Modern diuretics and the kidney

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It is particularly appropriate to discuss the pharmacology and mechanisms of action of diuretic drugs in a symposium on diseases of the kidney, since diuretics were the first group of drugs to be developed for the control and manipulation of selected aspects of renal function. Despite a relatively short history of less than three decades since the discovery of the first orally acting diuretic, chlorothiazide, modern diuretic therapy continues to enjoy an ever-expanding clinical demand: worldwide expenditure has been estimated at around US \$800 million per annum during the last five years. Though originally introduced for the treatment of oedematous conditions it is interesting that application of diuretics to the treatment of hypertension has outstripped their use in oedema. This has happened notwithstanding the parallel discoveries of other major antihypertensive agents such as the beta-adrenoceptor blocking drugs and powerful vasodilators.

The advent of novel, orally effective diuretics following on the prototype benzothiadiazine, chlorothiazide, has had a major influence in stimulating progress in the basic sciences relating to nephrology or electrolyte transport in body tissues in general. Advances in fundamental knowledge have in turn given the impetus to further discoveries of new and, in some cases, unique substances possessing diuretic activity. By and large these compounds have attained a remarkable degree of sophistication while, at the same time, remaining relatively safe during long term treatment of patients. The widespread use and, at times, the regrettable abuse of any group of drugs whose primary effect is directed toward interference with the renal handling of electrolytes must inevitably generate secondary disturbances in body homeostasis which have particular relevance to the chemical pathologist. To understand these, demands an understanding of how diuretics work in the context of modern views of the functional organisation of the kidney.

Renal regulation of sodium excretion^{1 2}

Four main mechanisms are believed to be involved in the control of sodium excretion by the kidney.

THE DISTRIBUTION OF GLOMERULAR FILTRATION BETWEEN OUTER CORTICAL AND JUXTAMEDULLARY NEPHRONS

The functional unit of each kidney comprises approximately one million nephrons which lack homogeneity in their structure. About 80% of them are in the outer cortex, have short loops of Henle, and have relatively low reabsorptive capacity for sodium. The remaining 20% are juxtamedullary, possessing long loops of Henle, and are largely responsible for creating the hyperosmotic interstitium in the medulla which mediates the process of urine concentration. Redistribution of blood flow from outer cortical to juxtamedullary nephrons can contribute to abnormal sodium retention, whilst predominance of the effect of outer cortical nephrons may lead to saluresis. A reduction in blood flow to the outer part of the cortex has been found to occur in some sodium-retaining states. Conversely, a drug which could shift blood flow from juxtamedullary to outer cortical nephrons would reduce sodium reabsorption and result in an effective diuretic response.

HAEMODYNAMIC AND PHYSICAL FACTORS³

Changes in physical forces within the peritubular capillaries have been shown to be important determinants of renal sodium reabsorption, especially in the proximal tubule. Thus, for example, renal arteriolar dilatation may, by increasing the hydrostatic pressure in the vasa recta, decrease net tubular reabsorption of sodium with resulting natriuresis, while the reverse occurs with any increase of plasma oncotic pressure. It seems probable that changes in the oncotic or hydrostatic pressures within the peritubular blood vessels achieve their effects on net tubular reabsorption of sodium by influencing the resistance of the intercellular channels or "shunt" paths through which sodium ions pass to reach the peritubular capillaries.

HORMONAL FACTORS

A number of hormonal mechanisms operate singly or together in encouraging sodium retention by the kidney.

Renin-angiotensin-aldosterone^{4 5}

The role of this humoral system in renal sodium regulation is considerable. Yet, because of the sluggish characteristics of the system, it is unlikely that these hormones, especially aldosterone, are involved in fine "moment to moment" modulation. Aldosterone influences epithelial transport of sodium through activation of DNA-dependent RNA synthesis, and the response to it follows a significant lag period which corresponds to the time needed for the induction of new protein synthesis; this may amount to an hour or more in experimental systems. Yet it is clear that changes in sodium excretion *in vivo* may be induced abruptly by various manoeuvres. Moreover, when excessive amounts of aldosterone or other mineralocorticoids are given experimentally sodium retention occurs, but is transient. The "escape" from the action of aldosterone cannot be accounted for by changes in glomerular filtration rate or renal blood flow, and it is one of the pieces of evidence supporting the presence of another humoral substance stimulating natriuresis—called Third Factor or natriuretic hormone.

Natriuretic hormone⁶

A growing body of evidence supports the existence of a natriuretic humoral agent which promotes the renal elimination of sodium, by inhibiting sodium reabsorption in the proximal tubule. It may also work in harmony with the renin-angiotensin-aldosterone system to provide fine control of sodium excretion by an action exerted mainly on the collecting ducts. The chemical characterisation of the natriuretic hormone remains incomplete and its role in the sodium-retaining states is also uncertain.

Prostaglandins and kinins^{7 8}

Many experimental studies have demonstrated the ability of renal tissues to generate prostaglandins and kinins. Whereas renin, angiotensin and aldosterone, like prostacyclin, are transported primarily in the vascular compartment, kallikrein-kinin and prostaglandins of the E series are associated with the renal interstitium and tubular lumen. One or more prostaglandins, primarily PGE₂, is probably responsible for mediating the increase in medullary blood flow that occurs in response to various stimuli including surgical trauma, salt loading, and the action of "loop" diuretics. The generation of kinins in the distal tubules and collecting ducts results in the release of prostaglandins which in turn inhibit the local effects of antidiuretic hormone (ADH) and thereby contribute to the tubular excretion of solute-free water.

FEEDBACK CONTROL SYSTEMS INVOLVING THE MACULA Densa⁹

A fourth intrarenal regulating system which has been proposed is that of a servo-mechanism operating between the macula densa cells of the ascending limb of Henle's loop and the glomerulus of the same nephron. This system functions by the release of renin and angiotensin II locally in response to the sodium concentration in the tubular fluid impinging on the macula densa, the feedback loop being completed by appropriate alterations in both GFR and proximal tubular reabsorption. The detailed mechanisms involved in such an autoregulatory system which couples distal salt delivery to the filtration rate in individual nephrons remain incompletely understood.

Organisation of tubular function^{10 11}

Diuretic drugs have actions on ion-transporting tissues as diverse as amphibian skin, intestinal epithelium, red and white blood cells and cornea. The primary target for the action of these drugs however is the kidney, where they promote the excretion of water and certain electrolytes such as sodium and chloride by interfering with tubular reabsorptive mechanisms. Because these reabsorptive mechanisms vary according to the degree of sophistication of different portions of the epithelium lining the tubule, a brief survey of the organisation of tubular functions is relevant to understanding diuretic action (Fig. 1).

GLOMERULUS AND PROXIMAL TUBULE

In normal man each day the renal glomeruli produce approximately 180 litres of filtrate, and urine is finally produced by the progressive reabsorption of 99% of this ultrafiltrate at various stages along the nephron. About two-thirds of the glomerular filtrate is reabsorbed iso-osmotically in the proximal tubule as a result of the active reabsorption of sodium chloride and sodium bicarbonate from the tubular lumen into the peritubular fluid. The mechanisms involved in transcellular ion movement are complex and involve a variety of energy dependent ion pumps as well as transfer paths or channels in between the loose-fitting cells of the proximal tubule. The resistance to these intercellular shunts of ions is influenced considerably by changes in the oncotic and hydrostatic pressures within the peritubular capillaries.

ASCENDING LIMB OF HENLE'S LOOP (MEDULLARY DILUTING SEGMENT)^{12 13}

There are two morphologically distinct kinds of nephron, namely the outer cortical nephrons

which are the more plentiful and have short loops of Henle, and the juxtamedullary nephrons which have long loops plunging down into the inner parts of the medulla. The suggestion that loops of Henle might act as a countercurrent multiplier system was first proposed in 1942 by Kuhn and Ryffel,¹⁴ but only received widespread attention in 1951 after Wirz, *et al*¹⁵ showed a striking osmotic gradient in the renal interstitium increasing from cortex to papilla. In the ascending limb of the loop of Henle two anatomically distinct portions can be distinguished, both of which are concerned with urinary dilution, namely a medullary portion lined by cuboidal cells and a cortical portion lined by flattened cells. The tubular epithelium of both portions is insensitive to ADH and is thus relatively impermeable to water but the ascending limb does possess the ability to reabsorb salt actively. Because, in mammals, the diluting segments of the loop of Henle do not reach the surface of the kidney, their functional characteristics have had to be deduced from micropuncture of distal convoluted tubules. Recent experiments employing techniques

of tubular microperfusion have shown that the transepithelial voltage in this part of the nephron is positive within the lumen and that chloride ion is absorbed against an electrochemical gradient. Ion substitution studies have lent support to the view that active chloride transport is the primary event and that sodium moves secondarily in this part of the nephron. Electrogenic sodium reabsorption from the tubular fluid may coexist with a separate neutral NaCl path (see later).

Hypertonicity of the inner medulla is the result of combined functions of the hairpin structure of the long loops of Henle acting as counter current multiplier systems and of their associated vasa recta acting as counter current exchange systems. It has been proposed that recycling of urea between the two limbs of the vasa recta and between the loop of Henle and the collecting duct contributes to the hypertonicity of the inner medulla on which concentration of the urine depends. In the presence of ADH¹⁶ the collecting ducts, which traverse the hypertonic medullary interstitium on their way to the renal pelvis, become permeable to water

Fig. 1 A diagram of the nephron showing the four tubular sites where diuretics act to block sodium chloride reabsorption.

Urinary dilution occurs as a result of sodium chloride reabsorption at two water-impermeable sites, the ascending limb of Henle's loop (site II; medullary diluting site) and the early part of the distal tubule (site III; cortical diluting site). During water diuresis, total urine volume can be divided into two moieties, the volume of urine required to excrete urinary solutes at plasma tonicity—that is, the osmolar clearance C_{osm} , and the volume of solute-free water generated at diluting sites II and III—that is, the solute-free water clearance C_{H_2O} . When fluid intake is restricted and hypertonic urine is formed, C_{H_2O} becomes negative and is referred to as $T^c_{H_2O}$. $T^c_{H_2O}$ reflects the passive reabsorption into the hypertonic medulla of solute-free water from the collecting ducts, stimulated by ADH (see footnote on page 1270).

Progressive extraction of solute-free water from the collecting ducts into the hypertonic medulla renders the residual tubular urine hypertonic.

CORTICAL DILUTING SEGMENT

As the diluting segment of the loop of Henle ascends out of the medulla to reach the cortex, continued reabsorption of sodium without water further dilutes the tubular fluid and enables a dilute urine to be excreted in the absence of ADH. The sodium that is reabsorbed in the cortex does not contribute to medullary hypertonicity, so that, whereas a reduction of sodium transport in this cortical segment will be reflected in a reduction of solute-free water clearance C_{H_2O} ,* it will not reduce solute-free water reabsorption $T^c_{H_2O}$,* by the collecting duct which depends on the hypertonicity of the medulla.

DISTAL CONVOLUTED TUBULE AND COLLECTING DUCT

Sodium reabsorption in this part of the nephron is also an active process and may be accompanied by chloride reabsorption or alternatively coupled to potassium and hydrogen ion secretion. The activity of these distal tubular exchange mechanisms is controlled to a large extent by aldosterone, whose modulating action involves the synthesis of mineralocorticoid-induced proteins capable of stimulating the sodium pump located at the peritubular border of distal tubular cells. Permeability to sodium at the luminal border of these cells and those of the cortical collecting ducts may also be increased.

The trans-tubular electrical potential difference in the distal tubule is negative within the lumen and largely generated by the active reabsorption of sodium. It amounts to about -10 mV in the early part of the tubule and reaches -45 mV more distally; it is increased further by the presence of poorly reabsorbable anions within the tubule.

In common with most other cells distal tubular cells are rich in potassium, and a chemical gradient exists for diffusion out of the cells across both the

luminal and peritubular membranes. Distal tubular potassium secretion is largely passive and follows a net electrochemical gradient generated by the active uptake of sodium, and probably also of potassium, from the luminal border of the cell. The luminal membrane possesses an active secretory pump for hydrogen ions which is fed by the intracellular hydrogen ion pool. This pool is replenished by several processes working in parallel, including catalysed and uncatalysed hydration of CO_2 within the cell, backdiffusion of CO_2 from the lumen and uptake of hydrogen ions from the peritubular space. Any diuretic acting proximal to the aldosterone-sensitive ion-exchange sites causes an increased delivery of sodium to the distal tubule and thereby leads to an increase in urinary loss of potassium.

The lining epithelium of the later part of the distal tubule gradually merges with that of the cortical part of the collecting duct where ADH-responsive-ness becomes a feature. The latter mechanism involves a stereospecific receptor at the basal or blood side of the tubular cell, a modulator which receives positive or negative signals—for example, PGE_1 , to be passed on to the catalytic step, phosphodiesterase, which converts cyclic AMP to 5'AMP. The cyclic nucleotide diffuses through the cytoplasm to reach the effector site—the luminal plasma membrane.

Sites of action of diuretic drugs in the nephron^{17 18 19}

Many techniques have been employed to localise the sites of action of diuretics in the nephron. These include various in vitro approaches such as stop-flow, free-flow micropuncture, autoradiography and single nephron micropfusion. The use of different species has made it difficult to interpret the results of such experiments and evidence from in vitro investigation may not be directly applicable to man. Understandably in vitro methods have been much more limited in scope but have nevertheless provided valuable evidence on the possible sites of action in the human nephron.

If it is recalled that of total sodium reabsorption in the nephron, approximately 65% takes place in the proximal tubule, 25% in the loop of Henle, 8-9% in the distal tubule and the remaining 1-2% in the collecting duct, the maximal natriuretic response to a diuretic can give a clue to its site of action (Table). Similarly the pattern of excretion of anions and cations evoked by diuretics can be related to the known functional characteristics of specific portions of the tubule.

Probably the most informative in vivo technique has been the study of diuretic effects on the mech-

*The free water clearance, C_{H_2O} , is defined as the volume of urine excreted in excess of that required to excrete the solute iso-osmotically with plasma. If the urinary solute concentration and volume are U_{osm} and V respectively, the total solute content = $U_{osm} \times V$, and the volume required to excrete this iso-osmotically is

$$\frac{U_{osm} \times V}{P_{osm}} = \text{the osmolal clearance } C_{osm}.$$

$$\therefore C_{H_2O} = V - C_{osm}$$

If there is net reabsorption of solute-free water, C_{H_2O} is negative; it is therefore expressed as

$$T^c_{H_2O} = -C_{H_2O} = C_{osm} - V$$

(Eds)

Table Classification of diuretics

Group	Predominant site of action*
High efficacy diuretics (> 15%)	
Organomercurials	Medullary diluting segment (site II)
Ethacrynic acid	
Furosemide (Furosemide)	
Bumetanide	
Piretanide	
Muzolimine	
Medium efficacy diuretics (5-10%)	
Chlorothiazide and Thiazide Family	Cortical diluting segment (site III)
Phthalimides	
Chlorthalidone, Clorexolone	
Quinazolinones	
Quinethazone	
Metolazone	
Benzenesulphonamides	
Mefruside	
Chlorobenzamides	
Clopamide	
Salicylamides	
Xipamide	
Weak or adjunct diuretics (< 5%)	
Xanthines	Glomerular arteriolar dilatation
Aminophylline	
Carbonic anhydrase inhibitors	Proximal tubule (site I)
Acetazolamide	
Osmotic agents	Proximal tubule (site I)
Mannitol	
Potassium-sparing compounds	Distal Na ⁺ /K ⁺ H ⁺ Exchange (site IV)
(a) Aldosterone antagonists	
Spironolactone and canrenoate	
(b) Pteridines and pyrazine-carboxamides-Triamterene and Amiloride	

Numbers in parentheses are the maximal fractional excretion of sodium (sodium excretion expressed as a percentage of the sodium filtered).

*In certain instances, experimental evidence has been adduced for secondary sites of diuretic action additional to the major locus within the nephron—for example, furosemide and piretanide may also exert some effects upon the proximal site I, as may the thiazide-like compound, metolazone.

reabsorption, a metabolic acidosis is engendered which has been found to limit the diuretic effect. There are other reasons why direct effects of diuretic drugs on the proximal tubule may be obscured or even annulled. When proximal tubular reabsorption of sodium chloride is blocked, compensatory increases in reabsorption further down the nephron may follow so that little additional saluresis occurs in the final urine. The reserve reabsorptive capacity of the diluting segments is considerable and can overshadow effects occurring in more proximal parts of the nephron. By contrast, a diuretic having a primary effect on the medullary diluting segment may lead to the excretion of a substantial amount of excess salt. The main reason for this is the limited reabsorptive capacity for salt of the distal tubule and collecting duct. The latter explains why diuretics acting primarily in the distal tubule such as the potassium-sparing compounds, evoke only a modest saluretic effect amounting, at most, to only 5% of the filtered load of sodium (see Table).

Subcellular mechanisms of diuretic drug action^{19 20}

With the exception of carbonic anhydrase inhibitors, the biochemical basis for the action of diuretics is still largely unknown. There is no doubt that the availability of diuretically active substances has stimulated considerable interest in the physiology and biochemistry of nephron function and has permitted the characterisation of intricacies of tubular function which had hitherto been unrecognised. Such studies have also emphasised, however, the unique anatomical and functional relations within the kidney which make it such a difficult organ to investigate experimentally.

Because tubular epithelium shares certain transport characteristics with other cellular systems, simpler tissues have been utilised to study the mechanisms of diuretic action with a view to explaining the latter in biochemical terms. This is the reason why such an assortment of non-renal tissues as—for example, amphibian skin, urinary bladder, blastocyst and corneal membranes have been used. Whilst undoubted parallels *can* be drawn from the findings in such diverse tissues, it is important that direct extrapolation to the function of the intact human kidney be made with caution. What may be demonstrable in isolated tissues in one species may be drastically altered by the many extra- and intrarenal mechanisms known to be operative in man. Despite these criticisms, the use of diuretics as "membrane probes" has yielded fascinating insights into some of the mechanisms whereby solutes are transferred across cell membranes. Numerous questions about the nature of

anisms for urinary concentration and dilution. The results of such investigations have permitted inferences to be drawn on the inhibitory effects of various diuretics on electrolyte transport within the medullary and cortical diluting segments of the loop of Henle. Four main tubular sites can be identified as of importance in the action of diuretics in the kidney (Fig. 1).

In view of the substantial reabsorptive capacity of the proximal tubule, it might at first sight appear to be useful to produce diuretics whose main locus of action lay proximally. However, those agents which do seem to work in the proximal tubule are either too weak to be effective alone—for example, inhibitors of carbonic anhydrase, or inconvenient, because of the need for intravenous administration—for example, mannitol. Furthermore, a major disadvantage of carbonic anhydrase blockade is that by inhibiting proximal bicarbonate

the "cell receptors" for diuretic drugs remain unanswered and await further exploration.

STRUCTURE:ACTIVITY RELATIONS^{21 22}

The history of the evolution of diuretic drugs reveals an interesting sequence based on chance observations of unwanted effects. Although the diuretic effects of mercury compounds were known to Paracelsus in the sixteenth century, it was Vogl who, as a medical student in Vienna in 1921, observed diuresis in a patient being treated with an organo-mercurial for syphilis and paved the way for the development of mercurial diuretics. By the early 1940s, it was known that various phenyl- and heterocyclic- substituted sulphonamides inhibited carbonic anhydrase and caused a natriuresis with the passage of alkaline urine but at the expense of inducing a metabolic acidosis. A major milestone in the development of the sulphonamide diuretics was the discovery of chlorothiazide in 1957. Subsequent molecular manipulation of the hydrogenated benzothiadiazine structure yielded many different thiazide and thiazide-like agents with progressively dwindling capacity to inhibit carbonic anhydrase (Fig. 2). Then, by opening the thiadiazine ring, and

Fig. 2 Structural relation between a classical carbonic anhydrase inhibitor, such as acetazolamide, and the benzothiadiazine (thiazide) group of diuretics. The three main positions for substitution in the thiazide molecule are depicted as R^2 , R^3 and R^6 , the latter invariably consisting of a halogen (eg, Cl) or trifluoromethyl (eg, CF_3) radical.

exploring derivatives of anthranilic acid, frusemide emerged in the early 1960s (Fig. 3). The discovery of ethacrynic acid, on the other hand, followed a quite separate avenue of research, in that it represented an orally effective "high ceiling" diuretic* which bound to sulphhydryl groups in renal tissue but did not contain any mercury.

It might, at first sight, appear that the biochemical mechanisms of actions of diuretics would be

*The term "high ceiling" is applied to diuretics which act on the loop of Henle because the dose/response curves continue to rise, even with very large doses, unlike the response to thiazides, which reaches a plateau.

Fig. 3 Comparison of two sulphamoyl loop diuretics, frusemide and bumetanide. Both are 5-sulphamoyl-carboxylic acids, but the change from the 4-chloro substituent in frusemide to a phenoxy group, as in bumetanide, results in a substantial increase in diuretic potency on a weight for weight basis.

especially straightforward in the case of the sulphamoyl radical (SO_2-NH_2). The converse is the case. Without doubt, the target enzyme for drugs like acetazolamide or dichlorphenamide is carbonic anhydrase, but precisely how the natriuretic effects of carbonic anhydrase inhibition are achieved is not clear. The evidence for involvement of a bicarbonate-activated ATPase coupled to sodium transport remains uncertain. Although all thiazide and thiazide-like diuretics possess a free sulphamoyl group, the hydrogenated derivatives which followed chlorothiazide have negligible carbonic anhydrase inhibitory capacities yet are potent saluretic agents. To date, no consistent biochemical mechanism of action has been worked out for this large group of diuretics. When high ceiling compounds such as frusemide and ethacrynic acid are compared in terms of their inhibitory effects on respiration and glycolysis in renal tissue it is again disappointing to find that natriuretic activity does not necessarily correlate with metabolic effects. For example, the concentration of diuretic needed to inhibit glycolysis in vitro may be higher than that which could be achieved in vivo within the renal tubular cells.

RENAL ION PUMPS AND DIURETIC ACTION^{10 18}

An impressive body of evidence now exists to show that the Na, K-ATPase of most cells represents or is an integral part of the sodium pump. The enzyme is found in high concentrations in the kidney and especially in the ascending limb of the loop of Henle. Not surprisingly, Na, K-ATPase has been considered a potential candidate as the cell "receptor" for diuretics, especially high ceiling "loop" compounds. Yet a number of important observations conflict with such a view. Although loop diuretics inhibit glycoside-sensitive Na, K-ATPase, they also inhibit that part of sodium transport that persists even in the presence of full doses of the cardiac glycoside, ouabain. The diuretic effects of "loop" compounds has been ascribed to blockade of active

chloride reabsorption in the medullary diluting segment, an action shared with organomercurials. The driving force for active chloride transport in renal tissue has not been fully defined nor has the relation of Na, K-ATPase or other Mg-dependent ATPases to chloride as opposed to sodium transport. A cyclic AMP-activated Na, K cotransport system has been described in avian erythrocytes which is inhibited by loop compounds, whilst a chloride self-exchange system in human erythrocytes has also been found to be inhibited by these diuretics. The relation between these transport systems in human red cells and the renal tubule remains to be clarified.^{23 24}

It is of considerable interest to note that about thirty years ago in the era when organomercurial diuretics reigned supreme, it was proposed that these compounds worked primarily by inhibiting chloride transport. This conclusion was encompassed in the view that "the relationship to plasma chloride is the significant one, and that if mercury specifically blocks one ion absorptive mechanism, that mechanism is the one for chloride absorption. Increased sodium excretion following mercurial diuretics is thus a more or less passive consequence of increased chloride elimination."²⁵ In retrospect, clearly the major role of chloride transport in the action of diuretics has been overshadowed over the years. Yet, it remains likely that the "uphill" reabsorptive transport of chloride in the loop of Henle may represent a transport process tied to the Na, K-ATPase. This means that translocation of chloride across membranes occurs by utilising the energy of the sodium gradient. This would still imply a degree of primacy in the operation of the electrogenic sodium pump.²⁶ Vanadate occurs naturally in cells throughout the body and has been found to inhibit the sodium pump reversibly from the cytoplasmic side of the cell membrane, in contrast with cardiac glycosides such as ouabain which bind to the exterior of cells.²⁷ Infusion of vanadate can induce saluresis by inhibition of tubular reabsorption and it has been proposed that vanadate may have a modulating role on the performance of renal Na, K-ATPase in normal as well as pathological states. The relation if any, between pump modification by vanadate and the cellular actions of diuretics remains obscure.

POTASSIUM-SPARING DIURETICS AND PASSIVE SODIUM-ENTRY CHANNELS^{28 29}

The use of non-renal tissues in the study of diuretic drug action has paid off most handsomely in the case of the potassium-sparing drugs, where most work on the mechanisms has been carried out on isolated amphibian epithelia. In the case of spiro-lactone, the close chemical similarity with

Fig. 4 Effects of 12 and 24 μg Amiloride (8 and $16 \times 10^{-7}\text{M}$) added to the Ringer solution bathing the external surface of isolated frog skin. The immediate fall in short circuit current is readily reversed by washing the skin surface with fresh Ringer solution (based on Baba *et al*²⁸).

aldosterone made it likely that diuretic effects are mediated by competitive binding to the mineralocorticoid-sensitive binding protein in cells located within the distal convoluted and cortical collecting tubules. Triamterene and amiloride act by blocking the passive entry of sodium into transporting cells in these same regions of the nephron. The evidence for this has been obtained for example in experiments where diuretics have been added to the outside surface of frog skin (Fig. 4) or the epithelial surface of the apex of the toad bladder. Triamterene and amiloride are unique among diuretics in blocking sodium entry mechanisms in a rapid and easily reversible manner. Both compounds have been extensively employed as membrane probes to investigate sodium-selective channels in a variety of transporting epithelia.

NEW GENERATION DIURETICS^{30 31}

The lack of any easily definable relation between chemical structure and mechanism of action has been emphasised clearly in the sulphonamide series of diuretics. At least three different modes of operation occur: classical carbonic anhydrase inhibition, benzothiadiazine action and high ceiling action. It is often questioned whether there is any merit in further work involving molecular manipulation within a drug family, when apparently all the useful variations in substitution have been carried out. Experience teaches us that the unpredictable can always emerge even from such mundane exercises. Thus, for example, the unexpected diuretic profile of frusemide emerged from molecular play

Fig. 5 Structural relation between ethacrynic acid and two polyvalent phenoxyacetic acid derivatives, tienilic acid and indacrinone, both capable of causing simultaneous saluresis and uricosuria. Indacrinone exists as a racemic mixture, isomerism occurring at the 2 position of the indanone ring.

with the well-explored benzothiadiazine series. Subsequent high ceiling developments which have followed include bumetanide and piretanide. Molecular manipulation of the ethacrynic acid molecule has led to the emergence of a new class of phenoxyacetic acid derivatives possessing an unusual combination of saluretic and uricosuric properties. The first of these so-called polyvalent diuretics was tienilic acid (ticrynafen) which unfortunately ran into major toxicological problems early in its clinical application to antihypertensive treatment. Development of human hepatotoxicity led to the withdrawal of tienilic acid from clinical use by its manufacturers in 1980. A further phenoxyacetic acid derivative, indacrinone lacks the thienyl substituent and has the unusual feature of stereo-isomerism, each of its constituent enantiomers possessing different pharmacological profiles in relation to saluresis and uricosuria.³²

Availability of this new generation of compounds has offered a means of investigating in greater depth the mechanisms of uric acid handling by the kidney and how these are affected by diuretics.

Current concepts of renal urate handling support the view that uric acid may undergo bidirectional transfer via one single transport system located at various sites along the nephron.³³ Such a mechanism is analogous to the forwards and backwards movement of sodium and potassium ions which has been demonstrated to occur through the conventional sodium pump.³⁴ Disturbance in the relative balance between the two processes of secretion and absorption of uric acid would be responsible for the predominance of either hyperuricaemia or uricosuria. Renal urate regulation probably involves a "four-component" system, namely, filtration, bidirectional

Fig. 6 Changes in handling of uric acid in normal man after a single dose of racemic indacrinone, 10 mg. The top half of the figure shows the plasma urate concentrations on control day (○) and drug day (●). A significant fall occurs at 1, 2 and 4 h after indacrinone compared to control. This parallels a significant increase in fractional renal uric acid clearance measured as $E_{urate} = \frac{U_{urate}}{C_{urate}} \times \frac{C_{creatinine}}{U_{creatinine}} \times 100$. At time of peak uricosuria (2-4h) E_{Na} increased from 0.55 ± 0.09 (control) to 2.92 ± 0.30 ($p < 0.001$)

□ control day; ▨ drug day
Results are expressed as mean values $\pm$ SEM ($n = 8$) (based on Brooks et al³²).

transtubular fluxes and also postsecretory reabsorption. The latter process is of particular importance in accounting for renal urate retention associated with chronic thiazide administration and occurs secondarily to diuretic-induced ECF volume contraction.

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