Ionic Permeability and Electrical Potential Differences in *Necturus* Kidney Cells

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ABSTRACT The cellular concentrations of Na, K, and Cl have been measured in kidney slices of the amphibian, *Necturus maculosus*. Permeability coefficients have been determined for Na, K, Cl, Rb, Cs, and choline, from studies both of the uptake of radioactive isotopes and the rate of cell swelling in anisotonic solutions. The results of both methods were found to agree well. Measurements were also made of electrical potential differences across the peritubular face of the kidney cells using bathing solutions in which the electrolyte composition and concentrations could be varied. The data obtained are consistent with a model cell in which the potential difference arises as a result of differences in Na permeability relative to K on the two faces of the cell. The intracellular Na concentration is considered to be regulated by a Na-K coupled pump located at the peritubular face of the cell.

Electrical potential gradients have been demonstrated between the interstitial fluid and the lumen of the proximal tubule of the kidney of the amphibian, *Necturus maculosus*, by Giebisch (1, 2), Whittembury (3), and Whittembury and Windhager (4). These authors have also shown that the electrical potential difference across the peritubular face of the cell is larger than that across the luminal face. The relationship between electrical potential differences and ionic permeabilities has been studied in nerve by Hodgkin and Katz (5). The present studies are concerned with similar relationships in *Necturus* proximal tubule cells.

For this purpose we have used kidney slices. The permeability of kidney slices to a variety of small ions and molecules has been studied using a combination of radioactive techniques and studies of cell swelling. It is possible to make chemical analyses of the slices before and after exposure to permeant molecules and to measure potential differences in the slices under the same conditions. The combination of the present results with those obtained *in situ* has enabled us to put forward a hypothetical model kidney cell which

seems to conform to most of the constraints which have so far been put upon it. The electrical potential differences in the model cell arise from differences in the ion permeability coefficients in the two cell faces, a situation analogous to that first put forward by Koefoed-Johnsen and Ussing to account for the differences observed across frog skin (6).

I. WATER AND ELECTROLYTE CONTENT OF NECTURUS KIDNEY

Chemical and radiochemical analyses have been carried out in whole kidneys and in tissue slices in order to determine both intracellular and extracellular concentrations of the electrolytes, Na, K, and Cl.

EXPERIMENTAL METHOD

The animals were of average size, the mean weight of a representative group of 11 animals being 135 ± 17 gm. (standard deviation). 42 kidneys from 21 animals had a mean weight of 312 ± 36 mg. (standard deviation). After the animals had been anesthetized with tricaine methanesulfonate (Sandoz), the abdomen was opened, the kidneys were quickly removed, divided into fractions (100–150 mg. each), gently blotted, and weighed. Usually the operation was finished in less than 3 minutes. During this time, evaporation was estimated to be less than 0.5 per cent and therefore neglected.

For analyses of tissue slices, both kidneys were sliced at once, using the Stadie-Riggs microtome (7), the blade cutting slices parallel to the dorsal aspect of the kidneys. Since the slices were blotted and weighed within 3 minutes after the kidneys had been excised, evaporation losses were negligible. After some practice, the weight and thickness of the slices were uniform (weight, 100 to 150 mg.; thickness, 0.3 mm. ± 0.05 mm.).

Water Content and Extracellular Space

The whole kidneys (cut in pieces), or the slices, were placed in watch glasses and allowed to dry to constant weight in an oven for 12 to 18 hours, at 105°C. The difference between the initial wet weight and the final dry weight was taken as the water content (kg. tissue water/kg. wet weight).

Extracellular space was determined on slices immersed in a standard medium with the following composition: 85.8 mM Na, 13.5 mM K, 1.2 mM Mg, 0.9 mM Ca, 71.4 mM Cl, 21.0 mM HCO_3, 3 mM PO_4, 1.8 mM SO_4, 5.5 mM glucose. The slices were aerated by bubbling with 95 per cent O_2–5 per cent CO_2. Under these conditions, the slices maintained a fairly constant weight (within 5 per cent) and electrolyte composition for about 60 to 90 minutes. pH remained between 7.3 and 7.4. All experiments were carried out between 18 and 22°C.

Both C^{14}-mannitol and C^{14}-inulin (New England Nuclear Corporation) were used to determine the extracellular space. Slices were immersed for periods of time ranging

from 15 to 60 minutes in standard medium to which trace amounts of the radioactive molecules had been added. Upon removal the slices were blotted and transferred to a test tube containing 2 ml. of H_2O. After shaking for 48 hours, aliquots of extraction fluid were pipetted onto aluminum planchets for determination of radioactivity according to the method described by Shipp *et al.* (8). The extracellular space (kg. extracellular space/kg. wet tissue) is determined from the ratio of the activity of the tissue extract to that of the bathing solution.

Electrolyte Analysis

After the dry weight had been obtained, the slices were immersed in 2 ml. of 1 N HNO_3 and shaken for 48 hours at room temperature. Control experiments indicated that extraction was complete in 24 hours so that the routine use of the 48 hour period gave a considerable margin of safety. Na and K concentrations were determined on aliquots of the extraction fluid using the flame photometer of Solomon and Caton (9). The standard deviations in sets of 10 replicates were 2.0 per cent for Na and 3.0 per cent for K. As a further check, recovery experiments were carried out in which the Na or K content was increased by addition of a known amount of NaCl or KCl (10 per cent of the normal ion content of the slice) to the vessel in which the extraction was carried out. When the analyses were completed 98 per cent of the added K or 100 per cent of the added Na was accounted for. In the Cl determinations, by potentiometric titration (10), aliquots of the extraction solution were dissolved in a 1:1 (by volume) mixture of ethanol and acetic acid (11). The standard deviation in a set of 10 replicates was 2.0 per cent. 97 per cent of the added Cl was recovered in a control experiment similar to that described for Na and K.

RESULTS

The mean water content of slices from 26 kidneys (13 animals) was 0.84 ± 0.01 kg. H_2O/kg. wet tissue (errors are standard errors of the mean). 20 whole kidneys from 10 animals also had a water content of 0.84 ± 0.01 kg. H_2O/kg. wet tissue. This value is in good agreement with the figure of 0.81 obtained in whole frog kidneys by Conway, FitzGerald, and Macdougald (12).

As shown in Table I, the extracellular space in kidney slices is the same whether determined by inulin or mannitol. The soaking time of 50 to 60 minutes for inulin was used because Conway, FitzGerald, and Macdougald (12) have shown no significant difference in inulin spaces between 60 and 120 minutes. For mannitol, whose diffusion coefficient is about 4 times larger than that of inulin, soaking times of 15 to 30 minutes appeared adequate. There is no apparent difference between the extracellular spaces obtained for the shorter time of soaking in mannitol and those obtained at the longer times, the ratio of the space determined at the shorter time to that at the longer time being 1.07 ± 0.07. Consequently, we have averaged the data obtained with inulin and with mannitol to give an extracellular space of 0.225 ± 0.007 kg.

TABLE I

EXTRACELLULAR SPACES IN NECTURUS KIDNEY

Substance	Animal	Soaking time	Extracellular space	Mean extracellular space for each animal
		min.	*kg./kg. wet tissue*	*kg./kg. wet tissue*
Mannitol	1	15	0.274	
		25	0.197	
		25	0.213	0.228
	2	19	0.186	
		21	0.244	
		25	0.163	
		25	0.244	0.209
	3	19	0.286	
		28	0.266	
		29	0.219	0.257
	4	22	0.227	
		24	0.170	
		29	0.211	
		31	0.246	0.213
Mean for mannitol experiments				0.227
Inulin	5	56	0.215	
		58	0.249	0.232
	6	51	0.200	
		60	0.217	0.208
	7	50	0.238	
		60	0.220	0.229
Mean for inulin experiments				0.223
Mean for all experiments*				0.225 ± 0.007

* Errors in this and subsequent tables are standard errors of the mean.

space/kg. wet tissue, a value which is in good agreement with the figure of 0.23 obtained by Conway, FitzGerald, and Macdougald in frog kidney slices.

Intracellular concentrations have been calculated on the basis of the following equation:

$$[X]_c = (X_T - [X]_o V_o)/V_c \qquad (1)$$

in which $[X]_c$ is the intracellular concentration of X in mM/kg. cell water, V_c is the relative volume of cell water (kg./kg. wet tissue), X_T is the amount of X in 1 kg. of wet tissue, $[X]_o$ is the concentration of X in the extracellular space in mM/kg. water (after correction for water content of serum and

Donnan factors), and V_o is the relative volume of the extracellular space (kg./kg. of wet weight). These calculations rest on the following implicit assumptions: (*a*) the extracellular space is homogeneous and has an electrolyte composition similar to that of blood serum; (*b*) the space of distribution of inulin and mannitol measures the total extracellular water; (*c*) the intracellular space is homogeneous and its volume equals total water minus inulin

TABLE II
WATER AND ELECTROLYTE COMPOSITION OF NECTURUS KIDNEY

Present studies	No. of animals	Water	Na	K	Cl	Units	
		kg. H_2O/kg. tissue	*mM*	*mM*	*mM*	*per kg.*	
Kidney tissue	13	0.841±0.008	44.9±0.5	67 ±1	37±1	Wet tissue	
Blood serum	11	0.96	96 ±2	3.1±0.2	75±2	Serum	
Extracellular water	7	0.225±0.007	98.3	3.1	76	H_2O	
Cell water		0.62 ±0.01	37 ±2	108 ±6	32±2	H_2O	
Kidney tissue lateral	8	0.836±0.002	43 ±2	70 ±2		Wet tissue	
Kidney tissue medial	8	0.846±0.004	49 ±1	67 ±2		Wet tissue	
Previous studies		**Water**	**Na**	**K**	**Cl**	**Units**	**Reference**
		kg. H_2O/kg. tissue	*mM*	*mM*	*mM*	*kg.*	
Necturus							
Blood serum					73	Serum	(13)
					76	Serum	(14)
			104.5	3.9		Serum	(15, 16)
			97.5	3.2	66.6	Serum	(8)
Frog							
Plasma			103.8	2.5	74.3	Plasma	(12)
Kidney tissue		0.816	41.2	60	30.1	Wet tissue	(12)
Cell water (calculated by us)		0.59	29.4	101	22	H_2O	

space. The results of our electrolyte analyses are given in the top part of Table II. As shown in the bottom section of this table, our results agree well with those given by other investigators.

Conway, FitzGerald, and Macdougald found that the kidneys of frogs did not behave as they expected on the basis of Boyle and Conway's observations on frog sartorius (17). To explain this discrepancy it was suggested that there might be two populations of cells in the kidney: the proximal cells, which are rich in K, impermeable to Na, and permeable to K; and the distal cells, rich in Na and impermeable to K. Chase's (18) study of histological sections of *Necturus* kidney shows that the lateral section is almost exclusively composed of proximal tubules. If Conway, FitzGerald, and Macdougald's suggestion holds for *Necturus* kidney, the lateral section of the kidney should contain a

high K concentration, and very little Na. The medial section which comprises distal tubules and glomeruli, as well as proximal tubules, should contain significantly less K and more Na. As Table II shows, there is no difference between the K concentrations (and little difference in the Na concentrations) in slices taken from the lateral and medial sections of the kidney, which leads us to conclude that Conway, FitzGerald, and Macdougald's suggestion is not applicable to *Necturus* kidney.

II. ELECTRICAL POTENTIAL DIFFERENCE MEASUREMENTS

Experiments were performed to measure the electrical potential difference (P.D.) across the membrane of kidney cells, both in slices and in whole kidneys *in vitro*.

EXPERIMENTAL METHOD

The apparatus, which has been described in detail in the previous paper (4), consists of two calomel electrodes connected to a high impedance voltmeter. The indifferent electrode is connected *via* a suitable agar bridge to the bathing solution. The exploring electrode is a micropipette (3 to 30 megohm resistance, tip diameter less than 0.5 μ, tip potential between 0 and -5 mv.), connected *via* a 3 M KCl solution to a calomel half-cell.

After excision, either the whole kidney or a slice was immersed in a bathing solution of known composition through which gas was bubbled slowly (95 per cent O_2–5 per cent CO_2). The exploring electrode was advanced with a micromanipulator and the tissue impaled under a dissecting microscope. When the precautions previously described (4) were observed, cells in the standard medium gave a potential difference of -50 mv. which was stable, on a single impalement, for 2 or 3 minutes and sometimes for as long as 5 or 10 minutes. Tip potentials and resistances were measured before and after each impalement; if a significant change was observed, the experiments were rejected.

When either the slice or the whole kidney is immersed in a bathing solution the cellular P.D. characteristic of the medium is established immediately. Once established, the P.D. appears to remain constant for 20 to 30 minutes as evidenced by repeated impalements of separate cells over this interval. After a cell is impaled, the P.D. is less stable, presumably as a result of the injury due to the micropipette. As routine, P.D.'s were not measured until 5 minutes had elapsed after placing the tissue in the bathing medium. All measurements made on tissues exposed to the medium for more than 30 minutes were discarded.

In vitro, the tubules cannot be seen in as fine detail and contrast as *in vivo*. The type of cell impaled was not clearly identified as proximal or distal; however, only the lateral part of the ventral surface of the kidney was punctured to increase the probability of impaling proximal tubule cells. Furthermore, the forward movement of the exploring electrode was kept to a minimum once the superficial membrane had

been penetrated in order to confine the P.D. measurements to the peritubular face of the cell. With these precautions it was hoped to make measurements comparable to those made *in situ*.

Potential differences were measured in a number of bathing solutions whose composition is given in Table III. Solutions B, C, D, E, and F were designed to maintain a constant product of K and Cl concentrations (300 mM²). In these solutions the pH was adjusted to 7.3 by alterations in the PO_4 composition; deficiencies in anions or osmolarity were made up by SO_4 and sucrose. The Ca concentration was increased

TABLE III

COMPOSITION OF BATHING SOLUTIONS*

Medium	Dominant cation	X	K	Cl	Na	HPO_4	H_2PO_4	Ca	SO_4	Sucrose
		mM	mM	mM	mM	mM	mM	mM	mM	mM
Standard‡			13	71	85	2.4	0.6	1.0	2	
Constant [K][Cl]										
B	K		4	75	86	1.9	0.6	3.0	9	20
C	K		10.5	28	67	1.4	0.4	6.0	27	62
D	K		30	10	41	1.2	0.4	7.5	35	81
E	K		60	5	10	1.1	0.3	7.5	37	85
F	K		130	2		1.1	0.3	7.5	70	
Cation chloride [XCl]										
G	K		96	96	4	1.9	0.6	1.8		
H	Choline	92	4	96	4	1.9	0.6	1.8		
I	Rb	92	4	96	4	1.9	0.6	1.8		
J	Cs	92	4	96	4	1.9	0.6	1.8		
Cation sulfate [X_2SO_4]										
K	Cs	128	4	4	2.5	1.1	0.3	7.5	70	
L	Na		4	4	130.5	1.1	0.3	7.5	70	

* The figures have been rounded to 0.5 mM except for Ca and PO_4 to 0.1 mM.

‡ The standard medium also contained 1.2 mM Mg, 21 mM HCO_3. All solutions contained 5 mM glucose.

at the higher SO_4 concentrations in order to keep the ionized Ca at a concentration near 1 mM. Solutions G, H, I, and J contain K, choline, Rb, and Cs chlorides; they were designed to study the effect of monovalent cations on potential difference. In solutions F, K, and L, Cl has been replaced by SO_4 in order to compare the effects of alkali cations with minimal interference due to Cl permeability.

RESULTS

The potential differences obtained are given in Table IV ($\Delta\psi = \psi_i - \psi_o$, in which *i* and *o* refer to inside and outside the cell). The results obtained with solution B, in which the K concentration is 4.0 mM, should be comparable with those obtained *in situ*, since the K concentration in serum is 3.1 mM.

Whittembury and Windhager (4) obtained a P.D. of -74 ± 1 mv. across the peritubular face of the tubular cell, in good agreement with Giebisch's (1) value of -72 mv. These figures are closely comparable to the value of -70 ± 2 mv. obtained in the whole kidney. The potential difference in the tissue slices is -57 mv., some 10 mv. lower than that obtained in whole kidney.

TABLE IV

POTENTIAL DIFFERENCES IN KIDNEY SLICES AND WHOLE KIDNEY

Medium	Dominant component		Slice		Whole kidney	
	Ion	Concentration	No. of observations	$-\Delta\Psi$	No. of observations	$-\Delta\Psi$
		mM		mv.		mv.
Standard	K	13	25	50 ±1		
Constant [K][Cl]						
B	K	4	35	57 ±1	10	70 ±2
C	K	10.5	7	45 ±1	13	57 ±1
D	K	30	15	33 ±1	9	35.9±0.8
E	K	60	19	17.6±0.8	8	19.8±0.5
F	K	130	25	4.0±0.5	13	5.6±0.4
Cation chloride						
G	K	96	18	17.0±0.5	32	15.8±0.7
H	Choline	92	8	25 ±1	8	36 ±1
I	Rb	92	12	15.6±0.2	10	21.6±0.8
J	Cs	92	9	23.1±0.5	8	39 ±1
Cation sulfate						
K	Cs	128	10	8.1±0.7	13	26 ±2
L	Na	130.5	29	51 ±2	41	64 ±1
Solution B + 2 gm. per cent protein						
	K	4	20	53 ±1	15	76 ±1
Solution B + 1 mM 2,4-dinitrophenol						
10 min.	K	4	10	42 ±1	9	55 ±1
20 min.	K	4	12	38 ±2	10	47 ±1

Typically, the P.D.'s in slices are lower than those in whole kidney, presumably as a result of damage done to the tissue in the slicing process.

There was considerable scatter in the measurements of slice P.D.'s. Although the lowest and the highest value in a series could differ by as much as 15 mv., the values were uniformly distributed and an average of 5 successive measurements differed at most by some 4 to 5 mv. from another group of 5 studied under similar conditions. The average standard deviation was 3.4 mv.

The relationship between the K concentration of the bathing medium and

the P.D. is shown in Fig. 1. It will be seen that the P.D.'s are related to the logarithm of the K concentration in a quasilinear fashion, as is the usual case for membranes with a dominant K electrode character. The linear portion of the curve is characterized by a slope of 48 mv./tenfold concentration gradient for the slices, and 51 mv. for the whole kidney. These figures are comparable to the figure of 55 mv. given by Giebisch (19) for the peritubular face of the cell in the doubly perfused *Necturus* kidney. The depolarizing effects of the higher concentrations of K are also exhibited by choline, Rb, and Cs as shown in Table IV. 2,4-Dinitrophenol at a concentration of 1 mM also depolarizes the cell, as shown by comparing the last two rows of Table IV with the results for

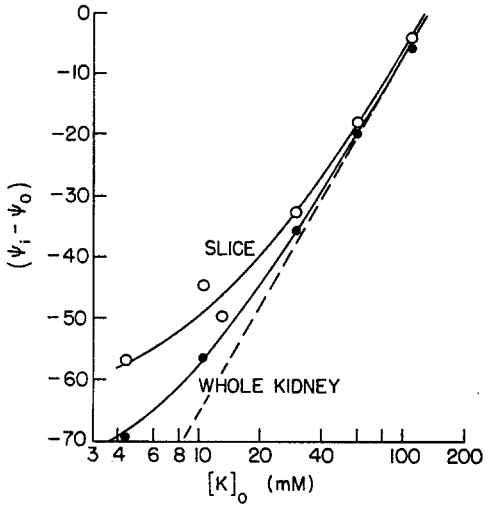

FIGURE 1. Relationship between measured potential difference and K concentration in the medium in solutions of constant [K] [Cl] product. The dashed line has been drawn with a slope of 58 mv. for a tenfold concentration difference. In order to take account of the low activity coefficient of K_2SO_4 at the highest K concentration, the activity, equivalent to 110 mM K, was plotted rather than the K concentration of 130 mM.

solution B. The effect of DNP seems to progress with time, since the P.D. after 20 minutes' exposure is significantly lower than that at 10 minutes. A similar effect has been found *in situ* by Whittembury and Windhager (4).

As shown in Table IV, the addition of protein to medium B increases the P.D., which may remain stable for as long as 1 hour. Kernan (20) has reported similar effects in frog sartorius. This is in accord with observations in a wide variety of tissues that the presence of protein in the bathing solution exercises a stabilizing effect on the membrane.

III. PERMEABILITY COEFFICIENT FROM ION FLUX MEASUREMENTS

The passive entrance of non-electrolytes into cells may be described by Fick's law. With large pored artificial membranes, the proportionality constant which relates the flux per unit pore area to the concentration difference is $D/\Delta x$, in which D is the diffusion coefficient and Δx, the path length through

the membrane. In biological membranes, the proportionality constant may be denoted as $D'/\Delta x$. We have used D' instead of D to emphasize that D' is *not* the diffusion coefficient in free solution, but includes implicitly all the restrictions to free diffusion offered by the membrane and its boundary layers. It is assumed that the only significant concentration gradients are at the membrane and that the activity coefficients are the same in the solutions on either side of the membrane. $D'/\Delta x$ has units of cm./sec. and is usually denoted by P, the permeability coefficient. In Einstein's terminology the permeability coefficient would be $RT/Nf'\Delta x$(N is Avogadro's number, and f' is the frictional resistance in the membrane per molecule; R and T have their usual meanings). When electrolyte diffusion is considered, and in particular when the permeability of a membrane to a single cation or anion is being investigated, this permeability coefficient, which we denote by P_Φ, is frequently given as $RTu'/zF\Delta x$, which also has units of cm./sec. (z and F have their usual meanings). The mobility, u, the free velocity of an ion moving in a field of 1 volt/cm. is replaced by u', the mobility in the membrane also given in units of cm^2./volt, sec. u'/zF is inversely proportional to the friction of the ion in the membrane, analogous to $1/Nf'$ in the Einstein diffusion equation. Thus u', like D', includes all the interactions with the membrane and is consequently denoted with a prime. Alternatively the permeability coefficient, P_Φ, may be defined as $RT\Phi/C\Delta\mu$, in which Φ represents the flux of the ion, as measured by tracers, C is the concentration of that ion in the solution of origin, and $\Delta\mu$ is the electrochemical potential difference driving the ion.

In order to obtain the permeability coefficient from ion movements, we have made the following assumptions, following Goldman (21) and Hodgkin and Katz (5): (*a*) the electric field is constant through the membrane, (*b*) the activity coefficient is constant throughout the system, (*c*) the membrane may be considered homogeneous so that the mobility, u', throughout the membrane is constant, and (*d*) the ions in the membrane move under the influence of concentration and potential gradients in a manner similar to free solution.

The flux of an ion, Φ, is given by the product of its concentration and driving force, divided by the friction, according to the following equation:

$$\Phi = -\frac{u'C}{zF}\left(\frac{RTd\ln C}{dx} + zF\frac{d\Psi}{dx}\right) \tag{2}$$

The solutions to the equation are

$$\Phi_{in} = \frac{P_\Phi\, zF\Delta\Psi C_o}{RT(1 - e^{zF\Delta\Psi/RT})} \tag{3}$$

$$\Phi_{out} = \frac{P_\Phi\, zF\Delta\Psi C_i\, e^{zF\Delta\Psi/RT}}{RT(1 - e^{zF\Delta\Psi/RT})} \tag{4}$$

in which the subscripts *i* and *o* refer to internal and external solutions, and in and out refer to influx and efflux. When the flux ratio, Φ_{in}/Φ_{out}, is obtained from these equations, it is seen to be identical with that given by Ussing (22) for independent passive movement of ions.

Ion fluxes and permeability coefficients have been determined in kidney slices using radioactive isotopes of Na, K, Cl, Rb, Cs, as well as C^{14}-choline.

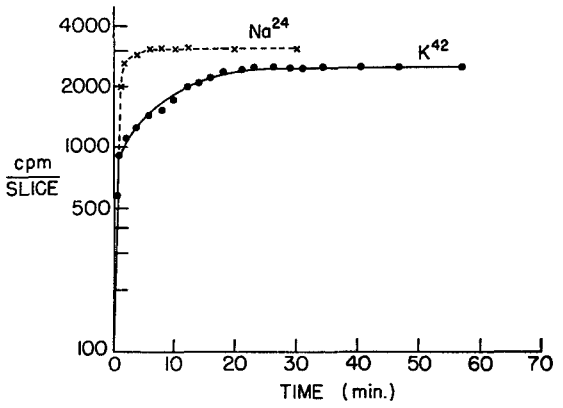

FIGURE 2.
Uptake of radioactivity by slices.

EXPERIMENTAL METHOD

Carey and Conway (23) have shown that tissues are maintained in the steady state for a longer period when the bathing solution contains proteins. Since the normal *Necturus* serum contains 2.35 gm. protein per 100 ml., 2 gm. of bovine serum albumin were added to each 100 ml. of medium for these experiments. Under these conditions, the kidneys did not lose weight and maintained a constant composition. When uptakes of Na^{24}, K^{42}, or Cl^{38} were studied, the standard medium was modified to contain 10 mM of K and 90 mM of Na in addition to trace amounts of isotopes. In the experiments with C^{14}-choline and Rb^{86}, the solution was modified to contain 8 mM K, 5 mM choline or Rb, and 86 mM Na. In order to maintain constant composition and specific activity, the volume of the bathing solution was made so large that its ion content was always at least 100 times that of the slice.

Radioactive Uptake Experiments

Since the slices comprise at least an extracellular and an intracellular compartment, the kinetic expression governing the uptake experiments will contain at least two exponentials. Fig. 2 shows typical uptake experiments for Na and K. In these experiments the slices were immersed in the radioactive (hot) solution, removed, blotted, counted, and then returned to the hot solution. Since the bathing media contained protein, it was not possible to bubble O_2

through the medium, so the flasks were aerated by shaking. In the experiments shown in Fig. 2, the fast exponential had a half-time of less than 30 seconds. In consequence it was possible to follow the procedure described by Mullins (24) in which the slices were washed for 60 seconds in a cold solution of identical composition before being counted in a scintillation well counter (Nancy Wood) for a 15 second period. The slices were then returned to the hot solution for further incubation. The initial incubation period was 3 minutes followed by 5 minute incubation periods until a plateau was reached. In the case of C^{14}-choline, extracts were prepared from the slices by shaking in water for 48 hours. Aliquots were counted using the same method previously described for the determination of extracellular space with C^{14}-inulin.

TABLE V
DIMENSIONS OF NECTURUS KIDNEY TUBULES

	Length	Radius		Volume		Surface		
		Lumen	Lumen + cells	Lumen	Cells	Luminal	Outer	Total
	mm.	*mm.*	*mm.*	*mm.*^{3}	*mm.*^{3}	*mm.*^{2}	*mm.*^{2}	*mm.*^{2}
Proximal	14	0.070	0.100	0.215	0.225	6.16	8.80	15.0
Distal	8	0.035	0.055	0.031	0.045	1.76	2.76	4.52
Total				0.246	0.270	7.92	11.6	19.5

Relationship between Slice Weight and Cellular Surface Area

In order to compare the present results with those obtained previously in this laboratory and elsewhere, it is necessary to express the fluxes in terms of cell surface. Windhager *et al.* (25) measured the length and diameter of the proximal tubule lumen both on histological sections and by the cast method of Bott (26). We have made further measurements on proximal and distal tubules in histological sections and have obtained values in good agreement with Windhager *et al.* Our results together with calculations of surface and volume, assuming uniform cylindrical tubules, are given in Table V. The length of the distal tubule is taken from Kempton (27). From the figures given in Table V and our value of 22.5 per cent for extracellular space, it is possible to calculate that each tubule weighs 0.38 mg. (cell volume, 0.27 mm.^{3}; specific gravity, 1.05; relative cellular volume, 77.5 per cent). This value is in good agreement with a weight of 0.40 mg./tubule derived from the mean weight of the kidneys used in our experiments, 312 mg., and the figure of 784 nephrons per kidney quoted by Richards and Walker (28). Thus the neglect of glomerulus, neck, and collecting ducts, and the heterogeneity of the tissue do not lead to gross errors in these calculations. We have taken a surface to weight ratio of 0.5 cm.^{2}/mg. (19.5 mm.^{2}/0.38 mg.) as characteristic of our

preparations of kidney slices. This figure neglects the contribution of the brush border, but serves as an operational figure for comparative purposes.

RESULTS

The fluxes of Na, K, and Cl have been determined on the basis of a two compartment system (29); they are presented together with the calculated permeability coefficients in Table VI. Since Rb, Cs, and choline are not normal constituents of the cell, the initial efflux of these substances is zero. The influxes are determined from the initial rate of uptake of radioactivity by the washed tissue. This initial rate remains linear for a period of 2 to 3 minutes.

TABLE VI
FLUXES AND PERMEABILITY COEFFICIENTS FOR ELECTROLYTES, AS DETERMINED BY TRACERS

Ion	Concentration	No. of experiments	Influx*	Permeability coefficient measured in slices		Permeability coefficient measured *in situ*	
			Φ_{in}	P_Φ	$P_{\Phi rel}$	P_Φ	$P_{\Phi rel}$
	mM		*pmols/sec.cm.²*	*cm./sec.* × 10^6		*cm./sec.* × 10^6	
Na	90	3	181	0.87	0.08	1.4	0.09
K	10	5	254	10.9	1.00	15.6	1.00
Cl	71	5	136	5.85	0.54	5.3	0.34
Cs	10	4	23.8	1.03	0.09		
Rb	5	4	27.4	2.37	0.22		
Choline	5	4	29.2	2.52	0.23		
Choline	90	4	1440	10.1	0.92		

* pmols = 10^{-12} mols.

In the case of choline, which enters more rapidly than any of the other substances studied, the specific activity of the cell was <5 per cent that of the medium at the end of 3 minutes which indicates that this approximate method of calculating the fluxes introduces only negligible error. The permeability coefficients, P_Φ, are obtained from Equation 3 using a potential difference of −50 mv. taken from Fig. 1 (10 mM K). A few check experiments with Cs, Rb, and choline (at its lower concentration) indicated that these substances did not affect the normal −50 mv. potential difference. At the higher choline concentration, the potential difference of −25 mv. given in Table IV was used. The permeability coefficients relative to that for K are denoted by $P_{\Phi rel}$.

The fluxes and permeability coefficients obtained from these tracer uptake experiments may be compared with permeability coefficients calculated from the ion fluxes previously measured *in situ* by the stopped-flow microperfusion method. Na fluxes have been measured by Oken *et al.* (30), K fluxes by Oken and Solomon (31), and Cl fluxes by Giebisch and Windhager (32). Their

influxes (flux into the lumen) are 204 pmols/cm.^{2}, sec. for Na, 68 pmols/cm.^{2}, sec. for K, and 301 pmols/cm.^{2}, sec. for Cl. The permeability coefficients, obtained from these influxes and Equation 3, are in good agreement with those found in the tracer uptake studies as shown in Table VI. The slice experiments measure the average permeability coefficients for both luminal and peritubular faces of the cell, whereas the *in situ* experiments measure the permeability of both cell faces in series. It is somewhat surprising that the results of these two separate kinds of experiments are in such good agreement.

The degree of depolarization produced by a given cation as compared to the depolarization produced by K can give an index of the relative permeability of the cell membrane to that cation—the larger the depolarization, the larger the permeability to the ion. The results of this comparison will only be qualitative since immersion of slices in bathing media that do not have a constant [K][Cl] product will cause changes in the intracellular concentrations. A study of Table IV shows that permeabilities in Cl solutions are arranged in the order K > Rb > Cs; in SO_4 (solutions F, K, and L) the order is K > Cs > Na. The combined order is K > Rb > Cs > Na, consistent with the relative permeability coefficients in Table VI. Comparison of the last two rows in Table VI shows that the permeability coefficient for choline is strongly dependent on choline concentration, so that no comparison may be made for this ion.

IV. PERMEABILITY COEFFICIENT FROM CELL SWELLING MEASUREMENTS

An alternative method of measuring permeability coefficients for solutes which pass through pores in cellular membranes depends upon a determination of Staverman's reflection coefficient, σ. For non-electrolytes, Durbin (33) has shown that $1 - \sigma = A_{sf}/A_{wf}$ in which A_f is the apparent area available for filtration in the membrane, and the subscripts, s and w, refer to solute and water filtration respectively. The permeability coefficient, $D'/\Delta x$, may be replaced by $(D/\Delta x)(A_{sd}/A)$ in which all the restrictions to diffusion offered by the membrane are taken into the term (A_{sd}/A). A_{sd} is the apparent pore area available for diffusion through the membrane, and A is the geometrical membrane area, usually taken as 1. The relationship between A_{sd} and A_{sf} depends upon a/r, the ratio of the radius of the solute molecule, a, to the equivalent pore radius, r, according to the equation, $A_{sd} = A_{sf}/[2 - (1 - a/r)^2]$, derived from Renkin's equations (34). Thus, the permeability coefficient, P_σ, derived from cell swelling experiments, is:

$$P_\sigma = [D_s A_{wf}(1 - \sigma)]/A\Delta x[2 - (1 - a/r)^2] \quad (5)$$

The subscript s has been added to D to emphasize that it refers to the solute

whose σ is being measured. In the case of the kidney slice, r has been found to be 5.6 Å (35), and a may be determined from molecular models. Since A_{wf} and Δx are constants of the specific membrane, it is not necessary to determine them explicitly if relative permeabilities are to be obtained.

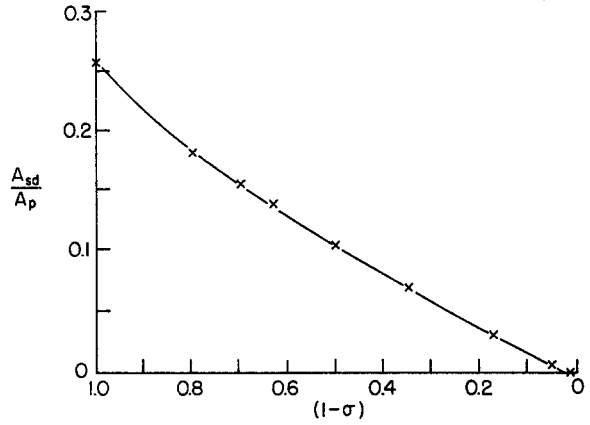

FIGURE 3. Relationship between A_{sd}/A_p and $(1 - \sigma)$ for a pore of 5.6 Å radius.

This method of determination of the permeability coefficient may be simplified by using a graphical calculation and the equation, $P_\sigma \Delta x = D_s A_{sd}/A$. Goldstein and Solomon (36) have shown $1 - \sigma$ to be a function only of a, r, and a_w, the molecular radius of water, according to the following equation:

$$1 - \sigma = \frac{[2(1 - a/r)^2 - (1 - a/r)^4][1 - 2.104a/r + 2.09(a/r)^3 - 0.95(a/r)^5]}{[2(1 - a_w/r)^2 - (1 - a_w/r)^4][1 - 2.104a_w/r + 2.09(a_w/r)^3 - 0.95(a_w/r)^5]} \quad (6)$$

A_{sd} is also a function of a, r, and A_p, the total apparent pore area, according to the following equation of Renkin:

$$A_{sd}/A_p = (1 - a/r)^2[1 - 2.104a/r + 2.09(a/r)^3 - 0.95(a/r)^5] \quad (7)$$

Thus it is possible to construct a graph showing the relationship between A_{sd}/A_p and $1 - \sigma$ for any specified equivalent pore radius, as given in Fig. 3 for pores of 5.6 Å equivalent radius. If σ and D_s are known, it is possible to find $P_\sigma A/(A_p/\Delta x)$ for non-electrolytes from this graph. Since $A_p/\Delta x$ is a constant of the membrane, relative values of P_σ may be obtained readily. If absolute values of P_σ are desired for a single membrane, $A_p/\Delta x$ may be determined from the rate of cell swelling under an osmotic pressure gradient. The method by which this is done and the assumptions underlying the equivalent pore theory of cellular permeability have been discussed by Solomon (37).

In the case of electrolytes, analogous expressions can be obtained. We will

replace σ by σ' to specify its application to electrolytes for which no theoretical expressions are yet available.[1] $P_{\sigma'}$, the permeability coefficient measured in this way, is given by:

$$P_{\sigma'} = D_s(A_p/\Delta x)(A_{sd}/A_p)/A \tag{8}$$

Since $P_{\sigma'}$ is an operational concept, it includes the hindrance arising from electrical interactions within the membrane as well as the other constraints previously discussed. Because of the restrictions of electroneutrality, D_s in Equation 8 is the diffusion coefficient of the dissociated salt in water at the concentration used.[2]

Determination of σ'

σ' has been determined from measurements of the zero time rate of cell swelling, following the method of Goldstein and Solomon (36). The index of cell volume in this case is slice weight; the specific technique has been described in detail by Whittembury, Sugino, and Solomon (35). When 86 mOsM of NaCl in the standard bathing medium was replaced by 83 mM of sucrose or mannitol, these authors found that kidney slices maintained constant weight for a 20 minute period. Since we have shown that mannitol is a satisfactory index of extracellular space, we may conclude that the cells are impermeable to sucrose for at least 20 minutes. When the sucrose is replaced by an electrolyte, σ' may be determined from the concentration of electrolyte required to make $(dw/dt)_0$ = zero. σ' is defined by the equation:

$$\sigma' \equiv (\sum_j c_j^i - \sum_j c_j^o)/(c_s^o)_{isos} \tag{9}$$

in which

$\sum_j c_j^i$ = the sum of the concentrations of all the "non-permeant" *intracellular* species

$\sum_j c_j^o$ = the sum of the concentrations of all the "non-permeant" *extracellular* species

$(\sum_j c_j^i - \sum_j c_j^o)$ = the concentration gradient created by the subtraction from the medium of 86 mOsM of NaCl

$(c_s^o)_{isos}$ = the initial extracellular concentration of the permeant species when $(dw/dt)_0 = 0$

[1] A theoretical expression for σ for electrolytes has been developed by Katchalsky and is now in press (Symposium on Membrane Transport and Metabolism, Prague, August 22–27, 1960). Katchalsky has shown that σ for electrolytes depends strongly on electrolyte concentration. The relative permeabilities determined from our values of σ' will not be altered greatly by this dependence, since the experiments were carried out over a concentration range which was as uniform as possible for the various cations employed.

[2] In some cases, values of D_s may not be available. Since the diffusion coefficients for small molecules are within one order of magnitude, approximate relative permeabilities may be obtained from the relative values of $1 - \sigma'$.

Slices were weighed and immersed in a number of test solutions. All the bathing solutions contained the same constituents as the standard medium, except for 86 mOsM of NaCl; this was replaced by one of the test solutes listed in Table VII at various concentrations. The final total concentrations ranged from 130 to 300 mOsM/liter. Changes in the slice weight were measured for 30 minutes. At 3 to 4 minute intervals each slice was taken out of the solution, blotted, weighed, and returned to the solution. Since the rate of change of weight was essentially constant during the first 5 to 10 minutes, an average

TABLE VII

PERMEABILITIES FOR ELECTROLYTES DETERMINED BY σ'

Solutes	No. of slices*	$(C_s^o)_{isos}$†	σ'	$1-\sigma'$	$P_{\sigma'}$§	$P_{\sigma'}$rel	$P\Phi$rel (cations)
		mOsM/liter			*cm./sec.* $\times 10^6$		
Sucrose	23 (12)	83	1.00	0.00	0.0	0.00	
NaCl	24 (11)	86	0.97	0.03	0.18	0.03	0.08
Na_2SO_4	8 (3)	87	0.96	0.04	0.18	0.03	
K_2SO_4	19 (8)	98	0.85	0.15			
KCl	19 (10)	143	0.58	0.42	6.4	1.00	1.00
LiCl	20 (5)	93	0.89	0.11	1.0	0.16	
RbCl	21 (6)	94	0.88	0.12	1.6	0.25	0.22
CsCl	16 (4)	86	0.96	0.04	0.30	0.05	0.09
Li_2SO_4	15 (4)	91	0.91	0.09	0.57	0.09	
Cs_2SO_4	14 (6)	86	0.96	0.04	0.24	0.04	
Choline Cl	12 (7)	106	0.78	0.22			0.92
Choline Cl		‖					0.23

* No. of animals in parentheses.

† The standard errors in $(C_s^o)_{isos}$ are ± 3 mOsM/liter.

§ The values of D required for this calculation have been taken from Robinson and Stokes (39).

‖ Standard medium modified to contain 8 mM K, 5 mM choline, and 86 mM Na.

rate during that period was determined and recorded as the per cent weight change per minute.

RESULTS

The zero time rate of change of slice weight, $(dw/dt)_o$, is obtained by extrapolation. It is shown in Fig. 4 as a function of the concentration of probing molecule in the medium. $(c_s^o)_{isos}$ is then obtained from the concentration at which $(dw/dt)_o = 0$. Each point is the average of data obtained from 8 to 12 slices in 4 to 6 kidneys. Since σ for sucrose has been shown to be 1.00, this compound serves as the reference substance. Values of $(c_s^o)_{isos}$ for other electrolytes, obtained from plots similar to Fig. 4, are given in Table VII.

When sucrose is replaced by electrolytes, there are important changes in the ionic strength of the medium. Furthermore, the Cl concentration gradient is sharply altered. These changes make it difficult to make exact comparisons

between the behavior of electrolytes and that of sucrose. Thus the difference between σ for sucrose, which equals 1.00, and σ' for NaCl, which equals 0.97, may be due in part to changes in the external ionic environment that affect the membrane but are not related to the Na ion itself. The experiments with Na_2SO_4 give essentially the same value for σ' as do those with NaCl; this suggests that the influence of the Cl concentration gradient is not important. Since the ionic strength of 87 mOsM/liter of Na_2SO_4 is about 50 per cent greater than that of the NaCl it replaces, it seems apparent that considerable shifts in ionic strength may be introduced without an appreciable influence on σ', unless the ionic strength effect tends to cancel the Cl gradient effect. The measured value of 0.97 for σ' for NaCl in proximal tubule cells fits well with

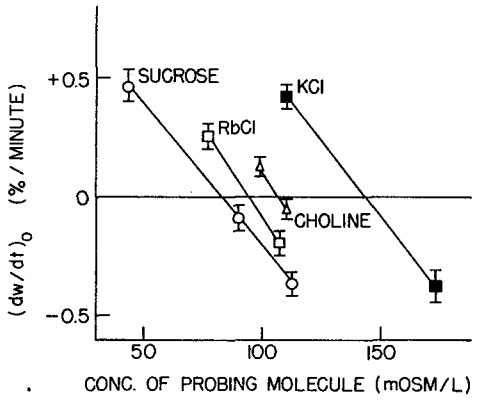

FIGURE 4. Initial rate of change of slice weight as a function of the concentration of sucrose and electrolytes in the external solution.

the value of 1 we have previously put forward based on indirect evidence (38). $P_{\sigma'}$ has been obtained from measurements of cell swelling in which the restrictions of electroneutrality prevent a cation from entering the cell without an anion. On the other hand $P_{\Phi rel(cations)}$ is derived from tracer experiments in which cation movement may be independent of anion movement, though it is restricted to studies with a common anion, Cl. Nonetheless, when the relative permeability coefficients obtained from the two kinds of measurements are compared, as in the last two columns of Table VII, the agreement is good.

V. DISCUSSION

When the data presented in this paper are coupled with results which have been obtained previously (3, 4), a model of the proximal tubule cell may be constructed, in which many of the ion transport processes are localized, as has been done by Koefoed-Johnsen and Ussing (6) for the frog skin. In contradistinction to the frog skin which is permeable to Na on the outer face only, the kidney tubule cell appears to be slightly permeable to Na on both faces. The evidence for this statement rests upon the observation of Oken *et al.* (30)

that the outward Na flux of 266 pmols/cm.^{2}, sec. is accompanied by an influx of 204 pmols/cm.^{2}, sec. Na exchange diffusion appears to be relatively unimportant since the influx into the lumen is essentially independent of the luminal Na concentration. The potential differences observed by Whittembury and Windhager (4) show that the cell potential is negative to its environment, as indicated in Fig. 5. Furthermore, the intracellular Na concentration is 37 mM, much lower than the normal external Na concentration of 100 mM at both faces. Consequently, Na moves down an electrochemical potential gradient from the lumen to the cell, and must climb an even higher gradient to reach the interstitial fluid, as pointed out by Giebisch (2).

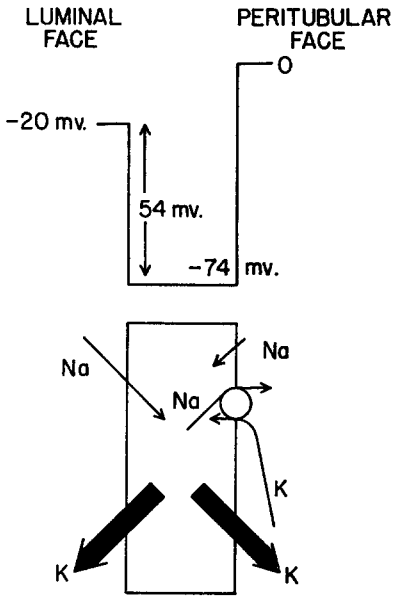

FIGURE 5. Schematic drawing showing ion permeabilities and potentials in proximal tubule cell.

Although the Na concentration in the lumen is normally equal to that in the interstitial fluid (40, 41), a different situation prevails for K, whose luminal concentration has been shown by Oken and Solomon (31) to be 1.8 times that of the interstitial fluid. This concentration ratio for K is equivalent to a -15 mv. transtubular potential difference if the cell behaves like a K electrode. Thus, much of the observed asymmetry in electrical potential difference across the two faces of the cell may be accounted for in terms of an asymmetry in K distribution. As shown in Fig. 1, the transcellular potential difference at high K concentrations is seen to approximate that expected for a K electrode.

At lower K concentrations, the potential difference departs from the ideal relationship, as is the case for nerve and muscle. Hodgkin and Katz (5) give the following variation of the Goldman equation which applies to a situation in which the ion pump is non-electrogenic, the outward current carried by the Na ions being equal to the inward current carried by the K ions.

$$-\Delta\Psi = \frac{RT}{F} \ln \frac{[K]_i + b[Na]_i}{[K]_o + b[Na]_o} \quad (10)$$

Here b is the permeability coefficient of Na relative to K. For such a neutral pump, Cl should be distributed passively between cell and environment. The points in Fig. 1 which represent potential differences across the peritubular face of the cell can be fitted by equations with the following coefficients:

$$-\Delta\Psi = 58 \log \frac{[K]_i + 0.10\,[Na]_i}{[K]_o + 0.10\,[Na]_o} \text{ for slices}$$
$$-\Delta\Psi = 58 \log \frac{[K]_i + 0.05\,[Na]_i}{[K]_o + 0.05\,[Na]_o} \text{ for whole kidney} \quad (11)$$

The values of $[K]_i$ used to obtain the empirical curves given in Fig. 1 were 120 mM for the slices, and 130 for the whole kidney; the value for $[Na]_i$ was 37 mM in both cases. The values for b in Equation 11 are considered to apply to the peritubular face since the potential differences were measured across that face. These results are closely similar to those that have been obtained by Giebisch (2, 19) on the doubly perfused *Necturus* kidney. He measured slopes of 48 and 55 mv. for tenfold changes in K concentration on the luminal and peritubular cell faces respectively. Giebisch fitted his data by giving b values of 0.10 and 0.03 for the respective faces, thus demonstrating that the relative cell permeability to Na on the luminal face is greater than that on the peritubular face.

Hodgkin and Katz (5) have shown that Equation 10 applies to situations in which there is no net current flow. In the *Necturus* proximal tubule cell, in which the cations are not in equilibrium with their environment, the absence of net current flow can be accounted for in terms of a neutral pump, presumably one in which Na is pumped out of the cell in exchange for K from the interstitial fluid. The electrochemical potential gradients in the cell suggest that the pump should be placed at the peritubular face of the cell. The evidence from the electron microscope is in agreement with a pump at this face of the cell since, in the mouse proximal tubule cell, all the mitochondria are at the basal end of the cell in close juxtaposition to the peritubular cell membrane (42). Similarly, in the frog proximal tubule cell Karnovsky (43) has reported that the mitochondria are concentrated near the peritubular face of the cell.

If the preparation were not in the steady state, Equation 10 would not describe the system; consequently, it is necessary to demonstrate that the intracellular ion concentrations and membrane potential difference remain constant. In *in vitro* experiments, the potential difference remained constant for a 20 to 30 minute period, and for as long as 60 minutes when protein was present. Furthermore, the volume of the cells, as evidenced by the weight of

the tissue, remained constant over the same period. Finally, the experiments were carried out at constant ion product, so that Cl shifts should not have followed changes in extracellular K concentration. The potential differences observed *in situ* were stable for periods longer than 1 hour; the peritubular face potential difference of −74 mv. measured in 123 observations is in close agreement with the potential difference of −70 mv. obtained with the whole kidney *in vitro*. Since this potential difference is some 20 mv. less than the K equilibrium potential, the Na-K exchange pump appears to play an important role in the determination of the observed peritubular potential difference.

As a corollary to Equation 10, the Cl distribution should be given by

$$\Delta\Psi = -(RT/F) \ln [Cl]_o/[Cl]_i \qquad (12)$$

When the slices are bathed in the standard medium, whose $[Cl]_o$ is 71 mM, $\Delta\Psi = -50$ mv., which corresponds to an internal [Cl] of 10 mM. This is markedly less than the measured intracellular [Cl] of 32 mM. A similar finding has been made by Whittam (44) in rat kidney cortex slices. This discrepancy is too large to be accounted for by errors in the analytical techniques employed. The extracellular space that has been used in the calculation is 0.23 ± 0.01. This figure would have to be raised to 0.45 to obtain an intracellular Cl concentration of 10 mM. Consequently, errors in the extracellular space determination would not seem to account for the discrepancy.

We may next ask whether the cellular space in the slices is homogeneous. The inhomogeneity could be of two kinds: the population of cells may include an appreciable number of cells characterized by low [K], low potential difference, and high [Cl], as suggested by Conway, FitzGerald, and Macdougald (12) or alternatively, the intracellular space may contain regions whose ionic composition is different from the rest of the cell. A correlation between K and Cl is evident from observations on the intracellular K concentration. As shown in Fig. 1, an extracellular K activity of 130 mM is required to bring the potential difference to zero. Thus we would expect to find an intracellular K concentration of 130 mM, rather than the figure of 108 observed analytically. The observed K concentration in the cells is too low by 22 mM, a figure equal to the excess Cl that has been found in the cells. This agreement, which is to be accepted only in its qualitative aspects, lends support to the existence of a region, or regions, with composition different from the rest of the tissue and also makes it unlikely that an adequate explanation can be given in terms of ion binding. Conway, FitzGerald, and Macdougald (12) have suggested that the distal tubular cells are characterized by a lower K concentration than the proximal cells. As previously stated, the results of our studies, given in Table II, exhibit no difference in the K concentration in lateral and medial slices of kidney, which suggests that this particular histological interpretation does not apply to the *Necturus* kidney.

On the assumptions that the Cl distribution is passive and the permeability coefficients are independent of the intracellular ion concentration, Equation 10 may be used to correlate the measured potential differences and permeability coefficients quite exactly with the model. Using the measured intracellular concentrations given in Table II, the potential difference at the luminal face is given by an equation in which $b_l = 0.09$ and that at the peritubular face by a similar equation in which $b_p = 0.03$, in close agreement with Giebisch's values of $b_l = 0.10$ and $b_p = 0.03$ (19). When the two equations are combined algebraically and the terms which make no significant contribution are dropped, the following equation is obtained:

$$-\Delta\Psi_{transtub} = 58 \log \frac{[K]_l + b_l[Na]_l}{[K]_p + b_p[Na]_p} \quad (13)$$

The absence of any terms which involve cellular ion concentrations indicates that the transtubular potential difference is independent of the Na and K concentrations in the cell. In contrast, the relative ion permeabilities for the inner and outer faces of frog skin epithelial cells are quite different so that the transcellular potential difference is sharply dependent on the intracellular Na and K concentrations.

The adjustable constants in Equation 13, b_l and b_p, may now be compared with the relative permeability coefficients for Na that have been determined by a variety of methods. These values, given in Tables VI and VII, range from a low of 0.04 to a high of 0.09. Thus it is clear that the present model fits with most of the measured quantities and provides an explanation of the potential difference in the tubule cell in terms of the relative permeabilities to Na and K. The passage of water out of the lumen, and the maintenance of the low intracellular Na concentration appear to be consequent to the action of a coupled Na-K pump located on the peritubular face of the cell.

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BIBLIOGRAPHY

1. GIEBISCH, G., *J. Cell. and Comp. Physiol.*, 1958, **51,** 221.
2. GIEBISCH, G., *Circulation*, 1960, **21,** 879 and Nierensymposium, Göttingen, August, 1959. Aktiver Ionentransport und Lokalisation des Stofftransports in der Niere, (K. Kramer and K. J. Ullrich, editors), Stuttgart, Georg Thieme, 1960, p. 49.

3. WHITTEMBURY, G., *J. Gen. Physiol.*, 1960, **43,** No. 5, suppl., 43.
4. WHITTEMBURY, G., and WINDHAGER, E. E., *J. Gen. Physiol.*, 1961, **44,** 679.
5. HODGKIN, A. L., and KATZ, B., *J. Physiol.*, 1949, **108,** 37.
6. KOEFOED-JOHNSEN, V., and USSING, H. H., *Acta Physiol. Scand.*, 1958, **42,** 298.
7. STADIE, W. C., and RIGGS, B. C., *J. Biol. Chem.*, 1944, **154,** 669.
8. SHIPP, J. C., HANENSON, I. B., WINDHAGER, E. E., SCHATZMANN, H. J., WHITTEMBURY, G., YOSHIMURA, H., and SOLOMON, A. K., *Am. J. Physiol.*, 1958, **195,** 563.
9. SOLOMON, A. K., and CATON, D. C., *Anal. Chem.*, 1955, **27,** 1849.
10. RAMSAY, J. A., BROWN, R. H. J., and CROGHAN, P. C., *J. Exp. Biol.*, 1955, **32,** 822.
11. WHITTEMBURY, G., RAMIREZ, M., FERNANDEZ, J., and MONGE, C., *Acta Physiol. Latinoamer.*, 1957, **7,** 76.
12. CONWAY, E. J., FITZGERALD, O., and MACDOUGALD, T. C., *J. Gen. Physiol.*, 1946, **29,** 305.
13. WALKER, A. M., HUDSON, C. L., FINDLEY, T., JR., and RICHARDS, A. N., *Am. J. Physiol.*, 1937, **118,** 121.
14. GIEBISCH, G., *Am. J. Physiol.*, 1956, **185,** 171.
15. BOTT, P. A., *J. Biol. Chem.*, 1955, **215,** 287.
16. BOTT, P. A., *J. Biol. Chem.*, 1943, **147,** 653.
17. BOYLE, P. J., and CONWAY, E. J., *J. Physiol.*, 1941, **100,** 1.
18. CHASE, S. W., *J. Morphol.*, 1923, **37,** 457.
19. GIEBISCH, G., *J. Gen. Physiol.*, 1961, **44,** 659.
20. KERNAN, R. P., *Nature*, 1960, **185,** 471.
21. GOLDMAN, D. E., *J. Gen. Physiol.*, 1943, **27,** 37.
22. USSING, H. H., *Acta Physiol. Scand.*, 1949, **19,** 43.
23. CAREY, M. J., and CONWAY, E. J., *J. Physiol.*, 1954, **125,** 232.
24. MULLINS, L. J., *J. Gen. Physiol.*, 1959, **42,** 817.
25. WINDHAGER, E. E., WHITTEMBURY, G., OKEN, D. E., SCHATZMANN, H. J., and SOLOMON, A. K., *Am. J. Physiol.*, 1959, **197,** 313.
26. BOTT, P. A., *Am. J. Physiol.*, 1952, **168,** 107.
27. KEMPTON, R. T., *J. Morphol.*, 1937, **61,** 51.
28. RICHARDS, A. N., and WALKER, A. M., *Am. J. Physiol.*, 1937, **118,** 111.
29. SOLOMON, A. K., *in* Mineral Metabolism, (C. L. Comar and F. Bronner, editors), New York, Academic Press, Inc.,1960, **1A,** 119.
30. OKEN, D. E., WHITTEMBURY, G., WINDHAGER, E. E., SCHATZMANN, H. J., and SOLOMON, A. K., *J. Clin. Inv.*, 1959, **38,** 1029.
31. OKEN, D. E., and SOLOMON, A. K., *J. Clin. Inv.*, 1960, **39,** 1015; and personal communication.
32. GIEBISCH, G., and WINDHAGER, E. E., *Fed. Proc.*, 1959, **18,** 52.
33. DURBIN, R. P., *J. Gen. Physiol.*, 1960, **44,** 315.
34. RENKIN, E. M., *J. Gen. Physiol.*, 1955, **38,** 225.
35. WHITTEMBURY, G., SUGINO, N., and SOLOMON, A. K., *Nature*, 1960, **187,** 699.
36. GOLDSTEIN, D. A., and SOLOMON, A. K., *J. Gen. Physiol.*, 1960, **44,** 1.
37. SOLOMON, A. K., Proceedings of the First National Biophysics Conference, Yale University Press, 1959, 314.

38. WHITTEMBURY, G., OKEN, D. E., WINDHAGER, E. E., and SOLOMON, A. K., *Am. J. Physiol.*, 1959, **197**, 1121.
39. ROBINSON, R. A., and STOKES, R. H., Electrolyte Solutions, New York, Academic Press, Inc., 1959, Appendix 11.2.
40. BOTT, P. A., Renal Function, Transactions of the Fifth Conference, New York, Josiah Macy Jr. Foundation, 1953, 42.
41. BOTT, P. A., *in* Proceedings of the Eighth Annual Conference on the Nephrotic Syndrome, (J. Metcoff, editor), New York, National Nephrosis Foundation, Inc., 1957, 39.
42. SJÖSTRAND, F. S., and RHODIN, J., *Exp. Cell Research*, 1953, **4**, 426.
43. KARNOVSKY, M. J., personal communication.
44. WHITTAM, R., *J. Physiol.*, 1956, **131**, 542.