ±SE. On the right is depicted the rpf and the response to mannitol in each animal. The variation in response to mannitol is greater with rpf than sngfr.

Determination of the profile of EFP profile along the length of the glomerular capillary (x^*), where $\text{EFP} = \Delta P - \pi$, requires a method of generating this curve accurately and integrating the curve to define a $\overline{\text{EFP}}$. A value for total L_pA can also be calculated by this technique ($\text{sngfr} = L_pA \cdot \overline{\text{EFP}}$). Deen, Robertson, and Brenner have described such a set of equations (10). We have also developed a method utilizing iterative techniques to determine the EFP profile and calculate L_pA. We have calculated $\overline{\text{EFP}}$ and L_pA by both methods in this study and utilized the iterative method to separate and quantitate the individual factors which act to increase the sngfr. Data required for such calculations are the C_A and C_E, rpf, and the ΔP. The iterative method is described in detail in the accompanying Appendix.

Statistical analysis. Calculation of the EFP profile and L_pA were conducted for each animal. Values for ΔP, rpf, C_A/C_E, and sngfr were analyzed by analysis of variance (25). Animal mean values for these computer input data were carried through the mathematical calculations were their estimates of variance to define the exact variance of both EFP and L_pA in each animal and overall. Statistical analyses of this type are also required to determine with confidence if filtration equilibrium occurs of if EFP_E was significantly positive.

RESULTS

With the infusion of mannitol, urine volume increased from 1.5±0.1 μl/min in hydropenia to 70.2±6.4. Serum sodium concentration was 133±2 meq/liter, potassium 5.0±0.3 meq/liter, and osmolality 273±5 mosmol after mannitol.

Effect of mannitol on the sngfr. Mannitol had a consistent and profound effect upon the sngfr in hydropenic animals, increasing the filtration rate from 32.0 ±0.9 ($n = 47$) to 42.0±1.6 nl/min/g kidney wt ($n = 46$) ($P < .001$). The results of filtration rates are displayed in Fig. 1 and Table I as a mean value for each

TABLE I

sngfr and rpf before and after Mannitol Infusion

Rat no.	Hydropenia		Mannitol	
	sngfr	rpf	sngfr	rpf
	(nl/min/g kidney wt	*(nl/min/g kidney wt*	*(nl/min/g kidney wt*	*(nl/min/g kidney wt*
9	27.8±1.7*	76.8±4.6	50.5±2.8	265.0±15.1
10	29.5±2.6	78.1±6.8	39.7±1.7	158.7±6.6
13	25.4±1.3	87.5±4.4	29.7±1.7	57.1±3.2
17	34.3±3.0	103.3±8.9	38.9±2.5	204.3±13.3
21	36.8±1.9	116.7±6.0	51.3±3.9	116.6±8.9
22	30.7±2.1	85.9±5.9	39.1±3.1	166.7±13.4
26	36.4±1.5	89.7±3.7	47.7±1.8	207.5±7.6
27	34.6±2.2	86.6±5.6	36.0±1.9	97.3±5.1
Overall mean	32.0±0.9	90.7±2.7	42.0‡±1.6	158.4‡±9.8
	(*n* = 47)	(*n* = 47)	(*n* = 46)	(*n* = 46)

*SEM.
‡ $P < 0.01$.

animal. Sngfr is expressed per gram kidney weight to normalize the variation between animals of varying size.

Hydrostatic factors affecting glomerular filtration. P_G during hydropenia was 43±1.0 ($n = 23$) and rose to 47.2±1.6 mm Hg ($n = 17$) after the infusion of mannitol ($P < 0.01$).[2] The ΔP between the glomerular capillary and Bowman's space was 30.5±1.1 ($n = 23$) during hydropenia and 28.7±1.4 mm Hg ($n = 17$) after mannitol infusion ($P > 0.05$). The individual values for P_G and ΔP in each animal are depicted in Table II. Therefore the ΔP across the glomerular capillary does not contribute to the increase in sngfr. Tubular pressure rose from 12.8±0.9 to 19.1±0.9 mm Hg after the infusion of mannitol ($P < 0.001$).

Hemodynamic effects of mannitol infusion. Single rpf increased from 90.7±2.7 ($n = 47$) to 158.4±9.8 nl/min/g kidney wt ($n = 46$) ($P < 0.01$). Although a statistically significant rise, the variation in the increase of plasma flow was rather marked from animal to animal (Fig. 1), in that rpf did not increase in an appreciable number of animals. Snbf increased from 204±7 to 312±19 nl/min/g kidney wt ($P < 0.01$). Hematocrit during hydropenia was 55±1 and fell to 49±1 volume % ($P < 0.01$) after the infusion of mannitol.

The arterial pressure in hydropenia was 134±5 mm Hg and after mannitol infusion was 125±6 ($P > 0.3$). Nephron AR was 0.43±0.03 mm Hg/ml/min/g kidney wt in hydropenia and was 0.28±0.04 ($n = 8$) after the infusion of mannitol ($P < 0.02$).

Influence of mannitol infusion upon C and π. C_A was 5.6±0.1 g/100 ml during hydropenia and fell to 4.1±0.2 g/100 ml after the infusion of mannitol. π_A therefore fell from 18.3±0.6 to 11.9±0.6 mm Hg ($P < 0.001$). The individual changes in π_A for each animals are demonstrated in Fig. 2. π fell consistently after mannitol in each study. C_E was 8.6±0.2 in hydropenia and was 6.0±0.3 g/100 ml after mannitol infusion. π_E generated was 35.7±1.4 in hydropenia and 20.7±1.7 mm Hg after mannitol ($P < 0.001$).

Mean snff did not change as a result of the infusion of mannitol 0.35±0.01–0.30±0.04, ($P > 0.4$). However, in those animals in which rpf increased substantially, snff tended to fall, and in those animals in which rpf alterations were minimal or did not change, there was an increase in snff. In summary, although sngfr rose in most animals, the increase in plasma flow was quite variable from animal to animal and was not a consistent determining factor in the increase in filtration rate.

Evaluation of the integrated $\overline{EFP}$ and L_pA before and after mannitol infusion. In hydropenia the EFP at the afferent end of the glomerular capillary (EFP_A) was 12.1±1.5 mm Hg. The π_E for each animal were compared with the mean ΔP and were not statistically different ($P > 0.05$) suggesting that filtration pressure equilibrium was attained. However, the mean efferent effective filtration pressure ($\overline{EFP}_E$) among animals was −5.3±2.0 mm Hg, a value less than SD from zero and therefore not different from zero. This negative value is of course biologically unrealistic and represents the range of errors in π_E and ΔP when analyzed as the mean of each animal. Since a smaller number of P_G observations was obtained in certain studies (Table I), we have also analyzed EFP_A and EFP_E as the mean of all observations ($n = 23$) and they are 12.4±1.2 (EFP_A) and −3.8±1.3 mm Hg (EFP_E). At filtration pressure equilibrium an infinite number of values for $\overline{EFP}$ may exist and only a maximum estimate can be determined. Also L_pA cannot be calculated uniquely under these conditions, but rather a minimum possible value. Maximum $\overline{EFP}$ in hydropenia was 4.2±1.1 mm Hg and the minimum possible L_pA 0.099±0.006 nl/s/g kidney wt/mm Hg. When these values were determined from all values of ΔP, maximum possible $\overline{EFP}$ was 5.3±1.0 mm Hg and L_pA 0.106+0.011 nl/s/g kidney wt/mm Hg (Table III).

[2] We have also conducted parallel estimates of P_G by stop flow technique ($P_{SFP} = SFP + \pi_A$) (9) in these same animals in both hydropenia and mannitol. P_{SFP} was significantly higher than the corresponding P_G in hydropenia at 51.2±1.2 mm Hg ($n = 30$) ($P < 0.001$), and P_{SFP} was 49.4±1.2 mm Hg ($n = 33$) after mannitol infusion ($P > 0.1$). Because of our greater confidence in the validity of direct P_G measurements, only these values were used in this study. In a previous study, were also noted that P_{SFP} in separate nephrons were higher than P_G in hydropenia but noted identical values when measured in the same nephron (9). The specific reason for this disparity is not known.

TABLE II

P_G and π during Hydropenia and after Mannitol Infusion

Rat no.	Hydropenia P_G	ΔP	π_A	π_E	EFP_E	Rat no.	Mannitol P_G	ΔP	π_A	π_E	EFP_E
	mm Hg	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*		*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*
9	51.2	40.0				9	47.1	29.6			
	41.7	30.5					44.0	26.5			
	42.1	30.9					37.2	19.7			
	45.3	34.1					43.2	25.7			
	34.6	23.4					—	—			
	36.9	25.7				Mean	42.9	25.4	11.3	15.2	+10.2
	—	—									
Mean	42.0	30.8	16.2	30.6	+0.2						
10	41.6	28.8				10	55.2	32.8			
	35.2	24.7					48.8	26.4			
	42.7	32.2					—	—			
	—	—				Mean	52.0	29.6	13.1	20.0	+9.6
Mean	39.8	28.6	18.4	35.7	−7.1						
13	41.6	28.8				13	42.8	26.0			
	41.6	29.6					42.4	24.4			
	50.4	39.3					40.0	20.8			
	—	—					—	—			
Mean	44.5	32.6	20.3	34.7	−2.1	Mean	41.7	23.7	9.0	25.1	−1.4
17	43.1	25.4				17	47.3	29.1			
	43.6	26.4				Mean	47.3	29.1	11.7	15.6	+13.5
	—	—									
Mean	43.4	25.9	20.2	38.0	−12.1						
21	40.0	24.5				21	62.6	41.1			
	43.2	27.7					49.7	35.1			
	45.6	30.1					—	—			
	—	—				Mean	56.2	38.1	10.4	23.7	+14.4
Mean	42.9	27.4	18.6	33.5	−6.1						
22	44.3	33.2				22	49.1	31.4			
	45.4	34.3					55.8	38.1			
	—	—					—	—			
Mean	44.8	33.8	16.8	33.1	+0.7	Mean	52.4	34.8	13.7	20.3	+11.1
26	44.8	29.6				26	48.0	28.8			
	54.4	43.2					50.4	31.2			
	40.0	29.6					—	—			
	—	—				Mean	49.2	30.0	11.5	16.6	+13.4
Mean	46.4	34.1	16.1	35.6	−1.5						
27	40.0	29.6				27	38.4	21.6			
Mean	40.0	29.6	19.8	44.0	−14.4	Mean	38.4	21.6	14.3	28.8	−7.2
Overall mean	43.0±1.0 (n = 23)	30.5±1.1 (n = 23)	18.3±0.6 (n = 8)	35.7±1.4 (n = 8)	−5.3±2.0 (n = 8)	Overall mean	47.2±1.6 (n = 17) $P < 0.01$	28.7±1.4 (n = 17) $P > 0.05$	11.9±0.6 (n = 8) $P < 0.001$	20.7±1.7 (n = 8) $P < 0.001$	+8.0±2.8 (n = 8) $P < 0.02$

After mannitol infusion, EFP_A was 17.2±2.1 mm Hg which was not significantly different from hydropenia ($P > 0.05$). When π_E and ΔP in each animal were compared after mannitol, ΔP was significantly higher by paired analysis ($P < 0.025$) and the mean EFP_E was +8.0±2.8 mm Hg, a value significantly different from the value in hydropenia and from zero ($P < 0.025$). Utilizing the mean of all values of ΔP ($n=17$), EFP_A was 17.2±1.4 mm Hg ($P < 0.02$ compared to hydropenia) and fell to and EFP_E of 8.6±1.8 mm Hg ($P < 0.001$).

Since the EFP_E was significantly greater than zero after mannitol, a specific value for both $\overline{EFP}$ and L_pA can be determined. The $\overline{EFP}$ by the iterative method was 12.8±0.5 and 12.7±0.5 mm Hg by the method of Deen et al. (10), significantly greater than the maximum possible value during the hydropenic condition ($P < 0.001$). The mean L_pA after mannitol was 0.065 ±0.003 nl/s/g kidney wt/mm Hg (0.066 by the method of Deen et al. [10]), which was significantly lower than the minimum possible value during hydropenia. Also, when this value for L_pA in mannitol was applied to the

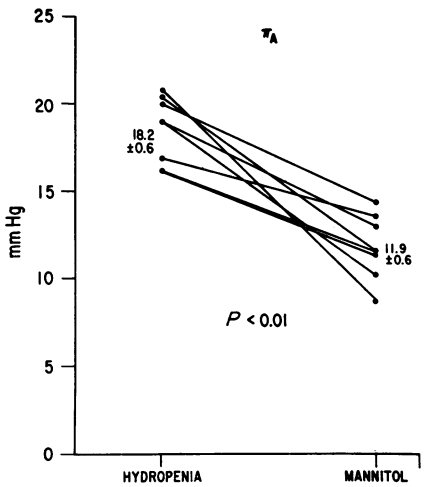

FIGURE 2 The π_A for each animal, as estimated from serum protein concentrations, are depicted for hydropenia and after mannitol infusion. Overall mean values are also given and the P value refers to the paired comparisons.

hydropenic values for π_A, rpf, and ΔP, the EFP_E that resulted was greater than zero, and the measured value for sngfr in hydropenia (32.0 nl/min/g kidney wt) could not be attained with this value for L_pA (23.2±4.1 nl/min/g kidney wt resulted).

This reduction in L_pA is unlikely the result of a decrease in surface area (A) but the exact mechanism for reduced L_pA is not known. Recently Deen et al. have suggested that a concentration polarization phenomenon might have a minor but real influence upon the effective permeability as calculated (26). The lower concentration of systemic protein after mannitol would tend to magnify the concentration polarization effect by permitting a higher rate of filtration at the capillary membrane. This effect would produce a calculated L_pA below the true permeability of the membrane. Also, reduced C might alter the configuration of pores or slits in the membrane if the π exerts its effect across the cell membrane of capillary endothelial cells and thereby alters cell volume.

TABLE III
$\overline{EFP}$ and Total L_pA during Hydropenia and after Mannitol Infusion

	Hydropenia	Mannitol
$\overline{EFP}$, *mm Hg*	4.2±1.1*	12.8±0.5§
L_pA, *nl/s/g kidney wt/mm Hg*	0.099±0.006‡	0.065±0.003§

* A maximum estimate at filtration pressure equilibrium.
‡ A minimum estimate at filtration pressure equilibrium.
§ $P < 0.001$.

Independent evaluation of the factors which increase EFP. There are two factors which have contributed to the increase in $\overline{EFP}$ and sngfr; increased rpf and decreased π_A. The ΔP and L_pA did not change in a direction which would act to increase the sngfr. Utilizing the iterative method for evaluation of the EFP profile, we have separated these two positive contributions to the increased $\overline{EFP}$ and quantitated their respective influences.

We have held π_A constant as in hydropenia but permitted all other factors to change as a result of mannitol infusion. The $\overline{EFP}$ which results was 7.0 mm Hg. When the opposite manuever was performed and rpf held constant while all other factors have changed to the postmannitol values, the $\overline{EFP}$ was higher at 9.7 mm Hg. The measured $\overline{EFP}$ after mannitol when all factors were changed from hydropenia was 12.8 mm Hg. Therefore, if we focus upon those two factors which directly increase filtration rate, more than one-half (58–66%) of this increase was directly a result of a decrease in π_A. This set of predictions is not dependent upon unique values for $\overline{EFP}$ and L_pA in hydropenia. This prediction can be conducted both at the maximum possible $\overline{EFP}$ in hydropenia and as $\overline{EFP}$ approaches zero as a limit, thus effectively defining the range of percentage influence of the respective positive factors (Table IV).

Decreased π_A mediates its effect by increasing the EFP and by allowing filtration fraction to rise to higher values even at a constant rate of plasma flow.

The effects of mannitol infusion on total glomerular filtration rate, plasma flow, and filtration fraction. Total kidney glomerular filtration rate was 1.00±0.05 ml/min/g kidney wt in hydropenia and was 1.02±0.07 ml/min/g kidney wt after mannitol infusion ($P > 0.8$). Total renal plasma flow was 2.90±0.15 in hydropenia and rose to 3.36±0.29 ml/min/g kidney wt ($P < 0.01$) after mannitol infusion. Renal blood flow was 6.48 ±0.34 in hydropenia and was unchanged at 6.57±0.55 ml/min/g kidney wt after mannitol infusion ($P > 0.3$). The total filtration fraction therefore was 0.35±0.01 in hydropenia and fell to 0.29±0.02 after mannitol infusion ($P < 0.05$). As noted previously, snff was unchanged after mannitol ($P > 0.4$).

TABLE IV
*Separation and Analysis of the Contribution of Decreased π_A and Increased rpf on the Increased $\overline{EFP}$ with Mannitol**

$\overline{EFP}$ after mannitol, *mm Hg*	12.8±0.5
$\overline{EFP}$ if π_A held constant, *mm Hg*	7.0
$\overline{EFP}$ if rpf held constant, *mm Hg*	9.7

* Maximum estimate of $\overline{EFP}$ in hydropenia is 4.2±1.1 mm Hg.

This finding of dissociation of changes in snff and total GFR has been observed by others in both expanded states and after reduction in renal blood flow (27–29). This finding suggest that filtration rate in superficial nephrons increases to a greater extent than do nephrons below the surface. This may be due to either (*a*) disproportionate reductions in ΔP due to either inadequate elevations of P_G or disproportionate increases in tubular pressure, or (*b*) that filtration equilibrium may not be attained in nephrons below the surface or equilibrium may be attained nearer to the end of the glomerular capillary. Either explanation might account for a lesser increase in sngfr.

DISCUSSION

The widespread use of mannitol in clinical medicine in an effort to increase, maintain, or restore glomerular filtration rate has inspired a large number of studies which have examined the mechanism of action of this useful agent (1–6, 11–14). Most investigators have favored an increase in either or both rpf and P_G as the major factors contributing to this beneficial effect. The postulated changes were attributed to a vasodilating effect of mannitol upon the AR vessels (11–13).

However, a careful examination of previous studies in both humans and laboratory animals reveals that glomerular filtration rate fell in some studies, often when bolus injections were made or urine volume replacement was potentially inadequate (1, 3). One explanation may be that large losses of urine volume resulted without adequate replacement and volume depletion could have produced this decrement in clearance.

Recent studies from this author and Brenner and coworkers have demonstrated that π_A increases to a value at the end of the glomerular capillary in hydropenia that approximates the ΔP, suggesting filtration equilibrium (9, 10, 15, 16). Under these conditions it is obvious that several EFP curves could exist and a unique value for L_pA cannot be calculated with confidence. Filtration equilibrium obtained during hydropenia in the present study, in that paired comparison of π_E and ΔP in each animal revealed that these values were not different.

Although the mean EFP_E was -5.3 mm Hg, when analyzed as the mean of each animal (-3.8 mm Hg when analyzed as the mean of all observations), these values fall within 1 SD of zero, and this negative value represents the errors involved in estimation of both π_E and ΔP. When three or more values for ΔP were obtained in a single animal, there was a significant variance in this determination among glomeruli, which represents both errors of measurement and true heterogeneity. At filtration equilibrium, this variation in ΔP should require a similar heterogeneity in π_E. Therefore, the accuracy with which filtration pressure equilibrium can be defined within an animal is enhanced with a greater number of evaluations of ΔP and limited with only single measurements.

Values for the L_pA calculated during hydropenia in this study remain minimum estimates of this value. However, after mannitol, in which a positive force occurred at the efferent arteriole, L_pA can be calculated with confidence. Therefore it seems likely that this value for L_pA is an accurate estimate and that mannitol increases sngfr while it decreases the calculated total glomerular permeability.

The increase in sngfr was entirely attributable to an increase in $\overline{EFP}$. Since the ΔP did not increase, hydrostatic forces were not a positive influence contributing to this increase in sngfr. Previous studies have demonstrated a high degree of filtration rate dependence upon changes in rpf, at least when filtration rate is increased by the infusion of isoncotic rat plasma (30). However, in those studies plus studies with aortic constriction by Robertson, Deen, Troy, and Brenner, C_A and π remain constant (31). The renal plasma flow response of animals to mannitol was quite variable in the present study and derived from considerable variability in alterations in the AR. This was not attributable to variations in extracellular volume, since net positive fluid balance did not exceed 1 ml before attaining a steady-state urine excretion in any of the eight studies. In several animals rpf did not increase, yet glomerular filtration rate rose in these animals.

When the increase in total $\overline{EFP}$ was analyzed by computer, it was noted that if renal plasma flow had not increased, then the sngfr would have increased more than 50% of the observed value after mannitol, due totally to the decrease in π. This consistent effect of decreased π observed in this study is not simply the result of an increased EFP_A. Because of the nonlinear relationship of C and π, for any given volume of filtrate produced along the glomerular capillary, the decay in the slope of the EFP due to the concentration of protein will be less at a lower C_A. Therefore the isolated effect of a decrease in π_A, at any EFP_A, will be an increase in the total $\overline{EFP}$, and therefore an increase in sngfr.

The second major effect of the decrease in π_A is to permit a higher snff and thereby a higher sngfr through an increase in $\overline{EFP}$, while plasma flow remains constant. At each value of π_A and ΔP, the maximum filtration fraction is defined by filtration equilibrium, or that value of π_E which equals the ΔP. Therefore we have two factors, decreased π_A and increased rpf, which both act to increase sngfr by increasing $\overline{EFP}$, but the decrease in π_A tends to increase filtration fraction, and at least at higher rates, increases in plasma flow tend to decrease filtration fraction. Both of these factors have combined to maintain the snff constant in this study,

but at a higher $\overline{EFP}$ and a positive value for EFP_E after mannitol.

The exact quantification of the respective influences of decreased π_A and increased rpf to the increased filtration rate apply, of course, to the specific conditions of this study. The relative contributions of these two factors may vary with markedly different quantities of mannitol used and possibly by the rate of administration. The effect of dilution of protein upon filtration rate is dependent only upon decreased π_A, a predictable occurrence. However, the efficiency of mannitol in producing afferent arteriolar dilatation and increased plasma flow is dependent upon the capacity of the renal vasculature to respond, which may vary markedly in differing clinical and experimental conditions.

The present observations suggest that the major effect of dilution of protein and reduced π with mannitol may serve to explain, in part, previous findings of restoration of filtration rate observed in certain states of low renal blood flow. In the studies of Flores, DiBona, Beck, and Leaf (12) and Morris, Alexander, Bruns, and Levinsky, the "no reflow" phenomenon was examined in detail. After a period of renal artery occlusion or during renal hypoperfusion, the infusion of hypertonic solutions containing mannitol produced significant restoration of filtration rate, as determined by direct methods (13) and as evidenced by a lesser rise in serum creatinine in the study of Flores et al. NaCl or Na_2SO_4 solutions of similar hypertonicity also had some beneficial effect upon filtration. The effect of mannitol was attributed to vasodilation at the afferent arteriole, possibly by reducing endothelial cell swelling. Although serum proteins were not measured in the studies (12, 13), an additive effect may have resulted from a large reduction in π_A with administration of hypertonic solutions which produce a redistribution of fluid from intracellular to extracellular compartments.

Therefore, a major beneficial effect of mannitol can be explained by mechanisms not totally dependent upon afferent arteriolar dilatation and the resultant increased plasma flow. An increase in the ΔP was not observed in this study. Mannitol produced a consistent increase in sngfr by augmenting plasma volume and diluting C at the expense of a decreasing intracellular fluid volume. This additional mechanism may explain the greater effect of mannitol on sngfr than observed with other agents which also vasodilate the renal vasculature.

APPENDIX

Measured data were analyzed by the method of Deen et al. (10) and by a set of equations which are described here are analyzed by computer at the University of California at San Diego Computer Facility. These equations are based upon the following relationship:

$$\text{sngfr} = (L_pA)\cdot\int_A^E EFP\ dx^*,$$

where A designates the afferent end of the capillary and E is the efferent end. Since a true glomerular capillary length is not known a dimensionless normalization of the length can be applied (x^*). A $\overline{EFP}$ can be determined by the following equation:

$$\overline{EFP} = \int_o^1 (\Delta P - \pi)dx^*,$$

and that:

$$\text{sngfr} = (L_pA)\cdot\int_o^1 (\Delta P - \pi)dx^*.$$

Utilizing the measurable parameters, the profile of the EFP and the corresponding total membrane hydraulic permeability can be determined through the use of a double iterative technique. Calculations require the nephron plasma flow (Q_o), C_A, C_E, and P_G and P_t. A block iteration technique is used to obtain an EFP profile for any particular L_pA. The initial step of the curve is determined by:

$$EFP_A = \Delta P - \pi_A.$$

Flux ($sngfr_1$) across any small distance Δx^*, where $\Delta x^* = x^*/n$ (n = 40–100), can be calculated as:

$$sngfr_1 = (L_pA\cdot\Delta x^*)EFP_A.$$

This determines the parameters at Δx_1^*, where $Q_{\Delta x_1^*}$, is equal to the initial nephron plasma flow (Q_o) minus $sngfr_1$. The new protein concentration ($C_{\Delta x_1^*}$) is defined by:

$$C_{\Delta x_1^*} = C_A(Q_o/Q_{\Delta x_1^*}),$$

which in turn can be used to determine $\pi_{\Delta x_1^*}$. EFP is then calculated by:

$$EFP_{\Delta x_1^*} = (\Delta P - \pi)_{\Delta x_1^*},$$

assuming that ΔP remains constant. The second flux increment is then:

$$sngfr_2 = (L_pA\cdot\Delta x^*)\cdot(\Delta P - P)_{\Delta x_1^*}.$$

The process of block iteration continues until the end of the capillary where $x^* = 1$. The result is a profile of EFP along capillary length x^* as long as the Δx^* is small. This profile depends on the L_pA used. To find a unique solution for L_pA an iteration of L_pA must be applied. Nesting the block iteration technique within the L_pA iterations then the following criterion must be satisfied with increasing values of L_pA:

$$\Sigma\ \text{sngfr} = Q_o(1 - C_A/C_E).$$

The final L_pA is then the value utilized to satisfy this condition. This particular method will provide a single solution for L_pA and $\overline{EFP}$ for any of the given parameters as long as the EFP profile does not reach equilibrium. When equilibrium attains, an infinite number of combinations of $\overline{EFP}$ and L_pA will meet the specified parameters. A minimum value for L_pA and maximum value for $\overline{EFP}$ is obtained in an equilibrated situation.

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