

## Perfusion of Isolated Renal Tubules

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The methods used for perfusing isolated renal tubules are generally different from micropuncture, as are the problems encountered. Although I will concentrate mainly on the technical aspects of perfusion, it seems to me most important to evaluate to what extent the results are pertinent to the study of kidney function. Are the tubules still "alive?" Are they still functioning as they did in the animal? My answer to the first question is that they are "alive," and I will later summarize the evidence for this by briefly reviewing some transport systems in proximal tubules. My answer to the second question is that function should differ *in vitro* from *in vivo* even with uninjured tubules since the environment of the tubule is altered. The transport mechanisms, however, should be basically the same, and the superior control *in vitro* should allow individual factors which influence tubule function to be evaluated more precisely than *in vivo*.

The most difficult part of *in vitro* perfusion is dissection of the tubules. Despite having tried many other animals, we have successfully dissected and perfused tubules only from rabbits(1) and flounders(2). We were unsuccessful with rats, mice, guinea pigs, hamsters, dogs, monkeys, frogs, toads, and *Necturi*. Even with rabbits there is variability in the ease of dissection from animal to animal which is unexplained. Persons learning the technique ordinarily require several months to dissect adequately, and continue to improve with practice for years. All of the segments of the rabbit nephron can be dissected in this fashion except for the papillary collecting duct which has multiple branches and tends to split open. The distal convoluted tubule has not been studied since it is very short (<1 mm) and the thin ascending limb of Henle's loop is difficult to identify. All the other segments have been dissected and perfused [proximal convoluted tubule(3), proximal straight tubule(3), descending limb of Henle's loop(4), thick ascending limb (Burg, unpublished observations), and cortical collecting tubule(5)].

Tubules which have been dissected are examined microscopically for defects

when they are first perfused. Since the tubules are ordinarily observed with an inverted microscope at magnification of up to  $400\times$  and the lighting conditions are favorable, we can often discriminate even a single damaged cell. Thus, we are reasonably certain whether the tubule is anatomically intact. Also, we have observed that the cells become visibly distressed, as evidenced by swelling, vacuolization, or other changes when the experimental conditions are too drastic, providing a further check on the condition of the tubule. Figure 1 is a photomicrograph of a portion of a perfused rabbit proximal convoluted tubule. We judged it to be acceptable for study since it is uniform in appearance, the luminal surface is smooth with the brush border clearly visible, and the vacuoles are mostly limited to the subapical region of the cells.

Provided that the dissected tubule is in good condition, the principal technical problem of perfusion has been to avoid leaks between the lumen and the bath. Some of the technical arrangements which we have used to perfuse tubules are shown in Fig. 2. The original procedure(1) (Fig. 2A) utilizes two concentric glass pipets. The outer pipet is attached to a syringe to provide suction for positioning the tubule over the inner pipet. The inner pipet contains perfusion solution and is attached to the pump. This arrangement is adequate for most studies, such as measurement of fluid absorption. There may be diffusion of ions between the tubule and the pipets, however, so the method is unreliable for electrical studies. For straight segments such as cortical collecting tubules the seal can be improved by advancing the inner pipet (Fig. 2A) so that its outside is in contact with the

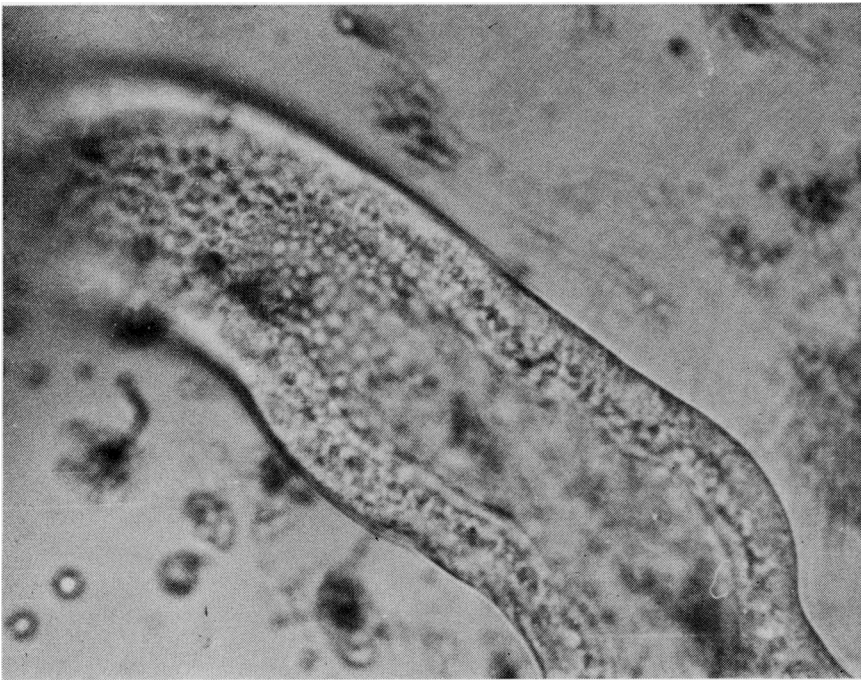


FIG. 1. Proximal convoluted tubule during perfusion.

tubule lumen over a distance of several hundred microns(6). Since this procedure carries a risk of damaging the tubule, and cannot be used for convoluted tubules, we now prefer to insulate with Sylgard 184, a liquid dielectric. Originally we placed it in a bulbous extension of the outer pipet(7) (Fig. 2B) but subsequently found it easier to use a separate pipet for the Sylgard 184 (Fig. 2C). A further improvement is the use of a fluid-changing pipet within the perfusion pipet (Fig. 2C). By the use of valves (not shown) the appropriate perfusion fluid can be placed within a few seconds into the tip of the perfusion pipet and the waste fluid drained from its rear. The obvious advantage is that a variety of perfusion fluids can be tested in a single tubule, whereas only one could be tested previously. In addition the pipets can be left assembled between experiments, greatly reducing the time previously spent assembling and disassembling them. Also, considerable care was previously required to prevent bath fluid from entering the tip of the perfusion pipet and diluting its contents while the tubule was being cannulated. With the new arrangement the correct perfusion solution can be placed in the pipet after the tubule is attached.

Leaks are an even greater problem at the collection end of the tubule. The original arrangement(1) is shown in Fig. 3A. Although it is possible to place a pipet within the lumen at the collection end(8), we found that the pipet plugged easily and that the use of an external holding pipet was more practical. In order to prevent leaks with the arrangement in Fig. 3A the holding pipet must be small enough to squeeze the tubule tightly without completely occluding it. Otherwise fluid tends to leak around the tubule into the holding pipet from the

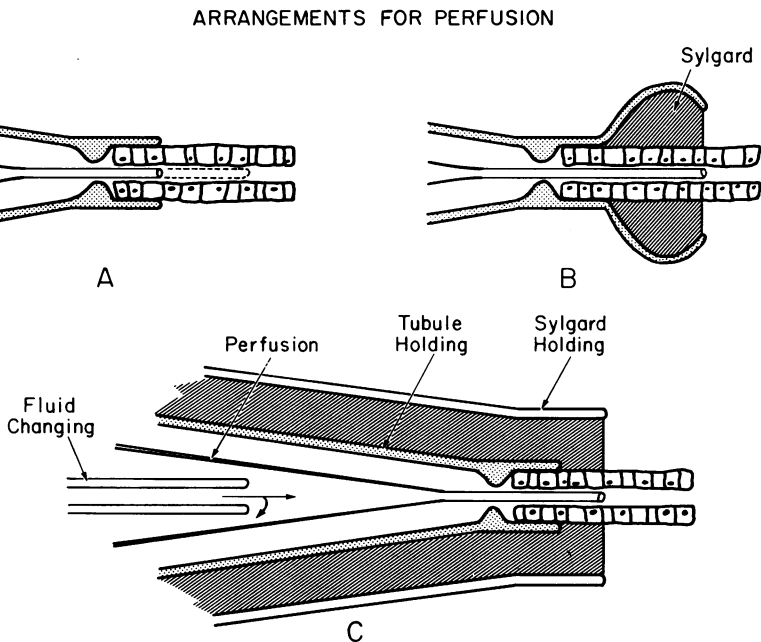


FIG. 2. Arrangements for perfusing renal tubules *in vitro*.

bath. Even with the utmost care it is not possible to get adequate electrical insulation in this fashion. Significant quantities of small molecules such as urea may be lost(9) and there is an electrical short circuit(6). To obviate these problems Sylgard 184 was used to insulate between the tubule and the glass (Fig. 2B). The Sylgard can be placed within the tubule-holding pipet as in Fig. 2B(9), but pockets of fluid develop if the Sylgard mixes with the mineral oil which is in the pipet to prevent evaporation. Therefore, we now prefer to use a separate pipet for the Sylgard (Fig. 2C), as at the perfusion end.

Originally, we used a calibrated capillary glass tube to collect and measure the perfused fluid(1) (Fig. 2A). A constriction pipet is more accurate (Fig. 2B), and can be used to collect multiple serial samples (Fig. 2C). We measure the time for each sequential filling of the pipet in order to calculate the rate of collection of the perfused fluid.

I would now like to return to the problem of whether the tubules are alive. The best evidence that we have that they are alive is that the cells in the various segments have a normal appearance both on light and electron microscopy during perfusion(10,11) (Fig. 1) and the transport systems continue to function at an apparently normal rate.

We have measured fluid and Na transport, and glucose absorption in isolated proximal convoluted tubules. The major problem in evaluating whether the measured rates of transport are "normal" is that there are no direct measurements of single proximal tubule function in rabbits. Rabbits are notoriously labile animals, so that it is difficult to evaluate even such relatively simple measures as

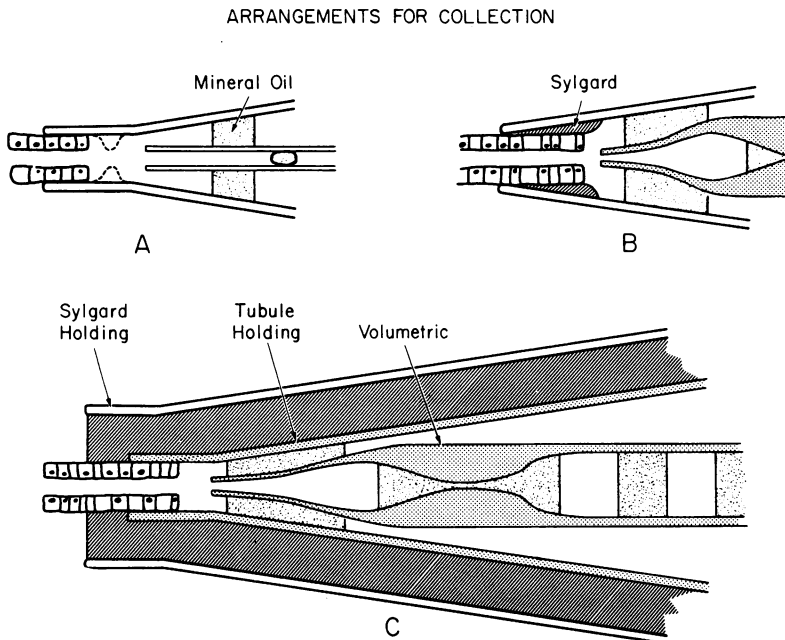


FIG. 3. Arrangements for collecting fluid from perfused renal tubules *in vitro*.

glomerular filtration rate(12) let alone perform micropuncture studies. Also, most of the results reported are from larger rabbits than the ones we use (approximately 1.75 kg weight). With these reservations in mind we have attempted to estimate rabbit single nephron GFR from the values for glomerular counts and creatinine clearance in the literature. Strassberg *et al.*(13) measured endogenous creatinine clearance and counted the glomeruli in the same rabbits of 1.76 kg average weight. The single nephron GFR was 14 nliters  $\text{min}^{-1}$ . We consider this to be the most reliable figure since all of the measurements were in the same rabbits which were equal in size to ours. The identical value results from the use of the GFR measurements in larger rabbits by Brod and Serota(14), [Smith cites these as most reliable(12)] and the glomerular count(15,16), provided the GFR measurements are corrected for body weight.

The measured rate of fluid absorption in isolated perfused rabbit proximal convoluted tubules is 1.18 nliters  $\text{mm}^{-1}$  tubule length  $\text{min}^{-1}$ (3). Thus, approximately 6 mm of tubule length is required to absorb half of the glomerular filtrate, which is the fraction absorbed in rats at the end of the accessible portion of the proximal tubule. The total length of the proximal tubule in rabbits this size is approximately 8 mm(17). Thus, the isolated rabbit tubules absorb fluid at what appears to be a reasonably normal rate. Further, the isolated rabbit proximal tubules are able to transport Na against a concentration gradient of approximately 30 mequiv liter $^{-1}$ (18) similar to the result in the rat, and other measures such as reflection coefficient for NaCl and permeabilities to water and salt are also in reasonable agreement between the two species(18).

The glucose Tm in isolated perfused rabbit proximal convoluted tubules is  $78.5 \times 10^{-12} M \text{ mm}^{-1} \text{ min}^{-1}$ (19). The ratio of glucose Tm to whole kidney GFR is 3.7 mg  $\text{ml}^{-1}$  in the intact rabbit(20). Thus, an estimated 4 mm of proximal convoluted tubule is necessary to account for glucose absorption of the intact kidney. This again is in agreement with the actual length of proximal tubules in rabbits this size.

Considering these and other similar results we believe that isolated perfused rabbit renal tubules survive *in vitro* and maintain reasonably normal function. Further, as the technical aspects of perfusion have evolved, the scope of the physiological problems which can be investigated with this preparation has increased.

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