

## Stationary Perfusion Methods

K. H. GERTZ

*Institut für Physiologie, Medizinische Hochschule Hannover, 3 Hannover-Kleefeld*

*Received for publication 19 November 1971*

The so-called split-drop method was developed to measure transtubular isotonic water reabsorption from tubular convolutions at kidney surfaces. In principle the method involves intratubular injection of viscous castor oil in amounts sufficient to block intratubular flow rate; the oil column remains in place. Subsequently the oil column is split into two oil columns by the injection of a small amount of isotonic saline solution. Since this saline droplet will be reabsorbed from the tubular lumen, the two oil columns will move together until they again merge. If the tubular diameter remains constant during reabsorption, the distance between the two oil columns, as a measure of intratubular volume, will decrease exponentially with time. Because of this exponential volume decrease, transtubular fluid reabsorption is expressed as half time of volume reabsorption instead of as milliliters per unit time and unit tubular length or as unit tubular area. This limitation has been a disadvantage of this method from the very beginning.

Half time of volume reabsorption under control conditions has been found in several laboratories to be about 10 sec. in proximal convolutions of rat kidneys and to be prolonged after administration of such inhibitors of the transepithelial sodium pump as diuretics.

Although the measurements of half time have been repeatable within small limits, doubts arose as to whether this method produced results consistent with fluid reabsorption under free flow conditions. One of the most frequent arguments against this method has concerned the influence of intratubular diameter on fluid reabsorption. The column of the saline droplet between the two adjacent oil columns has a diameter of about 30  $\mu$  compared to an intratubular diameter of about 20  $\mu$  under free flow conditions. For several reasons, such as inaccuracy of the microphotographs or the effects of the brush border, a direct evaluation of the influence of the tubular diameter on volume reabsorption is difficult to perform and therefore the data are conflicting.

In order to determine indirectly whether the halftime of volume reabsorption measured with the split-drop method is related to free-flow reabsorption, halftime has been correlated with the change of volume flow along tubes. According to the principles of hydrodynamics, volume flow  $\dot{V}$  through a tube with constant cross-sectional area  $r^2 \pi$ , constant length  $l$ , and no reabsorption is given by the equation

$$\frac{\Delta V}{\Delta t} = \dot{V} = \frac{r^2 \pi l}{T} \quad [1]$$

$T$  is the time necessary for fluid to move from the beginning to the end of the tube. Flow velocity across tubular diameter is believed to be constant and not parabolic. This simplification may be appropriate if convoluted instead of straight tubes are concerned.

The relationships expressed in Eq. [1] lead to the conclusion that changes in tubular flow rate  $\dot{V}$  are proportional to intratubular volume ( $r^2 \pi l$ ) if  $T$  remains constant, or in the case of a constant intratubular volume, a change in volume flow is proportional to  $\frac{1}{T}$ ; that is, if flow rate increases,  $T$  must decrease.

To adapt the above relationship to proximal convolutions of kidneys, a factor must be introduced to account for transtubular water reabsorption. This factor should somehow depend on the fraction of filtered fluid reabsorbed by proximal tubular cells. This factor in the following discussion is called  $R$ . Therefore, in the case of reabsorption, Eq. [1] is extended to Eq. [2]

$$\dot{V}_0 = R \cdot \left( \frac{r^2 \pi l}{T} \right) \quad [2]$$

$\dot{V}_0$  is the flow rate at the beginning of the proximal convolution, that is, single-nephron filtration rate. Since in proximal convolutions the fractional reabsorption was found to be fairly constant under a variety of experimental conditions,  $R$  in a first approximation in the subsequent discussion is taken as a constant value.

A discussion of Eq [2] leads to the same conclusions as Eq [1].

- (a) if  $T$  remains constant during experimental procedures, intratubular volume increases proportional to single-nephron filtration rate.
- (b) if intratubular volume remains constant, then  $T$  has to decrease with increasing filtration rate.

Furthermore, since fractional reabsorption remains constant, the total amount of fluid reabsorbed per unit time has to increase with increasing filtration rate. In case (a), the transtubular flow rate must increase proportional to intratubular volume. In case (b), the reabsorbed amount per unit time has to increase with constant tubular diameter. Therefore, tubular transit time, the time available for tubular cells to reabsorb, has to decrease with increasing  $\dot{V}_0$ .

(c) If  $r$  and  $T$  are changing together, then  $r^2/T$  has to change proportional to  $\dot{V}_0$ .

From the above statements it can be concluded that with changing transit times during an experiment, the reabsorption rate by no means can be expected to be proportional to intratubular volume.

These simple hydrodynamic relationships have been correlated to half-time of volume reabsorption during stop-flow conditions. The mathematical treatment of this problem is published elsewhere(9) and, therefore, will not be repeated here.

$$\ln F/P = 0.693 \frac{T}{t_{1/2}} = \frac{C}{\pi r^2} \cdot T = (1 - P/F) \frac{\dot{V}_0}{\pi r^2 l} \cdot T. \quad [3]$$

$F/P$  is the ratio of the inulin concentration of tubular fluid at the end of proximal convolution over plasma concentration, and  $C$  is the amount of fluid reabsorption per unit time and unit tubular length. The other dimensions have been introduced above.

The relationships of Eq [3] lead to the equation

$$\dot{V}_0 = \frac{\ln F/P}{1 - P/F} \frac{r^2 \pi l}{T} \quad [4]$$

that says that the factor  $R$  in Eq. [2] equals  $\frac{\ln F/P}{1 - P/F}$ . Since this factor changes

from 1.22 to 1.65 when  $F/P$  changes from 1.5 to 3.0, Eq. [4] is not very sensitive to changes in fractional reabsorption. It should be noted that Bojesen and Leyssac's theoretical treatment(2) leads to Eq. [4]. On the other hand Eq. [3] differs from those used by Schnermann *et al.*(3) by a factor of two. This arises from the fact that these investigators multiplied the equation of an earlier paper(4) by  $r$ ; in this paper the underlying theory differs from the second one in that the reabsorption *a priori* was expected to be proportional to surface area, that is  $2 \pi r$ , which leads to calculated transit times quite different from those measured. Furthermore,  $C$  in the Munich group is the reabsorption rate out of the total proximal convolution instead of unit tubular length.

The experimental data of several laboratories have been in reasonable agreement with the first part of Eq. [3]

$$\ln F/P = 0.693 \left( \frac{T}{t_{1/2}} \right), \quad [3a]$$

indicating that reabsorption rate increases proportional to  $\pi r^2$  as long as transit time remains constant.

Little agreement between the correlations given in [3] was found when values were compared with experiments that included measurement of tubular flow rates (free-flow conditions).

This disagreement occurs because measured single-nephron filtration rates are generally twice as high as those calculated by Eq. [4]. As long as this dis-

crepancy is not resolved, there is equal weight to arguments in favor or against the statement, that volume reabsorption measured with the split-drop method is quantitatively the same as volume reabsorption measured under free-flow conditions.

The above discussion is merely a description of what can be expected if fluid passes along proximal convolutions. The equations say nothing about driving forces that lead to fluid movement.

## REFERENCES

1. Gertz, K. H., Mangos, J. A., Braun, G., and Pagel, H. D., On the glomerular tubular balance in the rat kidney. *Pflügers Archiv.* 285, 360-372 (1965).
2. Bojeson, E., and Leysac, P. P., Proximal tubular reabsorption in the rat kidney as studied by the occlusion time and lissamine green transit time technique. *Acta Physiol. Scand.* 76, 213-235 (1969).
3. Schnermann, J., Wahl, M., Liebau, G., and Fischbach, H., Balance between tubular flow rate and net fluid reabsorption in the proximal convolution of the rat kidney. I. Dependency of reabsorptive net fluid flux upon proximal tubular surface area at spontaneous variations of filtration rate. *Pflügers Archiv.* 304, 90-103 (1968).
4. Gertz, K. H., Transtubuläre Natriumchloridflüsse und Permeabilität für Nichteletrolyte im proximalen und distalen Konvolut der Rattenniere. *Pflügers Archiv.* 276, 336-356 (1963).