JCI The Journal of Clinical Investigation

Control of fluid absorption in the renal proximal tubule

Maurice B. Burg, Jack Orloff

J Clin Invest. 1968;47(9):2016-2024. https://doi.org/10.1172/JCI105888.

Research Article

Glomerulotubular balance was investigated in isolated, perfused rabbit proximal tubules in vitro in order to evaluate some of the mechanisms proposed to account for the proportionate relationship between glomerular filtration rate and fluid absorption generally observed in vivo. The rate of fluid transport from lumen to bath in proximal convoluted tubules in vitro was approximately equal to the estimated normal rate in vivo. The absorption rate in proximal straight tubules however was approximately one-half as great. If the mechanism responsible for maintenance of glomerulotubular balance is intrinsic to the proximal tubule, as has been proposed on the basis of micropuncture studies, the rate of fluid absorption in vitro should be directly related to the perfusion rate and/or tubule volume. In the present studies absorption rate was only minimally affected when perfusion rate was increased or the tubule distended. Thus, glomerulotubular balance is not mediated by changes in velocity of flow of the tubular fluid or tubular diameter and therefore is not an intrinsic property of the proximal tubule. It has also been proposed that glomerulotubular balance results from a humoral feedback mechanism in which angiotensin directly inhibits fluid absorption by the proximal convoluted tubule. In the present experiments, angiotensin was found to have no significant effect on absorption rate.

Control of Fluid Absorption in the Renal Proximal Tubule

MAURICE B. BURG and JACK ORLOFF

From the Laboratory of Kidney and Electrolyte Metabolism, National Heart Institute, Bethesda, Maryland 20014

ABSTRACT Glomerulotubular balance was investigated in isolated, perfused rabbit proximal tubules in vitro in order to evaluate some of the mechanisms proposed to account for the proportionate relationship between glomerular filtration rate and fluid absorption generally observed in vivo. The rate of fluid transport from lumen to bath in proximal convoluted tubules in vitro was approximately equal to the estimated normal rate in vivo. The absorption rate in proximal straight tubules however was approximately one-half as great. If the mechanism responsible for maintenance of glomerulotubular balance is intrinsic to the proximal tubule, as has been proposed on the basis of micropuncture studies, the rate of fluid absorption in vitro should be directly related to the perfusion rate and/or tubule volume. In the present studies absorption rate was only minimally affected when perfusion rate was increased or the tubule distended. Thus, glomerulotubular balance is not mediated by changes in velocity of flow of the tubular fluid or tubular diameter and therefore is not an intrinsic property of the proximal tubule. It has also been proposed that glomerulotubular balance results from a humoral feedback mechanism in which angiotensin directly inhibits fluid absorption by the proximal convoluted tubule. In the present experiments, angiotensin was found to have no significant effect on absorption rate.

INTRODUCTION

In view of the importance of the kidneys in controlling body water and salt content, the nature of the renal regulatory mechanisms involved is of considerable interest. From the results of numerous investigations of this subject it is apparent that a multiplicity of factors are involved which may not be readily separable. In order to reduce the number of variables and provide more direct measurements of absorption, a method for isolating single renal tubules and perfusing them in vitro has been developed (1, 2). This method has been used in the present studies to evaluate factors affecting fluid absorption in the proximal tubule. Flow rate, tubule diameter, and the hormone angiotensin, each of which is purported to be of importance in maintaining glomerulotubular balance, were tested and found to have no important effect on absorption.

METHODS

The method employed in these studies has been previously described in detail (1, 2) and is summarized below with additions and modifications.

Female rabbits weighing 1.5–2 kg were used in all experiments. 15 min before sacrifice 15 ml of a 15% mannitol solution was injected intravenously into the animal. Immediately after death a small segment was removed surgically from one kidney and immersed in chilled (0–5°C) rabbit serum [1] that was gassed with 95% O_2 plus 5% CO_2. A fragment of proximal convoluted tubule (1–2 mm long) or proximal straight tubule (2–4 mm long) was dissected free and transferred to a perfusion chamber containing the identical medium, though at 37°C. During perfusion the tubule was observed through a stereoscopic microscope at magnification of × 60.

The shapes of the micropipets used (Fig. 1) were slightly modified from those previously described (1). Injection of mannitol and maintenance of low temperature during dissection delayed the onset of collapse of the proximal tubule that ordinarily follows interruption

Received for publication 29 January 1968 and in revised form 5 April 1968.

[1] Microbiological Associates, Bethesda, Md.

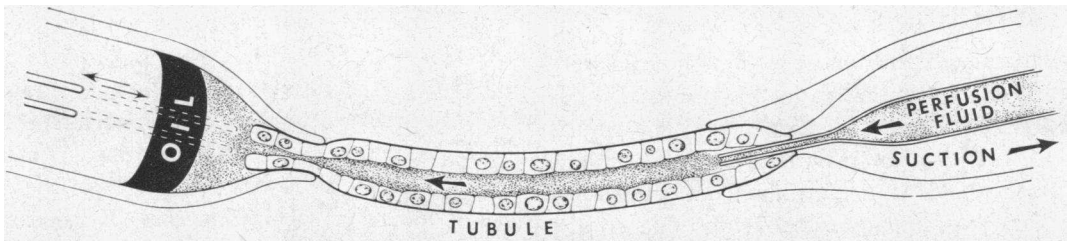

FIGURE 1 Arrangement for perfusing kidney tubules.

of the circulation (3) and which may be accompanied by structural damage to the tubule cells (4). The tubules were usually only partially collapsed at the time of transfer to the perfusion chamber and could ordinarily be perfused before complete collapse occurred at the higher temperature. Each tubule was examined microscopically at the beginning of the perfusion and was discarded if there appeared to be any leak or cellular damage.

The perfusion fluid was prepared by pressure dialysis through Visking cellulose tubing from the same lot of serum as that used to bathe the tubule. Albumin-^{131}I [2] was added to a final concentration of approximately 20 μc ml^{-1}. To correct for any minor changes in concentration during ultrafiltration, the freezing points of both the serum and perfusion fluid were measured and water was added, as necessary, to equalize the osmolality of the two solutions.

The concentration of ^{131}I was measured in the perfusion solution and in the collected tubule fluid. Calibrated glass capillaries were used to measure the volume of each sample and a Packard well scintillation counter was utilized to measure radioactivity. The perfusion rate (V_0, nl min^{-1}) for each period was calculated as

$$V_0 = \frac{^{131}I_L}{[^{131}I_0]t}$$

were $[^{131}I_0]$ is the concentration of radioactivity in the perfusion fluid (cpm, nl^{-1}); $^{131}I_L$ the total amount of radioactivity in the collected tubule fluid sample (cpm); and t the duration of the collection period (min). The rate of collection (V_L, nl min^{-1}) was determined directly from the volume and duration of each collection. The absorption rate C (nL mm^{-1} tubule length min^{-1}) was calculated as

$$C = \frac{V_0 - V_L}{L}$$

where L is the length of tubule perfused (mm).

After each collection of tubule fluid the serum bathing the tubule was changed and its radioactivity measured. The mean "leak" of ^{131}I from lumen to bath was less than 2% of that in the perfusing volume and was relatively constant in all studies. (Although the albumin-^{131}I was dialyzed against a physiologic saline solution within a few days of each use, it is possible that at least part of the "leak" represented free ^{131}I.)

[2] E. R. Squibb & Sons, Cheverly, Md.

In experiments in which the role of tubule volume was examined, the tubules were distended by increasing outflow resistance, as illustrated in Fig. 2. The tubule was held in a collecting pipet identical to that shown in Fig. 1 and the perfused fluid was permitted to accumulate under mineral oil in the usual fashion. After collection of an initial control sample, the special inner pipet shown in Fig. 2 was inserted and its tip used to compress the end of the tubule. The compression was adjusted until a desired degree of distension was achieved. Fluid that accumulated under the oil during the period of adjustment was aspirated into the lumen of the special pipet to be discarded, and a timed collection was then begun. After approximately 11 min the special inner pipet was rapidly removed and an experimental sample was immediately collected with the usual calibrated pipet. After the release of compression, the tubule diameter returned to the control value and another control collection was made at the smaller tubule volume. In this manner, several alternate collections could be made in a single tubule.

For measuring tubule diameter a single objective microscope fitted with a Leitz 6× objective and photographic attachment was used. A frosted glass disc was placed between the light source and the sample to improve the definition of the tubule lumen. Tubule diameter was measured on prints at 400× magnification at the same points in a given tubule each time.

Summary data are given as mean ± SEM (number of experiments).

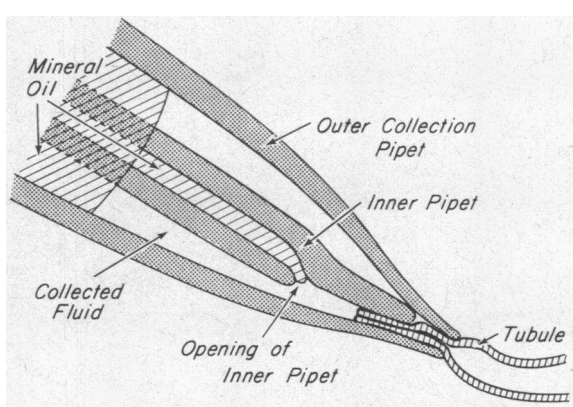

FIGURE 2 Arrangement for increasing tubule diameter (see Methods for explanation).

RESULTS

In preliminary experiments proximal tubules were dissected and perfused with the previously described artificial saline solutions (1). The results of these studies were unsatisfactory. The tubule cells, particularly at the proximal end, often become darkly granulated, apparently due to the development of vacuoles. It was determined that this could be avoided in most studies by substituting rabbit serum and ultrafiltrate, as described in Methods. In addition, the mean absorption rate with artificial saline solutions was less than that later found with serum and ultrafiltrate. Presumably, some factor present in rabbit serum helps to maintain proximal tubule integrity during dissection. No systematic attempt was made to identify this factor.

With rabbit serum in the bath and by perfusing with an ultrafiltrate of the serum, we found the mean rate of absorption in control periods during the 1st hr of perfusion was 1.18 ± 0.07 (40) nl mm^{-1} tubule length min^{-1} in the proximal convoluted tubule. For comparison, the in vivo absorption rate may be estimated from the total filtration rate and the number of nephrons in the rabbit and the assumption that a large fraction of the glomerular filtrate is absorbed in the proximal tubule. The glomerular filtration rate per nephron for rabbits weighing 1.76 kg is approximately 14.1 nL min^{-1} (5). Rats absorb approximately one-half of the glomerular filtrate in the accessible portion of the proximal convoluted tubule, whereas guinea pigs absorb somewhat less (6). In order to absorb one-half of the estimated filtrate in the rabbit at the rate observed in vitro, 6 mm of tubule length (7.05 nL $min^{-1} \div 1.18$ nL min^{-1} mm^{-1}) would be required. The measured total length of the proximal tubule in a rabbit this size (2 months old) was 8 mm (7). (It is approximately twice as great in a fully grown rabbit (7).) Thus, the measured rate of fluid absorption in rabbit proximal tubules in vitro seems to be approximately the same as in vivo and is considered to be reasonably "normal."

Fluid absorption rate in the straight portion of the proximal tubule was 0.42 ± 0.07 (10) nL mm^{-1} min^{-1}, which is less than one-half that of the convoluted portion. It had not been possible to make this comparison in vivo, since the straight portion does not approach the surface of the kidney and is therefore inaccessible for micropuncture study. However, a lower rate of absorption in the straight portion had been suspected. It had been calculated that, if the higher rate of absorption measured in the accessible convoluted portion were to continue, all of the fluid would be absorbed before the end of the proximal tubule, which is obviously not the case (6, 8, 9).

When temperature was decreased to 10–13°C (Fig. 3), the rate of fluid absorption was reduced to zero. The effect was reversible, since when the temperature was increased, fluid absorption was restored to normal levels. This suggests that the

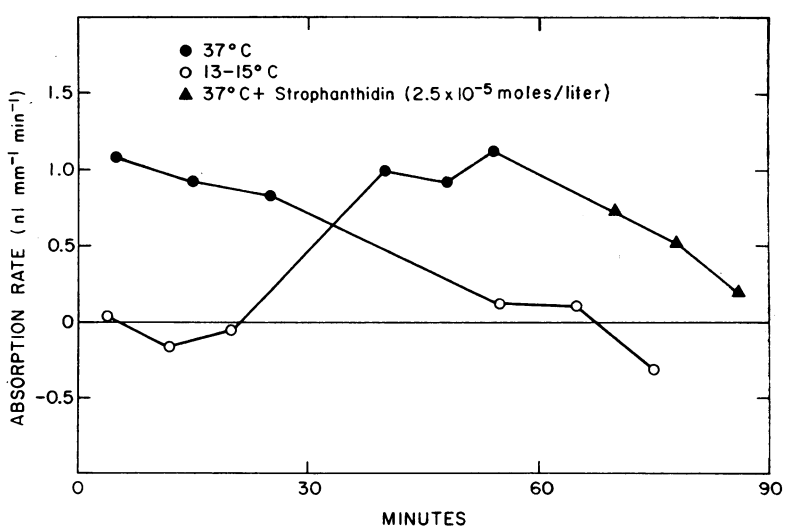

FIGURE 3 Inhibition of fluid of absorption in proximal convoluted tubules.

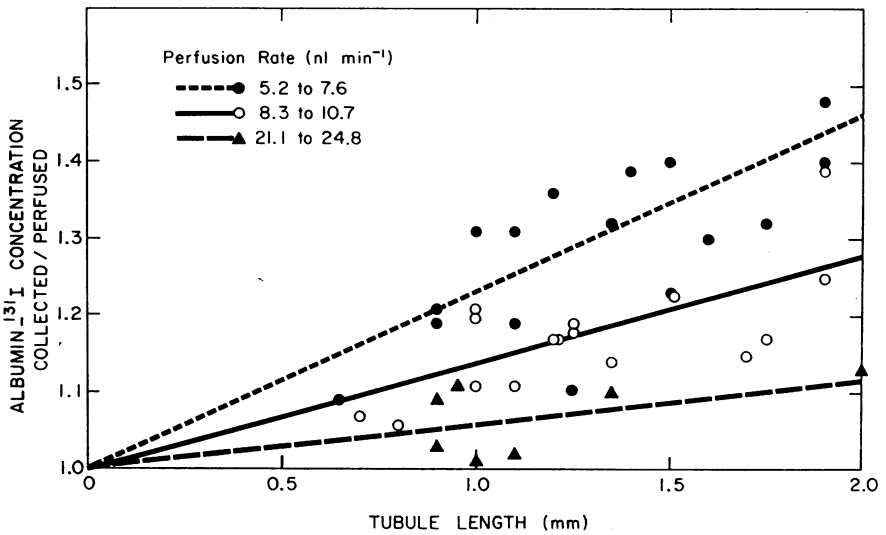

FIGURE 4 Albumin-^{131}I concentration ratio from isolated perfused proximal convoluted tubules. Each symbol is mean of the control measurements during the 1st hr of perfusion of a single tubule.

observed fluid absorption is an active, metabolically dependent process.

Fig. 4 shows the mean concentration ratio of albumin-^{131}I (collected/perfused fluid) in proximal convoluted tubules during control observations. The data are arbitrarily divided into three groups, depending on the perfusion rate. At any given perfusion rate the albumin ratio tended to be greater in the longer tubules, reflecting cumulative absorption with length. However, the ratio at a given length of tubule depended on the perfusion rate, being lowest at the highest perfusion rate. This differs from results of most micropuncture studies. When glomerulotubular balance is present in vivo, tubule fluid to plasma inulin ratio (and thereby fractional reabsorption) is constant or nearly so at a given point in the tubule regardless of the perfusion rate (6, 9–17 and footnote 3).

In order to test this point further, experiments were performed in which a single tubule was perfused at different rates. Each tubule was perfused for two to four control collection periods at a slow rate, for two to four experimental periods at a faster rate, then at the slow rate again. Since there was no significant difference in absorption between the early and late periods at the slow speed, the mean of all the results for the slow perfusion rate for each tubule was used for comparison with the results at the high rate. Increasing the perfusion rate approximately twofold (Table I) caused the fraction of fluid absorbed to decrease by approximately one-half. Thus there was little, if any, increase in the absolute rate of absorption when the perfusion rate was doubled. The results with proximal straight tubules were similar, except for a lower absolute rate of absorption (Table II).

TABLE I

Effect of Flow Rate on Fluid Absorption by Proximal Convoluted Tubules

	Control	Experimental
Perfusion rate, *nl min⁻¹*	8.0	19.0
Fraction absorbed	0.23	0.11
Tubule diameter, μ	21.0	22.0
Absorption, *nl mm⁻¹ min⁻¹*	1.24	1.37

Means of results for eight experiments are shown, except for tubule diameter that was measured in only the last three experiments. The mean of the differences in absorption between control and experimental periods (pairing the results from each tubule) is $+0.13 \pm 0.10$, $P > 0.2$.

[3] Lewy, J., and E. Windhager. Peritubular control of proximal tubular fluid reabsorption in the rat kidney. Submitted for publication.

TABLE II

Effect of Flow Rate on Fluid Absorption by Proximal Straight Tubules

	Control	Experimental
Perfusion rate, *nl min*$^{-1}$	6.0	18.0
Fraction absorbed	0.19	0.09
Tubule diameter, μ	17.0	24.0
Absorption, *nl mm*$^{-1}$ *min*$^{-1}$	0.42	0.57

Means of results for four experiments are shown. The mean of the differences in absorption between control and experimental periods (pairing the results from each tubule) is $+0.15 \pm 0.08$, $P > 0.10$.

TABLE III

Effect of Luminal Diameter on Fluid Absorption by Proximal Convoluted Tubules

	Control	Experimental	ratio E:C*
Tubule diameter, μ			
Outside	50.2	54.0	1.07
Inside	15.0	26.0	1.73
Flow rate, *nl min*$^{-1}$	9.6	9.6	1.00
Absorption, *nl mm*$^{-1}$ *min*$^{-1}$	1.00	1.19	1.19

Mean of results for 10 experiments are shown. The mean of the differences in absorption between experimental and control periods (pairing the results from each tubule) is $+0.19 \pm 0.08$, $P = 0.05$. The mean of the differences in outside diameter is $+3.8 \pm 0.6$, $P < 0.05$.

* E: C, Experimental: Control.

Previous investigators (14, 18) have ascribed altered fluid absorption in the proximal tubule under conditions of glomerulotubular balance to changes in tubule volume rather than to changes in flow rate, and have concluded that absorption increases in proportion to tubule volume (the square of tubule radius). The effect of tubule volume on absorption could not be evaluated in the experiments described in Table I, since there was little to no change in tubule diameter when flow was increased. Therefore the effect of tubule volume per se on absorption rate of the proximal convoluted tubule was tested with the apparatus illustrated in Fig. 2 to alter tubule diameter. In each experiment two or more control observations were compared with two or more alternating periods of tubule distension. Photographs representative of those used for measuring tubule diameter are shown in Fig. 5. The tubules were distended from a mean control diameter of 15 to 26 μ without change in perfusion rate (Table III). Absorption increased by only 19%, considerably less than tubule diameter that increased 73%. This point is emphasized in Fig. 6 in which the mean of the results from each of the 10 tubules is shown individually. It can be seen that in no experiment was there an increase in absorption in proportion to the increase in tubule diameter (Fig. 6, line D) or volume (Fig. 6, line D^2).

When the tubules were distended, the outside diameter increased, although to a lesser extent

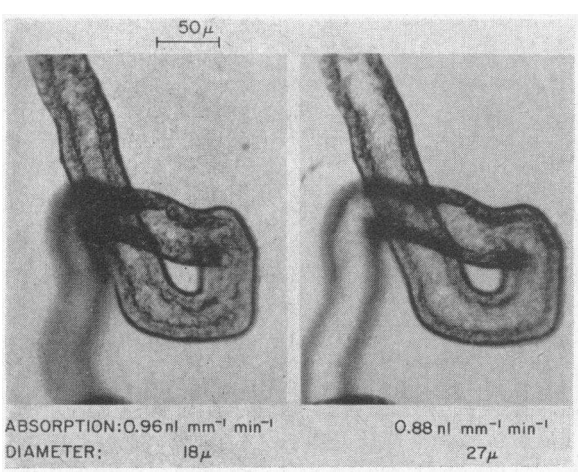

FIGURE 5 Effect of distension of a proximal convoluted tubule on absorption rate. Photographs and data are from two successive perfusion periods.

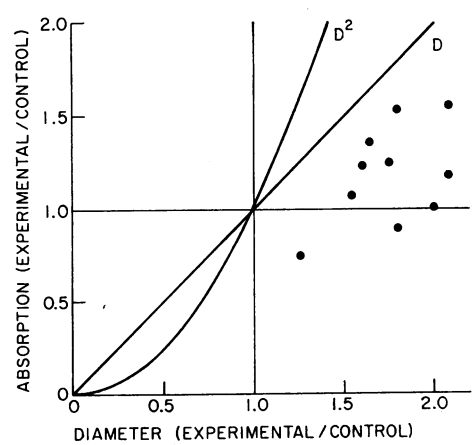

FIGURE 6 Relation between diameter and fluid absorption rate in proximal convoluted tubules.

than the increase of the inside diameter (Table III). The increase in outside diameter was not previously noted in studies in the rat (19). Cross-sectional area of the cells remained constant when the tubule was distended (mean of control and of distended tubules, 1800 μ^2), which suggested that in the absence of a change in tubule length there was little if any change in cell volume. Although tubule length did not appear to change appreciably, it could not be measured precisely, owing to the convoluted shape of the tubules. Thus, a change in cell volume cannot be excluded with certainty.

Leyssac (20, 21) concluded that angiotensin acts directly on the proximal convoluted tubule to inhibit fluid absorption. The effect of angiotensin on fluid absorption by the isolated proximal tubule was tested in three experiments. After two control periods, angiotensin (2.5 μg ml^{-1}) was added to the bath during each of three experimental periods. Then the bath was changed several times to wash off the angiotensin and three additional periods were collected. The mean absorption rate without angiotensin during the initial and final periods was 0.95 nl mm^{-1} min^{-1}. In the presence of angiotensin, absorption rate was 0.90 nl mm^{-1} min^{-1}. The difference was not statistically significant [-0.05 ± 0.07 (9)] when each experimental period was compared with the mean of the corresponding initial and final control periods. (There was also no significant difference when we compared each experimental period to either the corresponding initial control periods or final control periods.) Thus, angiotensin had no detectable effect on fluid absorption.

DISCUSSION

The primary purpose of the present studies was to evaluate current concepts regarding the nature of glomerulotubular balance. Smith (22) originally conceived glomerulotubular balance with respect to salt and water as a proportionate relationship between glomerular and tubular activity, such that salt and water balance is maintained over a wide range of activity of both filtration and reabsorption. Thus, the large changes in excretion of salt and water, which might be caused by variations of glomerular filtration rate, are blunted. It was noted in early micropuncture studies that virtually all of the filtered salt and water was reabsorbed in the proximal tubule. Furthermore, changes in glomerular filtration rate were accompanied by compensatory changes in proximal tubular reabsorption, such that the fraction of filtered salt and water reabsorbed remained relatively constant (6). From this it was apparent that adjustments of absorption in the proximal convoluted tubule are of prime importance for over-all glomerulotubular balance. Subsequently, interest has been focused on this segment of the nephron and constancy of fractional absorption in the proximal tubule has become synonymous with glomerulotubular balance.

There are two principal types of mechanisms which have been proposed to account for glomerulotubular balance in the proximal tubule, the one intrinsic to the proximal tubule, the other extrinsic. With respect to the first, in which it is assumed that adjustment in reabsorption is an intrinsic property of the tubule, it has been suggested that fluid absorption is directly affected by the velocity of flow of the tubular fluid (23) or by changes in tubule volume (14, 18). With respect to a possible extrinsic mechanism, it has been suggested that a humoral feedback system (20, 21, 24), or changes in functional state of the peritubular capillaries or interstitial space,[3] are responsible for the regulation of absorption rate. These alternatives can be evaluated on the basis of the present studies. If an extrinsic mechanism were acting, it would necessarily be interrupted when the tubule was dissected from the kidney and glomerulotubular balance would not occur in vitro. It has been observed that rabbits have glomerulotubular balance in vivo. Kruhoffer (25), using clearance techniques, found that sodium reabsorption varied directly with filtration rate and this was attributed to reabsorption of a fairly constant percentage of filtered water and sodium in the proximal tubule at varying filtration rates. In the present experiments, in contrast, when rabbit proximal tubules were studied in vitro, no glomerulotubular balance was found. This result is most consistent with the existence of an extrinsically regulated mechanism of glomerulotubular balance in vivo.

The intrinsic mechanism first proposed (23) was that linear velocity of flow directly controls absorption rate in a fashion similar to that of a catalytic flow reactor. In the present experiments, however, there was little if any change in absorp-

tion rate with perfusion rate at constant tubule diameter (Table I), which excluded this possibility.

Other investigators (14, 18) concluded that absorption rate in the proximal tubule is directly proportional to tubule volume, and that when filtration rate is altered tubule diameter changes, so that tubule volume (and thereby reabsorption) remains proportional to filtration rate (14). The relationship between tubule volume and absorption rate has not been confirmed in the present studies. When the tubules were distended by increasing the outflow resistance there was little change in absorption rate (Table III, Fig. 6), certainly much less than would be required by this theory. In view of this difference in results, it is pertinent to examine the basis for the previous theory more closely.

Gertz, Mangos, Braun, and Pagel (18) originally proposed a causal relation between tubule volume and absorption rate on the basis of comparison of simultaneous measurements of transit time (T) and inulin concentration ratio (TF/P) at a given point in the tubule with absorptive halftime when using the split oil drop technique ($t_{\frac{1}{2}}$). The relationships are (28):

$$\frac{\ln (TF/P)}{T} = \frac{C}{\pi r^2} \quad (1)$$

$$\frac{0.693}{t_{\frac{1}{2}}} = \frac{C}{\pi r^2} \quad (2)$$

where C is the reabsorptive rate per unit of tubule length and r is the tubule radius. Gertz et al. (18) found that $C/\pi r^2$ when calculated from the separate experiments represented by equations 1 and 2 was the same. This has been confirmed by others (Table IV, Nos. 1 and 2). In order to measure the $t_{\frac{1}{2}}$ the tubule must be filled with oil, distending it so that its observed radius is considerably greater than it would be under free flow conditions (Table IV, Nos. 6 and 7). For $C/\pi r^2$ to be constant despite the increase in radius, C must have increased in proportion to r^2. If it had not, $C/\pi r^2$ would have decreased enough for the difference to have been detected. Hence, the theory that absorptive rate is proportional to proximal tubule volume.

This interpretation of the results requires that C be less in free flow (equation 1) than with the split oil drop (equation 2). In fact there is independent evidence that C is actually the same in the two situations and that the equality of $C/\pi r^2$ may therefore be fortuitous, a result of systematic errors in one or more of the measurements. Although Gertz et al. (18) rejected this interpretation as being highly improbable, additional calculations suggest that it may actually be correct. C can be estimated independently for free flow from the relation

$$C = \frac{V_0}{L}\left(1 - \frac{P}{TF}\right) \quad (3)$$

where V_0 is the filtration rate per nephron and L is the length of the tubule to the point at which

TABLE IV

Proximal Tubule Absorption Rates and Diameters in the Rat

$C/\pi r^2$		
1. Free flow	$\frac{\ln (TF/P)}{T}$	3.54 (28) 3.70[3], 3.7 (17) 5.76 (18)
2. Split drop	$\frac{0.693}{t_{\frac{1}{2}}}$	3.99 (28), 4.25[3] 4.25 (29), 4.48 (19) 5.22 (18)
C		
3. Free flow	$\frac{\pi r^2 \ln (TF/P)}{T}$	1.33[3]
4. Free flow	$\frac{V_0}{L}\left(1 - \frac{P}{TF}\right)$	2.74*[3], 3.38* (17) 3.70‡ (30), 4.11 (6)
5. Split drop	$\frac{\pi r^2\, 0.693}{t_{\frac{1}{2}}}$	2.89, (19) 3.20 (29) 3.67 (26), 4.57[3]
Diameter		
6. Free flow		19.0 (32) 21.4[3], 21.6 (19), 22.8 (31), 23.0–28.0 (24)
7. Split drop		27.6 (19), 31.0 (29), 33.0 (26) 37.0[3]

Each value is taken directly from or calculated from control data in the reference cited, using the formulas given and changing of units as required.

* L, 0.5 cm. Micropunctures made at the end of the accessible portion of the proximal tubule are approximately one-half (14, 17) the total length of 1 cm (7).

‡ Total tubule length is 1 cm (7).

fractional fluid absorption (1 − P/TF) is measured. The values of C in free flow measured in this manner vary from 2.74 to 4.11 nL mm^{-1} min^{-1} when compared with 2.89 to 4.57 based on measurements with the split drop technique (Table IV, Nos. 4 and 5). Thus, C measured in free flow by the method in equation 3 is approximately equal to that measured with the split drop technique, despite the large difference in tubule radius, whereas with the relation in equation 1 it had been concluded that C during free flow must be less, because r was observed to be less (compare Nos. 3 and 4 in Table IV). The inconsistency in the results is not due to selection of the data or differences in experimental conditions, since there is good agreement between different investigators (Table IV).

Because the results with equations 4 and 5 in Table IV are in agreement, the most likely explanation for the inconsistency is a systematic error inherent in one or more of the measurements in equation 3.

Other evidence for the proposed relationship between tubule volume and absorption rate came from the effect of elevating ureteral pressure (14, 16, 26). Under these conditions, changes in tubule volume are dissociated from those in perfusion rate. Tubule volume increases, whereas the perfusion rate is decreased (14, 26) or unchanged (in pump-perfused tubules [16]). Therefore, if tubule volume is critical for the regulation of absorption rate, glomerulotubular balance should be disrupted, and absorption rate increased relative to perfusion rate. The actual results were that inulin TF/P (and, presumably, absorption) increased, suggesting that tubule volume is the primary factor controlling absorption. Other investigators (17) have repeated the same experiments under free flow conditions and have found essentially no change in inulin TF/P. They attributed the earlier result to technical inadequacy of the micropuncture method. When a sample of tubule fluid is aspirated by micropuncture during elevation of ureteral pressure, there may be reverse flow in the tubule, causes fluid to arrive at the micropuncture site from more distal segments where inulin is more concentrated. Reflux of this nature may occur even when precautions are taken by placing an oil drop distal to the collecting pipet (14, 17). Since contamination from more distal segments also results in a spurious elevation of measured filtration rate per nephron, gross contamination can be suspected if nephron filtration rate (V_0) is unreasonably high. Ureteral clamping should cause V_0 to decrease, since glomerular filtration rate is decreased. However, in some studies V_0 was found to increase, which indicated contamination of the collected samples. When all results in which V_0 *increased* more than 50% were excluded from consideration, there was no increase in inulin TF/P during ureteral clamping (17), and thus no increase in absorption. Actually there might have been a significant decrease in absorption which was not detected, since the criterion used for selecting the data may not be sufficiently sensitive to exclude all contaminated samples.

In the present studies, rabbit proximal tubules perfused in isolation lost the property of adjusting absorption rate to flow rate, even when changes in tubule diameter were taken into account. Therefore, it is likely that some factor extrinsic to the proximal tubule had been necessary for glomerulotubular balance *in vivo*. Leyssac (20, 21) suggested that in glomerulotubular balance the rate of proximal tubule absorption is regulated by angiotensin. According to his view, angiotensin specifically inhibits fluid absorption by a direct effect on the proximal tubule and thus causes a prolongation of the "collapse time." However, others have not found any effect of angiotensin on proximal tubule fluid absorption (27), and in the present studies proximal absorption rate was also unaffected by angiotensin. On the basis of the evidence, it appears unlikely that angiotensin is directly involved in this fashion in glomerulotubular balance. The possibility remains, however, that glomerulotubular balance might be regulated by a feedback system involving a hormone other than angiotensin.

None of the other current views are completely adequate to explain glomerulotubular balance. Even if we accept that regulation of proximal reabsorption is not intrinsic to the tubule itself, the nature of the responsible extrinsic mechanism remains to be established.

REFERENCES

1. Burg, M., J. Grantham, M. Abramow, and J. Orloff. 1966. Preparation and study of fragments of single rabbit nephrons. *Am. J. Physiol.* **210**: 1293.

2. Grantham, J. J., and M. B. Burg. 1966. Effect of vasopressin and cyclic AMP on permeability of isolated collecting tubules. *Am. J. Physiol.* **211**: 255.
3. Hanssen, O. E. 1960. Early postmortem renal changes studied in mice with one kidney exteriorized. *Acta Pathol. Microbiol. Scand.* **49**: 297.
4. Longley, J. B., and M. S. Burstone. 1963. Intraluminal nuclei and other inclusions as agonal artifacts of the renal proximal tubules. *Am. J. Pathol.* **42**: 643.
5. Strassberg, J., J. Paule, H. C. Gonick, M. H. Maxwell, and C. R. Kleeman. 1967. The quantitative estimation of perfusible glomeruli and the collagen and non-collagen nitrogen of the normal kidney. *Nephron.* **4**: 384.
6. Walker, A. M., P. A. Bott, J. Oliver, and M. C. MacDowell. 1941. The collection and analysis of fluid from single nephrons of the mammalian kidney. *Am. J. Physiol.* **134**: 580.
7. Sperber, I. 1944. Studies on the mammalian kidney. *Zool. Bidrag. Fran. Uppsala.* **22**: 249.
8. Gottschalk, C. 1963. Renal tubular function: lessons from micropuncture. *Harvey Lectures, Ser.* **58**: 99.
9. Giebisch, G., and E. E. Windhager. 1964. Renal tubular transfer of sodium, chloride, and potassium. *Am. J. Med.* **36**: 643.
10. Lassiter, W. E., M. Mylle, and C. W. Gottschalk. 1964. Net transtubular movement of water and urea in saline diuresis. *Am. J. Physiol.* **206**: 669.
11. Dirks, J. H., W. J. Cirksena, and R. W. Berliner. 1965. The effect of saline infusion on sodium reabsorption by the proximal tubule of the dog. *J. Clin. Invest.* **44**: 1160.
12. Glabman, S., H. S. Aynedjian, and N. Bank. 1965. Micropuncture study of the effect of acute reductions in glomerular filtration rate on sodium and water reabsorption by the proximal tubules of the rat. *J. Clin. Invest.* **44**: 1410.
13. Cirksena, W. J., J. H. Dirks, and R. W. Berliner. 1966. Effect of thoracic cava obstruction on response of proximal tubule sodium reabsorption to saline infusion. *J. Clin. Invest.* **45**: 179.
14. Rector, F. C., Jr., F. P. Brunner, and D. W. Seldin. 1966. Mechanism of glomerulotubular balance. I. Effect of aortic constriction and elevated ureteropelvic pressure on glomerular filtration rate, fractional reabsorption, transit time, and tubular size in the proximal tubule of the rat. *J. Clin. Invest.* **45**: 590.
15. Watson, J. F. 1966. Effect of saline loading on sodium reabsorption in the dog proximal tubule. *Am. J. Physiol.* **210**: 781.
16. Wiederhalt, M., K. Hierholzer, E. E. Windhager, and G. Giebisch. 1967. Microperfusion study of fluid reabsorption in proximal tubules of rat kidneys. *Am. J. Physiol.* **213**: 809.
17. Brenner, B., C. Bennett, and R. Berliner. 1968. The relationship between glomerular filtration rate and sodium reabsorption by the proximal tubule of the rat nephron. *J. Clin. Invest.* **47**: 1358.
18. Gertz, K. H., J. A. Mangos, G. Braun, and H. D. Pagel. 1965. On the glomerular tubular balance in the rat kidney. *Arch. Ges. Physiol.* **285**: 360.
19. Hayslett, J. P., M. Kashgarian, and F. H. Epstein. 1967. Changes in proximal and distal tubular reabsorption produced by rapid expansion of extracellular fluid. *J. Clin. Invest.* **46**: 1254.
20. Leyssac, P. P. 1964. The in vivo effect of angiotensin on the proximal tubular reabsorption of salt in rat kidneys. *Acta Physiol. Scand.* **62**: 436.
21. Leyssac, P. P. 1965. The in vivo effect of angiotensin and noradrenaline on the proximal tubular reabsorption of salt in mammalian kidneys. *Acta Physiol. Scand.* **64**: 167.
22. Smith, H. W. 1951. *In* The Kidney: Structure and Function in Health and Disease. Oxford University Press, Inc., New York. 331.
23. Kelman, R. B. 1962. A theoretical note on exponential flow in the proximal part of the mammalian nephron. *Bull. Math. Biophys.* **24**: 303.
24. Leyssac, P. P. 1963. Dependence of glomerular filtration rate on proximal tubular reabsorption of salt. *Acta Physiol. Scand.* **58**: 236.
25. Kruhoffer, P. 1950. Studies on water-electrolyte excretion and glomerular activity in the mammalian kidney. Rosenkilde and Bagger, Copenhagen.
26. Brunner, F. P., F. C. Rector, Jr., and D. W. Seldin. 1966. Mechanism of glomerulotubular balance. II. Regulation of proximal tubular reabsorption by tubular volume, as studied by stopped flow microperfusion. *J. Clin. Invest.* **45**: 603.
27. Horster, M., W. Nagel, S. Schnermann, and K. Thurau. 1966. Zur Frage einer direkten angiotensinwirkung auf die Natrium resorption im proximalen Tubulus und in der Henleschen Schleife der Ratteniere. *Arch. Ges. Physiol.* **292**: 118.
28. Rector, F. C., Jr., J. C. Sellman, M. Martinez-Maldonado, and D. W. Seldin. 1967. Mechanism of suppression of proximal tubular reabsorption by saline infusions. *J. Clin. Invest.* **46**: 47.
29. Gertz, K. H. 1963. Transtubuläre Natriumchloridflüsse und Permeabilitat für Nichtelektrolyte in proximalen und distalen konvolut der rattennierre. *Arch. Ges. Physiol.* **276**: 336.
30. Flanigan, W. J., and D. E. Oken. 1965. Renal micropuncture study of the development of anuria in the rat with mercury-induced acute renal failure. *J. Clin. Invest.* **44**: 449.
31. Steinhausen, M., I. Iravani, G. E. Schubert, and R. Taugner. 1963. Auflichtmikroskopie und Histologie der Tubulusdimensionen bei verschiedenen Diuresezuständen. *Arch. Pathol. Anat. Physiol.* **336**: 503.
32. Krause, H., H. Th. Dume, K. M. Koch, and B. Ochwadt. 1967. Intratubularer Druck, glomerularer Capillardruck und Glomerulumfiltrat nach Furosemid und Hydrochlorothiazid. *Arch. Ges. Physiol.* **295**: 80.