RECENT DEVELOPMENTS OF MICROMETHODS FOR THE STUDY OF RENAL PHYSIOLOGY AND PHARMACOLOGY

K. J. ULLRICH

The Physiological Institute Göttingen

CHIEFLY, I want to talk about the micromethods used by our group* in the Physiological Institute of the University of Göttingen for the last six years, to study the transport processes in the different parts of the nephron. From our results I will show only a few of those which are concerned with pharmacological effects.

I. MICROPUNCTURE, MICROCATHETERIZATION, MICROCUVETTE

The initial micropuncture experiments with analysis of tubular fluid were performed by Richards and co-workers[13]. Of particular note were the experiments of Walker[24] which are milestones in our knowledge

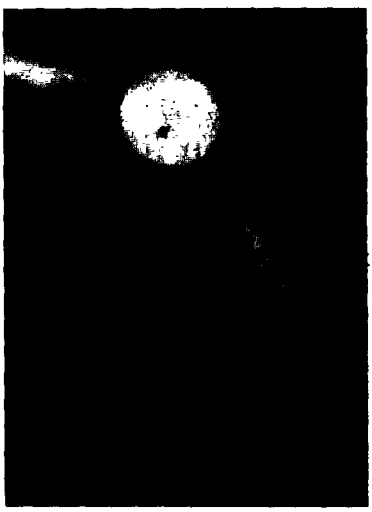

FIG. 1. Renal papilla of golden hamster with a polyethylene catheter introduced into one collecting duct. By adhesion a wax drop holds the catheter in this position.

* The work was started by Dr. K. H. Jarausch and continued by Dr. H. H. Hilger, Dr. J. D. Klümper, Dr. F. M. Eigler, Dr. W. Karger, Dr. K. H. Gertz, Dr. H. Stöckle and Miss G. Pehling.

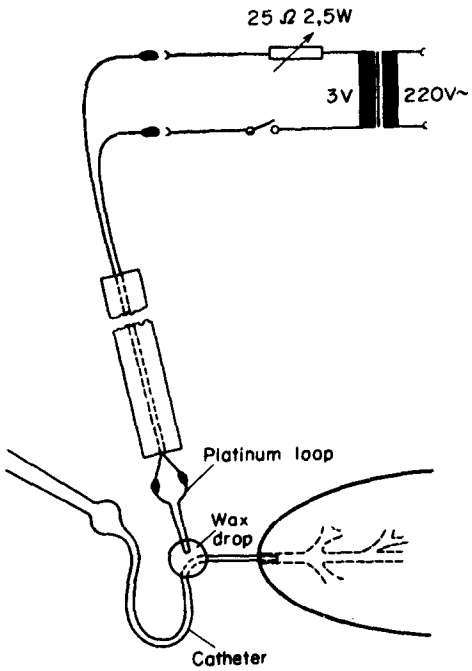

FIG. 2. Method for catheterization of a collecting duct. A polyethylene capillary is connected with a platinum loop by a wax drop so that the open end of the catheter can be introduced into a collecting duct.

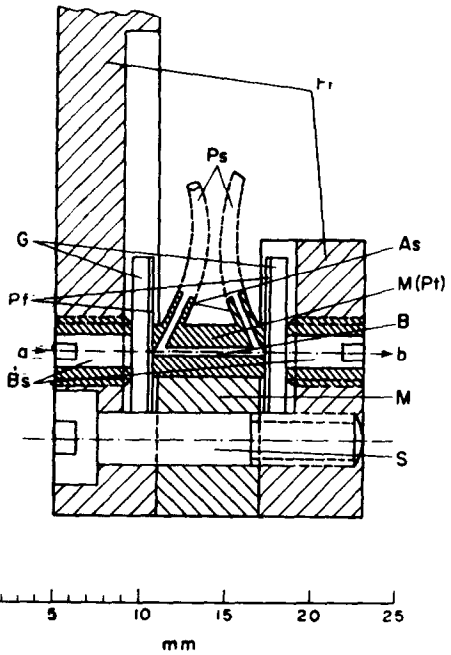

FIG. 3. Schematic drawing of a microcuvette. a → b = light path; Platinum tube (M (Pt) incl. B) with nozzles (as); glass windows (G) and sheets of polyethylene (Pf) for tightening. BS and S = Screws, Fr and M = brass housing.

of the function of the different parts of the mammalian kidney. This type of work, which gives us information concerning the localization and rough quantification of the transport processes has been continued in more recent years by Gottschalk[7], Wirz[26] and Giebisch and Windhager[6,25]. We have worked in the same field in respect to the transport processes in the collecting ducts[9,8,12,17,18]. For this we catheterized the collecting ducts with small polyethylene catheters (Fig. 1). From a polyethylene tube of 4 mm diameter small tubes of 1 mm diameter were first drawn and then in a second step they were pulled into capillary filaments 20-40 μ outside diameter. The microcatheters were then introduced into the collecting ducts frehand using a wax drop on a platinum loop (Fig. 2). On heating the loop the wax melted and, on cooling, it connected the loop with the polythylene microcatheter, so that it could be handled during the catheterization procedure. Upon subsequent heating the loop and catheter were disconnected and the latter remained in its position within the collecting duct. Subsequently this method has been used with slight modification by neurophysiologists to introduce microelectrodes into the cerebrum and to disconnect the electrodes from the manipulator. Thereby, the leads become flexible and pulsatile and respiratory artefacts are avoided[1].

Fig. 3 shows the microcuvette we use[19]. The main part is a platinum tube, closed tightly on both sides with a piece of glass and a little sheet of polyethylene. Through two nozzles, inflow and outlet, the cuvette can be filled. The capacity of the cuvette is 2·5 μl so that 7–10 μl total volume for cleaning and filling is sufficient. The lightpath within the cuvette is 6 mm. We used the cuvette first with the Zeiss-Spectrophotometer but for the past 5 years have used it with the Beckman Spectrophotometer model DU. Using this microcuvete we have determined inulin[8], urea[12], ammonia[17], glucose[14], lactic acid[14], protein[20] and haemoglobin[21]. Since, as already mentioned, the method of micropuncture allows only the localization and rough quantifications of transport processes I do not want to go into further details about it. The method is not particularly suitable for detecting small effects of drugs on the tubules.

II. APPLICATION OF USSING'S SHORT CIRCUIT METHOD ON KIDNEY TUBULES

More profitable for pharmacological work on kidney tubules is the application of Ussing's short-circuit method[22]. Therefore I shall talk about the modification of this method by Karger[10,10a]. The active transport of ions through a membrane may be demonstrated by the following scheme. (Fig. 4). E_a is the electromotive force of the ion battery, R_a is its internal resistance. The EMF moves a current of passive ions I_p through the membrane thereby overcoming a resistance R_p. The electrical potential (V) which can be measured across both sides of the

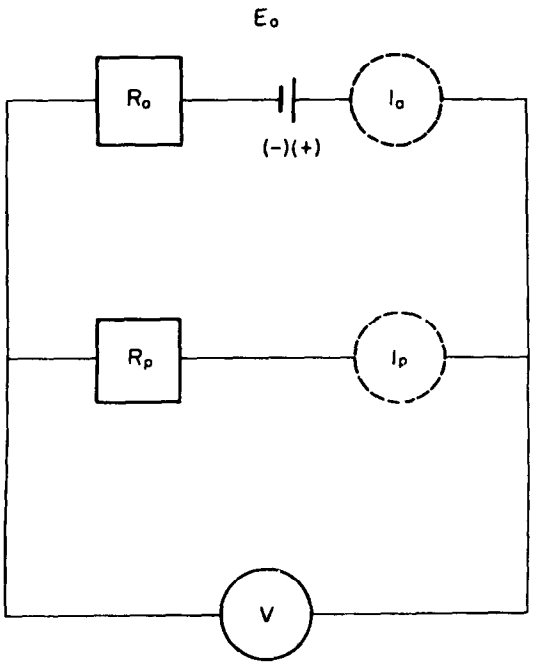

FIG. 4. Model of a transport mechanism for ions.
E_a = electromotive force acting on the actively transported ions;
R_a = resistance for the actively transported ions;
I_a = current of actively transported ions;
I_p = current of passively transported ions;
V = membrane potential; $V/E_a = R_a/(R_a + R_p)$.

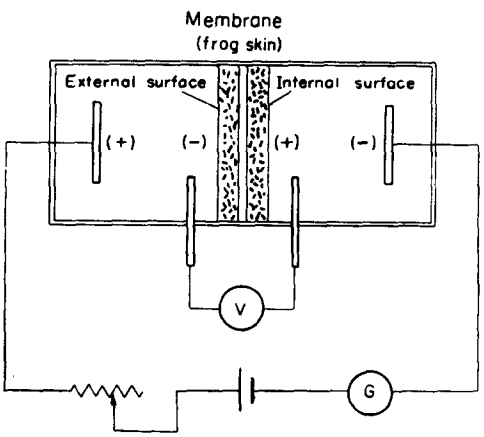

FIG. 5. Arrangement for short circuiting a membrane by applying a countercurrent so that the transmembrane potential becomes zero. The current of the galvanometer (G) corresponds then to the short circuit current i. e. the net amount of actively transported ions.

membrane is smaller than E_a. It is smaller, the smaller the resistance for the passive ions. The membrane is electrically short circuited by definition if the transmembrane potential is zero. In practice the transmembrane potential can be reduced to zero by applying an electrical counter potential (Fig. 5). In this case the membrane has in effect no resistance for passive ions. The ionic battery within the membrane must only overcome the internal resistance (R_a) since the external battery which creates the counterpotential supplies energy to overcome the external resistance of the whole circuit. Of course there is only one current value at which the case of electrical short circuiting of the membrane exists. In the experimental setup described by Ussing and Zehran[22] for the frog skin, two electrodes were placed at each side of the membrane, one pair for applying the counterpotential, and one pair for measuring the electrical potential.

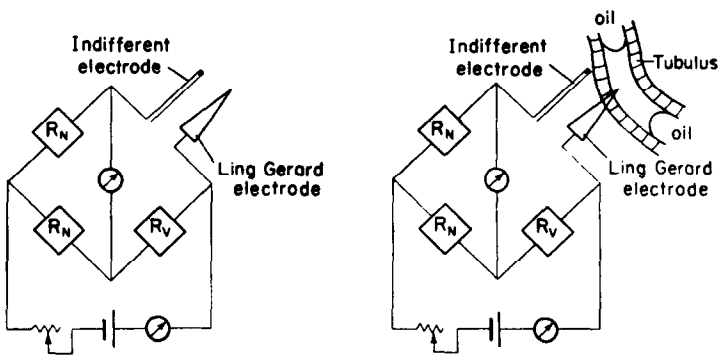

FIG. 6. Modification of Ussing's short circuit method: (a) In a bridge arrangement the resistance of the electrodes in saline is measured. (b) Then the tubule is punctured and by a variable voltage source the current changed until the resistance of the electrodes plus tubular membrane is the same as the resistance of the electrodes alone. In this case the current going through this bridge part corresponds to the short circuit current.

Because it is not easy to place two Ling-Gerard electrodes within the tubular lumen, Karger[10,11] worked out a modification where only one electrode is necessary on each side of the tubular membrane (Fig. 6). The resistance of the electrodes themselves is measured before impalement of the tubular lumen. Then the tubule is punctured with the Ling-Gerard microelectrodes and one searches for the current at which the resistance of the tubular membrane plus electrodes is as large as the resistance of the electrodes alone before impalement. In this case the tubular membrane has effectively no resistance and the current corresponds to the short circuit current. Unfortunately, the resistance of the Ling-Gerard capillaries is not independent of the current so that the current-resistance characteristics must be measured before, during

and after the puncture of the tubule. The intersection of the curve before (and after, which must be the same) and the curve taken during the puncture corresponds to the short circuit current (Fig. 7). The short circuit current is due to the net transport of the actively transported

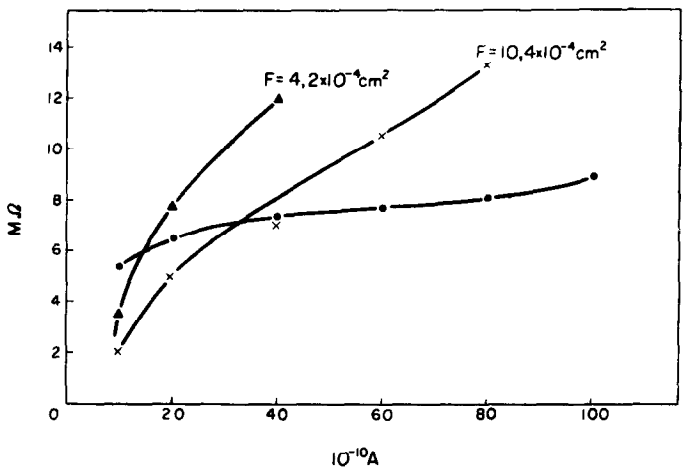

FIG. 7. Example of determination of circuit current from intersections of characteristic curves obtained in bathing solution, circles, and in tubular lumen, triangles and crosses. Electrical resistance in 10^6 Ω is plotted against electrical current in 10^{-10} A. F is area shortcircuited.

ions. With this method my colleague Dr. Eigler[2] has measured the active transport of ions through the proximal tubule of *Necturus*. More recently, Giebisch[5] performed similar measurements on rat tubules.

If the net transport of actively transported ions through the proximal tubules wall is chiefly a sodium transport, this method would be suitable to check pharmacological effects on the sodium transport.

I should mention that $I_s = E_s/R_s$ so that the ratio electromotive force to internal resistance is obtained by this method.

III. MEASUREMENT OF THE TRANSPORT OF NaCl AND PERMEABILITY TO NONELECTROLYTES IN SINGLE KIDNEY TUBULES

To study the mechanism of action of the saluretic sulphonamides, the method of short circuit measurements seemed to us too difficult to start with. Therefore, my colleague, Dr. Gertz, and I[3] have elaborated a simpler method for measuring the NaCl transport through the proximal and distal tubule.

The tubule is punctured by a double-barrelled Pyrex capillary, whose tip is ground with diamond paste. Then the lumen of the tubule is filled with coloured oil and a small amount of saline or tyrode solution is injected. The rate of reabsorption of this solution is photographed.

Because the solution in the lumen of the proximal tubule is always isotonic with the plasma, the rate of reabsorption of fluid also indicates the rate of reabsorption of the solutes. Figure 8 shows the reabsorption of injected NaCl solution from the proximal tubule.

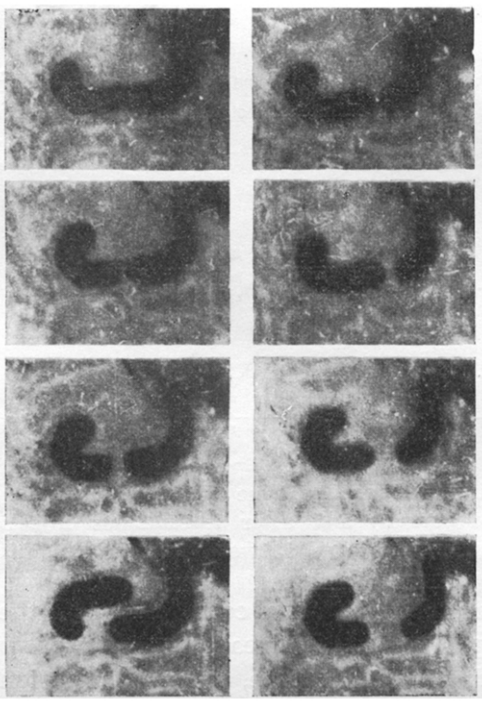

FIG. 8. Reabsorption of saline from a single proximal tubule (2 series). The gap between the columns of coloured oil is due to the injected fluid. The pictures were taken at 5 sec intervals.

The process, as expected, follows an exponential curve. The half volume reabsorption time is 10 sec. With this method the action of saluretic diuretics on the proximal NaCl reabsorption was tested. We used Hygroton in a dose of 40 mg/kg intravenously. The measurements 20–120 min after administration showed that sodium chloride reabsorption is diminished about 25% (Fig. 9).

Now I want to show you an example of how the method of Dr. Gertz[3] may be used to obtain measurements of the permeability of the tubules for nonelectrolytes which may have interesting aspects for pharmacological studies. If one injects instead of isotonic NaCl or Tyrode-solution an isotonic solution of a nonelectrolyte, i.e. mannitol or sorbose, then one observes first an elongation of the injected fluid column, apparently due to an inward flow of NaCl and water. If the injected solute cannot pass through the tubule wall, the size of the fluid column finally remains unchanged. But if the injected nonelectro-

lyte penetrates through the tubule wall the injected fluid column becomes smaller again. The quicker the nonelectrolyte diffuses out of the tubule, the quicker the fluid column disappears. This process also has an exponential time course. In this case, it is supposed that there is

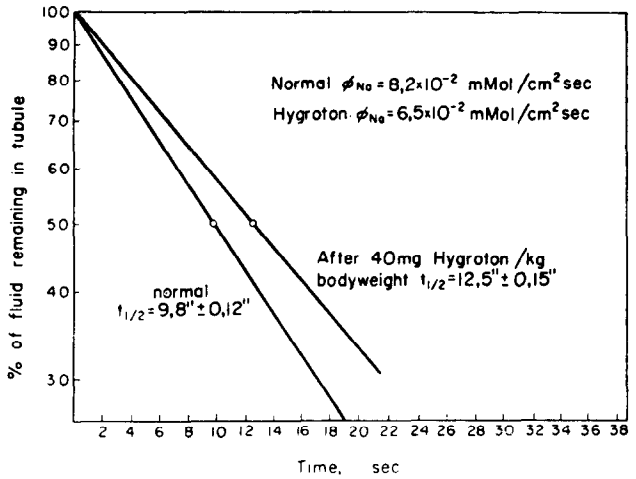

FIG. 9. Half value reabsorption time of saline from the proximal tubule of rats. Before (normal) and after intravenous injection of Hygroton 40 mg/kg. Φ Na = Outward transport of Na ions.

a steady state, meaning that the composition of the fluid within the lumen is unchanged during the reabsorption process. By withdrawing fluid from the tubule at that time, the concentration of nonelectrolyte can be determined indirectly by conductivity measurements. Consequently the permeability coefficient may be calculated from the time course of outward diffusion from the tubular lumen and from the concentration of the particular substance within the shrinking fluid column.

Experiments concerning the influence of pharmacological agents on the permeability of the tubular walls have not yet been done.

IV. MEASUREMENT OF ACTIVE TRANSPORT POTENTIALS (E_a) ON SINGLE KIDNEY TUBULES

For the diffusion of an electrolyte through a membrane we have the equations $M_o = P\,c_i$ (1) and $M_i = P\,c_o\,e\,\exp\,(\pm\,zFE)/(RT)$ (2) (P = permeability for the particular ion, c_i and c_o = concentrations at the inside and the outside respectively, M_i and M_o = unidirectional fluxes.)

(1) divided by (2) is:

$$\frac{M_o}{M_i} = \frac{c_i}{c_o\,e\,\exp\,\frac{\pm\,z\,F\,E}{RT}}$$

if we rearrange this equation we get:

$$0{\cdot}058 \lg \frac{M_o}{M_i} = 0{\cdot}058 \lg \frac{c_i}{c_o} \pm E$$

If an ion species follows this equation its flux is passive, if not, its flux is active. One can test the equation by making $M_o = M_i$ i.e., making the net flux zero. This condition can be obtained in the kidney tubule with sufficient accuracy if one injects an isotonic Raffinose-Tyrode solution into the tubular lumen. After an equilibration period, the injected volume remains almost unchanged, so that we have a net flow of approximately zero.

Use of sodium data from the rat proximal tubule in this equation yields an inequality equivalent to 29 mV. This deviation can be considered as the EMF acting on the sodium ions. This force was named by Ussing as $E_{Na}{}^{23}$. The same value could be calculated from the data, which A. K. Solomon[15] and Giebisch[4] obtained from experiments on the proximal tubule of *Necturus*.

SUMMARY

(1) It is possible with single tubules of the kidney: (a) to measure the efflux of sodium chloride, (b) to measure the short circuit current created by the net transport of actively transported ions, (c) to determinate whether an ion species is transported actively or passively, and how large the force is which acted on the particular actively transported ions, and (d) to measure the permeability of the tubular wall for nonelectrolytes.

(2) It is shown that Hygroton (40 mg/kg i. v.) diminishes the outflux of NaCl about 25% in the proximal tubule.

REFERENCES

1. R. v. BAUMGARTEN; Zur Technik der Mikroableitung am pulsierenden Gehirn. *Naturwissenschaften* **44** 22 (1957).
2. F. W. EIGLER; Short circuit current measurements in proximal tubule of Necturus kidney. *Amer. J. Physiol.* **201** 157 (1961).
3. K. H. GERTZ und K. J. ULLRICH; Methode zur Analyse des Stofftransportes am einzelnen Tubulus der intakten Rattenniere. *Pflügers Arch. Ges. Physiol.* **274** 61 (1961).
4. G. GIEBISCH; Measurements of electrical potentials and ion fluxes on single renal tubules. *Circulation* **21** 879 (1960).
5. E. E. WINDHAGER and G. GIEBISCH; Comparison of short circuit current and net water movement in single perfused proximal tubules of rat kidney. *Nature* **191** 1205 (1961).
6. G. GIEBISCH; Measurements of pH, chloride and inuline concentrations in proximal tubule fluid of Necturus. *Amer. J. Physiol.* **185** 171 (1956).
7. C. W. GOTTSCHALK; Micropuncture studies of tubular function in the mammalian kidney. *Physiologist* **4** 35 (1960).

8. H. H. HILGER, J. D. KLÜMPER und K. J. ULLRICH; Wasserrückresorption und Ionentransport durch die Sammelrohrzellen der Säugetierniere. *Pflügers Arch. Ges. Physiol.* **267** 218 (1958).
9. K. H. JARAUSCH und K. J. ULLRICH, Zur Technik der Entnahme von Harnproben aus einzelnen Sammelrohren der Säugetierniere mittels Polyäthylenkapillaren. *Pflügers Arch. Ges. Physiol.* **264** 83 (1957).
10. W. KARGER; Über eine Variation der Kurzschlußmethodik von H. H. Ussing zum aktiven Ionentransport. *Z. Naturforsch.* **14b** 118 (1959).
10a. W. KARGER; Über eine Modifikation der Kurzschlussmethode von H. H. Ussing zur Messung von aktiven Ionentransport Vorgängen an röhrförmigen Epithel-Membranen; Nervensymposium, Göttingen, Ed. K. Kramer and K. J. Ullrich.
11. W. KARGER, F. W. EIGLER und A. HAMPEL; Über eine Meßanordnung zur Bestimmung elektrischer Größen an biologischen Membranen mit aktiven Ionentransport. *Pflügers Arch. Ges. Physiol.* **272** 187 (1960).
12. J. D. KLÜMPER, K. J. ULLRICH und H. H. HILGER; Das Verhalten des Harnstoffs in den Sammelrohren der Säugetierniere. *Pflügers. Arch. Ges. Physiol.* **267** 238 (1958).
13. A. N. RICHARDS; *Methods and Results of Direct Investigations of the Function of the Kidney* Williams and Wilkins, Baltimore, 1929.
14. A. RUIZ-GUINAZU, G. PEHLING, G. RUMRICH und K. J. ULLRICH; Glukose- und Milchsäurekonzentration an der Spitze des vaskulären Gegenstromsystems im Nierenmark. *Pflügers Arch. Ges. Physiol.* **274** 311 (1961).
15. A. K. SOLOMON; The method of isotopic tracers applied to the study of active ion tranpsport. ler Colloque de Biologie de Saclay 1958.
16. H. STÖCKLE; *Analyse der Transportvorgänge an cortikalen Rattentubuli Pflügers Arch. Ges. Physiol.* **247** 62 (1961).
17. K. J. ULLRICH, H. H. HILGER und D. J. KLÜMPER; Sekretion von Ammoniumionen in den Sammelrohren der Säugetierniere. *Pflügers Arch. Ges. Physiol.* **267** 244 (1958).
18. K. J. ULLRICH, F. W. EILGER und G. PEHLING; Sekretion von Wasserstoffionen in den Sammelrohren der Säugetierniere. *Pflügers Arch. Ges. Physiol.* **267** 491 (1958).
19. K. J. ULLRICH und A. HAMPEL; Eine einfache Mikroküvette für Monochromator Zeiß und Beckman Modell DU. *Pflügers Arch. Ges. Physiol.* **268** 177 (1958).
20. K. J. ULLRICH, G. PEHLING und M. ESPINAR-LAFUENTE; Wasser- und Elektrolyfluss im vaskulären Gegenstromsystem des Nierenmarks. *Pflügers Arch. Ges. Physiol.* **273** 562 (1961).
21. K. J. ULLRICH, G. PEHLING und H. STÖCKLE; Hämoglobinkonzentration, Erythrocytenzahl und Hämatokrit im vasa recta Blut. *Pflügers Arch. Ges. Physiol.* **273** 573 (1961).
22. H. H. USSING and K. ZEHRAN; Active transport of sodium as the source of electric current in the short-circuit isolated frog skin. *Acta Physiol. Skand.* **23** 110 (1951).
23. H. H. USSING; The alkali metal ions in isolated systems and tissues. *Handb. der. exp. Pharmakologie* (1960).
24. A. M. WALKER, P. A. BOTT, J. OLIVER and M. C. Mac DOWELL. The collection and analysis of fluid from single nephrons of the mammalian kidney. *Amer. J. Physiol.* **134** 580 (1941).
25. E. E. WINDHAGER and G. GIEBISCH; Micropuncture study of renal tubular transfer of sodium chloride in the rat. *Amer. Physiol.* **200** 581 (1961).
26. H. WIRZ; Quoted from *The Neurohypophysis* p. 157. Edited by H. HELLER Proc. VIII Symposium Colston Res. Soc. N.Y. Academic Press. (1957).

DISCUSSION

Dr. T. A. MAREN: Does this technique give any evidence for active transport of chloride?

Dr. ULLRICH: I did not stress in my paper the question of the behaviour of chloride ion in the proximal tubule. To answer the question of Dr. Maren, I must say that the data we have so far agree with the results obtained by A. K. Solomon and his group on the proximal tubule of *Necturus* some years ago. If there is no net flux of Cl^-, and $M_i = M_o$, we have 18–20 mV transtubular potential and a ratio Cl_i/Cl_o of approximately 0·9. If Cl^- is passively transported the ratio Cl_i/Cl_o should be 0·5. The deviation means — it is strange to say — that an active force acts on the Cl^- ions pushing them into the lumen with a force of 15 mV.

Dr. J. E. BAER: Dr. Berliner has proposed that the saluretic sulphonamides have a primarily distal site of action. Do your experiments resemble those of Wilde *et al.* in that they are of a "stop flow" type? Have you any information concerning a distal action of these compounds?

Dr. ULLRICH: Our experiments have more in common with the "stop flow perfusion" experiments of A. K. Solomon and his group, than with the "stop flow" experiments of Malvin and Wilde. We have not done experiments concerning the effect of the saluretic sulphonamides on the NaCl transport through other parts of the nephron than the proximal tubule or concerning the permeability of the tubule for nonelectrolytes. But I suppose that the saluretic sulphonamides also have an effect on the distal tubule, which should be demonstrable with our methods.

Dr. Walter S. WILDE: May I first congratulate Dr. Ullrich on this excellent work. Relative to Dr. Baer's question, Dr. Berliner had noted that the failure of urine to dilute as much after carbonic anhydrase inhibition seemed inconsistent with the results of stop flow, indicating that the capacity of the tubule to lower the concentration of sodium at the distal minimum point is not impaired. Vander has since shown in our laboratory that when there is distal impairment it is best demonstrated when plasma sodium is at a normal to high level. Plasma sodium is often diluted during the mannitol diuresis imposed during stop flow. Our experiments should be repeated after administering a sodium load.

Dr. E. J. CAFRUNY: This is the second symposium today in which a distal action of thiazides has been alluded to. Recently, my coworkers

and I have shown that large doses of these drugs (equivalent to 20 mg chlorothiazide per kg) do elevate the distal sodium minimum of stop-flow urine. Our results were statistically significant. The work has just been completed and will be submitted for publication in the near future.