ON THE RELATION BETWEEN GLUCOSE REABSORPTION AND FILTRATION RATE IN THE DOG KIDNEY

by

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A THESIS

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INTRODUCTION

Purpose of this Investigation

It was first observed in 1941 (55) that the fractional reabsorption of fluid in the proximal tubule was relatively constant during spontaneous changes in the filtration rate. Water or fluid reabsorption in the proximal tubule is a passive process. Water follows the active reabsorption of sodium and its anions. So the constancy of the fractional reabsorption of water is a consequence of the constancy of the fractional reabsorption of sodium and its anions. Even during experimental reductions in glomerular filtration rate (GFR) produced by aortic clamp, fractional reabsorption of sodium and water in the proximal tubule remains constant (8,35). This phenomenon of the constancy of the fractional reabsorption in the proximal tubule is termed glomerulotubular balance (26).

One factor that is thought to have an important influence on glomerulotubular balance is the reabsorptive surface area of the proximal tubule (43). If the tubular volume and consequently the luminal surface area of the proximal tubule increases when filtration rate increases, then the passive flux of sodium ions from luminal fluid into the cells may be increased. This could in turn stimulate an increase in the active component of sodium ion reabsorption. On the other hand if luminal surface area decreases when GFR decreases, then passive flux of sodium ions may also decrease and consequently bring about a reduction in the active component of sodium ion reabsorption.

If changes in the reabsorptive surface area have such an effect on the reabsorption of sodium ions, one would predict that it should affect other solutes that are reabsorbed in the proximal tubule. Glucose is reabsorbed actively in the proximal tubule and only in this portion of the nephron (54,55). The luminal border of the proximal tubular cell is composed of microvilli (39). As the tubular volume increases, the microvilli might separate and expose more glucose transport sites to the luminal fluid. The rate of glucose reabsorption has been shown to have an upper limit (46). This upper limit may be a function of the number of active sites exposed to the tubular fluid. Increasing the number of exposed sites for active glucose transport could bring about an increase in the maximum rate of glucose reabsorption (Tm_G). On the other hand decreasing the tubular surface area by decreasing the GFR could reduce the number of glucose transport sites that are exposed to the tubular fluid. This would tend to bring about a reduction in Tm_G.

The purpose of the present investigation was to study the role of luminal surface area on the maximum rate of glucose reabsorption. I attempted to change the tubular surface area by two different methods: 1) Partial clamp of the renal artery in which case tubular surface area presumably decreases directly with the GFR and 2) Partial clamp of the ureter in which case luminal surface area increases as GFR decreases. Changes in Tm_G were compared with changes in GFR.

It should be noted here that in this thesis the Tm_G is not considered to be a fixed constant. This is in contrast to the use of Tm_G as expressed in most of the literature. Evidence and arguments supporting the idea that Tm_G is a variable are presented in the results and discussion of this thesis.

An Historical Review of the Study of Glucose Reabsorption in the Kidney.

As late as the second decade of the 20th Century the nature of the mechanisms involved in urine formation by the kidney was still not clearly defined. There were two opposing views. One claimed that the kidney operated by filtering plasma and then reabsorbing most of the filtrate. The other view claimed that urine was formed by means of active secretion from the cells of the uriniferous tubules. However, in the early 1920's Atkinson, Clark, and Menzies (1) and Clark (9) demonstrated in perfused frog kidneys that sulfate and glucose would appear in the urine only when present in the arterial perfusate. When these substances were in the renal portal perfusate only, neither appeared in the urine. If the uriniferous tubules secreted sulfate and glucose these substances should have been found in the urine when perfused through the renal portal vein. Then in 1924, Wearn and Richards (56) described in their now classic paper a method of obtaining fluid from individual Bowman's capsules in amphibian kidneys by means of a micropipette. Chemical analyses of their samples indicated that the capsular fluid contained all the plasma constituents except proteins and blood cells. The fluid always contained glucose even though the bladder urine did not. These investigators concluded that urine was formed by the process of filtration of plasma with the subsequent reabsorption of its useful constituents.

To characterize the filtration reabsorption theory more completely it was necessary to quantitate the amounts of various substances filtered and reabsorbed. Rehberg (37) noted that creatinine was concentrated to a greater extent than any other substance found in the urine. He reasoned that this substance would either have to be secreted by the tubules or filtered and because of the degree to which it was concentrated, not reabsorbed. He evidently believed secretion and filtration were mutually exclusive. On these assumptions he concluded that all of the creatinine filtered appears in the urine. Letting GFR represent the glomerular filtration rate, P_{Cr} the plasma creatinine concentration, V the urine flow, and U_{Cr} the urine creatinine concentration, then

$$GFR \cdot P_{Cr} = U_{Cr}V \qquad (1)$$

and

$$GFR = V(U/P)_{Cr} \qquad (2)$$

With a method that allowed calculation of filtration rate, Rehberg was then able to calculate reabsorptive rates of filtered water and solutes. Reabsorptive rate of water is simply the difference between the volume filtered and the volume excreted per unit time, i.e.,

$$\text{Volume reabsorbed} = GFR - V$$

Rate of reabsorption, T_s, of solute, S, would be:

$$T_s = GFR \cdot P_s - U_sV. \qquad (3)$$

For his experiments Rehberg ingested enough creatinine in the morning to raise his plasma concentration to about 8 mg/100 ml. Hourly blood samples were obtained in each experiment, the plasma creatinine concentration measured, and then a curve relating the

concentration to time was constructed. He marked the time a urine sample was obtained on this curve and used the corresponding plasma concentration for his calculation. The plasma creatinine concentration thus obtained underestimated the actual plasma concentration at the time the filtrate was being formed. Rehberg was aware of this and corrected for it by attempting to maintain a diuresis of sufficient magnitude to minimize time between urine samples. He found from his experiments that the amount of creatinine excreted varied directly with the plasma concentration. The slope of the line relating the excreted creatinine with the plasma concentration is the filtration rate, (equation 1). Rehberg's GFR varied between 80 and 200 ml/min, but 73% of 86 separate determinations ranged between 100 and 145 ml/min, the average being approximately 120 ml/min. The important result was the estimation of the order of magnitude of the GFR. Rehberg argued on the basis of results of experiments in other animals that the kidney had more than sufficient surface area to reabsorb 99% of this filtered volume. In addition because of resistance to flow through Henle's loop and distal parts of the nephron he hypothesized that from 60-80% of this volume would have to be reabsorbed in the proximal convoluted tubule.

The equation he derived to calculate GFR is known today as the clearance equation. According to Smith (48) the term clearance originated with D.D. Van Slyke who reasoned that the equation represented a virtual volume of plasma that would have contained the amount of substance that appeared in the urine in one minute. This volume of plasma would have been completely "cleared" of that

substance.

Poulsson (35) and Ni and Rehberg (30) using creatinine to calculate GFR studied glucose reabsorption in the dog nephron. They found that all of the glucose filtered (filtered load), was reabsorbed until a certain plasma concentration (threshold), was reached (about 350 mg/100 ml). Above threshold,glucose appeared in the urine at increasing rates as the plasma concentration increased.

Richards and Walker (40) developed a method of collecting fluid from and perfusing fluid through specific regions of the nephron of the amphibian kidney. Then Walker and Hudson (54) using these techniques discovered that in amphibian kidneys glucose was reabsorbed only in the proximal convoluted tubules. Walker, Bott, Oliver,and MacDowell (55) analyzed fluid from individual nephrons in the kidneys of rats and guinea pigs and found that all of the glucose was reabsorbed in the proximal convoluted tubule and most of it in the first half of the tubule. When the plasma glucose concentration was increased from normal to threshold levels, thereby increasing the filtered load of glucose, there was an increased reabsorption of the sugar in the first half of the tubule. These workers confirmed Rehberg's (37) prediction that approximately 2/3 of the filtered fluid was reabsorbed in the proximal tubule and in addition showed that the fraction of the filtered volume that was reabsorbed was constant. Hence, in their experiments when GFR increased spontaneously the volume of fluid reabsorbed in the proximal

tubule also increased. The fluid samples obtained from various regions of the proximal tubule were isosmotic with plasma, but the concentration of most of the solutes varied widely from that of the plasma.

Shannon and Fisher (46) quantitated their results of glucose reabsorption studies in dogs. A constant creatinine concentration was maintained in the plasma of trained unanesthetized dogs by means of an intravenous infusion. Urine samples were obtained from an indwelling bladder catheter. In decerebrate dogs the urine was collected from catheters placed in the ureters. Arterial blood samples were taken at the midpoint of a clearance period. Their experiments consisted of several groups of two or three observations made at successively higher plasma glucose concentrations. Their calculations revealed that the rate of glucose reabsorption exhibits an upper limit. Below threshold no glucose appeared in the urine and tubular reabsorption of glucose was complete. However, when the filtered load of glucose exceeded its maximum rate of tubular reabsorption (Tm_G), glucose appeared in the urine. Tm_G was determined at both normal and reduced filtration rates in decerebrate dogs with a denervated kidney. Glomerular filtration rate was reduced approximately 50% by partially clamping the aorta proximal to the origin of the renal arteries. This decrease in GFR was accompanied by about a 10% reduction of the Tm_G in the denervated kidney. The authors believed that the experimental Tm_G was not significantly different from control values obtained from the same kidney. These

investigators thus concluded that Tm_G was independent of filtration rate.

Goldring, Chasis, Ranges, and Smith (16) and Shannon, Farber, and Troast (47) described experimental results indicating that Tm_G was constant and independent of GFR over long periods of time in both man and the dog. Shannon's group used creatinine to determine GFR in the dog while Goldring, et al. used inulin. Inulin has been shown to be neither secreted nor reabsorbed by the vertegrate nephron [(49), pages 47-52]. Because of the observed constancy of Tm_G both groups of investigators hypothesized that the Tm_G could be used to characterize the amount of functional tubular tissue. In addition the Tm_G would be an indication of the total amount of tubular tissue with functioning glomeruli since before glucose can be reabsorbed it must first be filtered. Since Tm_G appeared to be constant, both groups of investigators concluded that normally all glomeruli in the kidney were perfused and functioning continuously. However, closer inspection of the results reported by Goldring, et al. (16) reveals that Tm_G may be related to filtration rate. The subjects for their study were hospitalized human volunteers free of symptoms of renal disease. Urine was collected from the bladder via a catheter. In six of the eight patients in which repeated experiments were done, Tm_G varied directly with the GFR. If the Tm_G does vary directly with GFR, the conclusion that the number of perfused glomeruli is constant may not be valid.

Forster (14) studied Tm_G in unanesthetized frogs. He used inulin for measurement of GFR. Sufficient glucose was injected into the dorsal lymph sac to raise plasma glucose concentrations well above threshold (so that filtered load of glucose was more than twice Tm_G). Timed urine samples were obtained and blood samples were obtained by heart puncture. In contrast to the results obtained by Shannon, et al. (46,47) in the mammal, Forster found that Tm_G in the frog was directly related to the filtration rate. His interpretation of these results was that the glomerular filtration rate in the frog depends upon the number of glomeruli which are open and filtering. Because of this direct correlation, Forster reasoned that the intermittency for any given glomerulus was "all or nothing", that is either it was filtering maximally or not at all.

Handley, Sigafoos, and LaForge (18) infused mercurial diuretics into dogs to bring about a reduction in the GFR. Mercurial diuretics block the reabsorption of sodium and water by the nephron. This tends to dehydrate the animal. The peak diuresis usually occurs about one hour after the drug is administered. The authors stated that Tm_G and GFR are not affected by the diuretics themselves. Nevertheless, after two hours, urine flow decreased and the GFR was also diminished presumably as a consequence of the dehydration. It was at this time that Tm_G and GFR were measured. They found that Tm_G varied directly with GFR. The ratio of Tm_G to GFR remained nearly constant under control and experimental conditions. The investigators interpreted their results to mean that dehydration

diminishes the number of glomeruli that are filtering. They further interpreted this loss of glomerular function to be an additional mechanism by which fluid balance is maintained.

However, Thompson, Barrett, and Pitts (51) were unable to confirm the results of Handley, et al. (18). Using creatinine clearance to estimate GFR they repeated the experiments with the diuretic agents. Thompson's group did not find that the diuretics consistently reduced the GFR. In none of their experiments were they able to find a decrease in Tm_G that was significantly different from control rates. They also reduced GFR by inflating a balloon catheter in the aorta just above the exit of the renal arteries. When the renal artery pressure was reduced to 80 mm of Hg by inflation of the balloon, the filtration rate decreased to about 65% of control. Tm_G was unchanged by this procedure. Further inflation of the balloon reduced the GFR to 40% of control. Tm_G then decreased by 10 to 15% of control values. The authors considered that the severe reduction of GFR to only 40% of control could indeed be due to loss of filtering glomeruli. However, they did not believe that the normal dog regulated its GFR by increasing or decreasing the number of perfused glomeruli since reducing the filtration rate by less than 50% did not bring about a concomitant decrement in Tm_G.

Handley and Moyer (19) re-investigated the relationship between GFR and Tm_G in dogs. Creatinine was used to measure GFR. Filtration rate was reduced using drugs such as epinephrine, norepinephrine,

and morphine, to reduce systemic blood pressure. In all cases Tm_G decreased in proportion to the decrement in filtration rate. However it should be noted that the depression of GFR and Tm_G outlasted the effects on reducing blood pressure. Handley and Moyer interpreted these data to be evidence for intermittency of glomerular perfusion in the mammal.

Selkurt, Bradfonbrener, and Geller (45) investigated the effects of increased ureteral pressure on Tm_G and GFR in the dog kidney. Creatinine clearance was used to estimate the GFR. Ureteral pressure was increased by elevating the ureteral catheter up to 52 cm above the kidney. During elevated ureteral pressure both the GFR and the Tm_G were decreased. The authors suggested that their results implied that the decreased GFR may be due to a reduction in the effective filtration pressure to the extent that filtration ceased in some of the glomeruli. However they felt that the decrement of both Tm_G and GFR was small and consequently stressed that their interpretation was provisional.

Malvin, Kutchai, and Osterman (29) studied glucose reabsorption in the dog kidney during partial obstruction of the ureter. As the ureteral pressure was increased the GFR decreased. When the ureteral pressure was increased to within 30% of the mean arterial blood pressure, there was a small decrease in Tm_G. Both glucose Tm and GFR decreased as ureteral pressure was increased to levels greater than 30% of the mean arterial blood pressure. If the glucose filtered load was reduced below Tm and all of the glomeruli were filtering,

then the ratio of filtered load to transport rate (T_G) would be unity. In their experiment the load to Tm_G ratio did not change appreciably during the clamp. Thus, the reduction in Tm_G is not due to unsaturation of the nephrons. However, if elevated ureteral pressure caused glomeruli to cease filtration, then the population of filtering glomeruli would be reduced as the ureteral pressure increased. As a consequence Tm_G would also be reduced. Thus, the authors concluded that when ureteral pressure was elevated sufficiently to reduce GFR and Tm_G, the functioning nephron population was diminished.

Glucose Tm in relation to the GFR was studied in rats by Van Liew, Deetjen, and Boylan (52). Inulin clearance was used to estimate the GFR. In their rats the GFR varied spontaneously in each animal over as much as a five fold range. In some experiments filtered load of glucose was reduced to values less than the Tm_G observed in a previous period. Glucose Tm, however, decreased in proportion to the GFR such that the ratio, Tm_G/GFR, was constant at glomerular filtration rates over 0.3 ml/min/g kidney. At GFR's below this value the ratio increased significantly (Fig. 8). The authors concluded that Tm_G is a linear function of GFR above 0.3 ml/min/g kidney. They hypothesized that the increased Tm_G/GFR ratio at GFR's below 0.3 ml/min/g kidney is a consequence of the increased contact time of the filtrate with the tubular cells. They reasoned that the time required for an elemental volume of

fluid to pass through the proximal tubule is increased when the GFR is low. This increased time supposedly increases the reabsorptive rate of glucose. Their argument will be examined in the discussion of the thesis.

Deetjen and Boylan (11) perfused single proximal tubules with a solution containing NaCl in concentrations of 150 or 138 mEq/l and glucose (216 mg/100 ml). Four rates of perfusion were used (12,16, 20 and 23 nl/min) and samples of perfusate were collected distal to the puncture site. Since the fractional reabsorption of glucose was much less than unity, the glucose reabsorptive mechanism presumably was saturated. They found that both glucose and water reabsorption per unit tubular length increased with each increment in perfusion rate. From a plot of glucose vs water reabsorption rates (in which each perfusion rate was identified by a separate symbol), they noted that the various perfusion rates were unsystematically scattered about the regression line. Deetjen and Boylan interpreted this finding as evidence that water reabsorption was a more important determinant of glucose reabsorption than was flow per se. In two rats the ureter was clamped ten minutes prior to perfusion in order to increase the tubular diameter. The dilated nephrons were perfused at the lowest of the four perfusion rates (12 nl/min). Glucose reabsorption per unit length was strikingly elevated for these nephrons over that for nephrons perfused at 16 nl/min without ureteral clamp. The authors concluded that Tm_G depends on the rate of glucose delivery to the transport sites.

They also believed that their results support the hypothesis that glucose reabsorption is coupled in some way to the sodium reabsorptive system.

These investigators injected ^{14}C-glucose into the peripheral circulation and perfused the nephron with unlabeled glucose. In four experiments they found that none of the labeled glucose was recovered from the perfusate and concluded that diffusion of glucose does not play a role in glucose reabsorption.

However, Loeschke, Baumann, Renschler and Ullrich (28) have found that the proximal tubule of the rat kidney is passively permeable to glucose. When proximal tubules were perfused with a solution containing 10^{-4} M phlorizin, active glucose transport was abolished and a small passive flux could then be measured. Since both influx and efflux were equal, the authors concluded that the passive flux was due to diffusion. In the absence of phlorizin but with normal tubular loads, the active and passive components were identical (i.e., zero net flux) at a peritubular to luminal concentration difference of 600 mg/100 ml. The authors concluded that normally the passive transport component of glucose plays essentially no role in glucose reabsorption.

METHODS

Surgical Procedures

Mongrel dogs weighing 20-25 kg were anesthetized with sodium pentobarbitol (30 mg/kg). Polyethylene catheters for urine collection were inserted into both ureters to the renal pelvis. The femoral and jugular veins were catheterized for infusion of solutions. Two to three hundred ml of Ringer's solution were infused during the surgical procedure. Blood pressure was monitored from the carotid or femoral arteries. In experiments involving partial clamp of the renal artery the left renal artery was dissected free at its point of emergence from the aorta. Partial clamping was accomplished by placing a screw clamp around the artery and tightening the clamp until urine flow was reduced to approximately 30-50% of control. For the renal blood flow studies the left renal vein was catheterized via the femoral vein with a cardiac catheter. The position of this catheter was checked before and after the experiment. The left ovarian or spermatic vein was always ligated for blood flow studies.

Partial ureteral obstruction was obtained by placing two stopcocks in tandem in one of the ureteral catheters and partially closing off the orifice of the more distal stopcock. The more proximal stopcock was connected to a pressure transducer for monitoring ureteropelvic pressure. The degree of obstruction was controlled by observing the change in ureteropelvic pressure and the urine flow. Partial ureteral obstruction and renal artery clamping were usually done in separate dogs.

Infusion Solutions

A 10% inulin solution was employed for priming (4 ml/kg BW), and sustaining (1.15 ml/min, Harvard Pump). The plasma inulin concentrations ranged from 100-175 mg/100 ml in all dogs. Four ml/kg BW of a 20% glucose solution was given as a priming dose and then infused at the rate of 6-8 ml/min from a Murphy drip bottle. Glucose given at this rate produces a moderate diuresis (3.8 ml/min/kidney). Plasma glucose concentration ranged from 400-900 mg/100 ml in all dogs. Ringer's solution was infused at a rate of approximately 1-2 ml during the experiment. Plasma sodium ion concentration remained relatively constant during the experiment (range 140-155 mEq/l in 4 dogs). In the renal blood flow studies Diodrast labeled with ^{131}I was given in a 1 μc priming dose and was infused at 0.42 μc/min with a Harvard pump. At least one half hour was allowed for equilibration of the infused substances before beginning the first control clearance period.

General Procedure

Clearance studies were done on 16 dogs. Each control or experimental procedure consisted of three consecutive clearance periods of 2-4 minutes. Arterial blood was drawn in a heparinized syringe at the midpoint of the second clearance period. Renal venous samples were drawn slowly in heparinized syringes during the second clearance period. An interval of at least fifteen minutes separated control and experimental clearance periods to permit re-equilibration to the new steady state. Urine flow was

measured with a graduated cylinder and a stopwatch. Blood was separated into two portions for analysis.

1. Two ml of arterial and venous samples were used for whole blood determination of Diodrast and hematocrit. Hematocrit was determined using the capillary microhematocrit method.
2. Four to six ml of arterial and venous blood were centrifuged to obtain plasma for determination of inulin, glucose, Diodrast, and sodium ion.

Centrifugation of renal venous blood samples was completed within seven minutes from the time the blood sample was obtained. This minimizes the total amount of leakage of Diodrast from red cells (15).

All plasma and urine samples were stored in capped sample tubes at 4° C. Chemical analyses of the samples were completed within four days of the experiment. No bacterial or mold contamination was seen in this time period.

Two methods were used to decrease the glomerular filtration rate (GFR): partial renal arterial clamp and partial ureteral obstruction. In two experiments the carotid arteries were clamped in an effort to increase blood pressure sufficiently to bring about an increase in GFR. However, the GFR remained constant despite a 10-15% increase in blood pressure. Consequently, the procedure was discontinued.

At the end of each experiment the kidneys were removed and weighed.

Chemical Methods

Standard methods were used for the determination of inulin (41), and glucose (22). Diodrast was determined spectroscopically for its ^{131}I content in a Nuclear of Chicago gamma well counter. Appropriate corrections for physical decay of ^{131}I and checks for instrument drift were made. Sodium determinations were done using a Baird-Atomic flame photometer, Model KY-3.

Glucose is known to interfere with the determination of inulin by the Roe procedure. Corrections for glucose interference were made in each experiment. Because of the interference by glucose, plasma inulin concentrations were maintained at 100 mg/100 ml or higher.

The Determination of Renal Plasma Flow

Diodrast (iodopyracet) labeled with ^{131}I was used in this investigation to estimate the renal blood flow. Para-aminohippurate (PAH) and Diodrast are substances that are actively secreted from the peritubular capillary blood into the proximal nephron (13,16, 24). Diodrast was used instead of PAH because high glucose concentrations interferes with the determination of PAH (5,10,23).

The blood flow was calculated according to the Wolf equation (57):

$$RBF = V(U-R)_D/(A-R)_D, \qquad (4)$$

where U, R, and A represent the concentration of Diodrast (D) in the urine, venous blood, and arterial blood respectively. Renal plasma flow was then calculated by multiplying the RBF by (1-hematocrit).

Renal plasma flow was obtained by this method (rather than by direct analysis of ^{131}I-Diodrast in plasma) because of slow leakage of Diodrast from red cells before centrifugation of the blood (15). This method of estimating renal blood flow agrees quite well with direct measurements of renal blood flow by timed collections of blood from the renal vein (20,32).

The plasma clearance of Diodrast is believed to be a measure of the renal cortical plasma flow (33,38). The assumption made in this case is that all of the Diodrast in the plasma flowing through the cortex is removed and excreted in the urine. A second assumption is that Diodrast in the red cells does not leak out and contribute to the urinary Diodrast while plasma is flowing through the cortex. These assumptions have been shown to be reasonably valid [(32),(49), pages 148-153].

The extraction ratio of Diodrast, E_D, is a measure of the fraction of Diodrast removed from the plasma. The extraction ratio for any substance is defined as follows:

$$E = (P_a - P_v)/P_a \qquad (5)$$

P_a represents the concentration of the substance in the arterial plasma and P_v the concentration of the substance in the renal venous plasma. If a substance is completely removed from the plasma as it passes through the kidney, P_v would be zero and E=1. In this case the clearance of that substance would be a measure of the renal plasma flow. On the other hand, for substances that are completely reabsorbed the extraction ratio is essentially zero at low urine flows.

RESULTS

The Effects of Ureteral Obstruction and Renal Arterial Occlusion on GFR and Tm_G

A sample protocol and the results obtained are shown in Table I. In every experiment in which the ureteral clamp effectively reduced the GFR, Tm_G was also simultaneously reduced. The contralateral control kidney showed no consistent change in GFR during ureteral obstruction of the experimental kidney. Figure 1 shows the results of three ureteral (A,B,C), and one renal artery (D), clamp experiments. Ipsilateral control points are the control points for the experimental kidney. The open circles are contralateral control points and show the relation between Tm_G and spontaneous changes in GFR. Again the Tm_G varies directly with the GFR.

Partial obstruction of the renal artery also caused a decrease in GFR and Tm_G (Fig. 1D). Even though filtered load decreased to values well below the control Tm_G, fractional reabsorption was always less than unity (Fig. 2). If fractional reabsorption had been unity, the slope of the line in Fig. 1D would have been equal to P_G (7.8 ± 0.2 SD mg/ml). The regression line for the data points of the experimental kidney in Fig. 1D has a slope of 4.38 ± 0.08 SE mg/ml. Hence, the renal reabsorptive transport system for glucose is saturated even at very low GFR.

During both control and clamp periods, filtered load of glucose (L_G), was always greater than Tm_G. This is shown graphically in Fig. 2 where Tm_G is plotted as a function of L_G. The 45° line

in Fig. 2 represents a fractional reabsorption of unity. If the filtered load of glucose had at any time decreased to a rate equal to or less than the Tm_G, the points would have fallen close to the 45° line. Only two points (both contralateral control points from dog 4), show fractional reabsorption greater than 0.66 which corresponds to a load to Tm_G ratio of less than 1.5. Thus, load was always greater than Tm_G even during the most severe reductions in GFR.

A mass plot of Tm_G against GFR is shown in Fig. 3. Comparison of Fig. 3 with Fig. 4 shows that the direct correlation between Tm_G and L_G in Fig. 2 is due to variation in GFR and not P_G. Plasma glucose concentration was reasonably constant during an experiment (Table I), and was always well above threshold.

Tm_G varied directly with spontaneous changes in GFR as well as with experimentally induced changes in GFR. Figure 5 shows the relation for the contralateral control kidneys. This same relation is also evident in individual dogs (Fig. 1).

The results shown in Figs. 3 and 5 suggest that Tm_G is a linear function of GFR. If the suggestion is true, then dividing each Tm_G by the GFR for that clearance period will give a ratio which is independent of filtered load. A plot of Tm_G/GFR against filtered load is shown in Fig. 6. This Figure shows that Tm_G/GFR is independent of filtered load and is constant (3.55 ± 0.31 SE mg/ml), except at low L_G. At these lower filtered loads (due to low GFR), the Tm_G/GFR ratio appears to be elevated. A comparison of the

Tm_G/GFR ratio during clamp with its own control ratios is presented in Table II. Tm_G/GFR increased during ureteral obstruction, but during renal arterial clamp the ratio is not different from its control value. Two exceptions are seen to this general statement in dogs 7 and 14 where the ratio appears to be decreased. In dog 14 the apparent decrease is due to a high ratio in the preclamp control period. This ratio is 3.96 ± 0.05 SD mg/ml. The postclamp control ratio was 3.47 ± 0.07 SD mg/ml. The mean of these two values (3.71 ± 0.25 mg/ml), appears as the control ratio for this experimental period. The Tm_G/GFR ratio during clamp was definitely higher for the clamp than for the postclamp control (3.64 mg/ml > 3.47 mg/ml). In dog 7 the decrease is real. One explanation for this decrease is examined in the discussion. Table II also shows the same comparisons for the contralateral control kidney. Note that in most cases clamping the ipsilateral kidney did not produce any consistent change in the Tm_G/GFR ratio in the contralateral kidney.

Results of Renal Plasma Flow Studies

The results obtained from the renal blood flow studies are shown in Tables I and III. Only those dogs that showed significant reductions in GFR with the clamping procedure are included in Table III. The renal blood flow, usually decreased spontaneously during the experiment, but Tm_G varied only with the changes in GFR (Table I). Changes in renal plasma flow and Diodrast clearance

did not always decrease during ureteral clamp and in fact increased in dog 14 (Table III). Both the renal blood flow and the Diodrast clearance decreased during renal artery clamp (Table III).

Renal plasma flow often decreased spontaneously during the experiment (Table I). In contrast, the Tm_G did not decrease except when the GFR also decreased spontaneously.

Sodium Reabsorption Studies

The results of the study on sodium reabsorption are presented in Fig. 7. The reabsorption of sodium, T_{Na}, varies directly with GFR. Normally 98-99% of the filtered load of sodium is reabsorbed by the kidney. This was true in the present study. The plasma sodium ion concentration in 4 dogs averaged 144 ± 3.8 SD mEq/l (N=18 determinations).

FIGURE 1. The Tm of glucose as a function of GFR in four individual dogs. Both contralateral and ipsilateral controls are shown. Each symbol represents the mean of 3 consecutive clearance periods. The symbols are explained in the key.

A, B, and C show the results obtained in 3 ureteral clamp experiments.

D shows the results obtained during partial clamp of the renal artery.

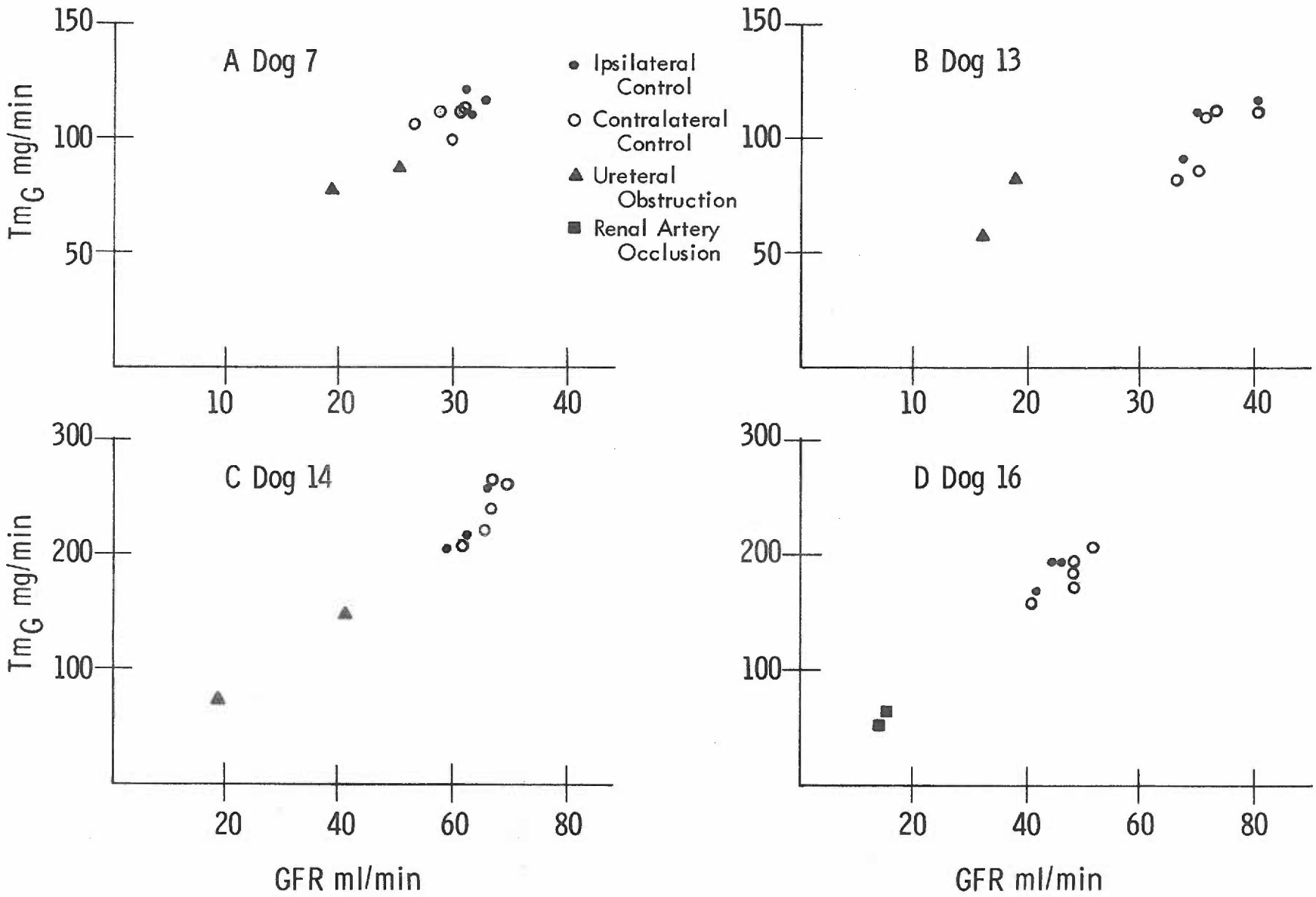

A Dog 7
B Dog 13
C Dog 14
D Dog 16
Ipsilateral Control
Contralateral Control
Ureteral Obstruction
Renal Artery Occlusion
Tm_G mg/min
GFR ml/min
150
100
50
300
200
10
20
30
40
60
80

FIGURE 2. Tm_G is shown as a function of filtered load of glucose (20 kidneys, 10 dogs). Note that the abscissa begins at 100 mg/min, not zero. The ordinate (Tm_G/100 g kidney) does begin from zero. The 45° line represents a fractional reabsorption of 1.0. Each point represents the mean of 3 consecutive clearance periods. Symbols are explained in the key.

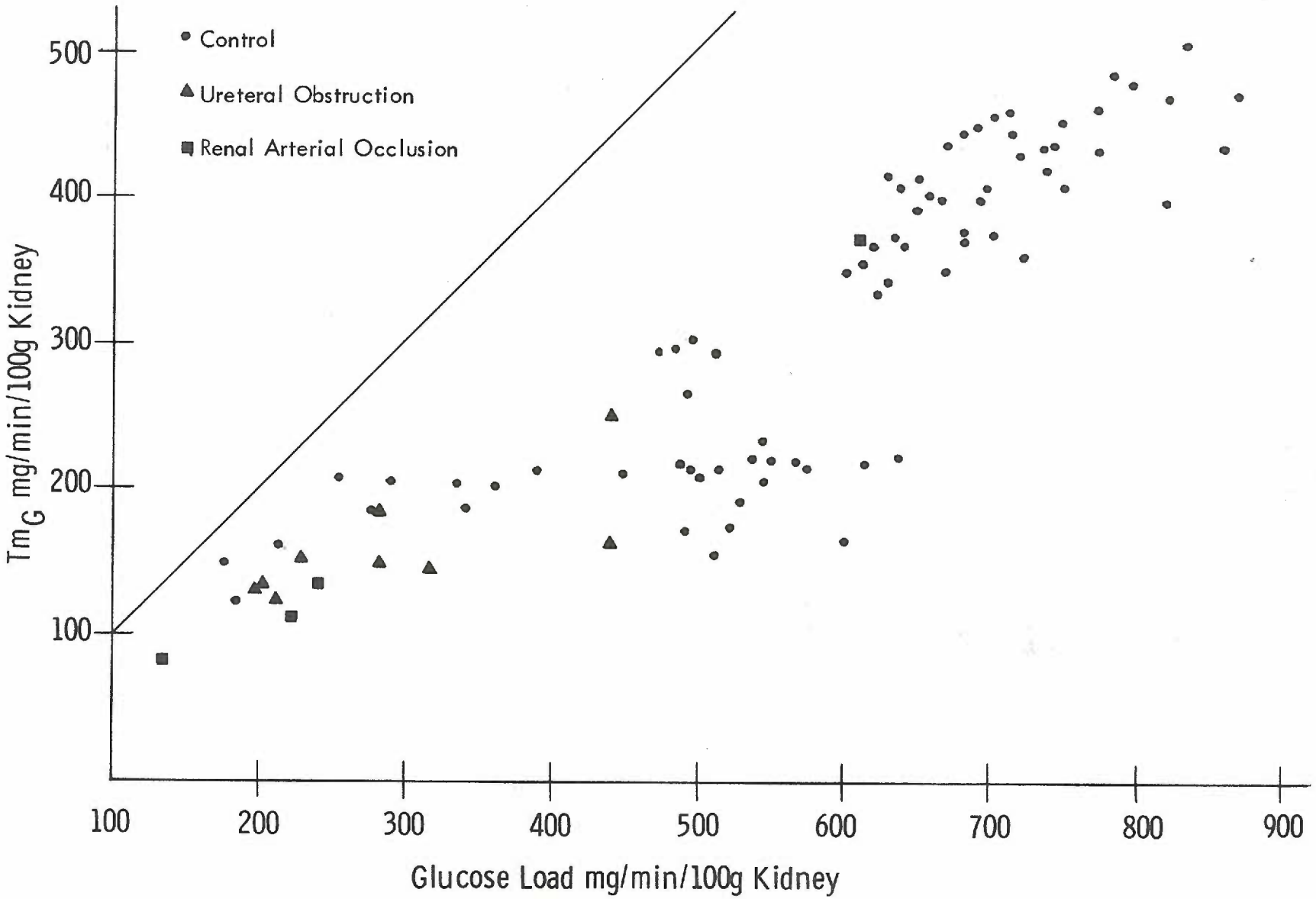

Control
Ureteral Obstruction
Renal Arterial Occlusion
500
400
300
200
100
Tm_G mg/min/100g Kidney
100
200
300
400
500
600
700
800
900
Glucose Load mg/min/100g Kidney

FIGURE 3. Tm_G/100 g of kidney as a function of GFR/100 g kidney (24 kidneys from 12 dogs). This Figure should be compared with Fig. 4. The correlation observed in Fig. 2 is clearly shown to be due to changes in GFR and not changes in P_G. Each symbol represents the mean of 3 consecutive clearance periods.

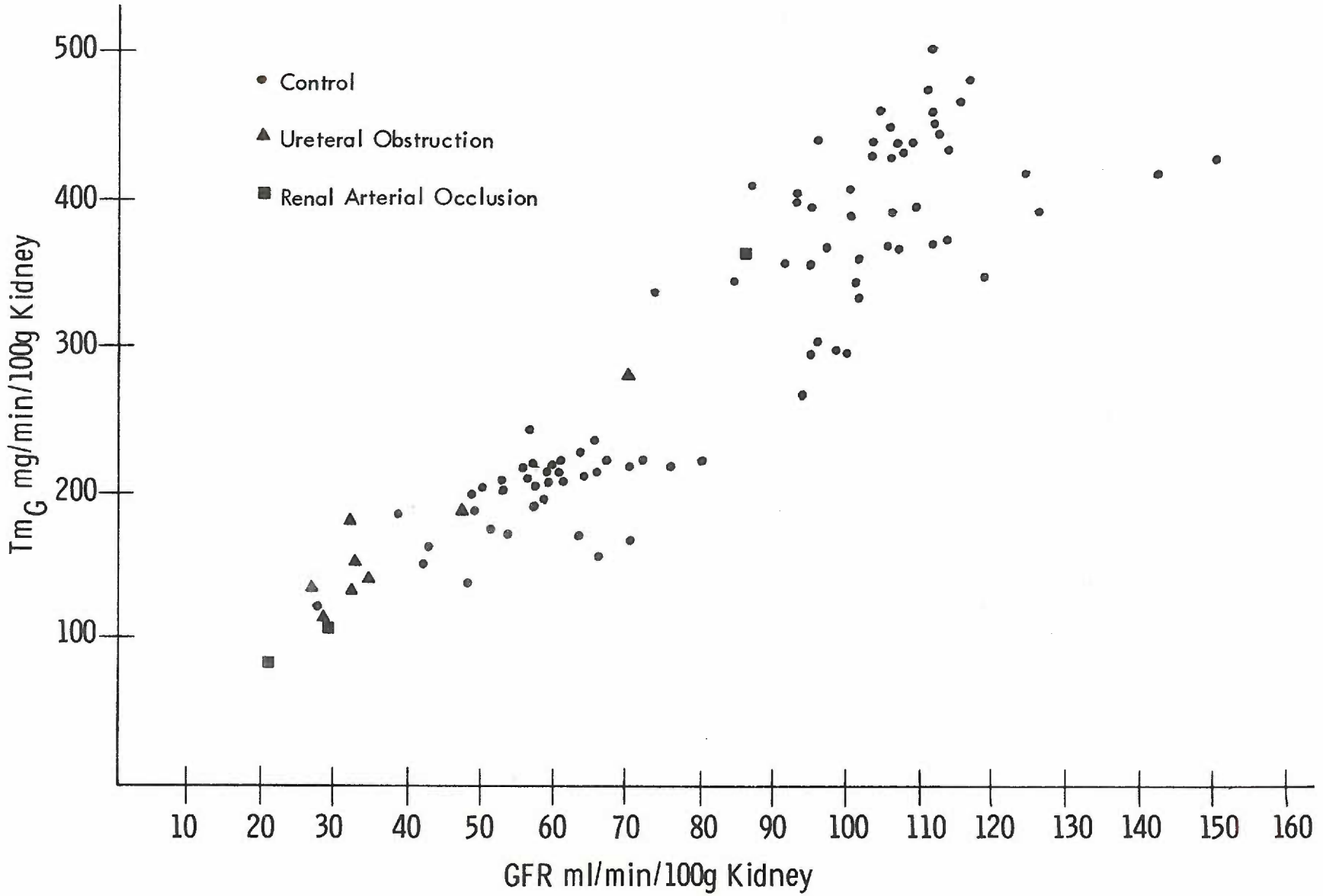

500
400
300
200
100
Control
Ureteral Obstruction
Renal Arterial Occlusion
Tm_G mg/min/100g Kidney
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
GFR ml/min/100g Kidney

FIGURE 4. Tm_G in relation to P_G. (Data from 12 dogs). Each symbol represents the mean of 3 consecutive clearance periods.

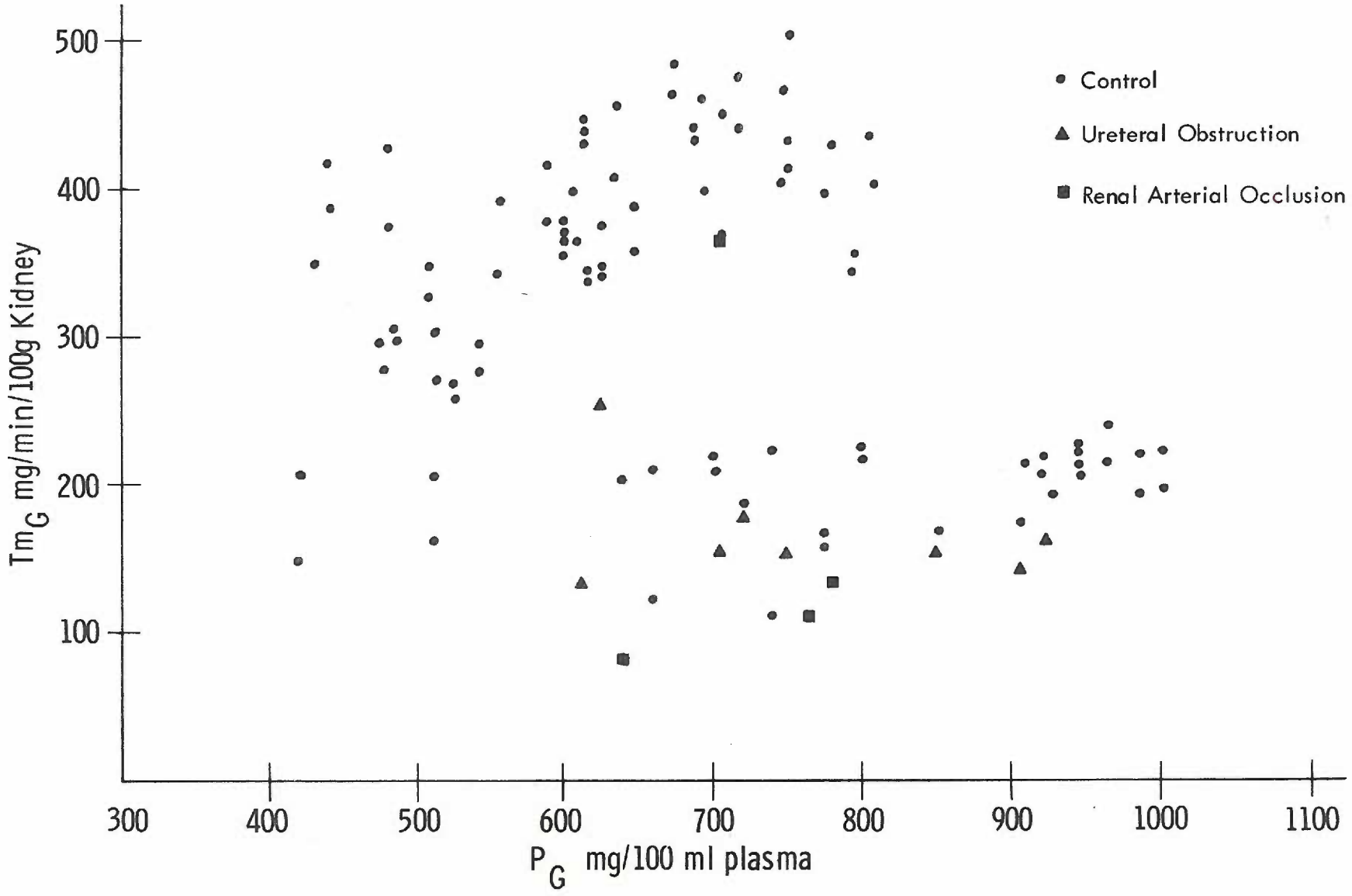

500
400
300
200
100
Tm_G mg/min/100g Kidney
300
400
500
600
700
800
900
1000
1100
P_G mg/100 ml plasma
Control
Ureteral Obstruction
Renal Arterial Occlusion

FIGURE 5. Tm_G as a function of spontaneous changes in GFR (12 dogs). The relation obtained suggests that Tm_G is a function of spontaneous as well as experimentally induced changes in GFR. Each symbol represents the mean of 3 consecutive clearance periods.

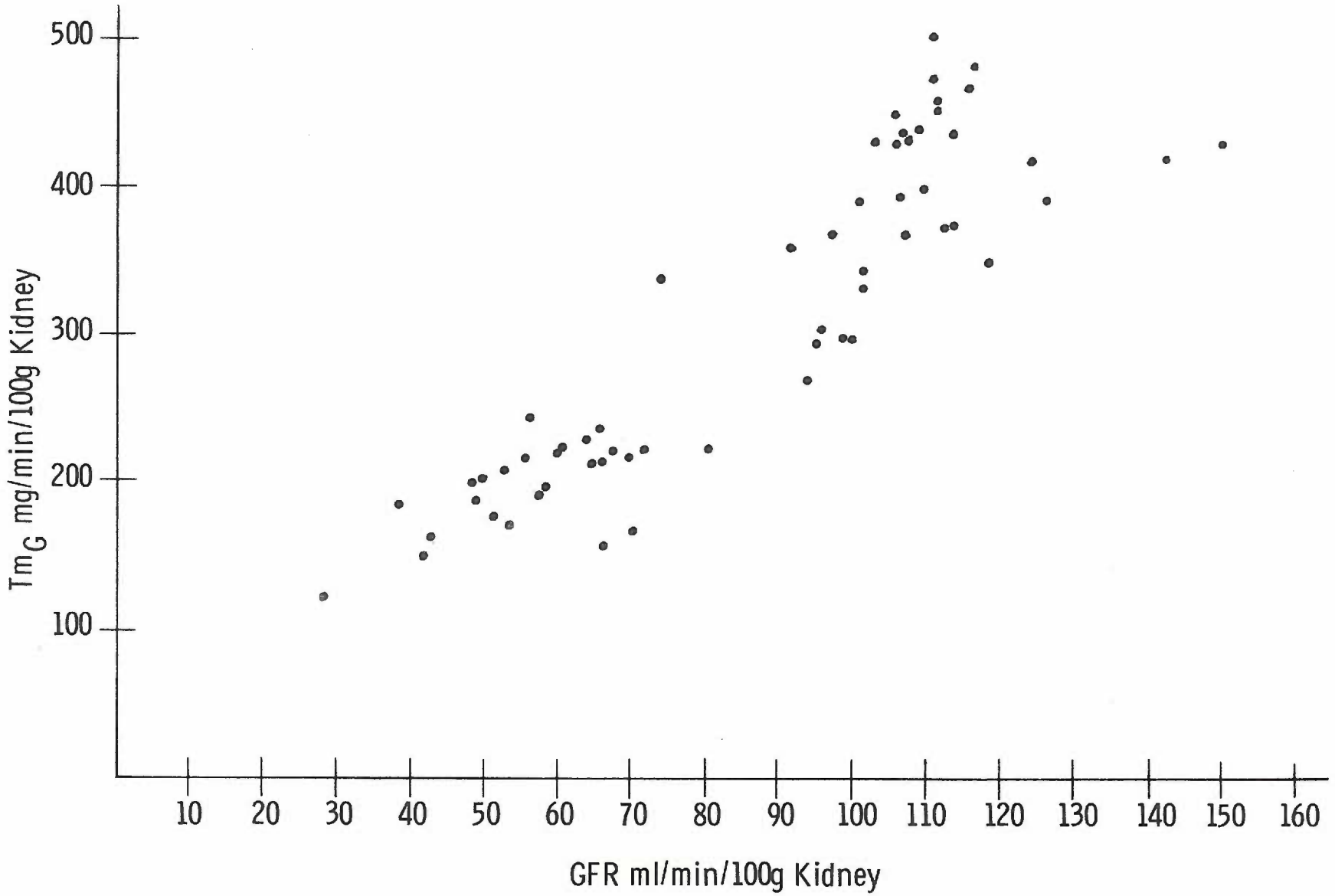

500
400
300
200
100
Tm_G mg/min/100g Kidney
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
GFR ml/min/100g Kidney

FIGURE 6. Tm_G/GFR as a function of glucose load (L_G). Data was taken from control and experimental kidneys of 12 dogs. The horizontal line represents the mean Tm_G/GFR ratio ± 1 SE (shaded area) obtained from control points only (see text). Each point represents the mean of 3 consecutive clearance periods.

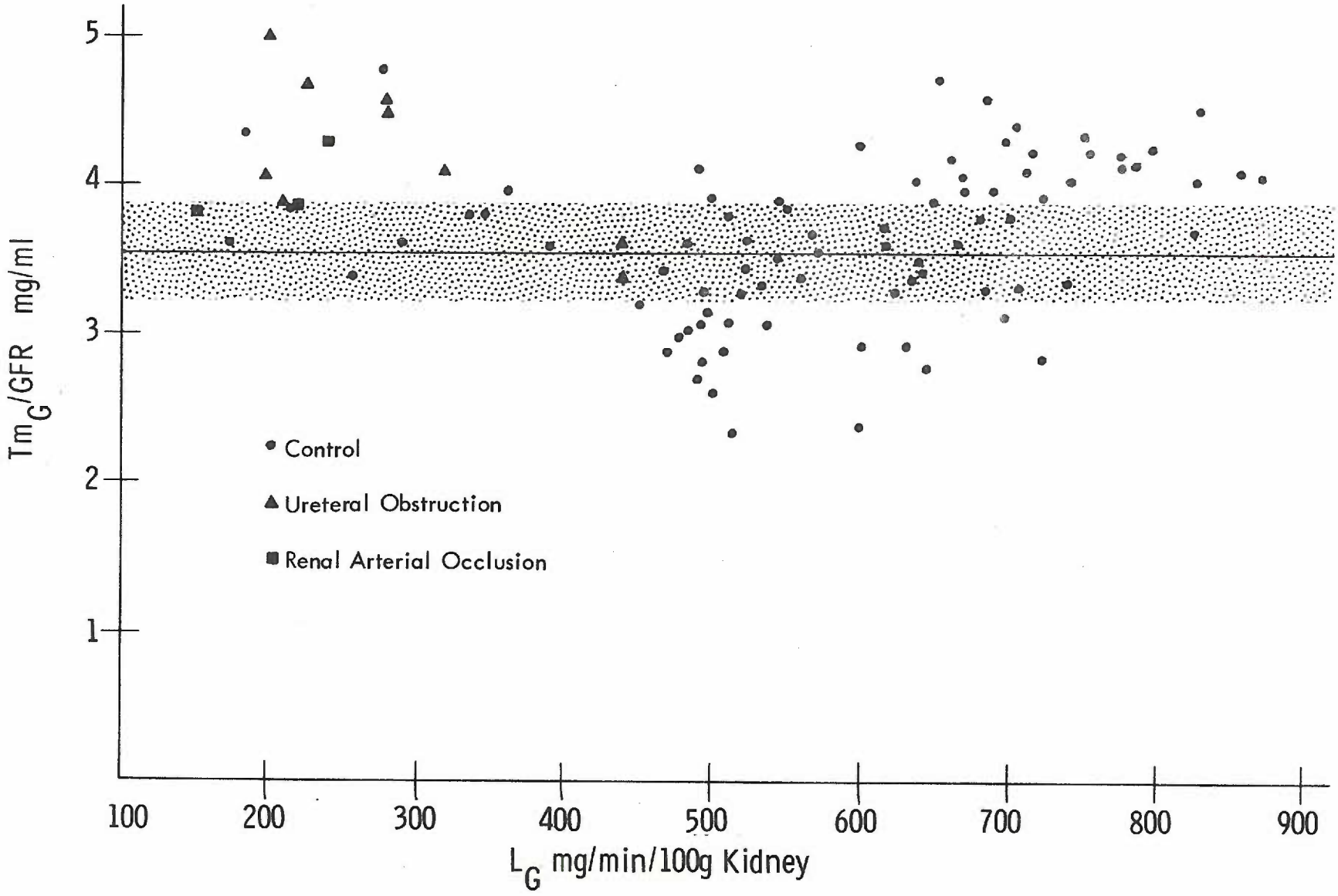

5
4
3
2
1
Tm_G/GFR mg/ml
Control
Ureteral Obstruction
Renal Arterial Occlusion
100
200
300
400
500
600
700
800
900
L_G mg/min/100g Kidney

FIGURE 7. T_{Na} as a function of GFR (8 kidneys, 4 dogs). One datum point not shown was off graph, but lined up with other points. Each point is a mean of 3 consecutive clearance periods.

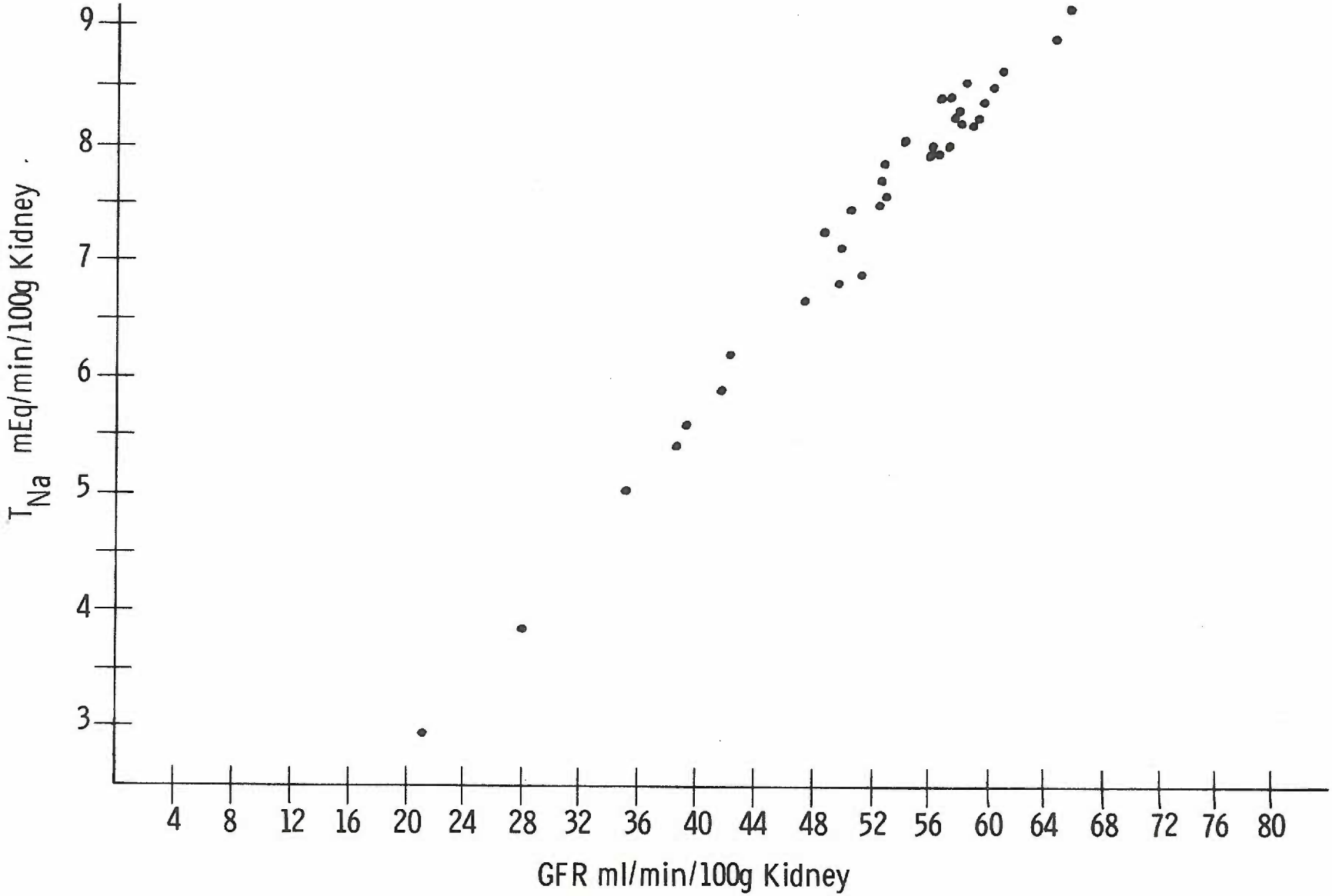

T_{Na} mEq/min/100g Kidney
9
8
7
6
5
4
3
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80
GFR ml/min/100g Kidney

TABLE I Sample Protocol of a Typical Experiment, Dog 13 (20 Kg Male)

Cumulative Time	V	P_G	P_{In}	GFR	Tm_G	Tm_G/GFR	C_D	RPF	E_D
Minutes	ml/min	mg/100 ml	mg/100 ml	ml/min	mg/min	mg/ml	ml/min	ml/min	
-46	Begin infusing priming and sustaining solutions								
0	Begin first control period								
0-3	2.92			42.2	120	2.83	164	-	
3-6	2.75	800	138	39.4	112	2.85	160	-	-
6-9	2.77			40.1	117	2.91	170	-	
9	Partial clamp applied to ureter								
26-29	1.07			18.3	84	4.57	98	205	
29-32	1.05	860	144	17.3	78	4.49	101	214	0.46
32-35	1.06			18.0	83	4.63	106	223	
35	Clamp released								
51-54	2.90			36.6	101	2.75	122	186	
54-57	2.67	776	149	32.4	79	2.45	118	178	0.66
57-60	2.40			32.5	91	2.81	112	170	
60	Partial clamp applied to the ureter								
71-74	0.73			15.1	54	3.58	53	81	
74-77	0.60	774	167	14.9	56	3.77	59	90	0.62
77-80	0.67			16.2	68	4.18	61	93	
80	Clamp released								
92-95	1.80			34.3	108	3.13	95	125	
95-98	1.73	700	162	34.3	111	3.23	94	125	0.70
98-101	1.65			35.0	118	3.38	94	124	

C_D is Diodrast Clearance
E_D is Extraction Ratio for Diodrast

TABLE II The Tm_G/GFR Ratio During Control and Clamping Procedures

Kind of Clamp	Dog	Tm_G/GFR Ipsilateral Control	Tm_G/GFR Experimental	P	P_G During Clamp	GFR % of Control	Tm_G/GFR Contralateral Control	Tm_G/GFR Contralateral Control During Clamp	P	GFR % of Control
		mg/ml	mg/ml		mg/ml		mg/ml	mg/ml		
Ureteral Clamp	4	3.70±0.17	4.58±0.02	<0.01	7.20	70.3	4.36±0.13*	4.78±0.03	<0.025	135
	7a	3.70±0.21	4.10±0.10	<0.05	9.08	59.9	3.78±0.16	3.77±0.19	NS	100.2
	b	3.52±0.07	3.40±0.02	<0.05	9.24	79.4	3.64±0.10	3.31±0.08	<0.01	95.9
	11a	4.37±0.22	4.72±0.04	<0.05	7.08	33.9	4.18±0.09	4.22±0.00	NS	95.2
	b	4.66±0.09	5.02±0.07	<0.01	7.50	29.9	4.38±0.14	4.16±0.02	NS	92.7
	13a	2.78±0.15	4.56±0.06	<0.01	8.60	48.1	2.56±0.26	2.38±0.17	NS	96.0
	b	2.96±0.32	3.84±0.25	<0.01	7.44	45.1	2.71±0.40	3.09±0.03	NS	105.9
	14a	3.71±0.25	3.64±0.06	NS	6.28	64.1	3.69±0.29	3.35±0.08	NS	93.6
	b	3.51±0.08	4.08±0.09	<0.01	6.12	31.3	3.51±0.15	3.77±0.10	<0.05	105.8
Renal Artery Clamp	4	4.32±0.48*	3.84±0.18	NS	6.40	52.7				
	15	4.13±0.12	4.19±0.04	NS	7.04	85.5	4.06±0.07	3.77±0.06	<0.01	88.4
	16a	4.31±0.04	3.83±0.08	<0.01	7.75	31.0	4.05±0.06	3.68±0.18	<0.01	95.5
	b	4.20±0.14	4.31±0.07	NS	7.79	35.2	3.97±0.08	4.03±0.06	NS	102.6

All ipsilateral control values are means of 6 clearance periods ± SD.
All experimental values and contralateral control values during clamp are means of 3 clearance periods ± SD.
* Only three clearance periods are included in these averages.
Levels of significance, P, were obtained from students t-test.
NS: Not significant.

TABLE III Comparison of Renal Plasma Flow with GFR and Tm_G During Control and Clamp Periods

Dog	Procedure	GFR	Tm_G	C_D	RPF	E_D
		ml/min	mg/min	ml/min	ml/min	
			Ureteral Clamp			
11	Control 1	51.0±1.7	223±13	155±10	196±14	0.80
	Clamp 1	17.3±0.2	82± 1	81± 2	170± 4	0.47
	Control 2	48.8±2.5	227±10	118± 1	148± 1	0.79
	Clamp 2	14.6±0.3	73± 2	59± 3	118± 7	0.36
14	Control 1	64.0±2.7	238±24	159± 8	179± 1	0.77
	Clamp 1	41.0±1.7	149± 4	169± 5	245± 7	0.65
	Control 2	60.0±1.9	213± 8	160± 7	213± 5	0.71
	Clamp 2	19.0±1.0	78± 4	53± 4	97± 7	0.54
			Renal Artery Clamp			
15	Control 1	63.3±2.0	262±14	140±11	188± 7	0.70
	Clamp 1	54.1±1.0	226± 5	136± 3	184± 5	0.72
16	Control 1	44.4±0.9	192± 4	109± 6	130± 6	0.78
	Clamp 1	13.8±0.0	53± 1	36± 0	57± 0	0.60
	Control 2	42.2±3.1	178±11	103± 8	137±12	0.70
	Clamp 2	14.9±0.2	64± 2	28± 0	61± 0	0.46

All values except E_D are mean values ± SD.
Control means are determined from 6 clearance periods.
Experimental means are determined from 3 clearance periods.

DISCUSSION

The Variation in Tm_G as a Function of Changes in GFR

The results presented in Table I and Figs. 1, 3, and 5 show that Tm_G decreases as GFR decreases. When ureteropelvic pressure is increased by ureteral obstruction, those glomeruli that are perfused at the lowest effective filtration pressure will tend to cease filtering (29). In the nephrons that are no longer receiving filtrate, glucose reabsorptive rate will fall to zero. Hence, reduction of GFR by ureteral obstruction will simultaneously tend to reduce the number of nephrons reabsorbing glucose by stopping filtration into those nephrons. Therefore, whole kidney Tm_G should decrease as GFR is reduced. An alternative argument used by Thompson, et al. (51) is that the reduction in GFR could reduce filtered load below Tm_G for some nephrons. This would also tend to lower the calculated Tm_G as GFR was reduced. Thompson, et al. (51) argued that the oversaturation of only a few nephrons could account for the glucose excreted. Had this occurred in the present study the Tm_G/GFR ratio would have approached the value of P_G in mg/ml. The data of Table I and II demonstrate that this did not occur. Although the Tm_G/GFR ratio did increase during ureteral clamp, the increase was not sufficient to approach the value of P_G. In addition, during partial clamp of the renal artery Tm_G also decreased as GFR decreased (Fig. 1D), but the Tm_G/GFR ratio did not change from control values (Table II). If the decreased Tm_G were due to the reduction of filtered load below

Tm_G for some nephrons, Tm_G/GFR should have increased during partial renal arterial occlusion also. There is an alternative explanation for the increase in Tm_G/GFR during ureteral clamp and is discussed later. Hence, the decrement in Tm_G which accompanied the reduced GFR during renal artery and ureteral clamp is due to the loss of functioning nephrons and not due to lack of saturation of the glucose transport system. In Fig. 1 both the contralateral control and experimental data are shown for individual dogs. Tm_G varied directly with GFR not only during experimentally induced changes in filtration rate, but also during spontaneous changes in GFR. All of the contralateral control values of Tm_G are plotted against their respective values of GFR in Fig. 5. This mass plot shows the striking direct correlation between Tm_G and GFR. These results may be interpreted as evidence indicating Tm_G is a direct function of GFR in the normal animal.

Other investigators (16,46,47) did not see this correlation in their experiments. One reason for this may be that these investigators reported their results in terms of T/Tm, i.e., observed rate of glucose reabsorbed/average Tm_G for the experiment. This method of presenting data tends to mask any relation between Tm_G and GFR. In the paper by Shannon and Fisher (46) describing a maximum reabsorptive rate for glucose, the authors plotted Tm_G/100 ml of glomerular filtrate against P_G and found

that the ratio was constant above threshold. Table 1D, below, shows Tm_G/GFR ratios calculated from their data obtained from one dog (Table I of their paper). The rate used for

Table (1D)

Period	Tm_G mg/min	GFR ml/min	Tm_G/GFR mg/ml	T/Tm_G
5	270	114	2.37	1.01
6	255	110	2.32	0.96
7	282	121	2.33	1.06
8	267	116	2.31	1.00
9	257	117	2.20	0.97

Tm_G used in calculating T/Tm_G was the mean rate obtained from that dog, 266 mg/min. It appears that Tm_G varied directly with GFR in their animals also.

An alternative explanation for the constant Tm_G/GFR ratio seen in the experiments of Shannon and Fisher is as follows. These investigators measured urine flow by measuring the volume of urine obtained from the dog and dividing that volume by the collection time. There could have been a rather substantial error introduced in the urine flow measurements. If even 5 ml of urine remained in the bladder after emptying, the error in urine flow could be 10% in periods 5 and 6. This error would be carried over into the calculation of Tm_G, since $Tm_G = V\,[P_G\,(U/P)_{Cr} - U_G]$. This error cancels if the Tm_G is divided by the GFR since $GFR=V(U/P)_{Cr}$. Hence, GFR and Tm_G may not have varied as much as was reported and the Tm_G/GFR ratio would have to be constant.

Thompson, et al. (51) believed that Tm_G was independent of the filtration rate within the normal range of variation of GFR.

They argued that at reductions in GFR of 40-50% of control values (produced by aortic constriction, dehydration, or hemorrhage), the filtered load of glucose would be reduced below the Tm_G of many of the nephrons. As discussed previously this did not occur in the present study. A point should be made about normal range. If a normal range of variation of GFR is seen in the contralateral control kidneys of this study, then the results obtained in these experiments disagree with their interpretation. However, if normal range includes the spontaneous variations in GFR that can occur in response to stress, then the results reported in their paper are not in agreement with their conclusions. The real issue under examination is the mechanism by which the kidney maintains fluid and electrolyte balance. Presumably Tm_G is a measure of the number of nephrons that are functioning in a given situation. If this is true then a decreased GFR with a decreased Tm_G should imply that there is a reduction in the number of functioning nephrons. To argue that the variations seen in the GFR are not normal is beside the point. If an animal can show large variations in GFR with concomitant and proportional changes in its Tm_G to various physiological stresses, then glomerular intermittency may be a mechanism by which the animal adjusts his renal function to changes in its internal environment. If glomerular shutdown can occur with large changes in GFR then it is possible that it could also occur with small changes in GFR.

The variations seen in Tm_G are not due to changes in plasma glucose concentration. Figure 4 clearly shows that Tm_G is not correlated with P_G in any of the experiments reported in this study.

If Tm_G is proportional to the GFR, then Tm_G divided by GFR should be constant and independent of the filtered load of glucose. A plot of Tm_G/GFR against filtered load is shown in Fig. 6. The horizontal line represents the mean Tm_G/GFR ratio determined from the contralateral control kidneys. The mean value was 3.55 ± 0.31 SEM mg/ml. Ratios for clamping periods were not included in the mean because there is evidence that the Tm_G/GFR ratio is elevated during the clamping procedure. Comparing Tm_G/GFR during ureteral obstruction and renal arterial occlusion with their respective ipsilateral controls revealed that Tm_G/GFR was significantly higher than the controls during ureteral obstruction (Table II). In contrast, the Tm_G/GFR ratio was not altered by the arterial clamping procedure (Table II). These results obtained from partial occlusion of the renal artery are in agreement with those obtained by others (18,19). In two cases (dogs 7 and 14, Table II) Tm_G/GFR did not increase during ureteral clamp. Dog 7 showed a significantly decreased ratio during the second ureteral clamp. In this animal the GFR was reduced to approximately 80% of control. This small a reduction in GFR implies that at best only a small percentage of the nephron population

ceased functioning. A possible explanation for the decreased Tm_G/GFR ratio in dog 7 is that those nephrons that are no longer functioning may have had a higher reabsorptive capacity for glucose than the mean for the whole kidney. The Tm_G/GFR ratio seen in the first clamp of dog 14 has already been discussed in the results section.

Tm_G as a Function of Changes in Renal Plasma Flow

The results of four separate experiments in Table III (dogs 11, 14, 15, 16) indicate that Tm_G is not a function of renal plasma flow (RPF). As shown in Table I (dog 13), the RPF decreased from 220 ml/min in the first control period to 125 ml/min in the last control period, yet Tm_G was virtually unaffected by this change in RPF. Furthermore, the RPF was unchanged or even increased during many of the ureteral clamp periods, yet the Tm_G declined whenever the GFR dropped (dogs 13 and 4). These results showing that changes in RPF are unpredictable with ureteral clamp are in agreement with those results obtained by others (20,21). Changes in plasma Diodrast clearance were also unpredictable during ureteral clamp. With renal artery occlusion both C_D and RPF decreased. This latter result is due to the increased vascular resistance caused by the clamp. It is concluded from the evidence obtained in this study that Tm_G is independent of the RPF.

One assumption that is made in estimating RBF (equation 4) using Diodrast is that the amount of Diodrast appearing in the urine

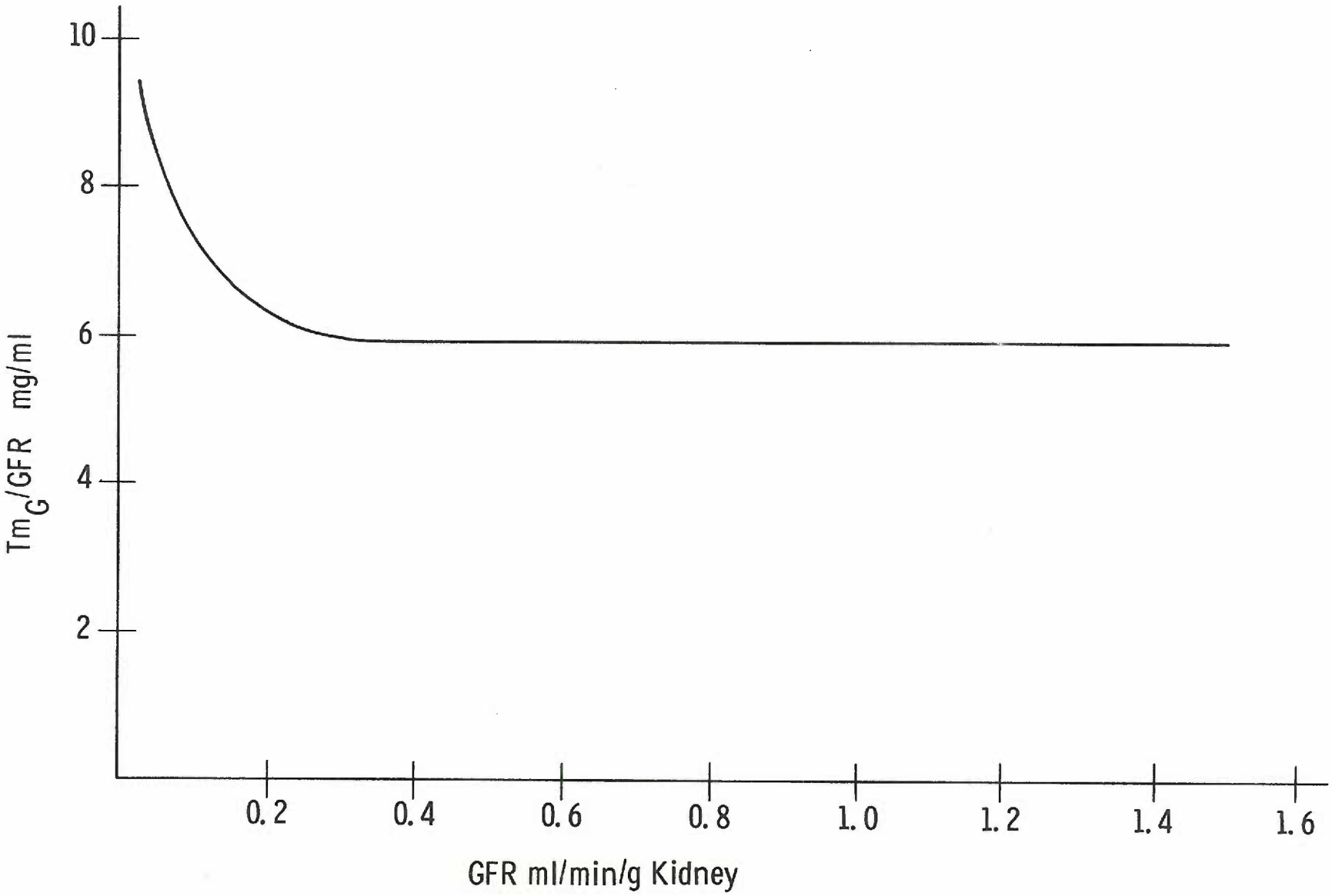
is equal to the amount removed from the plasma, i.e., a steady state exists. When a ureteral or arterial clamp is first applied or released, non-steady state conditions will prevail temporarily. After sufficient time for equilibration, a new steady state should be reached in which the rate of removal of Diodrast from the plasma equals the rate of appearance of Diodrast in the urine. In support of this, Hársing, Szánto, and Bartha (20) and Hársing, Bartha, Harza, and Köver (21) have found good agreement between the ratio of the cortical blood flow estimated from ^{86}Rb uptake to total blood flow measured directly from venous outflow and E_{PAH} during stop flow in dogs. Hence the values shown for RPF in Table III seem reasonable.

However, suppose for the moment that non-steady state sequestration occurred during clamp. How would this affect the interpretation of the results reported in Tables I and III? During clamp the reported RPF's would be less than the actual RPF's. Thus the conclusion that Tm_G is independent of RPF is strengthened, not weakened by non-steady state sequestration.

An Analysis of Glucose Reabsorption in Terms of the Ratio of Tm_G to GFR

Van Liew, et al. (52) found that the Tm_G/GFR ratio in rats increases at low filtration rates (Fig. 8). These authors believed the increased ratio was due to increased contact time of filtrate with the proximal tubule cells. They reasoned contact time should increase when the GFR decreases. There is evidence that the contact time or transit time, is not increased with spontaneous changes in GFR (43). Even if the contact time were prolonged over that seen at

FIGURE 8. Tm_G/GFR as a function of GFR. This Figure was redrawn from the results of Van Liew, et al. (52). GFR is expressed per gram of kidney weight instead of per 100 grams. The mean ratio obtained by Van Liew, et al. (52) in rats (5.9) was higher than that obtained for dogs (3.55) in the present study.


10
8
6
4
2
Tm_C/GFR mg/ml
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
GFR ml/min/g Kidney

higher filtration rates, Tm_G would not necessarily increase. Tm_G is a reabsorptive rate, i.e., reabsorption in milligrams per minute. Increasing the contact time will increase the time that an elemental volume remains in contact with the internal surface, and might allow for a greater fractional reabsorption of glucose and filtrate. Nevertheless, there is no reason to believe that increased contact time would increase the rate at which glucose was reabsorbed.

If Tm_G were a fixed constant, then a plot of Tm_G/GFR against GFR would appear as shown in Fig. 9. As can be seen from equation 6

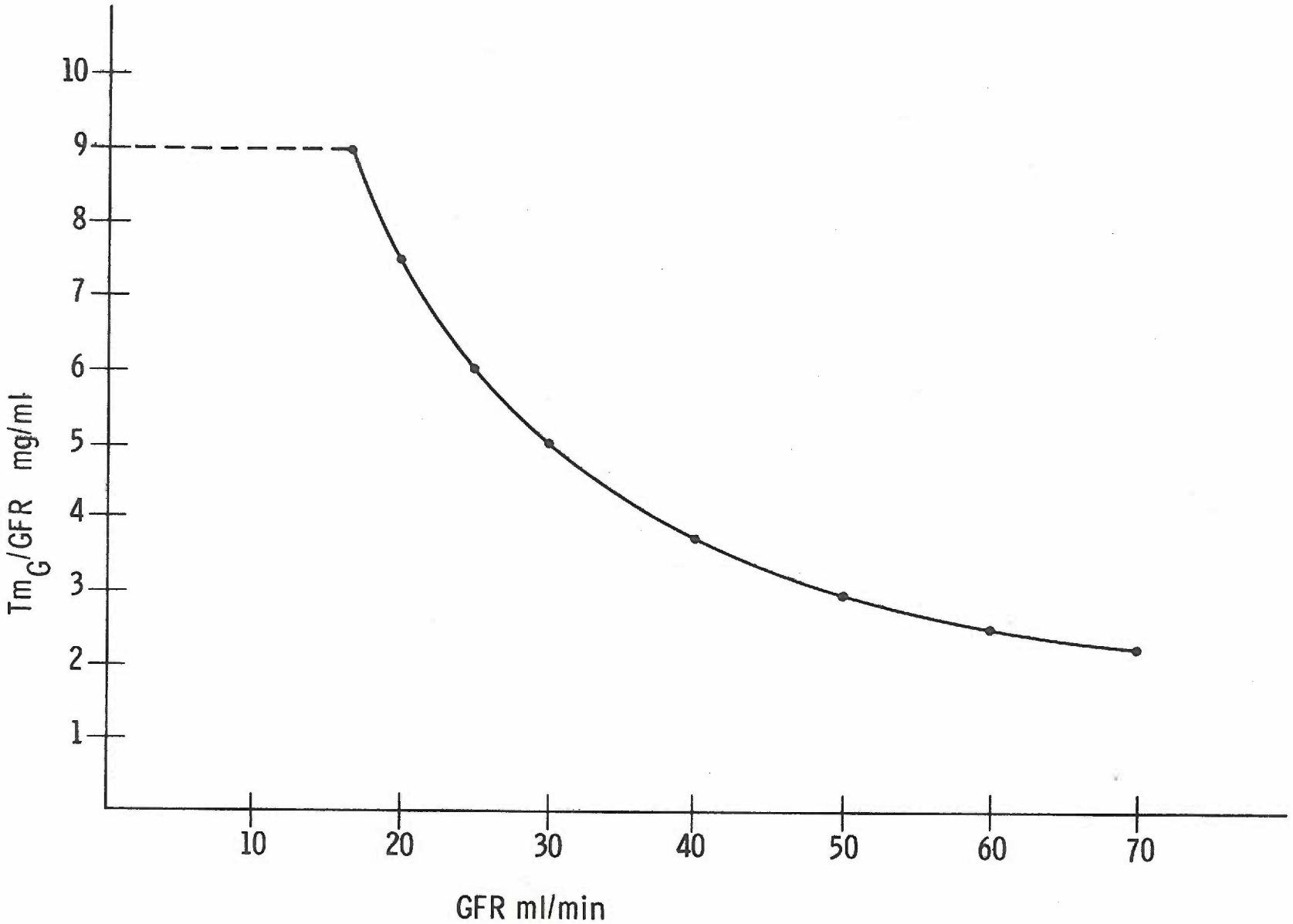
$$Tm_G/GFR = P_G - U_G V/GFR \qquad (6)$$

Tm_G/GFR is less than P_G and will theoretically approach zero asymptotically. However, the evidence presented in this study and elsewhere (18,19,52) indicates that Tm_G is not a fixed constant, but is a variable dependent on the GFR. Above a certain minimum GFR, Tm_G/GFR is constant for the whole kidney during periods of spontaneous changes in GFR (52) and reduced GFR produced by clamping the renal artery (Table II). The tm_g/gfr* ratio has also been shown to be constant for the perfused nephron (11). Below the critical rate of glomerular filtration, Tm_G/GFR increases with further reductions in the filtration ratio (52).

In order to consider some of the mechanisms that can be invoked to explain the results in both the present study and that of Van Liew, et al. (52), the discussion of the Tm_G/GFR ratio will be divided into

* Lower case letters will be used when referring to parameters in individual nephrons.

FIGURE 9. This Figure illustrates how the Tm_G/GFR ratio would be affected by changes in GFR if Tm_G were a fixed constant. This curve was not obtained in the present study nor in that of Van Liew, et al. (52).


10
9
8
7
6
5
4
3
2
1
Tm_G/GFR mg/ml
10
20
30
40
50
60
70
GFR ml/min

two parts. First, the events relating glucose reabsorption to filtration rate in individual nephrons will be considered in terms of a model in which the luminal surface area of the nephron changes with gfr. The second part will describe how individual nephron activity relates to the whole kidney Tm_G and GFR. In addition, in this part of the discussion the results of the present study and those of Van Liew, et al. (52) will be interpreted in terms of the surface area model.

The Constancy of tm_g/gfr with Spontaneous Variations in gfr

In the first part of this discussion two assumptions are made: (1) The filtered load of glucose for an individual nephron is greater than the tm_g for that nephron and (2) changes in gfr are accompanied by proportional changes in tubular diameter by an unspecified mechanism. As discussed in the introduction there is evidence that tm_g is directly proportional to gfr. Luminal diameter, and therefore surface area, is directly proportional to gfr (43), or perfusion rate (11). A change in the reabsorptive surface area could affect tm_g by at least two mechanisms. First if the canine proximal tubule is passively permeable to glucose, then decreasing the surface area would decrease the diffusive component of glucose reabsorption. In support of this argument it has been found that the proximal tubule of rats has a rather low permeability to glucose (6,11,28). When the filtered load of glucose is greater than the tm_g, the transtubular concentration gradient which arises as a consequence of water reabsorption would favor diffusion from the tubular lumen to the

peritubular capillary. However, it is likely that the permeability of the dog proximal tubule to glucose is not as high as that reported for the rat. Swanson and Thompson (50) found that the proximal tubule of the dog kidney was essentially impermeable to glycerol, a polar solute that is smaller than glucose. Peña and Malvin (31) were unable to demonstrate any passive permeability of the proximal tubule to glucose in stop flow studies in dogs. It appears, therefore, that passive diffusion cannot be invoked to explain the proportionality between tm_g and gfr.

On the other hand, changes in the gfr might be associated with changes in the degree of separation of the microvilli that form the luminal surface of the proximal tubule cells (39). When gfr decreases spontaneously in rats the tubular volume is diminished (43). The reduced surface area brought about by the reduced gfr might compress the microvilli more closely together and thereby decrease the total number of transport sites that are exposed to the tubular fluid. Thus, changes in tubular diameter could bring about proportional changes in the total number of sites for active transport of glucose. Therefore, it is predicted from the surface area model that changes in gfr are associated with proportional changes in surface area and hence, sites for transport of glucose. As the gfr changes, then tm_g will also change in the same direction thereby keeping tm_g/gfr constant for an individual nephron.

The tm_g/gfr Ratio at Low gfr

If glucose reabsorption is a direct function of tubular surface

area, then tm_g will decrease in proportion to gfr only as long as the reabsorptive surface area also decreases.

This model of the nephron is analogous to a piece of compliant rubber tubing which is filled with enough water to stretch its walls and then clamped. Now, if a slow leak is produced by puncturing the wall with a needle both the diameter of the tubing and its luminal surface area will decrease as fluid leaves via the puncture site. The luminal surface area will decrease with decreasing volume until the walls of the tubing reach their unstretched circumference. From this circumference further decrements in volume of the tubing would occur by collapse of the walls, but at a constant and minimal luminal surface area.

In the nephron, at this minimum surface area, a certain number of active sites would remain exposed to the filtrate thereby maintaining a constant tm_g. Further reductions in gfr would not be accompanied by corresponding reductions in tm_g, i.e., the tm_g would be constant. Under these circumstances the tm_g/gfr ratio would increase as gfr is decreased. Therefore, both the constancy of the tm_g/gfr ratio and the increase in this ratio below a critical gfr may be explained on the basis of a surface area model. In support of this model, Schnermann, Wahl, Liebau, and Fischbach (43) have shown that in rats the rate of sodium reabsorption is directly proportional to luminal surface area of the proximal tubule. As pointed out in the introduction, a more detailed analysis of the experiments reported by Deetjen and Boylan (11) showed that tm_g per unit length of

nephron divided by the perfusion rate was constant.

Further support for the model comes from microperfusion studies on glucose reabsorption in the rat (11). This paper was reviewed in the introduction. These investigators found that tm_g increased as the perfusion rate was increased. I reanalyzed the data presented in Table I of their paper. When I divided the tm_g per unit length of nephron by the perfusion rate, V_o, a value of $tm_g/length/V_o$ was obtained that is analogous to tm_g/gfr. The units for this ratio are moles/mm tubular length/nl perfusion rate. The ratios calculated from nephrons not dilated by ureteral clamp were not significantly different from one another for any of the perfusion rates used ($P > 0.1$). However, during ureteral clamp the $tm_g/length/V_o$ ratio was significantly greater than ratios obtained from experiments without ureteral clamp ($P < 0.02$). Thus the results obtained from microperfusion of individual nephrons confirm experimentally the predictions made from the model.

An experiment that should be done to test the validity of the model is outlined below. The protocol is similar to that used by Deetjen and Boylan (11), but differs from their technique in that each nephron is to be perfused at more than one rate. Individual segments of proximal tubules should be perfused with Ringers solution with glucose at a concentration of 800 mg/100 ml. The high concentration of glucose would insure complete saturation of the reabsorptive mechanism over a wide range of perfusion rates. Perfusion rates should be varied in increments from approximately 10 nl/min (low perfusion rate) to as high as 50-50 nl/min (high perfusion rate).

This range of perfusion flows was selected from computations made from values of GFR and the number of nephrons per kidney and values of gfr obtained in the literature (7,42). The results obtained from this study should be expressed in terms of tm_g/V_o per unit nephron length and plotted against glucose load. This should give a curve similar to that of Fig. 8 if the tubular surface area is a key factor in glucose reabsorption.

Tm_G/GFR in the Whole Kidney

Glomerular filtration rate is dependent on the net pressure difference across the glomerular capillary wall. This net pressure difference is called the net effective filtration pressure (NEFP). The factors determining the NEFP and their relation to each other are shown in equation 7:

$$NEFP = (P_C - P_B) - (\pi_C - \pi_B) \qquad (7)$$

where P represents the hydrostatic pressure, π the osmotic pressure in C, capillary plasma and B, Bowman's capsule fluid. Since, normally all constituents in the plasma with the exception of cells and plasma proteins are freely filterable, equation 7 becomes:

$$NEFP = P_C - (P_B + \Pi) \qquad (8)$$

where Π represents the colloidal osmotic pressure of the plasma.

Spontaneous changes in GFR can occur via changes in the net effective filtration pressure by either constriction or dilation of the afferent and efferent arterioles. The diameter of individual nephrons increases and decreases in direct proportion to the nephron

gfr (43). Whole kidney GFR can be spontaneously reduced when a fraction of the glomeruli cease filtering or when all glomeruli have a reduced rate of filtration. However, as discussed previously there is ample evidence (18,19,29) that glomerular shutdown may play a role in the normal variation of GFR. It is not assumed that glomerular shutdown excludes the possibility that the GFR can be reduced in all glomeruli simultaneously. Probably the GFR may be reduced normally by both methods.

It may be predicted from the surface area model that the tm_G/gfr ratio should increase before complete cessation of filtration occurs. This increase in tm_G/gfr was not reflected as an increase in Tm_G/GFR at GFR values above 25 ml/min/100 g kidney. One reason the Tm_G/GFR ratio did not increase could be that at GFR's above 25 ml/min/100 g kidney, so many nephrons are still left functioning that the increase in the ratio is masked. At GFR's less than 25 ml/min/100 g kidney, the number of functioning nephrons is severely reduced. At the lower GFR, masking would presumably not occur to the extent that it would at the higher GFR and the predicted increase in the Tm_G/GFR ratio would be more readily detected. This increase in the Tm_G/GFR ratio at low GFR has been observed by Van Liew, et al. (52).

The Tm_G/GFR ratio would also increase if filtered load of glucose became less than the Tm_G. The ratio would become constant and equal to P_G (equation 6). In all of the experiments in this study the Tm_G/GFR ratio was always much less than P_G (Tables I and II).

The ratio may have been close to P_G in one or two instances in the results obtained by Van Liew, et al. (52) but, generally the Tm_G/GFR ratio was much less than P_G. During partial occlusion of the renal artery in the present study no increase in Tm_G/GFR was observed. This could be due to an insufficient lowering of the GFR. In dog 4 the GFR was reduced to 50% of the control during the arterial clamps (Table II). It is possible that GFR may have to be reduced more in the dog than in the rat to produce an increase in the Tm_G/GFR ratio.

A decrease in the proximal tubular volume has been observed in rat kidneys during constriction of the renal artery (8,36). This has not been confirmed by others (7,53). In dogs reductions in GFR produced by reducing arterial blood pressure with drugs are associated with a decreased tubular volume (18,19). There are not as many micropuncture studies of dog kidneys as there are of rat kidneys. The only evidence available for dogs indicates that tubular volume decreases as GFR decreases spontaneously or after arterial clamp (25).

Tm_G During Partial Ureteral Obstruction

Increments in the ureteropelvic pressure will elevate nephron hydrostatic pressure and, as can be seen from equation 3D, reduce the effective filtration pressure. When the ureteropelvic pressure is increased so that the hydrostatic pressure in the nephron at Bowman's capsule is equal to $P_C - \Pi$, gfr will cease for that nephron (29) and (equation 8). Unlike the case for partial renal arterial

occlusion, the nephrons should not collapse or decrease in diameter as GFR is decreased. In fact, proximal tubular diameter increases during ureteral obstruction in rats (7,8,36). If tm_g is a function of tubular surface area, then the tm_g/gfr ratio in an individual nephron should be higher during partial ureteral obstruction than it is during control periods. Also for a whole kidney, the Tm_G/GFR ratio during partial ureteral obstruction should be greater than the control ratio. Table II shows that the Tm_G/GFR ratios during ureteral obstruction were significantly elevated above their control values while the contralateral control kidney showed no consistent variation in Tm_G/GFR during the clamp. In contrast to these results, during partial renal arterial occlusion no consistent changes in the Tm_G/GFR ratio were noted and for the most part these were not different from control values. During ureteral clamp the luminal surface area does not decrease in proportion to the decrement in GFR. Hence, Tm_G for the kidney was actually greater than it would have been had the surface area decreased in proportion to GFR as it did during renal artery constriction.

As noted above the Tm_G/GFR ratio did not change during partial occlusion of the renal artery. This constant Tm_G/GFR ratio is explained as being partially due to the simultaneous reduction in reabsorptive surface area with reductions in gfr. During ureteral obstruction reabsorptive surface area cannot decrease as gfr decreases, in fact it increases. Thus, Tm_G/GFR should increase as GFR is reduced. An increase was indeed observed (Table II). If the GFR is then reduced further by increasing the degree of ureteral obstruction, then

the Tm_G/GFR ratio should be increased even more. This is predicted from the model since luminal surface area of a nephron cannot decrease during ureteral obstruction. In the study reported by Malvin, et al. (29), the Tm_G/GFR ratio* was elevated from 3.47 during control to 3.83 during an elevation of ureteropelvic pressure to 24% of the mean blood pressure (GFR reduced 15%). When the ureteropelvic pressure was elevated to 41% of the mean blood pressure (GFR reduced 40%), the Tm_G/GFR ratio was elevated to 4.85. Similar results were obtained in this study (dog 14, Table II). Thus the prediction made from the model that Tm_G/GFR should increase as ureteropelvic pressure is increased has been confirmed experimentally.

Sodium Ion Reabsorption in Relation to the Surface Area Model

As stated in the introduction the original purpose of this investigation was designed to determine the role of changes in luminal surface area on reabsorptive rates in the proximal tubule. Investigations directed to the role of tubular geometry on sodium ion reabsorption have yielded conflicting results. Nevertheless, the results from some of these investigations suggest that luminal surface area plays an important role in the reabsorption of sodium ions.

In most of the studies using rats, the tubular volume decreases when the GFR is caused to decrease by aortic or renal artery constriction (4,7,8,17,36). However, Baines, Gottsschalk and Leyssac (4)

* The Tm_G/GFR ratios reported above were calculated from the results presented in Table I of the paper by Malvin, et al. (29).

reported that the volume reduction is only temporary. These authors found that the tubules began to dilate after 10 minutes and reached their normal diameter by 60 minutes despite the continued low GFR. As discussed previously, the proximal tubular volume in dog kidneys decreases as GFR decreases (25).

Both an increased (7,53) or unchanged (7,36) fractional reabsorption (estimated from the proximal TF/P ratio of inulin in rats) after arterial clamp have been reported. Those investigators who reported an increased $(TF/P)_{In}$ maintained that tubular volume was the same as control volumes (17,53). In one paper an increased $(TF/P)_{In}$ associated with a decreased tubular diameter during aortic constriction was reported (7). This has not been documented by others. Schnermann, et al. (43) found that $(TF/P)_{In}$ remained constant despite spontaneous and proportional changes in GFR and tubular volume. In addition these investigators reported a direct correlation between t_{Na} and luminal surface area of the proximal tubule. Wiederholt, Hierholzer, Windhager, and Giebisch (58) found a similar correlation in microperfusion studies. It should be pointed out that the authors of some of these papers do not believe that t_{Na} is a function of luminal surface area (4,7,17,44). Nevertheless, their results can be interpreted as evidence to support the hypothesis that the luminal surface area exposed to tubular fluid plays a direct role in sodium ion reabsorption during aortic or renal artery constriction. It should be emphasized that in addition to luminal surface area, there are other important factors that

affect sodium ion reabsorption, e.g., according to Earley and Daugharty (12) the oncotic pressure of the peritubular capillaries may be such a factor.

When the ureteropelvic pressure is elevated,the proximal tubules dilate, but gfr decreases (7,8,36,44). Rector, Brunner, and Seldin (36) found that the $(TF/P)_{In}$ ratio increases during ureteral clamp. However, such an increase has not been confirmed by other investigators (7). In addition, Schnermann, Levine, and Horster (44) were unable to see a significant increase in the $(TF/P)_{In}$ ratio during partial obstruction individual nephrons. However, an elevated t_{Na} was clearly observed by Schnermann, et al. (43) when tubular surface area increased following spontaneous changes in GFR.

The details of glucose and sodium ion mechanisms differ and should not necessarily be compared directly. For example, the site for active reabsorption of glucose is thought to be the luminal membrane whereas that for sodium ion is the peritubular membrane of the proximal tubule cell (34). The first step in sodium ion reabsorption consists of diffusion across the luminal membrane into the proximal tubular cell and thence to the active transport site (peritubular membrane). A change in the surface area exposed to the tubular fluid will affect the rate of diffusion, but may have little effect on the active component of reabsorption. Another difference between sodium ion and glucose reabsorption is that the glucose reabsorptive mechanism becomes saturated at high P_G whereas saturation

of the sodium transport system has never been demonstrated [(57), pages 262-266]. Therefore the predictions made from the surface area model for glucose reabsorption must be applied with caution to the reabsorption of sodium ions. Because of the somewhat less complicated nature of glucose reabsorption, it appears that glucose rather than sodium ion is a better solute with which to study the effects of luminal surface area on solute reabsorption.

A New Interpretation of Tm_G

A new interpretation of the significance of the Tm_G seems justified. The results presented in this study indicate that Tm_G is not a fixed constant in dogs. Rather it is a variable indirectly dependent on the GFR. The GFR can be reduced by either reducing filtration in all nephrons (with a concomitant reduction in tubular surface area) or glomerular shutdown of a fraction of the nephrons. In either case, Tm_G should vary directly with the GFR. Other investigators consider Tm_G to be a fixed constant (16,46,47,49,51). Because the Tm_G did not change as GFR changed in their studies, these investigators concluded that normally all of the nephrons receive filtrate. Reductions in GFR were thought to be brought about by reductions in gfr. They believed glomerular shutdown was not a mechanism that was used to change the filtration rate over the range observed in their investigations. The results of the present study suggest that glomerular shutdown occurs during renal artery clamp and partial ureteral obstruction. There is no reason to doubt that

glomerular shutdown may occur even with spontaneous reductions in GFR.

Tm_G is independent of the filtered load only if the GFR is constant. Within a narrow range of GFR's Tm_G might appear to be constant. However, over a wide range of GFR's the Tm_G varies directly with filtration rate (Figs. 1, 3, 5). Therefore a better way to characterize the functional nephron population would be to report the GFR, the Tm_G, and the ratio, Tm_G/GFR. That is, all three parameters should be included in such a characterization. The first two parameters, Tm_G and GFR, are currently used to characterize renal function, but the Tm_G/GFR ratio is not generally used. The ratio of reabsorptive rate per 100 ml of filtrate is frequently used for describing the reabsorption of sodium and bicarbonate ions whose reabsorptive rates have long been known to vary directly with the filtration rate. Since the present study shows that glucose reabsorption varies directly with the GFR, it is appropriate to use the Tm_G/GFR ratio to describe the characteristics of glucose transport by the kidney. The Tm_G/GFR ratio is a parameter of kidney function which is normally independent of: 1) P_G, 2) GFR, and 3) tubular surface area. The exceptions to the preceding statement include: 1) very low GFR's in which tubular surface area is independent of GFR and 2) elevated ureteral pressure, in which case tubules may dilate in the face of a reduced GFR.

Summary and Conclusions

Glucose Tm was studied in dogs by the clearance method. Tm_G was found to be directly proportional to the GFR. This direct relation was observed during spontaneous reductions in GFR as well as during reductions in GFR produced by partial renal artery clamp and partial ureteral obstruction. The ratio produced by dividing Tm_G by the GFR was essentially constant when the GFR was reduced spontaneously or by renal artery clamp. However the Tm_G/GFR ratio was increased over control values when reductions in GFR were produced by ureteral clamp. No correlation between Tm_G and renal blood flow was observed.

These results are interpreted to indicate that Tm_G is not a fixed constant but instead is a function of the GFR. The results are further interpreted as evidence suggesting that the correlation of Tm_G with GFR is in fact a correlation of Tm_G with luminal surface area of the proximal tubule. A model based on tubular surface area is described. Evidence from the literature and this thesis are used to support the model. Suggestions regarding the definition and implications of the Tm_G are also discussed.

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