Effect of Extracellular Fluid Volume Expansion on Maximum Glucose Reabsorption Rate and Glomerular Tubular Balance in Single Rat Nephrons

ANDREW D. BAINES with the technical assistance of JOHN H. V. BISHOP

From the Department of Pathological Chemistry, University of Toronto, Toronto 181, Ontario, Canada

ABSTRACT Extracellular fluid volume expansion with isotonic saline (7.5% of body weight) decreased maximum glucose reabsorption rate by rat kidneys at plasma glucose concentrations greater than 30 mM. Glucose reabsorption rate was 30.2 ±1.6 (SE) μmoles/min·g kidney in nonexpanded rats; it was 18.4 ±1.5 μmoles/min·g in volume-expanded rats. Glucose reabsorption determined by micropuncture was 92% complete at the end of accessible superficial proximal convolutions. Volume expansion resulted in a slight but statistically insignificant reduction of maximal glucose reabsorption rate in superficial nephrons from 786 ±35 μμmoles/min·g kidney in nonexpanded rats to 720 ±30 μμmoles/min·g in volume-expanded rats. Superficial nephron filtration rate was increased by volume expansion from 28.8 ±1.2 nl/min·g to 36.6 ±1.5 nl/min·g kidney. In nonexpanded rats, the ratio of glucose reabsorption to glomerular filtration (tmg/sgfr) was similar in superficial and juxtamedullary nephrons. In volume-expanded rats superficial nephron tmg/sgfr was greater than juxtamedullary nephron tmg/sgfr. Juxtamedullary nephron function was measured by puncturing loops of Henle in the exposed papillae of small rats.

Volume expansion increased sgfr without much effect on tmg in superficial nephrons while it decreased tmg without much effect on sgfr in deep nephrons. Physical changes produced by volume expansion seem to exert their greatest effect on proximal tubular function in the inner cortex. The increase in heterogeneity of glomerular-tubular balance could account for increased splay of glucose titration curves previously reported to accompany volume expansion.

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INTRODUCTION

Expansion of extracellular fluid volume decreases the maximum rate of glucose reabsorption (TmG)[1] and increases splay of the glucose titration curve in rats (1). Similar changes in glucose reabsorption occur in uremia (2). Robson, Srivastava, and Bricker (1) postulated that these changes in glucose transport are produced by the same physical (3)[2] or hormonal (4) factors which reduce sodium transport in volume-expanded or uremic animals, this possibility being supported by the interrelationship of glucose and sodium transport in many cell systems (5).

Extracellular fluid volume expansion could reduce TmG by a uniform effect on all nephrons or by a nonuniform effect. A nonuniform effect could either increase heterogeneity of nephron function (6, 7) or reduce the number of functioning nephrons (8). These possibilities were examined by measuring the effect of extracellular fluid volume expansion on maximal glucose reabsorption rates in single superficial and juxtamedullary nephrons (tmg) of the rat kidney.

METHODS

Male Wistar rats weighing between 180 and 300 g, fed Rockland mouse rat diet (Rockland Farms, N. Y.) and tap water, were starved overnight but allowed access to

[1] *Abbreviations used in this paper:* Cin, inulin clearance; TF/P, tubule fluid/plasma ratio; tg, glucose reabsorption per nephron; tmg, maximum glucose reabsorption per nephron; TmG, maximum rate of glucose reabsorption for whole kidney; tmg/sgfr, ratio of glucose reabsorption to glomerular filtration in single nephrons.

[2] See (3) for additional references.

water before being anesthetized with Inactin (90 mg/kg)[3] given intraperitoneally. A heated animal board on which the rats lay supine maintained their body temperature at 37.7 ±0.7°C (mean ±SD) throughout the experiment. Polyethylene catheters (PE50) were inserted into the left carotid and jugular veins and the trachea was intubated. The abdomen was opened through a midline incision extended laterally in a T shape below the left ribs. The left ureter was cannulated with PE50 tubing.

The kidney was immobilized *in situ* with a semicircular holder. The region between holder and kidney was packed gently with cotton wool soaked in warm mineral oil. Warm saline-soaked gauze overlaid with Saran Wrap covered exposed portions of the abdominal contents while warmed mineral oil (38°C) bathed the kidney surface. An air curtain incubator (Sage Instruments, Inc., White Plains, N. Y.) maintained a similar temperature in the air above the kidney. A Grass polygraph (Grass Instrument Co., Quincy, Mass.) and Statham pressure transducer (Statham Instruments, Oxnard, Calif.) recorded blood pressure in the carotid artery.

Two experimental protocols were used. One group of 14 rats (nonexpanded) were infused intravenously with 10–40% D-glucose in water at 0.076 ml/min. At the beginning of the experiment they received a volume of isotonic salt solution[4] equivalent to 1% of their body weight. After this, a priming injection of 40 μCi tritiated inulin (New England Nuclear Corp., Boston, Mass.) and a sustaining infusion of tritiated inulin (2 μCi/min) in isotonic salt solution at 0.02 ml/min were administered. The glucose and inulin solutions were delivered by separate pumps connected by a Y tube to the infusion catheter.

The other group of 13 volume-expanded rats were infused with 2–5% glucose in isotonic salt solution at 0.49 ml/min until they had received a volume equivalent to 7.5% of their body weight. The infusion was then reduced to 0.25 ml/min and the glucose concentration doubled to 4–10%. Radioactive inulin priming and sustaining infusions were the same as for the first group. Four rats were infused first with glucose in water at the low rate and then with glucose and saline at the higher infusion rates.

Urine collections and micropunctures began after an equilibration period of 45–90 min for the nonexpanded rats and 30–60 min after beginning the infusion at 0.25 ml/min for the volume-expanded rats. At least three and as many as six sequential urine collections of 10–20 min duration were obtained at each glucose infusion rate. Arterial blood samples (50 μl) were taken from the carotid catheter near the midpoint of each urine collection.

Urine and blood were stored in ice until analyzed. Urine volume was determined by weight corrected for glucose content. In some experiments the concentration of glucose in the infusate was raised and a second set of collections taken after 30 min equilibration. At the end of the experiments the kidney was removed, decapsulated, blotted, and weighed.

Sharpened micropipettes with a tip diameter of 9 μ were used to obtain samples from superficial proximal and distal tubules. A column of Sudan black–stained castor oil 5–10 times the tubule diameter in length was injected into the tubule; 5–10 sec later, when the oil column had moved downstream beyond the tip of the pipette, collections were initiated by a briefly applied suction. During most of the collection, the pipette was open to the air although occasionally slight negative pressure was used to maintain the oil drop stationary. Collections lasted 3–9 min except for a few 1 to 2 min collections from proximal tubules of volume-expanded rats. Samples were stored over ice until analyzed.

The effect of tubular fluid collection on intraluminal pressure in proximal convolutions of volume-expanded rats was determined using the Landis technique (9). A pipette filled with 1% Fast green solution was inserted into a mid-proximal convolution. The pressure required to maintain the dye column in balance at the tip was recorded on the polygraph through a Statham transducer (P23BC, Statham Instruments) attached by a Y tube to the screw-operated syringe used to regulate pressure in the micropipette. A second pipette was then inserted into the late proximal or distal tubule, oil was injected, and tubular fluid collection carried out as described above. Intraluminal pressure was measured throughout the collection. Capillarity of the micropipette was measured in saline at 37°C.

The site of puncture was identified in several ways. Some tubules were filled with latex and the puncture site marked with nigrosin. Those tubules were subsequently microdissected and measured on a camera lucida drawing (10). In other experiments, late proximal tubules were identified by injecting 0.02 ml of 5% Fast green FCF (Fisher Scientific Co., Pittsburgh, Pa.) intravenously and observing the kidney surface for proximal convolutions which stained last. In still other experiments, the abrupt disappearance of a small amount of Fast green injected into a superficial tubule was taken as evidence of its terminal position.

Collecting ducts and thin loops of Henle were punctured in the papillae of rats weighing 80–115 g. In these experiments, the left kidney was exposed through a flank incision and the kidney immobilized in a plastic cup. The nonexpanded rats were treated as were the large rats described above; however, volume expansion to 7.5% of body weight was carried out by infusing at half the rate for large rats (0.27 ml/min) followed by a sustaining infusion given at 0.145 ml/min. A sample was collected from an adjacent collecting duct before or after each collection from a thin limb of Henle's loop. A few collections from superficial convolutions were also obtained.

The total micropuncture sample volume was measured as a function of its length between two columns of a silicone oil : chloroform mixture (6 : 1) in a constant bore capillary tube. The capillary tube was advanced from one oil-fluid meniscus to the other using a fixed line in the microscope eyepiece as a reference. The length of the tubular fluid column was read from the screw micrometer of the micromanipulator (Narashigi [Eric Sobotka Co., Inc., Farmington, N. Y.]). The capillary tube was calibrated with a tritiated inulin solution. After measurement of its total volume, the sample was transferred to a plastic trough under mineral oil saturated with water.

A microinulin transfer apparatus (Bunton Instruments, Rockville, Md.) was used to transfer portions from the plastic trough to microcuvettes (Drummond microcaps 100 μl, [Drummond Scientific Co.]). Calibrated constriction pipettes made with a DeFonbrune Microforge (The Welch Scientific Company, Weston, Ont.) and calibrated with tritiated inulin solution were used to obtain known volumes between 2 and 30 nl.

For the measurement of inulin, 10–25 nl portions were pipetted into 5 μl of water in a glass capillary tube. The contents of the tube were then washed into 0.4 ml Soluene

[3] (Na-ethyl-(1-methyl-propyl)-malonyl-thio urea), Promonta, Hamburgh, West Germany.

[4] Na, 150 mEq/liter; K 4 mEq/liter; Cl 129 mEq/liter; and HCO_3 25 mEq/liter (1).

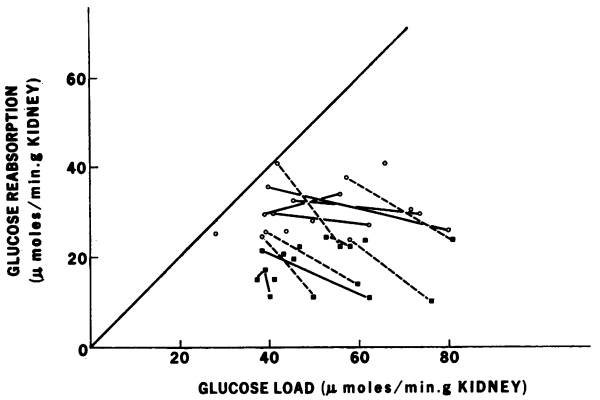

FIGURE 1 Whole kidney glucose reabsorption rate compared with glucose load (Cin × plasma glucose concentration) in nonexpanded rats (open circles) and expanded rats (closed squares). Each point is the mean of three or more measurements at similar plasma glucose concentrations. Solid lines join measurements made in the same rat at different plasma glucose concentrations. Hatched lines join measurements made in the same rat first, in the nonexpanded state and second, after volume expansion. The diagonal solid line indicates reabsorption rate equal to load.

TABLE I

*Clearance Data for Rats Infused with Glucose in Water**

Body weight	Plasma glucose	C Inulin‡	T Glucose§
g	μM		
216‖	39 ±0.0	1.01 ±0.01	25.6 ±0.7
196‖	46 ±3.0	1.24 ±0.08	37.5 ±2.8
229‖	33 ±0.0	1.26 ±0.05	41.8 ±2.1
183‖	59 ±2.2	0.98 ±0.02	23.8 ±1.0
215‖	36 ±0.8	1.11 ±0.06	24.0 ±0.8
215	64 ±1.2	1.03 ±0.0	40.7 ±1.3
194	30 ±0.0	1.31 ±0.06	35.2 ±1.9
	68 ±2.1	1.16 ±0.02	25.5 ±1.9
197	73 ±1.7	0.98 ±0.03	30.0 ±1.7
300	45 ±1.5	0.97 ±0.05	25.5 ±1.4
267	36 ±0.8	1.12 ±0.04	29.5 ±1.7
	53 ±1.7	1.17 ±0.02	26.6 ±0.8
206	36 ±0.0	1.25 ±0.09	32.3 ±0.8
	62 ±4.0	1.19 ±0.05	29.4 ±1.7
267	45 ±1.2	1.10 ±0.02	28.1 ±1.2
256	38 ±2.0	0.74 ±0.01	25.0 ±0.8
245	43 ±1.5	0.91 ±0.04	29.5 ±2.0
	54 ±0.9	1.03 ±0.08	33.5 ±2.0
Means		1.07 ±0.03	30.2 ±1.6

* Mean ±SE.
‡ Inulin clearance ml/min × g kidney weight.
§ Glucose reabsorption μmoles/min × g kidney weight.
‖ These rats were reexamined after volume expansion, see Table II.

(Packard Instrument Co., Downers Grove, Ill.) in a counting vial and after 30 min, 10 ml of scintillation fluid was added (5 g 2,5-diphenyloxazole (PPO), 0.25 g dimethyl *p*-bis[2-(5-phenyloxazolyl)]benzene (POPOP) in 1 liter of toluene). Only samples containing more than twice background activity in a Packard scintillation counter were used.

Glucose was measured by transferring 2–5 nl of tubular fluid to 5 μl of an enzyme mixture in microcuvette sealed at one end. The enzyme mixture contained 0.014 M $MgCl_2$, 0.02% albumin, 0.4 mM NADP, 0.8 mM ATP and approximately 1 IU/ml each of glucose-6-phosphate dehydrogenase and hexokinase (11). After centrifugation, the fluorescence of the sample was determined in Aminco fluoro-microphotometer with micro-attachment (American Instrument Co. Inc., Silver Spring, Md.). Determinations were done in duplicate or triplicate.

Radioactivity in urine and plasma was measured by adding 2 μl portions to 0.4 ml Soluene, adding scintillation fluid, then counting in a Packard scintillation counter. For glucose measurements, 1 μl of plasma or diluted urine (1 : 5–1 : 10) was added to 2 ml of enzyme mixture and read in the fluoromicrophotometer. Urine and plasma, sodium and potassium were measured with an IL flame photometer (Instrumentation Laboratory, Inc., Lexington, Mass.).

Clearances and tubular glucose reabsorption rates were calculated without correction for plasma water content.

Cin	= Uin × V/Pin
TG	= Cin × Pg–Ug × V
sgfr	= Tin × v/Pin
tg	= sgfr × Pg–v × Tg
Pin and Uin	= plasma and urine inulin concentration
Pg and Ug	= plasma and urine glucose concentration
V	= urine flow; milliliters per minute
v	= tubular fluid flow; nanoliters per minute
Tg	= tubular fluid glucose concentration
Tin	= tubular fluid inulin concentration

Standard statistical methods were used (12).

RESULTS

The macroglucose assay yielded plasma glucose recoveries of 102.2 ±1.3 (SE) and urine glucose recoveries of 104.0 ±1.9. With the microassay, the relationship between fluorescence and glucose concentration from 10 to 200 mM was linear with a SE less than 3%. Four plasma samples were assayed with micro and macromethods; the ratio microassay/macroassay was 1.05 ±0.04.

This report deals primarily with data obtained from rats with plasma glucose concentrations greater than 30 mM, the level at which glucose reabsorption appeared to reach a maximum rate (Table I and II). At lower concentrations of glucose, the data were insufficient to delineate splay in glucose titration curves and they have, therefore, been omitted from Discussion, Tables, and Figures. That total renal glucose reabsorptive capacity was saturated at plasma glucose concentrations greater than 30 mM may be assumed since TG was constant or decreased in all but one experiment while glucose load exceeded TG to a considerable extent (Fig. 1). In single nephrons neither tg nor tg/sgfr (Fig. 2) increased as plasma glucose rose from 30 to nearly 80 mM and, there-

fore, reabsorption rate seems to have been maximal. The terms TmG for whole kidney and tmg for single nephrons will be used to specify these maximum reabsorption rates.

Blood pressure was similar in the two groups; 145 ±4/111 ±4 for volume-expanded and 139 ±4.7/102 ±4.5 for nonexpanded rats. Hematocrit and plasma potassium concentration were significantly lower and plasma sodium, urine flow, and urine sodium and potassium concentrations were higher in the volume-expanded rats (Table III).

In four rats studied before and after volume expansion, there was a 3–19% increase in inulin clearance (Cin) associated with the infusion of large amounts of saline. Mean Cin of volume-expanded rats was higher than that of nonexpanded rats (Tables I and II, $P = 0.05$).

Single nephron filtration rate (sgfr) in superficial nephrons increased after volume expansion both in absolute terms (28.8–36.6 nl/min·g kidney weight) and relative to total kidney Cin (Table IV). A greater increase from 29.0 ±1.6 to 45.5 ±3.4 nl/min was found in recollection experiments (Fig. 3). There was no difference between initial and recollection sgfr when both samples

TABLE II

*Clearance Data for Rats Infused with Glucose in Isotonic Salt Solution after Volume Expansion**

Body weight	Plasma glucose	C Inulin‡	T Glucose§
g	*μM*		
216‖	49 ±1.2	1.20 ±0.08	14.9 ±2.1
196‖	56 ±1.7	1.44 ±0.01	24.6 ±2.4
229‖	40 ±0.0	1.39 ±0.03	23.3 ±2.2
183‖	74 ±1.2	1.01 ±0.01	10.9 ±1.4
215‖	36 ±0.2	1.36 ±0.03	9.2 ±1.5
210	47 ±1.2	0.85 ±0.01	11.1 ±1.0
	51 ±2.3	0.76 ±0.03	16.8 ±0.5
	51 ±1.2	0.73 ±0.02	15.1 ±0.3
186	36 ±1.7	1.07 ±0.03	21.6 ±0.8
	73 ±1.7	0.85 ±0.03	10.9 ±0.6
297	48 ±0.5	1.20 ±0.02	23.5 ±1.5
216	34 ±1.2	1.26 ±0.02	20.8 ±0.8
190	36 ±1.2	1.44 ±0.01	24.1 ±1.0
	47 ±1.2	1.30 ±0.02	23.4 ±0.9
220	36.7 ±0.9	1.23 ±0.04	23.2 ±1.7
214	30.2 ±0.3	1.35 ±0.03	14.0 ±0.7
236	30.1 ±0.6	1.48 ±0.02	21.0 ±2.1
Means		1.23 ±0.05	18.4 ±1.5

* Means ±SE.

‡ Inulin clearance ml/min × g kidney weight.

§ Glucose reabsorption μmoles/min × g kidney weight.

‖ These rats examined first without volume expansion, see Table I.

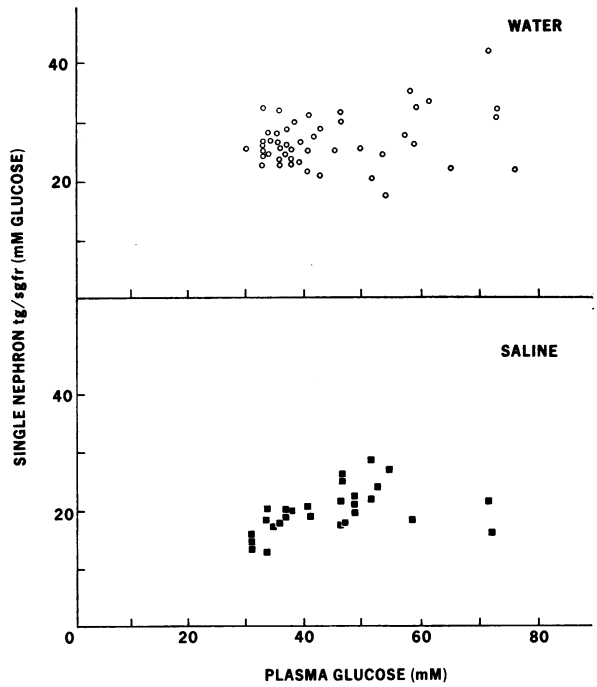

FIGURE 2 Glucose reabsorption rates factored by single nephron filtration rates in superficial proximal and distal tubules compared with plasma glucose concentration. The upper graph (water) shows results from nonexpanded rats and the lower graph shows results from volume-expanded rats (saline).

were collected either during glucose and water infusion or after volume expansion (28.5 ±3.1 and 27.7 ±3.0 nl/min·g kidney weight). Some micropuncture collections from mid to late proximal sites are included in the recollection experiments (Figs. 3 and 4), otherwise all collections were from late superficial proximal or distal micropunctures.

Proximal intraluminal pressure rose from 15.1 ±1.2 mm Hg in nonexpanded glucosuric rats to 20.8 ±0.6 mm Hg in volume-expanded rats. The method of collection used in routine collections was associated with changes of between plus and minus 4 mm Hg (Table V). In volume-expanded rats, the mean sgfr was the same during pressure measurement as it was in the routine collections. However, a problem in the use of the Landis method to monitor intraluminal pressure was apparent. It was difficult to prevent some Fast green from entering the tubule lumen and staining the collected tubular fluid to a variable extent. Glucose reabsorption was considerably lower in these doubly punctured tubules than in other routinely punctured tubules with similar sgfrs in the same rat or in other volume-expanded rats. Inhibition of glucose reabsorption seemed to be proportional to the intensity of green stain in the tubular fluid. A similar dye, lissamine green, has been found to inhibit sodium

TABLE III

Blood and Urine Composition in Nonexpanded and Volume-Expanded Glucosuric Rats

	Hematocrit	Plasma Na	Plasma K	Urine flow	Urine Na	Urine K
	%	*mEq/liter*		*μl/min*	*mEq/liter*	
Glucose-water	49.6 ±0.5	145.0 ±1.4	4.0 ±0.1	49 ±7	8 ±1.5	6.2 ±2.7
Volume expansion, Glucose-isotonic salt solution	43.9 ±0.9	152.6 ±1.7	3.4 ±0.1	142 ±15	108 ±8.6	10.5 ±4.3
P‡	<0.001	<0.005	<0.001	<0.001	<0.001	<0.01

* Means ±SE

‡ Probability, *t* test.

TABLE IV

*Micropuncture Data from Rats Infused with Glucose in Water (W) and with Glucose in Isotonic Salt Solution after Volume Expansion (S)**

	sgfr‡			Cin/sgfr × 10^{-3}			t Glucose§			tg/sgfr / TG/Cin		
	W	S	*P*‖	W	S	*P*	W	S	*P*	W	S	*P*
Proximal	30.3 ± 1.6 (27)	36.7 ± 2.3 (15)	0.05	36.7 ± 1.6 (27)	31.1 ± 2.5 (15)	NS	794 ± 49 (27)	710 ± 45 (15)	NS	0.92 ± 0.02 (27)	1.15 ± 0.06 (12)	0.001
Distal	26.7 ± 1.6 (21)	36.4 ± 1.9 (12)	0.005	39.4 ± 2.5 (16)	32.8 ± 1.5 (12)	0.05	776 ± 48 (21)	731 ± 45 (12)	NS	1.02 ± 0.04 (21)	1.24 ± 0.08 (12)	0.01
P‖	NS	NS		NS	NS		NS	NS		NS	NS	
Total	28.8 ± 1.2 (48)	36.6 ± 1.5 (27)	0.001	37.8 ± 1.2 (43)	31.9 ± 1.5 (29)	0.01	786 ± 35 (48)	720 ± 30 (27)	NS	0.97 ± 0.02 (48)	1.20 ± 0.05 (24)	0.001

* Mean ±SE.

‡ Single nephron filtration rate 10^{-9} liters/min × gram kidney weight.

§ Single nephron glucose reabsorption rate 10^{-12} moles/min × gram kidney weight.

‖ *t* test.

transport in the rat kidney (13) and proximal tubule (14).

Fractional water reabsorption in proximal tubules tended to decrease as plasma glucose rose (Fig. 5); the negative correlation between TF/P inulin and plasma glucose, between 30 and 77 mM, was significant after volume expansion ($r = 0.619$ $P < 0.05$) but not in nonexpanded rats ($r = 0.291$).

Fractional water reabsorption in the proximal tubule was lower in volume-expanded than in nonexpanded rats (Fig. 5), but absolute water reabsorption in single proximal tubules was not significantly less (15.6 ±1.3 nl/min·g kidney weight in volume-expanded rats; 17.5 ±1.1 nl/min·g kidney weight in nonexpanded rats). Distal TF/P inulin ratios were lower in volume-expanded rats (3.02 ±0.30) than in nonexpanded rats (4.26 ±0.4).

Volume expansion was associated with a 39% decrease in glucose reabsorption from the TmG of 30.2 μmoles/min·g kidney weight in nonexpanded rats to 18.4 μmoles/min·g in volume-expanded rats. The validity of this observation is reinforced by four experiments in

which TmG was measured before and after volume expansion in the same rats. (Tables I and II, first four rats; Fig. 1). Here also TmG decreased by 40%. That these large decreases of TmG after volume expansion are not due to deterioration of the rats can be seen by comparing them with the small changes in three out of four nonexpanded rats infused with two different concentrations of glucose in water (Fig. 1, Table I). There was a pronounced fall of TmG in one volume-expanded rat infused with two different concentrations of glucose. In this case the fall was associated with a 21% decrease in Cin and a very high plasma glucose concentration (Table II, 186 g rat).

If, as has been assumed since the work of Walker, Bott, Oliver, and MacDowell (15), glucose is reabsorbed by the entire proximal tubule, then late superficial proximal micropunctures at 50–60% of proximal tubule length could not be used to measure tmg. However, in the present experiments, maximum glucose reabsorption at 50–60% of proximal tubule length was 92% of that in the distal tubule for both nonexpanded and volume-expanded rats. This is shown in Table IV where for comparison between different rats, tmg is expressed in terms of sgfr and whole kidney function (tmg/sgfr ÷ TmG/Cin). Further evidence of the virtually exclusive role of the pars convoluta in glucose reabsorption comes from microperfusion experiments on

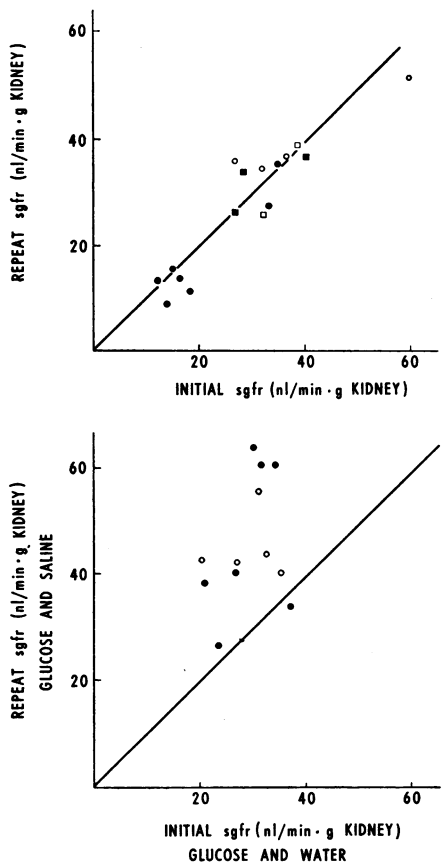

FIGURE 3 Recollection measurements of single nephron filtration rates. The upper graph shows the results of experiments in which both collections were made either in the nonexpanded state (circles) or in the volume-expanded state (squares). The lower graph shows the results when the initial collection was made in the nonexpanded state (glucose and water) and the recollection was made after volume expansion (glucose and saline). Closed symbols represent proximal; open symbols represent distal micropunctures.

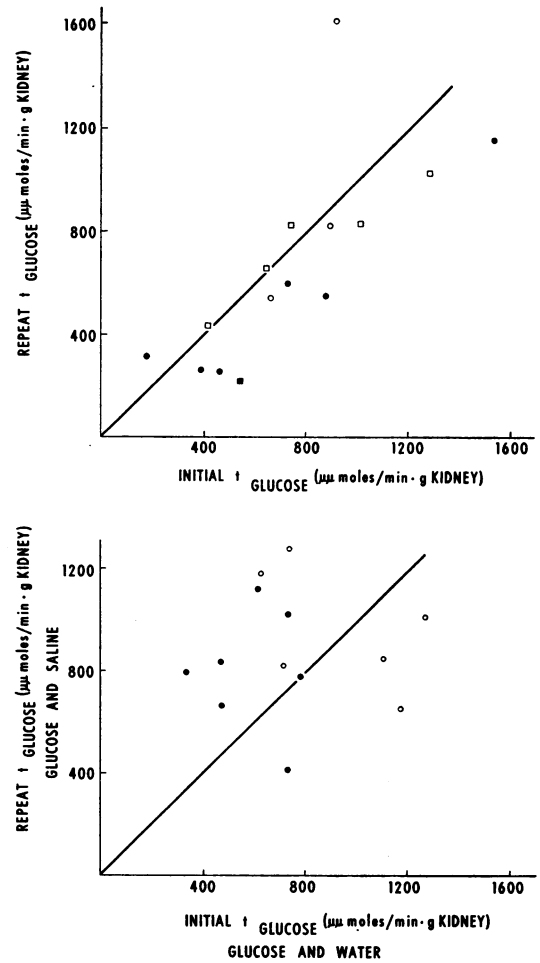

FIGURE 4 Recollection measurements of single nephron glucose reabsorption rates. The upper graph shows results when both collections were made in the nonexpanded state (circles) or when both collections were made in the expanded state (squares). Closed symbols represent proximal micropunctures and open symbols represent distal micropunctures. The lower graph shows the results when the initial collection was made in the nonexpanded state (glucose and water) and the recollection was made in the expanded state (glucose and saline).

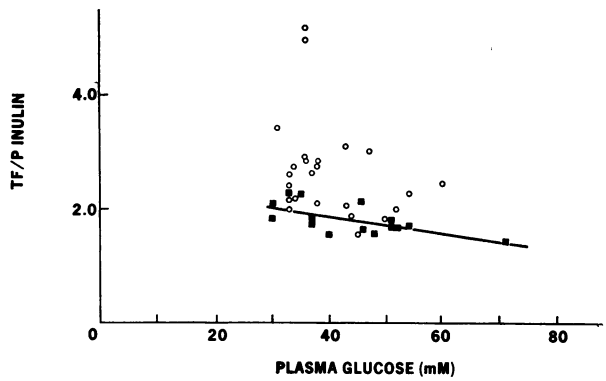

FIGURE 5 Late proximal TF/P inulin ratios related to plasma glucose concentration. Open circles represent micropunctures of nonexpanded rats; closed squares represent micropunctures of volume-expanded rats. The solid line is the regression of TF/P inulin on plasma glucose concentration above 30 mM for volume-expanded rats: $y = -0.015x + 2.42$, $r = -0.619$. There was not a significant correlation for nonexpanded rats ($r = -0.291$).

isolated rabbit proximal tubules.[5] The first part of these tubules reabsorbed glucose at 78.5 ±5.1 μμmoles/min·mm while reabsorption in the terminal portion was only 5.9 ±0.6 μμmoles/min·mm.

The 8.5% decrease of tmg in single superficial nephrons after volume expansion was not statistically significant. In recollection experiments, measurements of tmg were similar when both samples were drawn during a glucose and water infusion or when both samples were drawn from volume-expanded rats (Fig. 4; initial collection 749 ±91 μμmoles/min·g kidney weight; recollection 668 ±99 μμmoles/min·g kidney weight). On the other hand, if the initial collection was made during glucose and water infusion and the recollection was made during volume expansion, then the second measurement of tmg was significantly higher than the first (Fig. 4; initial collection 703 ±69 μμmoles/min·g kidney weight; recollection 862 ±71 μμmoles/min·g kidney weight; $P < 0.005$). Recollection experiments are not included in Table IV and Figs. 2, 5, and 6.

Single nephron tmg was significantly correlated with sgfr (nonexpanded $r = 0.778$; volume-expanded $r = 0.58$ (Fig. 6). Needless to say, there were also correlations between tmg and glucose load (sgfr × plasma glucose concentration), but they were less significant than those between tmg and sgfr.

The ratio tmg/sgfr has been used as theoretical index of glomerular/tubular balance for single nephrons and the ratio TmG/Cin as an index of the mean glomerular tubular balance for the whole kidney (16, 17). Comparing these two indices (Table IV) we find that in nonexpanded rats tmg/sgfr in superficial distal tubules was the same as the mean for the whole kidney while in superficial distal tubules of volume expanded rats it was 24% higher than the mean for the whole kidney. The ratio tmg/sgfr is calculated as Pg–Tg/(Tin/Pin), without the inclusion of a volume term. The data in Table IV, therefore, indicates a shift in the proportion of total kidney glucose reabsorption provided by superficial nephrons after volume expansion. This conclusion does not depend upon accurate measurements of sgfr. The validity of this observation was borne out by finding that in small volume-expanded rats with exposed papillae, tmg/sgfr in juxtamedullary nephrons was consistently lower than tmg/sgfr in collecting ducts (Table VI). The ratio tmg/sgfr in superficial nephrons was consistently higher than in collecting ducts. In nonexpanded rats the ratio in both superficial and juxtamedullary nephrons was similar to that in collecting ducts.

[5] Tune, B. M., and M. B. Burg. 1970. Glucose transport by proximal renal tubules. *Amer. J. Physiol.* **221**: 580.

DISCUSSION

For 30 yr, physiologists have argued for and against a dependence of TmG on GFR. Although the argument is not yet resolved, it should be considered before discussing changes in filtration and reabsorption produced by volume expansion.

Glomerular intermittence, often suggested as the explanation for a correlation between TmG and GFR (8) could not produce the correlation between tmg and sgfr observed in single nephrons (Fig. 6). There are three other possible explanations: the correlation could be ar-

TABLE V

Superficial Nephron Filtration Rates from Volume-Expanded Rats Measured with Monitored Proximal Intraluminal Pressure

Site	TF/Pin	sgfr*	ΔP‡
P§	1.57	45.7	+3
P	1.32	40.2	+4
P	1.52	24.6	−2
P	1.87	35.7	−3
P	1.37	31.2	0
P	1.68	41.4	−4
		36.5 ±3.1	
D‖	4.88	27.3	−2
D	2.77	35.9	−4
D	3.00	45.7	0
D	2.08	38.8	−4
		36.9 ±3.8	

* Single nephron filtration rate nl/min g kidney weight.
‡ Mean proximal intraluminal pressure during collection minus pressure before insertion of collecting pipette (mm Hg).
§ Late proximal.
‖ Distal.

tifactual, it could be due to a structural correlation between filtering and reabsorbing portions of the nephron, or it could result from a functional dependence of tmg on sgfr or the factors determining sgfr. These three possible explanations are considered in the ensuing paragraphs.

Sgfr contributes a numerically large portion to the calculated tmg, thus errors in sgfr measurement will produce comparable errors in tmg. The contribution of this common error to the total correlation between sgfr and tmg cannot be precisely assessed; however, the reproducibility of recollection sgfr and tmg measurements over a wide range of values (upper part of Fig. 3 and Fig. 4 [18]) indicates that a considerable fraction of the variation represents true functional diversity.

As structural correlates for glomerular tubular balance, Oliver and MacDowell (16) postulated that glomerular capillary surface area determines sgfr and that proximal tubular cell volume determines tmg. They found variations in structural glomerular tubular balance (glomerular volume)/(proximal tubular volume) similar to the variations of functional glomerular tubular balance (sgfr/tmg) predicted from a mathematical analysis of splay in glucose titration curves (16, 17).

Consistent with their hypothesis are the correlations of sgfr to glomerular volume and proximal tubule length in rat and psammomys kidneys studied with Hanssen's radioactive ferrocyanide technique (7, 10) and, in the present study, the small but significant correlation between sgfr (micropuncture) and length of the pars convoluta ($r = 0.579$, $P < 0.01$). The latter was measured in 22 latex-filled tubules microdissected from nonexpanded rats. There is good reason then to expect that long proximal tubules have large glomeruli with high sgfr's and that short proximal tubules have small glomeruli with low sgfr's. Length of the proximal tubule should influence the number of transport sites in a nephron, hence a structurally determined correlation between sgfr and tmg is probable.

There remains the possibility of a functional relationship between sgfr and tmg. Deetjen and Boylan (19) deduced from microperfusion experiments that proximal tubular glucose reabsorption was influenced by flow rate into the perfused tubule. Their experiments, however, are not directly related to the regulation of maximal glucose reabsorption since the concentration (12 mM) of glucose and maximum infusion rate (23 nl/min) they used were insufficient to saturate transport capacity. They observed a maximum reabsorption rate of 63 μμmoles/min·mm tubule length, whereas in our experiments, tmg was 160 μμmoles/min·mm assuming an average length of 5 mm for the pars convoluta. With reabsorption less than maximal, a correlation between load of glucose delivered to the tubule and tg is to be expected. Tune and Burg[6] found that tmg in isolated, perfused rabbit proximal tubules was independent of perfusion rate if the glucose load exceeded 200 μμmoles/min.

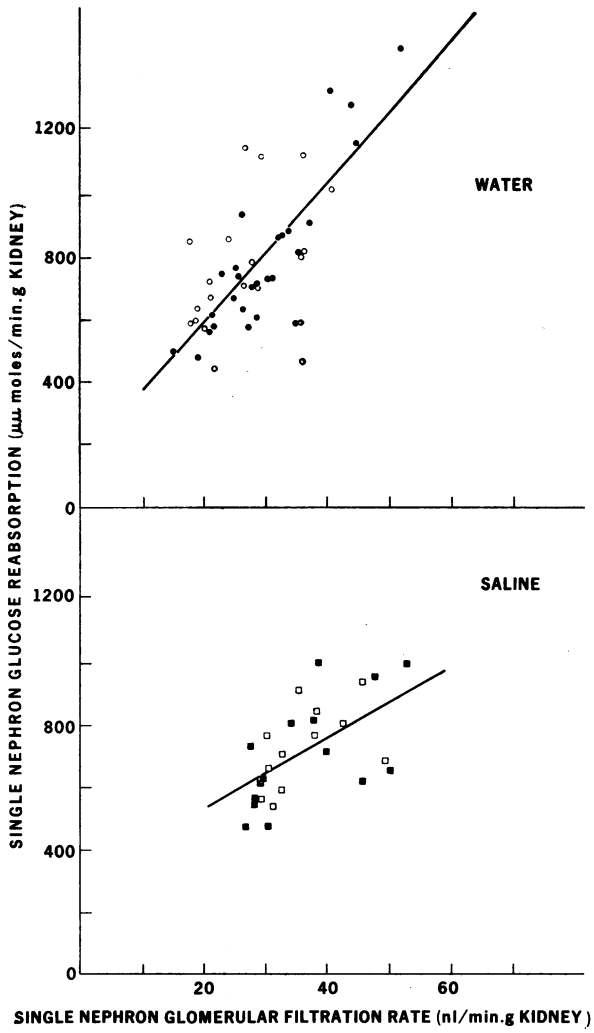

FIGURE 6 Relationship of single nephron glucose reabsorption rate to single nephron filtration rate in late proximal (closed symbols) and in distal tubules (open symbols). The upper figure shows results obtained from nonexpanded rats (water). The regression line depicts the equation $y = 22.8\ x + 132.8$, $r = 0.778$. The lower graph shows results from volume-expanded rats. The regression equation is $y = 11.4\ x + 304.6$, $r = 0.580$.

Other information related to the problem of functional relationships between maximal glucose reabsorption rates and glomerular filtration rates comes from clearance experiments. In numerous experiments (20–28) in which GFR in humans, dogs, and rats varied spontaneously or was altered experimentally, a correlation between TmgG and GFR was found, but Shannon and Fisher (29), and Thompson, Barrett, and Pitts (8) reduced GFR by partial obstruction of the abdominal

TABLE VI

Micropuncture Data Obtained from Small Rats with Exposed Papillae

	T/Pin*	T/P$_G$‡	sgfr	tmg	tmg/sgfr	tmg/sgfr§ tmg C.D./gfr C.D.	
			nl/min g	*μμM/min g*			*n*
Nonexpanded							
Superficial distal	3.68 ±0.30	1.50 ±0.1	29.2 ±2.7	890 ±88	33.1 ±3.2	1.02 ±0.02	(9)
Loop	2.69 ±0.18	1.06 ±0.07	39.3 ±1.9	1317 ±84	33.6 ±1.8	1.02 ±0.05	(9)
C.D.	9.39 ±1.61	3.89 ±0.79	—	—	31.7 ±2.0	—	(6)
Volume-expanded							
Superficial distal	2.42 ±0.11	1.55 ±0.10	—	—	16.9 ±0.8	1.19 ±0.01	(4)
Loop	1.92 ±0.11	1.42 ±0.10	46.1 ±5.0	622 ±90	14.0 ±1.4	0.73 ±0.07	(9)
C.D.	4.49 ±0.22	2.89 ±0.14	—	—	17.9 ±0.8	—	(8)

* Tubular fluid to plasma inulin ratio.

‡ Tubular fluid to plasma glucose ratio.

§ The ratio of (P_G–T_G/[T/Pin]) in loop or superficial distal tubule as a ratio of the same value in a paired collection from a collecting duct.

aorta without producing a proportional decrease in TmG. If there is functional glomerular tubular balance for glucose reabsorption similar to that for sodium and water reabsorption in the proximal tubule (30) it is not yet proven.

There are probably three components to the correlations between tmg and sgfr: first, technical errors; second, a structural correlation between glomerular and proximal tubular size; and third, a possible functional dependence of tmg on sgfr or the factors determining sgfr. The contribution of each component to the total correlation cannot be determined.

In the experiments of Robson et al. (1), volume expansion increased the GFR of conscious rats on the average 0.25 ml/min·kidney. Comparable increases in GFR were produced by volume expansion in three out of four anesthetized rats examined before and after volume expansion in our experiments (Tables I and II). TmG, which in the conscious nonexpanded rats of Robson et al. was approximately 28 μmoles/min·per kidney (1), was reduced by volume expansion (10% of body weight) to 22 μmoles/min (1). In our hands volume expansion to 7.5% of the body weight reduced TmG in anesthetized rats from 30.6 to 18.5 μmoles/min·g kidney, a reduction of 39%. Thus our experiments confirm those of Robson et al., although volume expansion seems to have had a more pronounced effect in anesthetized rats.

The small decreases of TmG observed in three out four nonexpanded rats examined at two different plasma glucose concentrations (Table I, Fig. 1) may have been the result of mild extracellular fluid volume expansion secondary to prolonged glucose infusions and hyperglycemia.

Studies using either micropuncture (6, 31) or Hanssen's (7, 10) techniques indicate that in nonexpanded rats on a normal or low-salt diet juxtamedullary sgfr is considerably greater than superficial sgfr. A similar distinction was also found in nonexpanded glucosuric rats. In their kidneys 37,800 ±1,200 nephrons producing glomerular filtrate at the rate of superficial nephrons would have been required to account for total kidney GFR but rat kidneys usually contain 30,000–32,000 nephrons (10), therefore, sgfr in deep nephrons must have been greater than in superficial nephrons.

Glomerular-tubular balance (tmg/sgfr) was similar in both superficial and deep nephrons of nonexpanded rats. This follows from the observation that superficial tmg/sgfr was the same as whole kidney TmG/GFR, that is the same as mean tmg/sgfr for all the nephrons (Table IV). Glomerular-tubular balance in juxtamedullary nephrons of small nonexpanded rats was the same as that in superficial nephrons and collecting ducts (Table VI), while both sgfr and tmg were higher in juxtamedullary than in superficial nephrons.

Superficial and deep sgfr's were also examined with Hanssen's ferrocyanide technique.[6] In nonexpanded glucose infused rats juxtamedullary sgfr was 1.24 times as high as superficial sgfr. On the other hand, in volume-expanded rats juxtamedullary sgfr was only 1.05 times as high as superficial sgfr. There is good agreement between the results of our micropuncture and ferrocyanide experiments (Table VII).

Recent observations of Mandin, Israelit, Rector, and Seldin (32) suggest that superficial sgfr is artifactually increased by recollection from the same nephron after volume expansion. The results of our recollection experiments after volume expansion are similar to theirs in that superficial sgfr increased by 57% after volume

[6] Baines, A. D., and I. Godi. 1971. Submitted for publication.

TABLE VII

Comparison of sgfr Measured by Micropuncture and by the Distribution of Radioactive Ferrocyanide

	Nonexpanded		Volume expanded	
	Superficial	Juxtamedullary	Superficial	Juxtamedullary
Micropuncture				
Large rats	28.8 ±1.2	—	36.6 ±1.5	—
Small rats	29.2 ±2.7	39.3 ±1.9	—	46.1 ±5.0
Ferrocyanide	30.6 ±1.2	38.0 ±1.2	37.5 ±0.9	39.2 ±0.9

expansion when measured by recollection but increased by only 21% when measured in intact tubules. Data from recollections are not included in Tables IV and VII, and Figs. 2, 5, and 6.

According to the literature the effect of volume expansion on the rat kidney differs from that on the dog kidney (32). In rat kidneys there is an increase in superficial sgfr (31, 32) with little change in juxtamedullary sgfr (31). Absolute rates of water reabsorption are not significantly reduced in superficial proximal tubules (32) but are lower in juxtamedullary nephrons (31). Despite the similarity of our results to this published data it was necessary (because of the crucial effect of sgfr on the calculated tmg) to establish that superficial sgfr was not spuriously high in volume-expanded hyperglycemic rats. The intraluminal pressure changes created by fluid collection from volume-expanded rats were within the range reported to be without effect on sgfr if the collection is made in late proximal or distal tubules (Table V), (18, 33). In addtion, 31,900 ±1500 nephrons filtering at the rates measured in volume-expanded rats would have been required to account for total kidney GFR (Table IV); this implies that there was little difference between superficial and deep nephron filtration rates which agrees with the distribution of ferrocyanide in volume-expanded rat kidneys[6] (Table VII). Taken together with the published data these findings support the conclusion that the measured superficial sgfr was representative of actual function.

Although glucose reabsorption by the whole kidney decreased 39% after volume expansion, superficial nephron tmg decreased little if at all (Table IV). Mean superficial tmg would have to be almost two standard deviations lower than that measured for there to have been a 39% decrease. Even assuming superficial sgfr had remained at 29 nl/min·g in volume-expanded rats, which is unlikely, we can see from the regression line in the lower part of Fig. 6 that tmg would have been 635 μμM/min·g—only 19% less than that in the nonexpanded state.

It can be calculated that glucose reabsorption in the 40% of nephrons which lie below the capsular surface must have decreased by 60 to 70% to account for the observed decrease in TmG without much change in superficial tmg. Juxtamedullary tmg was found to be lowered to almost this extent by volume-expanded small rats, from 1317 ±84 to 622 ±90 μμM/min·g ($P < 0.001$). In nonexpanded rats tmg/sgfr was similar in collections from superficial and juxtamedullary nephrons and collecting ducts or ureter, whereas in volume-expanded rats tmg/gfr was higher than that in ureter or collecting duct when the fluid came from superficial nephrons and lower when the fluid came from juxtamedullary nephrons (Tables IV and VI). Since superficial sgfr increased while juxtamedullary sgfr remained relatively unchanged, one can only conclude that volume expansion inhibited glucose reabsorption primarily in deep nephrons.

It does not appear likely that a change in the concentration of an inhibitory substance in blood bathing all nephrons could inhibit glucose transport in deep but not superficial nephrons; however, as we have previously suggested (7), a nonuniform change in one or more of the physical factors known to influence tubular reabsorptive capacity could be responsible. Renal blood flow increases during volume expansion (34). The resulting peritubular vascular and interstitial engorgement should be greatest where all blood enters and leaves the kidney in the inner cortex and outer medulla. Compression of juxtamedullary proximal tubules in this region might impede sodium, water, and glucose reabsorption (7). Reabsorption from proximal tubules situated in the outer cortex would be less affected by elevated peritubular hydrostatic pressure.

A disproportionate increase in peritubular hydrostatic pressure in the inner cortex and outer medulla could also account for redistribution of sgfr. Afferent arterial tone probably decreases during volume expansion producing an increase in glomerular capillary hydrostatic pressure. Filtration rate may not increase in deep nephrons because pressure in tubular lumens rises as much or more than capillary pressure. Intraluminal pressure would rise because the tubules are compressed and reabsorption of water from them is decreased. The results obtained

with Hanssen's technique support this argument by demonstrating visible compression of deep proximal tubules, decreased fractional water reabsorption in deep nephrons, and redistribution of sgfr (7).[6]

Dogs do not appear to increase their superficial sgfr after volume expansion (32). Structural differences between rat and dog kidneys may account for this discrepancy. Nevertheless the physical effects of volume expansion might still inhibit reabsorption more from deep than from superficial proximal tubules in dog kidneys.

In summary, volume expansion led to redistribution of sgfr with an increase in superficial nephrons and to redistribution of proximal tubular function with a decrease primarily in deep nephron tmg. The resultant increase in heterogeneity of glomerular-tubular balance could explain the increased splay of glucose titration curves observed by Robson et al. (1) in volume expanded rats. It is possible that a mechanism smiliar to that postulated is responsible for the depression of phosphate and bicarbonate reabsorption by volume expansion (35, 36).

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