Some New Developments in Continuous Microperfusion Technique

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Nine years ago we designed the prototype of a microperfusion pump for calibrated perfusions of tubular segments in vivo (1). The apparatus consists, in principle, of a metal pin which is driven into the fluid-filled shaft of a micropipet. Since the base of this pipet is sealed, the fluid may escape with a constant flow rate only through the tip of the capillary.

In the meantime many improvements were developed both in the apparatus and in the perfusion technique. I should like to start this review with a brief description of new details in the equipment. Formerly the pump was driven by an electric synchronous motor and the pump rate was adjusted by regulating the speed of the motor. This resulted in a reliable reproducibility of the pumping rate. However, the constancy of perfusion rate was dependent on a highly stable electric power supply. Furthermore, calibration of the pump was somewhat troublesome since it had a sigma-shaped calibration curve with only a relatively short region in which the pumping rate could be precisely established (Fig. 1). In the new system, constancy of perfusion rate is stabilized by a self-regulating electronic device. Because the calibration curve has a linear characteristic, the calibration procedure is simplified. The new apparatus was built by V. Rosliceck and Wolfgang Hampel, the engineer who previously had helped us to develop the first type of microperfusion equipment. The new principle is depicted schematically in Fig. 2.

In spite of the great advantages which the new apparatus provide, it should be emphasized that the most frequent pitfalls encountered with the microperfusion pump remain the same. The most critical point is the careful mounting of the microcapillary to the pump. When the base of the glass capillary does not exactly fit into the Teflon seal around the forward-moving metal pin, then the fluid displaced by the pin does not quantitatively escape through the tip of the
capillary and the perfusion rate is uncontrolled. To avoid this, we first fill the chamber above the Teflon seal with mineral oil before we mount the capillary holder. Thus, any air bubble will be ejected from the system when the holder is screwed down. After this, the capillary is inserted and fixed. Even if the base of the micropipet is not completely closed off at the Teflon seal, no fluid may escape in a retrograde direction since the capillary base is immersed in incompressible fluid.

Another pitfall which leads to inaccuracy of the pump occurs when the metal pin is distorted causing rotatory movement of the pipet. Enhanced by the long lever, movement of the pipet tip is in the range of several microns and necessarily will lead to leakage around the perfusion capillary.

![Graph](image)

**Fig. 1.** Calibration curves of the microperfusion pump; left: old prototype; right: newly developed apparatus.

![Diagram](image)

**Fig. 2.** Scheme of the self-regulating electronic device to stabilize the speed of the microperfusion pump.
Next, I should like to mention a few improvements in the tubular perfusion technique. In our hands, it proved to be of advantage to work with three micro-manipulators. The classical procedure depended on two manipulators and two pipets. After filling the tubular segment between the two capillaries with mineral oil, the oil capillary was withdrawn and reinserted distally to collect perfusate for analysis. We now leave the first oil capillary in position and use a second one for collecting downstream (Fig. 3). The proximal oil capillary is used to aspirate glomerular fluid and to adjust intratubular pressure in front of the oil column in order to maintain position of the oil block. At the end of the collection period, the first oil capillary is used to inject as many oil droplets into the perfused section as necessary to make a precise drawing of nephron topography for later identification during microdissection. These advantages of a third capillary have greatly increased the number of successful microperfusions.

The last point to which I should like to draw your attention concerns handling of the experimental animal during the microperfusion experiment. In our first description of the microperfusion method, we noted that it is an inevitable requirement of a reliable micropuncture experiment to control the body temperature of the experimental animal(1). We are now able to substantiate this early observation with precise data.

Regulation of body temperature of anesthetized rats is rather poor. At a room temperature of 22°, rats cool down in 30–50 min from their normal body temperature of 37–38° to about 34°. The mean arterial blood pressure is unaffected and remains constant at 100–110 mm Hg. Cardiac output is significantly reduced by about 25% (Fig. 4). The most marked effect, however, is a decrease in renal cortical blood flow (RCBF).

To measure RCBF rats were anesthetized and prepared in the usual way for micropuncture experiments(2). The left kidney was exposed through a flank incision and immobilized in a Plexiglas cup. As shown schematically in Fig. 5, a small area of the kidney surface was illuminated through the tip of a conical glass rod which was positioned a few microns above the kidney surface by a mi-

Fig. 3. Technique of microperfusion in a proximal tubular segment.
cromanipulator. A second similar glass rod was focused on the illuminated spot. Its upper end was connected to a photo-sensitive resistor. For optical insulation, the rod was covered with light-reflecting material as far as its tip where an area of not more than 1 mm² permitted the entrance of reflected light from the kidney surface. The photoresistor was sensitive at 805 mμ. This corresponds to an isosbestic point of hemoglobin. Thus, the measured light reflection was independent of oxygen saturation. After injection of 50 μl of a cyanine dye (indocyanine

![Graph](image)

**Fig. 4.** In anesthetized rats spontaneous fall in body temperature is accompanied by a proportional fall in cardiac output while mean arterial blood pressure (MABP) remains unchanged.

![Scheme](image)

**Fig. 5.** Scheme of the photoelectric device for recording cortical mean-transit times.
green, Cardio-Green) into the aorta cranial to the renal arteries, dye-dilution curves were recorded from the kidney surface and mean transit times were determined. To calculate RCBF from mean transit time, it is necessary to know the vascular volume of this area. According to earlier experiences in our laboratory(3) this value was estimated by measuring vascular hematocrit and hemoglobin concentration of cortex tissue from rat kidneys under the same experimental conditions. Tissue for analysis was obtained from kidneys frozen instantly by pouring liquid nitrogen into the abdominal cavity. Before freezing, vascular hematocrit was controlled by puncturing peritubular capillaries with micropuncture pipets. We never observed any systematic differences between the hematocrit of peritubular and systemic arterial blood.

As summarized in Table 1, vascular volume in a well-heated rat was 17.6 ml blood/100 g. tissue while the average mean transit time amounted to 0.93 seconds. This results in an average RCBF of about 11.4 ml/g.min. Since the hematocrits averaged 48%, renal cortical plasma flow had a mean value of 5.9 ml/g.min. Glomerular filtration rates in these experiments were measured to be between 1.0 and 1.5 ml/g.min. Calculated filtration fractions, therefore, were in the normal range of 0.26.

In an animal with a body temperature of 34°, vascular volume decreases by about one third and mean cortical transit time increases by a factor of 2.3, both changes apparently due to renal cortical vasoconstriction. The resulting decrease in renal cortical blood flow is in the range of 70% compared to only 25% decrease of cardiac output at this temperature. The results of 122 measurements of RCBF at body temperature between 28.2 and 39.2° are demonstrated in Fig. 6. The exponential fall of the curve documents quantitatively an event which we

![Fig. 6. Relation between spontaneous decrease in body temperature in anesthetized rats and in renal cortical blood flow (RCBF).](image-url)
have to realize as a factor influencing functional parameters predominantly just in the region where most micropuncture experiments are performed.

**TABLE 1**

| Body temp. (°C) | Mean passage time (sec) | Vascular volume (ml/100 g tissue) | RCBF (ml/g/min) |
|----------------|-------------------------|----------------------------------|-----------------|
| 38             | 0.93                    | 17.6                             | 11.4            |
| 34             | 2.11                    | 11.8                             | 3.3             |

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