Sources of Error in and Limitations in the Use of $t_{1/2}$ as a Measure of Tubular Reabsorptive Capacity

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The actual technique and theory behind the shrinking-drop method as originally described by Dr. Gertz(1) is well known and does not require a detailed description here. This paper will deal only with the method as applied to measuring transepithelial volume and sodium fluxes in segments of isolated tubular epithelium. However, as the years have gone by, the poor reproducibility of this method has incurred more disfavor than other micropuncture techniques. The reasons for this relatively poor reproducibility of $t_{1/2}$ as a measure of tubular reabsorptive capacity will be examined here.

The fundamental and only measurement in this technique involves the photographic recording of the rate at which two opposing oil columns approach each other as the fluid interposed between them is being reabsorbed. That is, the reabsorptive capacity of the epithelium depends on the rate of shrinkage of the droplet, which in turn is intimately related to the geometry of the epithelium as will be shown here. Various methods have been employed to measure the rate of disappearance of the fluid from the tubular lumen from this photographic recording; all arrive at $t_{1/2}$, the time taken for half of this volume to be reabsorbed, as an expression of this rate. Until now this has generally been considered a measure of reabsorptive capacity, but small alterations in the geometry of the tubule can lead to relatively large variations in $t_{1/2}$, large enough as shall be shown here, to explain entirely the poor reproducibility obtained with this technique.

The first major source of error is the meniscus error, one which had been noted earlier by a number of investigators, but had not been elucidated until recent work with both the dog and the rat(2,3). It had been previously realized

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that the meniscus error probably cause uncertainties in the measured $t_{1/2}$, but the actual size of the error was never really determined. Figure 1 shows in graphic form the effect of the meniscus error on $t_{1/2}$. Volume change is here equated with rate of change of the length between the tops of the approaching menisci. When volume change is recorded in this manner one ignores the re-absorptive surface covered by the menisci and, as can be seen, the smaller the droplet becomes the faster it shrinks. That is, if a small volume of test solution is injected, a faster half-time will be obtained than when a larger volume is injected. This results in: first a nonlinear rate of change of length, and second, as an expression of the first, a positive correlation between length and $t_{1/2}$. Figure 1 also shows that when the length is measured between the bases of the approaching menisci (i.e., when one tubular diameter is added to each length measured between the tops of the menisci) linearity is restored and, therefore, the correlation between initial length of injected fluid and $t_{1/2}$ is no longer present. In addition, the correction with one tubular diameter, that is, measuring between the bases of the menisci, also "slows" the $t_{1/2}$ thus measured.

From a comparison of a number of $t_{1/2}$ values measured with and without the correction(3), it became apparent that the smaller the ratio of initial length be-

**Fig. 1.** The graph part shows the semilogarithmic relationship between $l$ and time to illustrate the effect of each correction on linearity and halftime. The diagram shows the various correction factors used.
tween tops of menisci to tubular radius became, the larger was the error made in \( t_{1/2} \) by not using the correction factor. Using the regression obtained between this ratio and percentage of error in \( t_{1/2} \) values measured with and without the correction of:

\[
\% \text{error in } t_{1/2} = -2.77 \times 1/r + 54.7
\]

for initial lengths between 80 and 230 \( \mu \), one could predict the error which would be made in \( t_{1/2} \) by not using the correction factor. Table 1 shows how this method can be used. Two hypothetical tubules of identical diameter are used, but unequal amounts of fluid are injected into the two, so that the length in one is 120 while in the other it is 80 \( \mu \). If both tubules would have a real \( t_{1/2} \) of 12.3 sec obtained with the correction factor, it can be predicted that, without the correction, the tubule with the more fluid would have a \( t_{1/2} \) of 8.1 while that with less fluid would have one of 7.3 sec. The variation thus introduced is \( \pm 6\% \). It should be stressed that the mean difference in injected fluids of \( \pm 20\% \) has been purposely kept low and that in practice differences of up to \( 100\% \) are by no means rare and may thus produce even larger variations.

The next source of error, and one of equal importance, was the observation that when the variation in \( t_{1/2} \) due to the volume of fluid injected was eliminated and \( t_{1/2} \) made more accurate, in a large number of tubules with naturally varying radii the \( t_{1/2} \) varied with the tubular radius. In a naturally smaller tubule the measured \( t_{1/2} \) was faster than in a larger tubule. Figure 2 shows the correlation between the corrected \( t_{1/2} \) values and radius in a group of tubules with naturally varying radii. Again, the effect of this on measured \( t_{1/2} \) is shown in Table 1. Here the tubule with less fluid is made narrower at a radius of 14 \( \mu \) while the other was made larger at a radius of 18 \( \mu \), thus introducing an average variation of \( \pm 13\% \) into the tubular radii. From the relationship between radius and corrected \( t_{1/2} \) (Fig. 2) it can be predicted that the larger tubule will have a \( t_{1/2} \) of

![Fig. 2. Relationship between internal tubular radius and the corrected half-times (\( t_{1/2} \)) in 44 tubules of naturally varying sizes. The thin dotted line represents the relationship when the origin is at zero while the solid line represents the regression obtained from the experimental points, whose intercept on the y axis was not significantly different from zero.](image-url)
13.5 and the smaller one of 11.1 sec. There is now a variation of \( \pm 10\% \) even in the corrected \( t_{1/2} \) values. The variation is even greater when no correction is applied and would result in \( t_{1/2} \) values of 8.6 and 6.8 sec, a difference of 1.8 sec. If the amounts of injected fluid are increased so that the initial length of the thicker tubule is now twice that of the thinner tubule, and the radii varied by about \( \pm 30\% \) (2 \( \times \) SD as a percentage of the mean of a large number of naturally varying radii), the variation introduced into \( t_{1/2} \) values without the correction is \( \pm 23\% \), and with correction is \( \pm 21\% \). Why these two errors are so similar will become evident later. It is obvious now, however, that small variations in injected fluid and tubular diameter can lead to relatively large and nonphysiological variations, whether or not the \( t_{1/2} \) is corrected, and, therefore, the \( t_{1/2} \) cannot and should not be taken as a measure of reabsorptive capacity of tubular epithelium.

What then can be taken to represent the reabsorptive capacity? The relationship between radius and corrected \( t_{1/2} \) in tubules varying in width by nature implies a certain degree of constancy of reabsorptive capacity calculated per apparent tubular surface area with the formula derived by Gertz(1):

\[
J_{(oa)} = \frac{0.347 \times \text{radius}}{t_{1/2}} \text{ (mm}^3/\text{mm}^2 \cdot \text{sec)},
\]

where \( J_{(oa)} \) is volume flux, and radius is measured in millimeters. And indeed

| TABLE 1 |
| --- |
| **Magnitude of Variations and Absolute Errors Introduced into the Measurement of** \( t_{1/2} \) **Consequent upon the Geometric Alteration of the Relationship Between Volume of Injected Fluid and Area of Epithelium in Contact with this Fluid** |
| --- |
| **Radius** & **16 \( \mu \)** & **16 \( \mu \)** & **18 \( \mu \)** & **14 \( \mu \)** & **Radius, “average”** & **“16 \( \mu \)”** & **“16 \( \mu \)”** |
| With correction factor (C.F.) | 12.3 sec | 4.52 | - | 11.1 sec | 4.52 |
| Without C.F. | 8.1 sec | 6.84 | \( \pm 6\% \) | 7.3 sec | 7.64 |
| Absolute error | 52% | | | 69% | |
| **Radius** | **18 \( \mu \)** & **14 \( \mu \)** & **Radius, “average”** & **“16 \( \mu \)”** & **“16 \( \mu \)”** |
| With C.F. | 13.5 sec | 4.52 | \( \pm 10\% \) | 11.5 sec | 4.81 |
| Without C.F. | 8.6 sec | 7.26 | \( \pm 12\% \) | 6.8 sec | 8.21 |
| Absolute error | 52% | | | 69% | |
when reabsorptive capacity was expressed as volume flux per apparent surface area, there was no longer any correlation between it and either radius or \( t_{1/2} \). It is recognized that internal tubular radius does not necessarily give the correct measure of actual reabsorptive surface area. Nevertheless, the constancy of reabsorption when calculated in this manner implies that internal tubular radius does maintain a relatively constant relationship to the real area of absorption whatever that may be. As shown in Table 1, with the correction factor when reabsorptive capacity is expressed as volume flux per surface area, it remains constant irrespective of the amount of injected fluid or the radius of the tubule.

Two additional points are revealed in this table. The first explains why not all previous studies employing \( t_{1/2} \) gave inconsistent results. If one was lucky or careful enough to “choose” the correct difference between injected fluid and tubular radii, one could have obtained a constant volume flux without the correction factor (see Table 1), but one that grossly overestimated the reabsorptive capacity. This is because if less fluid is injected into a narrower tubule, the ratio of length to radius can become by chance similar to that in the larger tubule with more fluid. When more fluid is injected into the narrower tubule than into the wider tubule, the \( t_{1/2} \) values will be approximately the same but volume fluxes will now differ. In other words, injecting more fluid into a narrower tubule than into a thicker tubule will result in two errors, each cancelling out the other producing apparent constancy of the determination, but in reality leaving a large absolute error.

It is also clear from Table 1 that one cannot become “lazy” and instead of measuring individual radii take an “overall” radius in one’s calculations of either the corrected \( t_{1/2} \) or of volume flux. If this is done for both the \( t_{1/2} \) and \( J_{v(a)} \), the variation introduced in our two hypothetical tubules will be \( \pm 5\% \) for both (Table 1), and an absolute error of the order of \( 3-6\% \) will be made. The reason for this is that when one uses an inappropriate radius for the correction factor, the \( t_{1/2} \) obtained will be altered but in the opposite direction from which the over- or underestimation of the radius will influence the calculation of the volume flux, thereby cancelling each other out to some extent but not entirely. Should one, under similar circumstances, obtain the correct \( t_{1/2} \) by using individually measured radii for each tubule, but then use an “overall” radius for calculating \( J_{v(a)} \), the errors will be \( \pm 10\% \), and absolute errors of the order of \( -9\% \) and \( +10\% \) will be made.

The calculations used in the “overall” radius and its consequent errors also provide an accurate indication of the possible errors made when radii are measured incorrectly: a \( \pm 13\% \) error in measuring a radius will produce an absolute error of \( 3-6\% \) and a variation of \( \pm 5\% \) in volume flux. In practice, however, by using only sharp and clear pictures a tubular radius can be measured to within \( 5-7\% \) thus producing even smaller errors.

A great number of measurements performed in a routine manner, employing only clear and sharp pictures of straight tubules and using a computerized statistical method to eliminate observer bias instead of the usual “eye-ball” method for obtaining \( t_{1/2} \), and using only those with a correlation coefficient of greater
than 0.99, duplicate measurements gave a coefficient of variation of $J_{v(e)}$ of between 10 and 14%, which compares rather favorably with all other micropuncture techniques.

In conclusion, we believe that all inconsistencies of the shrinking drop technique can be explained on the grounds of reabsorptive capacity being equated with reabsorptive half-time which is systematically influenced by the geometry of the tubule. These inconsistencies can be overcome by correcting the $t_{1/2}$ and by comparing reabsorptive capacity of different tubules with naturally varying diameters as volume flux per apparent surface area. Comparing them per unit tubular length introduces an additional source of variation no matter how reabsorptive capacity is measured: by the shrinking drop technique or by TF/P inulin ratios. To allow comparisons to be made between different publications, the mean radii of the tubules used should also be recorded.

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