

Potassium and Sodium Transport across Single Distal Tubules of *Amphiuma*

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ABSTRACT The transport properties of potassium (K) and sodium (Na) were studied in single distal tubules of *Amphiuma* using free-flow micropuncture techniques and stationary micropfusion methods. The transepithelial movement of labeled potassium was measured utilizing a three-compartment system in series in which the time course of tracer disappearance from the lumen was followed. Under control conditions, in blood- and doubly-perfused kidneys, extensive active net reabsorption of sodium and potassium obtains along single distal tubules. Tubular potassium reabsorption is abolished by ouabain at a concentration of 5×10^{-6} M. Significant net secretion of K can be induced by exposing *Amphiuma* to a high K environment (100 mM KCl) or by adding acetazoleamide (1×10^{-4} M) to the perfusion fluid. Transepithelial movement of potassium involves mixing with only a small fraction of total distal tubular cell potassium. This transport pool of potassium increases significantly with the transition from tubular net reabsorption to net secretion. Indirect evidence is presented which indicates that increased active K uptake across the peritubular cell boundary may be of prime importance during states of net K secretion.

Micropuncture studies on mammalian distal tubules have defined this nephron segment as a site at which sodium ions are actively reabsorbed against increasing transepithelial concentration gradients and as that part of the nephron which determines the rate of urinary potassium excretion (Windhager and Giebisch, 1961; Giebisch and Windhager, 1964; Ullrich et al., 1963; Malnic, Klose, and Giebisch, 1966 *a, b*; Giebisch, 1969). In particular with respect to potassium transport the distal tubular epithelium shows a wide range of variation including even the reversal of the direction

of net transport. Tubular net reabsorption can effect the almost complete abstraction of this ion species from the tubular fluid against sizeable concentration gradients. Alternatively, a powerful secretory mechanism can add potassium ions to the tubular fluid and achieve urinary excretion rates in excess of filtered potassium.

Information on the role of the distal tubule in sodium and potassium excretion by amphibian nephrons is much less extensive (Bott, 1962; Maude et al., 1966), particularly with respect to changes in transport patterns induced by different metabolic demands. The recent observation by Sullivan (1968) that the ventral portion of *Amphiuma* kidneys is made up predominantly of distal tubules has prompted the present investigation which takes advantage of a number of unusual properties of this amphibian kidney. These are, first, the relatively large size of individual distal tubules permitting collection of adequate amounts of tubular fluid for both chemical analysis of electrolytes and for measuring the rate of disappearance of isotopes from the lumen. Second, the large size of distal tubule cells allows for the measurement of electrical potential differences across individual cell boundaries and thus permits an accurate assessment of the electrochemical potential difference across both luminal and peritubular cell membranes. Third, the *Amphiuma* kidney can be artificially perfused via the aortic and portal circulation without significant functional compromise. This makes possible extensive changes in the extracellular environment and a study of the effects of these changes upon tubular transport of sodium and potassium.

The studies to be described deal with two aspects of distal tubular function. First, micropuncture and stationary microperfusion studies have been carried out to define the transport pattern of the distal tubular epithelium with respect to sodium and potassium ions. Second, microperfusion studies with radioactive tracers and measurements of electrical potential differences across individual tubular cell membranes have been done and have provided information on unidirectional ion fluxes across the luminal and peritubular cell membrane, on some of the mechanisms involved in the transport of sodium and potassium across these cell boundaries, and on the size of the intracellular transport pool of these ions under conditions of widely differing net transport patterns.

METHODS

Preparation of Animals Experiments were carried out on male *Amphiuma* (110–440 g) during the months of May to March. The animals were obtained from the Waubun Laboratories (Schriever, La.) or from the Lemberger Company (Oshkosh, Wis.). They were kept at room temperature in plastic containers receiving running tap water and fed frozen shrimp once a week. Animals were anesthetized by intraperitoneal injection of 30–50 mg Inactin per kg body weight and were prepared for micro-

puncture or microperfusion experiments as described by Sullivan (1968). Experiments were carried out in vivo (blood-perfused), 30–120 min after induction of anesthesia, or in animals in which both kidneys were perfused via the aortic and portal circulation (Sullivan, 1968; Giebisch, 1961; Cullis, 1906). Perfusion was carried out via an aortic polyethylene catheter (arterial perfusion reservoir 30–40 cm above the level of the animal) and via the tail vein which communicates with the renal portal system (venous perfusion reservoir 10–15 cm above the animal). Effluent was collected from the vena cava at a rate varying between 1 and 2 ml/min. Experiments on perfused kidneys were carried out 2–4 hr after anesthesia had taken effect. The composition of the perfusion fluid is given in Table I and is similar to that used by Sullivan (1968). The perfusion fluid prepared freshly before each experiment was equilibrated for 1 hr prior to the experiment with a gas mixture containing 2.5% CO₂ and 97.5% O₂. It had a pH varying between 7.2 and 7.4 and an osmolality of 200 to 210 milliosmols/liter. Experiments were carried out at room temperature (20–22°C).

TABLE I
COMPOSITION OF PERFUSION FLUID

NaCl	75.0 mm/liter
KCl	3.0 mm/liter
CaCl ₂	1.8 mm/liter
MgCl ₂	1.0 mm/liter
Na ₂ HPO ₄	0.56 mm/liter
NaH ₂ PO ₄	0.14 mm/liter
NaHCO ₃	20.0 mm/liter
Glucose	2.0 g/liter
Glycine	250 mg/liter
PVP*	15 g/liter
Heparin	2 mg/liter

* Polyvinylpyrrolidone, Plasdone C grade, General Aniline and Film Corp., New York.

Four groups of animals were used: (a) Control animals in which experiments were carried out in the anesthetized state (blood-perfused) or in which the kidneys were perfused with the solution described in Table I. (b) Potassium-loaded animals. In these animals an attempt was made to stimulate kaliuresis by exposing *Amphiuma* for a period of 3–5 days to an ambient solution containing 100 mEq/liter KCl, care being taken that the animals had minimal opportunity for breathing air. This pretreatment resulted in an increase in the plasma potassium concentration to about 5.0 mEq/liter. In this group, experiments were carried out on blood-perfused kidneys and on kidneys of animals pretreated as described and perfused with a solution as given in Table I with the exception that the potassium concentration was increased to 5.0 mEq/liter. (c) Acetazoleamide-treated animals. In this group kidneys were perfused with the solution as described in Table I to which acetazoleamide (Diamox) was added to give a final concentration of 1.0×10^{-4} M. (d) Ouabain-treated animals. Kidneys were perfused with the control perfusion fluid (Table I) to which ouabain (Eli Lilly) had been added to give a final concentration of 5.5×10^{-6} M.

Microperfusion and Microperfusion Methods. Free-Flow Microperfusion Collection of tubular fluid from individual distal tubules during free-flow conditions was carried out by means of micropipettes ground from Pyrex capillaries (Corning 7740) having a tip diameter of some 15μ . Tubules were blocked with castor oil (stained with Sudan Black) and the collection rate adjusted so that the position of the oil block remained just distal to the puncture site. Localization of the puncture site was achieved by means of the Latex method (Windhager, 1968). The total length of single, dissected distal tubules varied between 7 and 10 mm. Puncture sites are given in per cent of the total length. Fluid reabsorption along individual distal tubules was measured by the extent to which inulin- ^{14}C was concentrated. In perfused kidneys, the concentration of inulin- ^{14}C in the perfusion fluid was $50 \mu\text{Ci}/100 \text{ ml}$. Samples of tubular fluid and of plasma or perfusion fluid were counted immediately after collection.

Stationary Microperfusion These experiments were done using the method of Shipp et al. (1958) and of Gertz (1962). This approach has been used widely (Kashgarian et al., 1963; Marsh et al., 1963; Malnic et al., 1966 *b*; Wiederholt et al., 1966, 1968) and permits the assessment of limiting transepithelial ionic concentration differences under different experimental conditions across isolated, well-defined segments of tubular epithelium. This method is particularly useful when both net fluid and solute movement are minimal. Experiments were carried out using single-barreled micropipettes filled with colored castor oil and the test solution. The latter contained 50 mM/liter NaCl , 3 mM/liter KCl , and enough raffinose to bring the total osmolality to $200 \text{ milliosmols/liter}$. This composition assures relative minor initial solute and fluid movement because it contains raffinose which is poorly permeant and Na and K at concentrations achieved under steady-state conditions. A small amount of fluid was injected between oil droplets into a distal tubule on the kidney surface and thereby was isolated effectively from the rest of the tubular contents. It was then withdrawn and analyzed for Na and K after 20 min, a time interval adequate to assure attainment of steady-state conditions. The method is schematically described in Fig. 1.

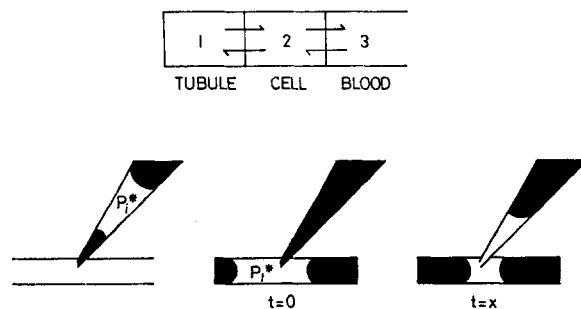


FIGURE 1. Schematic presentation of stationary microperfusion method. Compartments 1, 2, and 3 denote tubular lumen, cell water, and infinite peritubular fluid compartment. A sample of known specific activity P_i^* is injected at time 0. Fluid is withdrawn after variable time intervals x either to measure ionic steady-state concentration differences or to observe the decay of P_i^* as a function of time.

Measurement of Tracer Fluxes Movement of ^{22}Na and ^{42}K across the tubular epithelium was measured by observing the rate of disappearance of tracer from the lumen of single distal tubules under conditions approaching zero solute and fluid movement. The theoretical considerations pertinent to the cell model used for analysis and the derivation of the equations for calculating unidirectional fluxes across individual tubular cell membranes and for estimating transport pools are given in the Appendix. Again, stationary microperfusion methods were used to measure the time course of disappearance of tracer from the lumen (Fig. 1). This was done by depositing appropriate test fluids between oil droplets and reaspirating them after time intervals varying between 10 sec and 20 min. The collected perfusate was then analyzed for radioactivity and its ionic content. The composition of the test solutions was that of "equilibrium solutions," i.e. solutions which in previous stationary microperfusion experiments had been found to result in the establishment of steady-state concentration differences with respect to Na and K (see Figs. 7 and 8). The solutions had the following composition: (mEq/liter) controls: 3.0 KCl, 50 NaCl; K loading: 9.0 KCl, 50 NaCl; acetazoleamide: 6.5 KCl, 70 NaCl; ouabain: 4.5 KCl, 85 NaCl. All solutions were brought to isotonicity by raffinose. In addition, they also contained inulin- ^{14}C (5 mg/ml). This allowed us to verify negligible transepithelial net fluid movement in individual perfusion experiments. Also, the fact that the concentration of inulin- ^{14}C remained unchanged in successful perfusions assured that no contamination of the perfusion fluid with fluid from the kidney surface or from other intratubular sites had occurred.

^{22}Na Carrier-free ^{22}Na (New England Nuclear Corp., Boston, Mass.) of a specific activity of 20 mCi/mg was used to prepare perfusion fluids having a final activity of 160 $\mu\text{Ci/ml}$. In individual perfusion experiments, some $10\text{--}15 \times 10^{-6}$ ml of the respective equilibrium solution were deposited intratubularly between oil droplets and subsequently withdrawn and analyzed.

^{42}K Equilibrium solutions were prepared utilizing ^{42}K (high specific activity, 2 mCi/mg at 12 noon of day of delivery) in such concentrations that the activity of the perfusion fluid during the experiment was approximately 205 $\mu\text{Ci/ml}$.

Analytical Methods The concentration of sodium and potassium in samples of tubular fluid and in the collected perfusate was measured by ultramicroflame photometry according to Malnic et al. (1964, see also Müller, 1958) using samples of about 1×10^{-6} ml. Radioactivity due to ^{14}C , ^{42}K , and ^{22}Na was determined in a liquid scintillation counter (Mark I, Nuclear-Chicago Corp., Des Plaines, Ill.) using 10 ml Bray solution as scintillation activator (Bray, 1960). Activity in individual samples was at least twice background, total counts exceeded at least 2000. Differential counting in mixtures containing ^{22}Na and ^{14}C resulted in 22% of ^{22}Na counts in the ^{14}C channel and 2.8% ^{14}C counts in the ^{22}Na channel. Only 2.8% of the counts were due to ^{14}C in the ^{42}K channel while the radioactivity due to ^{14}C was estimated from the counts of the sample after 20 half-times of ^{42}K had elapsed.

Electrical Potential Measurements Microelectrodes were drawn from 1.0 mm o.d. Pyrex capillaries in a mechanical pulling device (Alexander and Nastuk, 1953) and filled with 3 M KCl by the technique of Caldwell and Downing (1955). Micro-

electrodes having a resistance between 5 and 35 megohms and tip potentials less than 5 mv were used for the measurement of transepithelial and peritubular membrane potentials of single tubule cells according to methods previously described (Sullivan, 1968; Giebisch, 1958, 1961). Since the potential difference between distal tubule lumen and peritubular fluid is made up of two finite potential steps (Sullivan, 1968; Giebisch, 1968; Windhager and Giebisch, 1965; Giebisch et al., 1966) the potential difference across the luminal cell membrane was calculated as the difference between the transepithelial and peritubular membrane potential differences.

RESULTS

Free-Flow Micropuncture Experiments

FLUID REABSORPTION ALONG THE DISTAL TUBULE

Fig. 2 graphically summarizes the progression of inulin- ^{14}C tubular fluid (TF) over plasma (P) concentration ratios along distal tubules of *Amphiuma* kidneys. Five Ringer-perfused animals were studied. It is apparent that significant fluid reabsorption takes place in all experimental conditions although to a different extent. In control animals, some 25% of the filtrate is reabsorbed as fluid passes along the distal tubule. Extrapolation to the very beginning of this nephron segment permits an estimate to be made of the

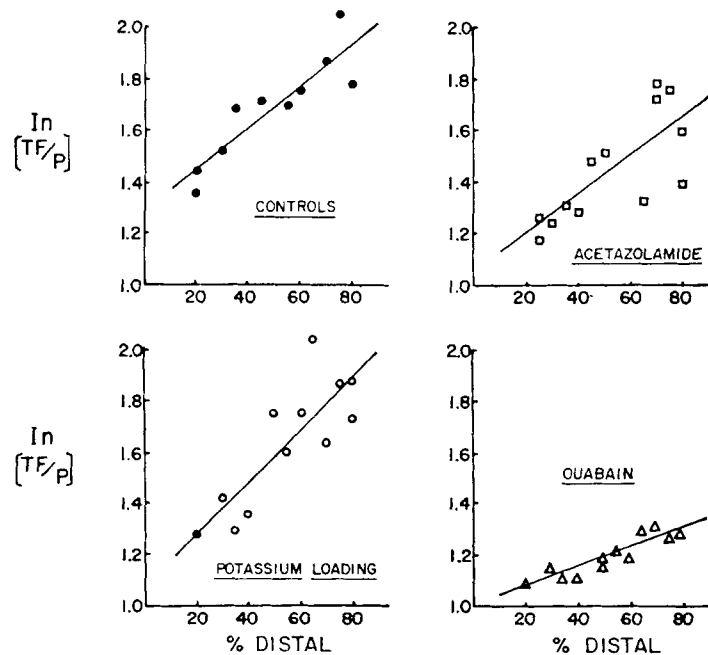


FIGURE 2. Progression with tubule distance of free-flow distal transtubular inulin- ^{14}C concentration ratios (TF/P inulin) under different experimental conditions.

extent of fluid conservation along the proximal tubule since only a very short intermediate segment is interposed between the proximal and distal tubule. Some 25–30% ($1-P/TF$) of the fluid filtered is reabsorbed along the proximal tubule. These results confirm previous observations (Walker and Hudson, 1937; Bott, 1952, 1962; Giebisch, 1956) made on another amphibian kidney, that of *Necturus*, in which tubular fluid reabsorption is about equally distributed between the proximal and distal tubule. This pattern is different from that observed in the mammalian nephron in which the proximal tubule is the main site of fluid reabsorption.

The slopes of the lines relating ^{14}C TF/P ratios observed in potassium-loaded (eight animals) and acetazoleamide-treated animals (five animals) were similar to the slope for control animals. Hence, fractional fluid reabsorption was not significantly affected. However, the smaller value of TF/P inulin- ^{14}C ratios at zero distal tubular length suggests that fluid reabsorption along the proximal convoluted tubule was moderately reduced in potassium-loaded, acetazoleamide- and ouabain-treated kidneys. Inspection of the inulin- ^{14}C data in the ouabain-treated animals (eight animals) clearly indicates a significant reduction of fractional fluid reabsorption along the distal tubule ($P < 0.01$). Compared to control kidneys in which some 50% of the filtrate had been reabsorbed by the end of the distal tubule, only some 30% had been reabsorbed in ouabain-treated kidneys. In view of the absence of a single ureter in *Amphiuma*, urinary excretion data were not obtained.

SODIUM REABSORPTION

Figs. 3 and 4 summarize data on transepithelial sodium concentration differences and fractional reabsorption rates. Inulin concentration ratios were not measured in all fluid samples collected. A significant decline in TF/P concentration ratios of sodium is apparent in both blood- and Ringer-perfused kidneys. At a mean plasma concentration of 97 mEq/liter, the intratubular sodium concentration was 75 mEq/liter at the beginning of the distal tubule, and declined to some 30 mEq/liter by the end of this tubular segment. No difference was observed between blood- and Ringer-perfused kidneys, a fact underscoring the well-maintained viability of the perfused kidney preparation. The values of transepithelial sodium concentration differences are quite similar to those observed by Bott (1962) along the distal tubule of *Necturus*. Inspection of Fig. 3 also indicates that the progressive decline of intratubular sodium concentrations along the distal tubule is not affected by exposure of animals to a high potassium medium for 3–5 days. This is of some importance since the direction of net potassium transport is that of extensive net reabsorption in control animals, whereas distal tubular net secretion is consistently observed in potassium-loaded animals.

The lower part of Fig. 3 graphically illustrates the extent of fractional distal

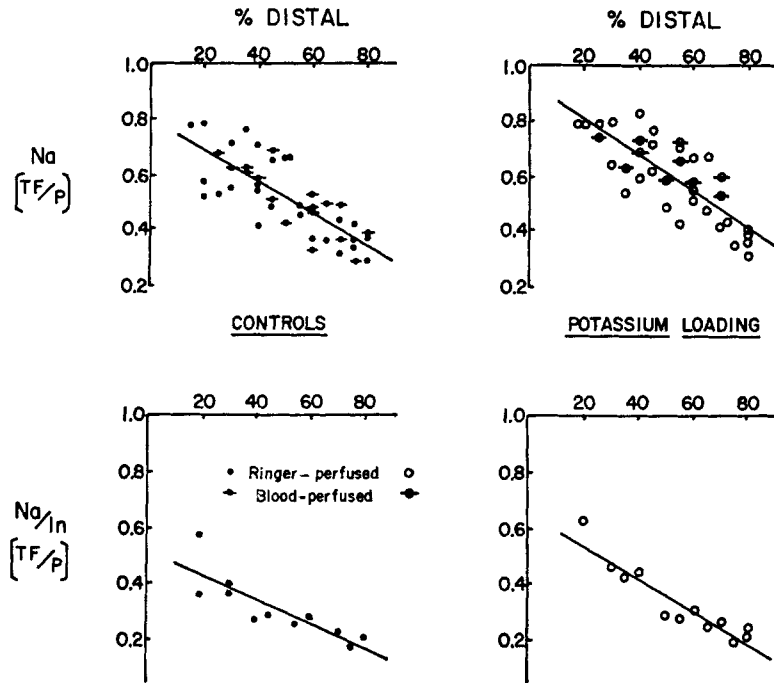


FIGURE 3. Progression with tubule distance of distal sodium and sodium/inulin concentration ratios in nonperfused and perfused *Amphiuma* kidneys under control and potassium-loaded conditions.

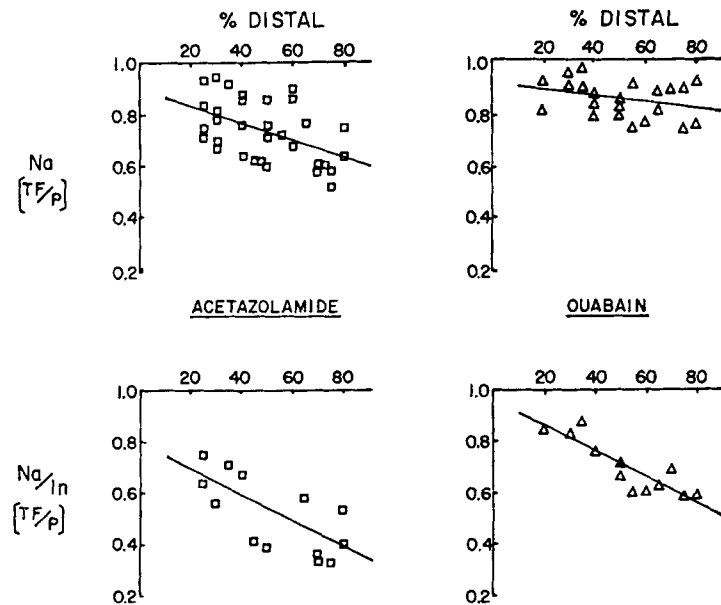


FIGURE 4. Progression with tubule distance of distal sodium and sodium/inulin concentration ratios in acetazolamide- and ouabain-treated kidneys. Perfusion was carried out with 1.0×10^{-4} M acetazolamide (Diamox) and with 5.5×10^{-6} M ouabain.

tubular sodium reabsorption as derived from simultaneously measured sodium and inulin- ^{14}C TF/P concentration ratios. Extrapolation of the regression lines to 100% length indicates that some 90% of the filtered sodium load has been reabsorbed by the end of the distal tubule. Of that moiety, some 40% is reabsorbed along the proximal tubule while 50% is abstracted from distal tubular fluid. The extent of fractional distal tubular sodium reabsorption was not significantly different from control values in potassium-loaded animals (see right lower panel of Fig. 3).

Fig. 4 summarizes data on transepithelial sodium concentration differences and on fractional reabsorption rates in acetazoleamide- and ouabain-treated kidneys. Notably, the sodium concentration ratios decline to a lesser degree than in control animals in both experimental conditions; i.e., most absolute values of intratubular sodium concentrations fall within a higher range than during control perfusions. Thus, values for early and late distal tubular sites were 92 and 50 mEq/liter in acetazoleamide-treated kidneys and 96 and 72 mEq/liter in ouabain-treated kidneys. The latter value is significantly higher ($P < 0.01$) than similar concentration values in control animals. Despite the marked elevation of intratubular sodium concentrations in both experimental conditions, fractional sodium reabsorption was not materially reduced in acetazoleamide- and ouabain-treated kidneys since the progression of Na/inulin TF/P ratios along the distal tubule is similar to that in control conditions. However, it is apparent from the higher than normal values of Na/inulin TF/P sodium ratios at very beginning of the distal tubule that a larger than normal fraction of the filtered sodium escapes proximal tubular reabsorption in the drug-treated kidneys. This observation and the fact that the fraction of sodium leaving the distal tubules is significantly larger than normal in both acetazoleamide- and ouabain-treated kidneys, indicate that proximal sodium reabsorption not only is inhibited but also that the excess sodium leaving the proximal convolution is significantly excreted. Thus, some 30 and 50%, respectively, of the filtered sodium enters the collecting duct system in these kidneys, a value greatly exceeding that of 10% in control kidneys.

POTASSIUM TRANSPORT

Figs. 5 and 6 summarize data on transepithelial potassium concentration differences and on fractional transport rates. Similar to the behavior of sodium ions, TF/P concentration ratios of potassium decline along the distal tubule. It should be noted that the potassium concentration at the very beginning of the distal tubule exceeds that in plasma or in the perfusion fluid. The extent of the elevation of potassium in early distal tubular fluid is equivalent to that expected from fluid reabsorption along the proximal tubule. This is also apparent from inspection of the left lower panel of Fig. 5 in which the progression of fractional tubular potassium reabsorption is illustrated. It is

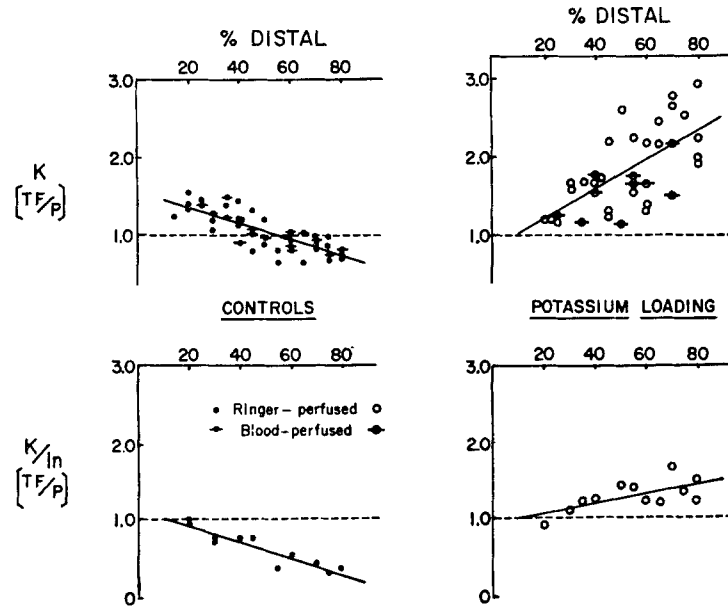


FIGURE 5. Progression with tubule distance of distal potassium and potassium/inulin concentration ratios in nonperfused and perfused *Amphiuma* kidneys under control and potassium-loaded conditions.

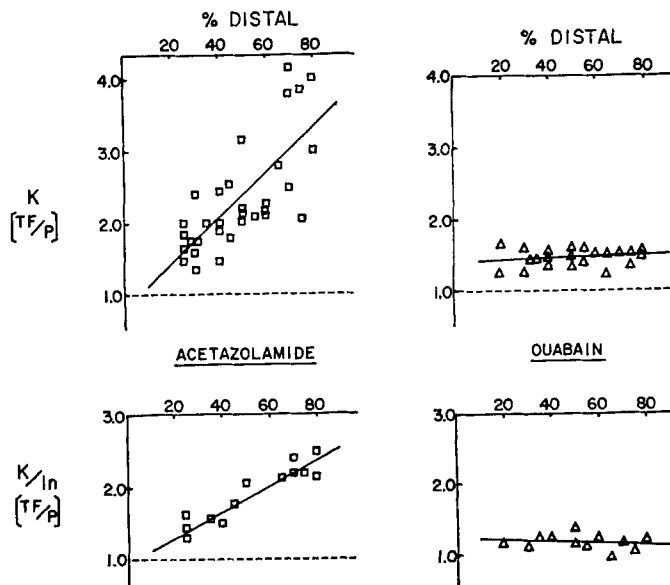


FIGURE 6. Progression with tubule distance of distal potassium and potassium/inulin concentration ratios in acetazolamide- and ouabain-treated kidneys. Perfusion was carried out with 1.0×10^{-4} M acetazolamide (Diamox) and with 5.5×10^{-6} M ouabain.

evident from the potassium-to-inulin TF/P ratio of unity at the earliest distal tubular level that the amount of potassium entering the distal tubule relative to that filtered has remained unchanged. The amphibian nephron lacks a loop of Henle and only a very short intermediate segment connects the proximal with the distal tubule. Accordingly, early distal K/inulin concentration ratios reflect end proximal values, and the fact that these ratios do not deviate from unity indicates absence of significant reabsorption of potassium ions along the proximal tubule. This functional pattern of the amphibian proximal tubule differs from that commonly observed in the mammalian nephron in which some two-thirds of the filtered potassium is normally reabsorbed during passage of fluid along the proximal convolution and in which only an amount equivalent to some 5–10% of the filtered potassium enters the distal tubule (Malnic et al., 1964, 1966 *a, b*). In contrast, in the amphibian proximal tubule no major modification of the filtered potassium occurs along the proximal tubule and quantitative modifications of the filtered potassium moiety are exclusively the function of the distal tubule.

The decline of both potassium TF/P and potassium-to-inulin TF/P ratios along the distal tubule indicates that net reabsorption of potassium is extensive. In both blood- and Ringer-perfused kidneys some 75% of the filtered potassium is reabsorbed along the distal tubule. With respect to the direction of the net transport of this ion species, the situation in *Amphiuma* differs from that in the rat on a normal dietary K intake in which significant net secretion of potassium has been shown to occur (Malnic et al., 1964, 1966 *a*). It is similar, however, to the behavior in the rat under conditions of maximal potassium conservation (Malnic et al., 1964) and may, accordingly, reflect the permanent state of intense electrolyte conservation typical of renal tubular adaptation to the freshwater habitat.

However, the present series of experiments shows that, similar to the distal tubular system in mammals, direction and magnitude of distal tubular potassium transport strongly depend upon the metabolic situation. This is apparent from experiments in which *Amphiuma* were exposed to an ambient solution containing 100 mEq/liter of potassium chloride. Inspection of Fig. 5 indicates that under these conditions, in contrast to those in control animals, there obtains a very marked increase in the potassium concentration along the distal tubule. Since fluid abstraction cannot account for the observed rise in potassium concentration significant tubular net secretion occurs. Inspection of Fig. 6 illustrates the situation in the acetazoleamide-perfused *Amphiuma* kidney. Again, the state of potassium reabsorption has been converted into significant net secretion as evidenced by the very dramatic increase in both tubular fluid-to-plasma potassium and potassium/inulin concentration ratios. A similar situation obtains in the mammalian distal tubule in which inhibition of carbonic anhydrase activity by a variety of

drugs induces both alkalization of distal tubular fluid and marked distal secretion of potassium (Malnic et al., 1964; Vieira and Malnic, 1968; Giebisch and Malnic, 1969).

It is also apparent from inspection of Fig. 6 that the cardiac glycoside, ouabain, abolishes any major modification of the filtered potassium along the entire nephron. The concentration of potassium fails to decline along the distal tubule and net reabsorption of potassium has been reduced to insignificant levels.

Stationary Microperfusion Experiments

The ability of distal tubules to establish limiting transepithelial concentration differences was tested in stationary microperfusion experiments. Fig. 7 contains a summary of collected perfusate-to-plasma concentration ratios of sodium under various experimental conditions. No effort was made to divide data according to puncture sites along individual tubules since a considerable and variable part of the distal tubular epithelium that extended beyond the collection site is exposed to the perfusion fluid. Hence, the steady-state concentration differences represent mean values of distal transtubular concentration differences.

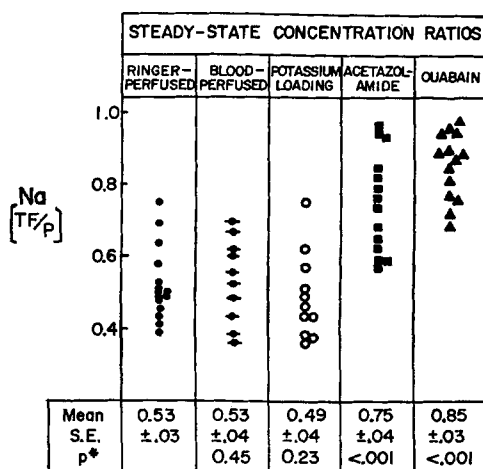
Inspection of Fig. 7 indicates that the transepithelial concentration differences of sodium were of similar magnitude in Ringer- and blood-perfused kidneys as well as in potassium-adapted animals. Also, it should be noted that the range of intratubular sodium concentrations in these experimental conditions did not differ significantly from the range of values observed during free-flow conditions. This observation, i.e. the attainment of similar transepithelial concentration differences in the presence of drastically different rates of net fluid reabsorption, argues against an important contribution of solvent drag to the establishment of distal transtubular sodium concentration differences. These results are essentially in agreement with relevant observations in the mammalian distal tubule in which, in the low and normal range of tubular volume flow, free-flow and steady-state concentration differences are of similar magnitude (Kashgarian et al., 1963; Hierholzer et al., 1965; Malnic et al., 1966)¹

Perfusion with the carbonic anhydrase inhibitor, acetazoleamide, and with ouabain elevated intratubular steady-state sodium concentrations significantly. Thus, both agents reduce the ability of the distal nephron segment to lower the sodium concentration. This effect is consistent with the well-established observation that these agents block sodium reabsorption across renal tubules (Orloff and Burg, 1960; Duarte et al., 1969; Maren, 1967) as well as across other epithelial structures (Ussing, 1960).

Fig. 8 provides a summary of relevant collected perfusate-to-plasma con-

¹ Khuri, R. N., N. Strieder, M. Wiederholt, and G. Giebisch. Unpublished observations.

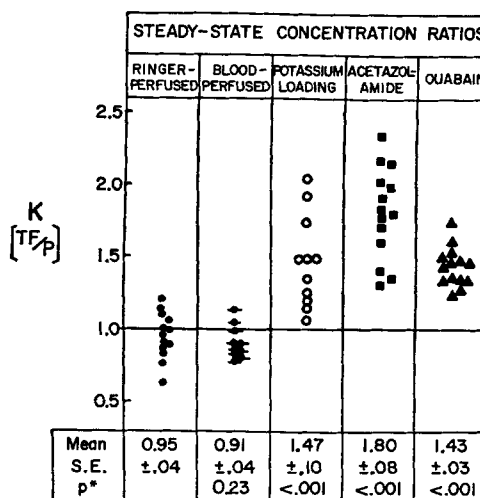
centration ratios of potassium during various experimental conditions. In both control groups (blood and Ringer perfusion) the concentration ratio was slightly below unity, indicative of the reabsorptive capacity of this nephron segment. The transepithelial concentration differences (TF/P ratios) exceed unity significantly under all other experimental conditions in agreement with the results obtained under free-flow conditions. This reflects the ability of the distal tubular epithelium to modify the intratubular potassium concentration independent of net solvent movement. The data underscore the secretory capacity of the distal tubular epithelium in potassium-loaded



* Compared with "RINGER-PERFUSED"

FIGURE 7

FIGURE 7. Summary of distal transepithelial sodium steady-state concentration ratios. Mean plasma Na concentration 97.0 mEq/liter.



* Compared with "RINGER-PERFUSED"

FIGURE 8

FIGURE 8. Summary of distal transepithelial potassium steady-state concentration ratios. Mean plasma K concentration 3.0 mEq/liter in all but the potassium-loaded condition in which the potassium concentration in the perfusion fluid was 5.0 mEq/liter.

and acetazoleamide-treated animals. The moderate elevation of the distal transepithelial concentration ratio during ouabain perfusion as compared to control conditions is evidence for extensive inhibition of distal tubular potassium reabsorption. The observed transepithelial concentration ratio approaches the value expected from passive transepithelial distribution according to the electrical potential difference (see Table II).

Electrical Potential Differences

Electrical potential differences across the distal tubular epithelium and across the peritubular cell membrane were measured under the different experimental conditions. Pertinent results are summarized in Table II. Also

shown are the maximal values of distal transtubular concentration ratios expected from passive distribution across the distal tubular epithelium for the respective transepithelial electrical potential differences. Inspection of the data in which the observed mean collected perfusate-to-plasma concentration ratios are compared with those expected on the basis of passive distribution (using the Nernst equation) indicates that the observed electrical driving force could quantitatively account for entry of potassium into the lumen to produce the observed concentration ratios. In all situations, with the notable

TABLE II
ELECTRICAL POTENTIAL MEASUREMENTS ON SINGLE DISTAL TUBULES

Experimental condition	Electrical potential difference				Transepithelial potassium concentration ratio (TF/P)	
	Transepithelial (lumen negative) mean \pm se	No. of observations	Peritubular transmembrane (cell negative) mean \pm se	No. of observations	Observed (mean \pm se)	Calculated*
Control	43.0 \pm 1.2	10	72.0 \pm 2.2	12	0.95 \pm 0.04 \ddagger 0.91 \pm 0.04 \S	5.50
K loading	30.2 \pm 1.3	12	54.7 \pm 3.4	11	1.47 \pm 0.10	3.32
Acetazoleamide	44.9 \pm 1.2	40	69.7 \pm 1.5	43	1.80 \pm 0.08	5.99
Ouabain	15.9 \pm 0.9	17	28.2 \pm 1.2	18	1.43 \pm 0.03	1.86

* Maximal transepithelial potassium concentration ratios were calculated according to the Nernst equation

$$\left(p d_{\text{observed}} = 60 \log \frac{K_{TF}}{K_P} \right).$$

\ddagger Kidneys perfused with Ringer.

\S Kidneys perfused with blood.

exception of the ouabain-perfused kidneys, the observed concentration ratios are considerably below the calculated ones.

Time Course of Disappearance of ^{22}Na and ^{42}K from Single Distal Tubules

Figs. 9 and 10 summarize results of experiments in control kidneys in which the disappearance of ^{22}Na and ^{42}K was measured as a function of time in stationary microperfusion experiments. Radioactivity due to ^{22}Na and ^{42}K is expressed as a fraction of that initially contained within the tubular lumen. Since the concentrations of sodium and potassium in the injected perfusate were those of an equilibrium solution, i.e. that to be expected after attainment of steady-state conditions, it was assumed that transepithelial movement of fluid, Na, and K was negligible. In order to test this prediction the change in inulin- ^{14}C concentration was followed in 16 individual recollections. In 10 experiments a small water influx (mean: 6.8%) and in 6 experiments a

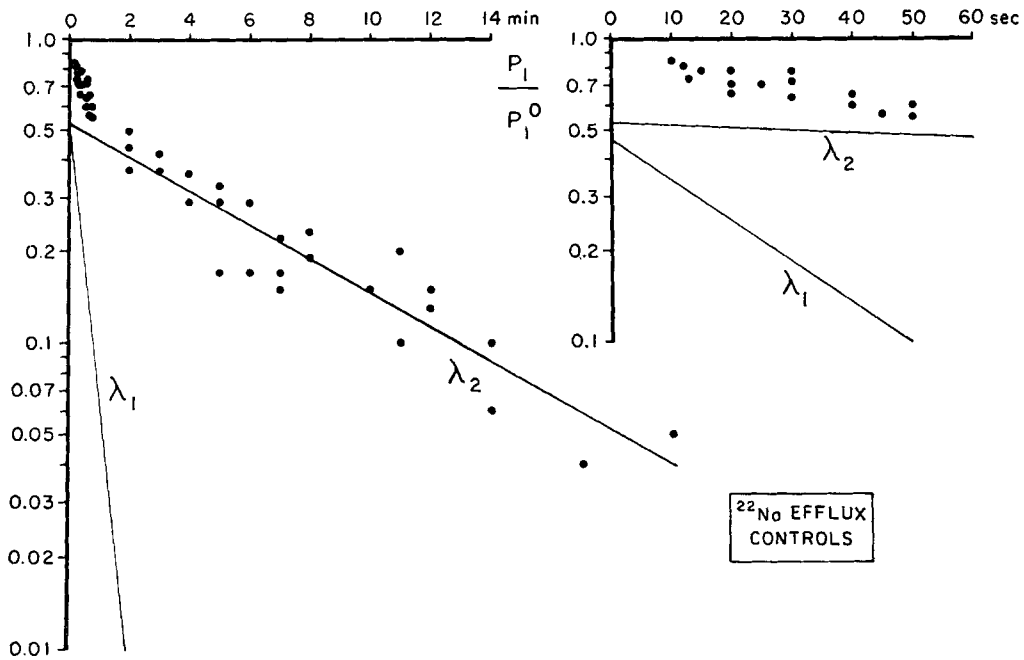


FIGURE 9. Disappearance of ^{22}Na as a function of time from the tubular lumen in control conditions. The smaller plot on the right illustrates the time course of tracer disappearance at an expanded time scale.

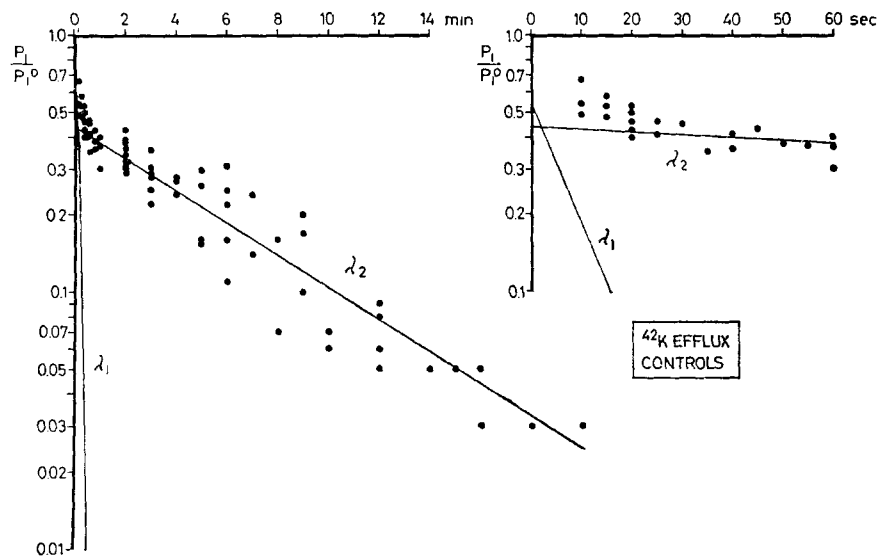


FIGURE 10. Disappearance of ^{42}K as a function of time from the tubular lumen in control conditions. The smaller plot on the right illustrates the time course of tracer disappearance at an expanded time scale.

similarly small water efflux was observed. It was concluded that the conditions of minimal net fluid and net solute movement were fulfilled.

From the distribution of individual measurements under control conditions it is apparent that the curve relating disappearance of ^{22}Na from the tubular lumen can be separated into at least two exponentials with coefficients λ_1 and λ_2 . The experimental points were fitted by a least-squares method to two exponentials as illustrated in Fig. 9. The slopes of tracer disappearance curves and y-intercepts in the different experimental conditions are summarized in Table III. Fig. 10 and Table III also contain data on ^{42}K disappearance. The magnitude of transepithelial net fluid movement was again assessed by comparing the radioactivity due to inulin- ^{14}C in the injected to

TABLE III
EFFLUX COMPONENTS OF ^{22}Na
AND ^{42}K AND γ -INTERCEPT OF λ_2 UNDER
DIFFERENT EXPERIMENTAL CONDITIONS

	$\lambda_1 \pm \text{SD}$ <i>min</i> ⁻¹	$\lambda_2 \pm \text{SD}$ <i>min</i> ⁻¹	$I_2 \pm \text{SD}$
$^{42}\text{Potassium}$			
Control	-7.84 ± 1.24	-0.145 ± 0.004	0.45 ± 0.015
K loading	-1.51 ± 0.34	-0.168 ± 0.013	0.44 ± 0.047
Acetazoleamide ($1 \cdot 10^{-4} \text{ M}$)	-8.05 ± 1.64	-0.199 ± 0.012	0.28 ± 0.016
Ouabain ($5 \cdot 10^{-6} \text{ M}$)	-7.55 ± 2.06	-0.159 ± 0.008	0.57 ± 0.024
$^{22}\text{Sodium}$			
Control	-1.89 ± 0.43	-0.130 ± 0.006	0.53 ± 0.034

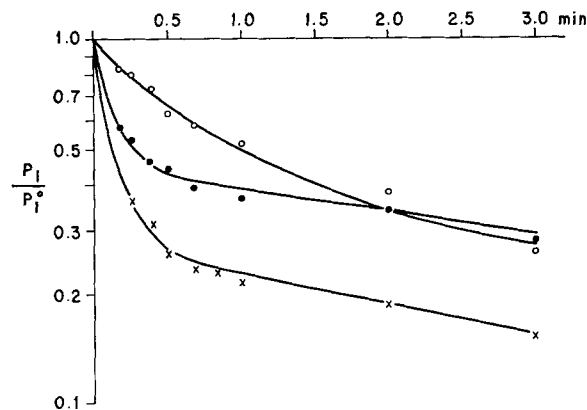


FIGURE 11. Early portion of curves for disappearance of ^{42}K from the lumen. Points are average values at each time and lines are calculated from equation 3 (Appendix) and the data given in Table III. Open circles, controls, x, acetazoleamide, solid circles, K loading.

that in the collected perfusate. In 15 of a total of 26 experiments a small inward fluid movement (mean: 3.2%) was observed, while in 11 experiments a small reabsorptive net movement (mean: 2.8%) of water occurred. The disappearance curve of ^{42}K from distal tubules could also be separated into two components. Pertinent information on individual slopes and intercepts is given in Table III. Fig. 11 shows the early portion of the ^{42}K disappearance curves for three conditions. The curves illustrate the differences among the three conditions (control, K loading, and acetazoleamide treatment) and show the calculated lines obtained from the least-squares fit to the complete set of data. Data from ouabain-treated animals are not included because they did not differ appreciably from control data. Figs. 9 and 10 are representative of fits obtained to data for times greater than 3 min.

DISCUSSION

The present series of experiments clearly establishes that in the amphibian nephron the distal tubular epithelium is the sole tubular site at which major modifications of the filtered potassium moiety take place. It is also apparent that the direction of net transfer of potassium across this nephron segment changes as a function of the metabolic situation. Furthermore, the results obtained during free flow of tubular fluid and in the series of stationary microperfusion experiments demonstrate that the distal tubular epithelium establishes potassium concentrations significantly lower than could be expected from the observed transepithelial potential difference (see also Maude et al., 1966). The observed transepithelial potassium concentration ratios are even considerably below those expected on the basis of passive distribution according to the electrical driving force under conditions of maximal potassium secretion; i.e., in animals which had been exposed to a high-K environment or had been given a carbonic anhydrase inhibitor. The similarity of the transepithelial concentration differences under free-flow and steady-state conditions argues strongly against an important role of solvent drag in determining the luminal potassium concentration. Net fluid movement is drastically different under these two experimental conditions, yet the transepithelial concentration differences achieved are quite similar.

These results may be interpreted as indicative of a reabsorptive force preventing potassium ions from distributing themselves across distal tubular epithelium according to electrochemical equilibrium. Since active net reabsorption against an electrochemical potential gradient (lumen electrically negative with respect to peritubular fluid) occurs under control conditions across the distal tubules, it is virtually certain that electrochemical equilibrium for potassium ions is prevented by an active reabsorptive potassium pump effecting reabsorptive extrusion from the distal tubular lumen. The different

magnitude of the distal transepithelial potassium concentration difference observed under different experimental conditions and responsible for the widely varying transport pattern of this nephron segment is thought to be the result of a shift in the balance between a reabsorptive potassium pump and a passive potassium leak from the peritubular into the luminal fluid compartment along the electrical potential difference. The situation is, in principle, similar to that in the mammalian distal tubule in which supportive evidence for a similar mechanism of potassium transport is available (Malnic et al., 1966 *b*; Giebisch et al., 1967; Giebisch and Malnic, 1969).

However, some significant differences between amphibian and mammalian distal tubular function should be noted. First, net reabsorption of potassium does not occur normally along the mammalian distal tubule as it does in the amphibian. In mammals, net potassium reabsorption can be demonstrated only in conditions of maximal potassium deprivation; i.e., subsequent to dietary potassium depletion (Malnic et al., 1964). However, the distal tubule of the amphibian nephron shares with the mammalian distal tubule the great flexibility in adjusting potassium transport to varying metabolic situations. Hence, it has the capacity to initiate significant potassium secretion when appropriately challenged. A second point of difference between the mammalian and amphibian distal tubule is the nature of the fluid entering this tubular segment. In the mammal the activity of the proximal convolution and the loop of Henle has led to significant reductions in both the sodium and potassium concentration. In contrast, in the amphibian nephron, no major modification in the amount of filtered potassium has occurred during passage of fluid along the proximal tubule. In view of significant fluid reabsorption along this nephron segment the concentration of potassium in tubular fluid entering the distal tubule is elevated above that in plasma water.

The distal tubule of *Amphiuma* responds to a variety of maneuvers with a dramatic change in the transport pattern of potassium. This is similar to the behavior of the mammalian distal tubule. Both potassium adaptation (Wright et al., 1969) and the administration of powerful carbonic anhydrase inhibitors (Malnic et al., 1964; Giebisch and Malnic, 1969) have been shown to induce a dramatic stimulation of distal tubular potassium secretion. With respect to the effects of cardiac steroids, recent micropuncture studies on distal tubules in rats on a low or normal dietary potassium intake have also established that ouabain increases the concentration of potassium, an effect consistent with its blocking distal tubular reabsorption of this ion species (Duarte et al., 1969).² Similar to the results obtained in *Amphiuma* experiments, ouabain promotes urinary potassium loss in the rat.

A consideration of both distal tubular free-flow and steady-state concentra-

² Strieder, N., R. N. Khuri, and G. Giebisch. Unpublished observations.

tions ratios of sodium confirms previous results (Bott, 1962; Maude et al., 1966) that this tubular segment establishes significant concentration differences for this ion species. Similar results obtain in the mammalian distal tubule (Malnic et al., 1966 *a, b*; Giebisch and Windhager, 1964). No consistent relationship between the transfer rates of sodium and potassium was found in our experiments. Fractional sodium reabsorption was similar under widely differing transport patterns of potassium. Thus, the transition from the state of distal tubular potassium reabsorption to that of either Diamox- or high potassium-induced potassium secretion was unaccompanied by any consistent modification of the distal tubular sodium transport system.

Additional information on the behavior of distal tubular electrolyte transport was obtained in a series of experiments carried out to examine some aspects of the kinetics of potassium and sodium movement across individual epithelial cell membranes. An effort was also made to assess the extent to which potassium ions mix with the cellular potassium pool during their transit through the distal tubular cell layer. This analysis has provided some insight into the mechanism underlying the dramatic reversal of the direction of net transport of potassium ions under different experimental conditions. The methods used to estimate rate coefficients and fluxes across individual membranes and the size of the cellular "transport pool" are given in detail in the Appendix. A summary of the most important kinetic parameters obtained from data such as those shown in Figs. 9 and 10 is presented in Table IV.

Some simplifying assumptions concerning the tubular cell model and some limitations of the approach should be considered. The distal tubular epithelium was analyzed as a three-compartment series system in the steady state, because the experimental data can be fitted adequately by two exponentials. This implies that both potassium and sodium ions solely traverse the luminal and peritubular cell membranes as they are either reabsorbed or secreted (Fig. 12). We have assumed that compartment 3 represents the peritubular fluid rather than other cellular compartments. Studies of the disappearance of isotope from the lumen alone do not necessarily distinguish between these possibilities. However, as discussed in the Appendix, the parameters of the model calculated from the disappearance of isotope adequately predicted transtubular movement of ^{42}K in the single successful attempt at making this measurement (see Fig. 14). This suggests that the model shown in Fig. 12 is a reasonable representation of the system.

Two additional limitations of the present analysis are noteworthy. First, there is no proof excluding participation of an extracellular fluid compartment in parallel with the cellular fluid space in the transepithelial transport path. The presence of such an additional transport route has recently been recognized in frog skin epithelium (Ussing and Windhager, 1964), and in *Necturus*

proximal tubule (Windhager et al., 1966; Boulpaep, 1967). However, the observation that the electrical transepithelial specific resistance of distal tubular epithelium is about one order of magnitude higher than that of proximal epithelium is consistent with our view that such extracellular parallel shunt paths are quantitatively less important in the distal tubule (Giebisch and Malnic, 1969). A second consideration concerns the significance of individual rate con-

TABLE IV
RATE CONSTANTS, EPITHELIAL TRANSPORT
POOL OF DISTAL TUBULE, AND UNIDIRECTIONAL
FLUXES OF POTASSIUM AND SODIUM UNDER
DIFFERENT EXPERIMENTAL CONDITIONS

	S_1	k_{12}	k_{21}	ϕ_{12}	S_2	k_{23}	ϕ_{23}
	10^{-8} mM mm tubule^{-1}	min^{-1}	min^{-1}	10^{-8} mM $\text{min}^{-1} \cdot \text{mm}$	10^{-8} mM mm tubule^{-1}	min^{-1}	10^{-8} mM $\text{min}^{-1} \cdot \text{min}$
Potassium							
Control	0.59	4.42	3.31	2.61	0.79	0.26	0.21
K loading	1.77	0.92	0.49	1.63	5.25	0.27	1.42
Acetazoleamide ($1 \cdot 10^{-4} \text{ M}$)	1.28	5.82	2.16	7.42	3.50	0.28	0.98
Ouabain ($5 \times 10^{-6} \text{ M}$)	0.85	3.34	3.85	2.85	0.74	0.36	0.27
Sodium							
Control	9.8	0.96	0.80	9.4	11.8	0.26	3.1

S_1 and S_2 , luminal and extraluminal transport pool. For the calculation of S_1 and S_2 a tubular volume of $1.96 \times 10^{-6} \text{ ml} \cdot \text{mm}^{-1}$ was used. This corresponds to a luminal diameter of 50μ . k_{12} , k_{23} denote rate constants, ϕ_{12} and ϕ_{23} are unidirectional ion fluxes. Note that $\phi_{12} = \phi_{21}$ and $\phi_{23} = \phi_{32}$.

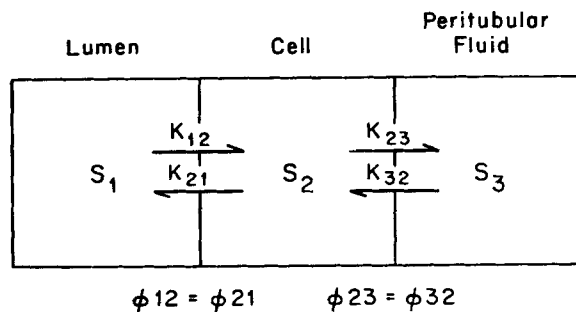


FIGURE 12. Schematic illustration of simple three-compartment system consisting of the tubular lumen, the cell compartment, and the peritubular fluid compartment. S_1 , S_2 , and S_3 denote amount of solute in individual compartment, k_{12} , k_{21} and k_{23} , k_{32} are rate constants defining unidirectional solute movement across the luminal and peritubular cell membrane, respectively. The ϕ_{ij} are unidirectional fluxes. The system is considered to be in the steady state and net transport of potassium and sodium to be zero.

stants of ion movements across the luminal and peritubular cell membranes. Evidence is available that an important component of active potassium reabsorption participates in the establishment of the observed transepithelial potassium concentration differences. This conclusion rests on the observation of extensive net reabsorption in the presence of a significant electronegativity of the distal tubular lumen. It is most likely that potassium is actively transported from the lumen into the cell across the luminal cell boundary of distal tubule cells. Under control conditions, the average potassium concentration in luminal fluid is 3.0 mEq/liter and the electrical potential difference 30 mv (cell negative). If potassium transport were exclusively passive the cell concentration could not exceed 10 mEq/liter, a value about one-tenth the potassium concentration found in distal tubular cells of amphibian kidneys (Sullivan, 1968). Admittedly, this argument assumes uniform distribution of potassium in one cell compartment and evidence to be presented indicates that this is an oversimplification. However, the view that active potassium uptake takes place across the luminal cell membrane is also supported by the marked inhibition of tubular net reabsorption of potassium by ouabain. This cardiac glycoside inhibits active potassium uptake in many cell systems (Ussing, 1960) and the observed increase in distal tubular potassium concentration in the present series of ouabain-perfused kidneys is quite consistent with the notion that one of the important cellular sites of action is the luminal membrane. Some considerations also support the notion that active uptake of potassium into the cell compartment also may take place across the peritubular cell membrane (Sullivan, 1968) since the electrical driving force across this cell boundary is insufficient to account for the observed cellular potassium concentration. These observations on active potassium transport indicate that rate constants and unidirectional fluxes from both luminal and peritubular extracellular fluid compartments include active and passive flux components.

The results of the present estimates of kinetic parameters of transepithelial potassium movement provide several interesting points. The data in Table IV on the transport pool for potassium indicate that only a small fraction of total cell potassium is involved in the over-all transport process. If we assume a mean potassium concentration of 100 mEq/liter cell water (Sullivan, 1968) and tubular dimensions of 50 μ inner and 80 μ outer diameter, the total cellular potassium pool is 30.6×10^{-8} mEq per mm length of tubule. However, as shown in Table IV, the transport pool (S_2) measured by the tracer technique is only $0.8-5.3 \times 10^{-8}$ mEq. A similar striking difference between cell potassium content and transport pool has also been observed in the colonic mucosa of rats (Edmonds, 1969) and in the midgut of *Cecropia* (Harvey and Zerahn, 1969). Thus, although the behavior of ^{42}K can be described adequately by three-compartment kinetics, the system does not appear to be simply lumen K- total cell K- peritubular K. Instead, the transepithelial potassium move-

ment appears to involve passage through a separate epithelial compartment of small capacity but with a considerably faster turnover rate than the main portion of cellular potassium. At present, the intraepithelial site of this transport pool is unknown. Since its size varies considerably and depends on the metabolic situation, we consider remote the possibility that this fraction of potassium is located extracellularly.

A comparison of the magnitude of the epithelial transport pool of potassium under different experimental conditions clearly shows a very marked increase in cellular potassium labeling in those experimental conditions in which potassium secretion had been induced. Thus, during both K loading and acetazolamide administration a four- to sixfold increase in the cellular potassium pool was observed. This very dramatic increase in the secretory potassium pool coincides with the reversal of the direction of net transport from net reabsorption to net secretion. It is of interest that Foulkes (1963) has also deduced that in the rabbit the cellular potassium pool with which potassium mixes during passage from plasma to urine increases considerably during renal adaptation to chronic high potassium loads. He showed that despite much higher potassium excretion rates in potassium-adapted animals, ^{42}K injected intraarterially appeared in the urine of K-adapted animals more slowly than in controls.

The very profound change in the transport characteristics of the distal tubular epithelium is not associated with significant alterations in the electrical potential differences across the luminal cell membrane (see Table II). This makes it unlikely that changes in the electrical driving force at this cell site are of primary importance in the transition from reabsorptive to secretory distal tubular potassium transport.

The present observation that only a variable and relatively small fraction of cell potassium exchanges with luminal ^{42}K makes difficult a precise assessment of the electrochemical potential difference of potassium across the luminal cell boundary. This is due to the fact that the volume in which the exchangeable potassium distributes is unknown. Accordingly, the uncertainty of the magnitude of this cell transport pool precludes the calculation of the effective intracellular potassium concentration which drives potassium from the cell into the lumen. It is also for this reason that we have not attempted to calculate the potassium permeability ($P_{\text{K}21}$ and $P_{\text{K}23}$) despite the fact that such measurements should mainly measure passive diffusion permeabilities. Obviously though, in the absence of dramatic changes in the fraction of cell water in which the transport pool is contained, the observed alterations in the size of the intracellular transport pool (S_2) would correspond to a very marked elevation of the effective intracellular potassium concentration. Conceivably this elevation is responsible for the enhanced tubular secretion since it would correspond to a significant elevation of the electrochemical potential difference of

potassium across the luminal cell border. It is also significant that the variation in the size of the potassium transport pool over a severalfold range (compare control value with values in K-loaded or acetazoleamide-treated animals of S_2 in Table IV) can obviously not be associated with similar changes in total cell potassium content.

The observation that the transport pool of distal tubular potassium increases after potassium adaptation and after acetazoleamide treatment raises the question of whether events at the peritubular or luminal cell membrane are primarily responsible. Although this problem is not completely resolved by the present experiments some clues have emerged. In the first place, both potassium loading and acetazoleamide treatment lead to substantial increases in the flux of potassium from peritubular fluid into the cells (ϕ_{32} equals ϕ_{23} in Table IV). This flux increase seems to be due primarily to stimulation of an active uptake of potassium rather than an increased permeability. If the exit of potassium from the cell across the peritubular membrane is due mainly to diffusion, the coefficient k_{23} should provide an estimate of the potassium permeability of that membrane although k_{23} will also be dependent on electrical potential difference. The results in Table IV show that k_{23} is unchanged in potassium-loaded and acetazoleamide-treated animals and since these conditions cause little change in potential difference across this membrane (Table II), we can conclude that potassium permeability is not markedly changed. Thus, the increase in the flux ϕ_{32} appears to be due to a stimulation of an active uptake of potassium. The observed increase in the intracellular potassium transport pool would be consistent with enhanced cellular uptake across the peritubular membrane in those conditions in which transepithelial potassium secretion is stimulated.

Similar arguments applied to the luminal cell membrane seem less clear-cut. The rather marked decrease in k_{21} in potassium-loaded animals coupled with a rather small change in potential difference across the luminal membrane suggests that the potassium permeability of this barrier has decreased significantly. This decrease in potassium permeability with increased extracellular potassium concentration may be a phenomenon similar to that occurring in *Necturus* in which the electrical resistance of the peritubular cell membrane of tubule cells increases with elevation of the potassium concentration in the external medium (Boulpaep, 1966). There also appears to be a relatively small decrease in potassium permeability of this membrane in acetazoleamide-treated animals if we assume that potassium movement from the cell into the lumen is governed primarily by passive diffusion. It is, however, more difficult to evaluate the nature of effects on potassium movement from the lumen into the cell. The observation that k_{12} increases in acetazoleamide treatment even though potassium permeability of the membrane is somewhat decreased suggests a stimulation of an active process of uptake by the cells. In potassium

loading, the decrease in k_{12} is of the same order of magnitude as the decrease in k_{21} so that there is no necessity to postulate a change in the active component. Thus, these data suggest that the primary effect leading to a change in potassium transport from net reabsorption to net secretion under the two conditions studied is an increase in transport of potassium from peritubular fluid into the cells. In both cases, this effect appears to be modulated by a decrease in potassium permeability of the luminal membrane. However, since the increase in potassium uptake at the peritubular side is greater than the decrease in exit at the luminal side, the net effect is a secretion of potassium into the lumen.

The interpretation of the results in ouabain experiments is complicated by the fact that the electrical potential difference across both the luminal and peritubular cell membrane changes markedly (see Table II). The changes in k_{12} , k_{21} , and k_{23} are in the direction to be expected from the observed depolarization. The decrease in k_{12} could also represent a decline in active potassium uptake across the luminal membrane. Such a reduction in reabsorptive potassium uptake is consistent with the observation made in stationary microperfusion studies in which the transtubular concentration difference approached values to be expected from passive distribution according to the electrical potential difference. Clearly, additional studies will be necessary to assess the contribution of various processes to the rate coefficients and unidirectional fluxes across the individual tubular cell membranes.

With respect to the sodium data a pertinent finding is the greater permeability of the luminal cell membrane to sodium ions than that of the peritubular cell membrane ($P_{\text{Na}_{\text{L,C}}}: 0.070 \times 10^{-2} \text{ cm} \cdot \text{min}^{-1}$, $P_{\text{Na}_{\text{P,C}}}: 0.0042 \cdot 10^{-2} \text{ cm} \cdot \text{min}^{-1}$).³ This difference is quite consistent with electrophysiological observations by Sullivan (1968) who has concluded from studies in which the selective sensitivity of the luminal and peritubular transmembrane potential difference to changes in the extracellular sodium concentration were compared, that the luminal membrane of the distal tubule is characterized by a considerably higher sodium permeability. Similar conclusions have also been reached by Giebisch and Malnic (1969) with respect to the mammalian distal tubule. Another relevant observation of the present experiments concerns the magni-

³ The permeability, P_{ij} , of an ion species across the luminal and peritubular membrane can be calculated according to (Hodgkin and Katz, 1949) as follows

$$P_{ij} = \frac{\phi_{ij} \left(1 - e^{\frac{-ZF\Delta\psi}{RT}} \right)}{\frac{ZF}{RT} \Delta\psi C_i}$$

where P_{ij} is the permeability of the ion species under consideration, ϕ_{ij} the unidirectional flux from compartment i to j , C_i the concentration of the ion in compartment i , $\Delta\psi$ the electrical potential difference, and R , T , F , and Z have their usual meaning.

tude of the intratubular sodium pool. With a concentration of 45 mEq/liter cell water a value of 13.8×10^{-8} mmole·mm tubule⁻¹ obtains. This value is similar to S_2 (11.8×10^{-8} mmole·mm tubule⁻¹) and indicates that intraluminal ²²Na exchanges with all the total distal tubular cell sodium.

In conclusion the present experiments have demonstrated that the distal tubular system in *Amphiuma* is the main site at which renal tubular potassium excretion is regulated. This nephron segment is endowed with the capacity for both net reabsorption and net secretion of potassium. Movement of potassium across the distal tubular epithelium takes place through only a small fraction of the total cell potassium. A very significant increase in this transport pool of potassium is associated with the transition of net reabsorption to net secretion and some evidence is compatible with the thesis that increased active potassium uptake across the peritubular cell membrane is of importance in those experimental conditions in which an elevated potassium secretion rate was observed.

APPENDIX

Analysis of Tracer Behavior

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We wish to consider the behavior of the system shown in Fig. 12 under two different conditions. (See Solomon, 1963, 1964 for a general discussion of tracer kinetics in this type of system.) In the first condition which corresponds to experiments described above, tracer is placed in compartment 1 (the tubular lumen) at zero time and the disappearance of tracer is determined as a function of time. If we assume that the system is in a steady state with respect to ion (sodium or potassium) and that the volumes of all compartments are constant, the system can be described by the following equations:

$$\frac{dP_1}{dt} = -k_{12}P_1 + k_{21}P_2 \quad (1)$$

$$\frac{dP_2}{dt} = k_{12}P_1 - (k_{21} + k_{23})P_2 \quad (2)$$

in which P_i is total tracer (cpm) in compartment i and k_{ij} is the rate coefficient (min⁻¹) for transfer of the ion from compartment i to compartment j . Compartment 2 represents the tubular cells and compartment 3 the vascular system. We assume

that the amount of tracer in compartment 3 is negligible at all times so that equation 2 does not contain a term $k_{32}P_3$.

Equations 1 and 2 can be solved to yield the following expression for P_1

$$P_1 = A_1 e^{\lambda_1 t} + A_2 e^{\lambda_2 t} \quad (3)$$

in which

$$\lambda_1 = -\frac{1}{2}(k_{12} + k_{21} + k_{23}) - \frac{1}{2}\sqrt{(k_{12} + k_{21} + k_{23})^2 - 4k_{12}k_{23}} \quad (4)$$

$$\lambda_2 = -\frac{1}{2}(k_{12} + k_{21} + k_{23}) + \frac{1}{2}\sqrt{(k_{12} + k_{21} + k_{23})^2 - 4k_{12}k_{23}} \quad (5)$$

$$A_1 = -\frac{P_{10}(k_{12} + \lambda_2)}{\lambda_1 - \lambda_2}$$

$$A_2 = \frac{P_{10}(k_{12} + \lambda_1)}{\lambda_1 - \lambda_2}$$

where P_{10} is the amount of tracer in compartment 1 at $t = 0$. The initial condition $P_{20} = 0$ at $t = 0$ has also been used. Equation 3 can then be written in the form

$$\frac{P_1}{P_{10}} = \frac{-(k_{12} + \lambda_2)}{\lambda_1 - \lambda_2} e^{\lambda_1 t} + \frac{(k_{12} + \lambda_1)}{\lambda_1 - \lambda_2} e^{\lambda_2 t}. \quad (6)$$

As shown in Figs. 9 and 10, the data for both Na and K appear to conform to the predictions of equation 6 since P_1/P_{10} can be described adequately in terms of two exponentials. Thus, these results can be used to obtain values of λ_1 , λ_2 , and the intercept, I of the slow component on the y-axis. From these values, the three rate coefficients, k_{12} , k_{21} , k_{23} , can be calculated easily. From the expressions for λ_1 and λ_2 we note that

$$\lambda_1 + \lambda_2 = -(k_{12} + k_{21} + k_{23})$$

$$\lambda_1 \lambda_2 = k_{12} k_{23}$$

and from equation 6,

$$I = (k_{12} + \lambda_1)/(\lambda_1 - \lambda_2).$$

The data for each experimental condition have been averaged to give single values of P_1/P_{10} at different times. The resulting curves were then fitted to two exponentials by least squares⁴ to obtain the values of λ_1 , λ_2 , and I given in Table IV and k_{ij} were calculated from the above equations. Unidirectional fluxes were then evaluated as follows: The flux from tubular lumen into the cells, ϕ_{12} , is given by

$$\phi_{12} = k_{12} S_1$$

⁴ The least-squares fit of the data to two exponentials was obtained using a digital computer and a program denoted SAAM developed by M. Berman and his collaborators at the National Institutes of Health. We are indebted to Dr. A. L. Finn for his help in using the program.

in which S_1 is the pool (mmoles) of ion in the tubular fluid contained in a 1 mm length of tubule of diameter 50μ . Since the system is in a steady state, efflux from the lumen, ϕ_{12} , must be equal to influx into the lumen ϕ_{21} . Thus,

$$\phi_{21} = k_{21}S_2 = k_{12}S_1 \quad (7)$$

in which S_2 is the cellular pool of ion. S_2 is then calculated from equation 7 and the flux from cell to peritubular space, ϕ_{23} , from the expression

$$\phi_{23} = k_{23}S_2.$$

The condition of a steady state also requires that $\phi_{23} = \phi_{32}$.

It seemed desirable to attempt to check the reliability of the parameters calculated by the above method by testing whether they could be used to predict the result of another type of experiment. This alternative experiment involves measurement of tracer influx into the lumen when the vascular system is perfused with solution containing ^{42}K at constant specific activity. At various times after addition of tracer to the vascular perfusate, nonradioactive solution is introduced into the lumen (compartment 1), left for 1 min, and withdrawn. The rate of tracer entry into this compartment (counts per minute per minute) is compared to the rate observed after a long perfusion of the vascular system with tracer (i.e., after the cell compartment has reached specific activity equilibrium). In order to describe this system, we must first obtain an expression for P_2 (tracer in the cellular compartment) as a function of time. With the assumptions of steady state and constant volume, the system is now described by the equations

$$\frac{dP_1}{dt} = k_{12}P_1 + k_{21}P_2 \quad (8)$$

$$\frac{dP_2}{dt} = -(k_{21} + k_{23})P_2 + k_{12}P_1 + k_{32}P_3 \quad (9)$$

in which P_3 is a constant. With the initial conditions $P_1 = 0$ and $P_2 = 0$ at $t = 0$, the solution of these equations is

$$P_2 = \frac{k_{32}P_3}{k_{23}} + \frac{k_{23}P_3}{\lambda_1 - \lambda_2} \left[1 + \frac{\lambda_2}{k_{23}} \right] e^{\lambda_1 t} - \frac{k_{32}P_3}{\lambda_1 - \lambda_2} \left[1 + \frac{\lambda_1}{k_{23}} \right] e^{\lambda_2 t} \quad (10)$$

in which λ_1 and λ_2 are given by equations 4 and 5. From equation 10 we can calculate P_2 at any time τ after the beginning of perfusion of compartment 3 with tracer. (Note that the quantity $k_{32}P_3$ appearing in equation 10 is equal to $\phi_{32}p_3^*$ where p_3^* is specific activity in compartment 3. Since $\phi_{32} = \phi_{23}$, $k_{32}P_3$ can be replaced by $\phi_{23}p_3^*$. The coefficient k_{32} cannot be evaluated because the pool size of the peritubular compartment is unknown.) At time τ we introduce nonradioactive solution into compartment 1 and we then require an expression for P_1 as a function of time with initial conditions $P_1 = 0$ and $P_2 = P_2(\tau)$ at $t = 0$. The general solution for P_1 obtained from

equations 8 and 9 is

$$P_1 = B_0 + B_1 e^{\lambda_1 t} + B_2 e^{\lambda_2 t}$$

in which

$$B_0 = \frac{k_{21} k_{32} P_3}{k_{12} k_{23}}.$$

In order to obtain B_1 and B_2 for these conditions, we make use of the initial conditions $P_1 = 0$ and $dP_1/dt = k_{21}P_2(\tau)$ at $t = 0$. (Note that $t = 0$ now refers to the time at which fresh solution is introduced into compartment 1.) The resulting expressions are

$$B_1 = \frac{k_{21} P_2(\tau) + \lambda_2 B_0}{\lambda_1 - \lambda_2}$$

$$B_2 = \frac{-k_{21} P_2(\tau) + \lambda_1 B_0}{\lambda_1 - \lambda_2}.$$

From the known values of λ_1 , λ_2 , and the k_{ij} 's, the amount of tracer entering compartment 1 in 1 min can be calculated and compared with the observed values. It is most convenient to express the results in terms of a ratio of the rate of entry at any time τ divided by entry when τ is very large.

The behavior of the system under these conditions is illustrated in Fig. 13. The upper solid line shows the relative value of P_2 ($P_2/P_{2\infty}$) as a function of time after beginning perfusion of compartment 3 if the system is left undisturbed. If the lumen (compartment 1) is suddenly filled with nonradioactive fluid at some time after initiation of perfusion, the progressive increase in P_2 is disturbed. This effect is illustrated in Fig. 13 at three time intervals. P_1 drops markedly as tracer moves from compartment 2 to 1 and then begins to increase again since tracer is continually supplied from compartment 3. As shown, P_1 initially rises sharply and then increases concomitantly with P_2 . The theoretical curve for relative influx into compartment 1 shown in Fig. 14 was constructed by using computations such as those shown in Fig. 13 to evaluate the amount of tracer in compartment 1 at 1 min after introduction of nonradioactive fluid. The resulting value for each time τ was expressed as the fraction of the maximum value which was obtained for $\tau = 100$ min. The points in Fig. 14 are experimentally observed values from a single experiment carried out with ^{42}K under control conditions. The agreement between observed and predicted values is reasonable suggesting that the analysis provides an adequate description of the system within the limits of experimental error.

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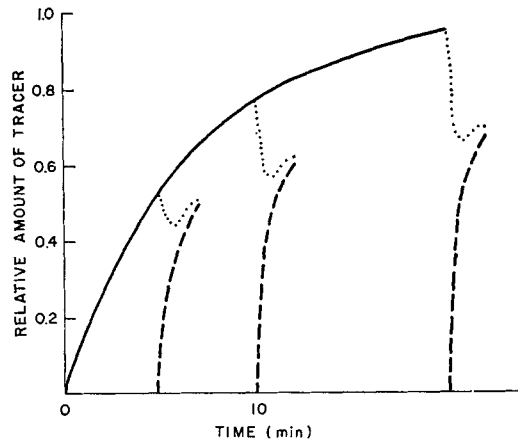


FIGURE 13. Behavior of system as described by equations 10 and 11. The solid line gives P_2 as a function of time after initiation of perfusion of compartment 3 for an undisturbed system. The dashed line and solid dots show the behavior of P_1 and P_3 , respectively when nonradioactive solution is introduced into compartment 1 at the time indicated.

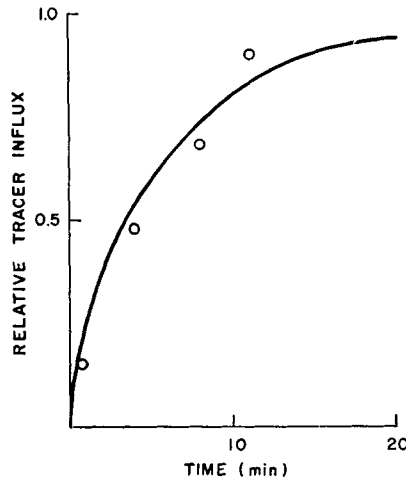


FIGURE 14. Relative ^{49}K influx into compartment 1 as a function of time after beginning perfusion of compartment 3. Solid line was calculated as described in the text and the points are observed values.

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