Chapter I

INTRODUCTION
1.1 POINTS TO PONDER REGARDING DIURETICS

A diuretic is any substance, which increases urine and solute excretion. The primary action of most diuretics is the direct inhibition of sodium transport at one or more of the four major anatomic sites along the nephron where sodium reabsorption takes place. Because the sodium-transport systems at each of these locations are unique, there is a different set of relatively rigid structural features that a diuretic must possess in order to inhibit sodium reabsorption at each site. Of additional importance are the secondary (or indirect) events that are triggered as a result of the diuretic's primary action. The nature and magnitude of many of the observed secondary effects are dependent on the locus of action of the diuretic and the response of nephron sites “downstream” to an enhanced delivery of fluid, sodium or other solutes. The secondary events are quite characteristic for each class of diuretics and are often highly predictable. Collectively, the primary and secondary effects induced by a diuretic determine its electrolyte excretion pattern. A diuretic usually possesses some combination of natriuretic, chloruretic, saluretic, kaliuretic, bicarbonaturetic, and calciuretic properties depending on whether it enhances the renal excretion of sodium, chloride, sodium chloride, potassium, bicarbonate, or calcium, respectively.

1.1A ANATOMY AND PHYSIOLOGY OF THE NEPHRON

The functional unit of the kidney is the nephron with its accompanying glomerulus (Fig. 1.1). There are approximately one million nephron in each kidney. The blood (or, more appropriately, the plasma), from which all urine is formed, is
Fig 1.1 The anatomy of the nephron with emphasis on the four major sites of sodium reabsorption.
brought to each nephron within the glomerular capillary network (Fig. 1.2). Most of the plasma components are filtered into Bowman’s space. During the process of urine formation, the resulting glomerular filtrate flows through proximal tubule (convoluted and straight portions), the descending limb of Henle’s loop, the thin and thick ascending limbs of Henle’s loop, area of the maculadensa cells, the distal convoluted tubule, the connecting tubule (sometimes referred to as the late or terminal distal tubule), and the cortical and medullary collecting tubules. Each of these nephron segments consists of ultra structurally and functionally unique cell types.

1.1B FUNCTION OF NEPHRON WHEN THE PLASMA VOLUME IS NORMAL (NORMOVOLEMIA OR EUVOLEMIA)

As blood is delivered to each glomerulus, many (but not all) of its components are filtered into Bowman’s space through the pores in the glomerular capillary loops. Several physicochemical properties of each blood component dictate the extent to which it is removed from the blood by glomerular filtration. These include the component’s (1) relative molecular mass (Mr), (2) overall charge (applies primarily to large molecules), and (3) degree and nature of binding to plasma proteins. For example, plasma proteins with a Mr in excess of 50,000 da and red blood cells are not readily filtered, where as low-Mr, non-protein-bound components (e.g., sodium, potassium, chloride, bicarbonate, glucose, and amino acids) are readily filtered.

The glomerular filtration rate (GFR) of plasma components that possess a Mr of less than 50,000 Da and not bound to plasma proteins is (1) directly dependent on the hydraulic
Fig 1.2 The juxtaglomerular apparatus (JGA). Urine is formed from the filtration of plasma through the glomerular capillary loops into Bowman’s space. The JGA is of paramount importance for the operation of the tubuloglomerular feedback mechanism, which allows a nephron to regulate the glomerular filtration rate of its own glomerulus.
(hydrostatic) pressure in the renal vasculature (created by the pumping heart), which tends to drive water and solutes out of the glomerular capillaries into Bowman’s space; (2) related inversely to the plasma oncotic pressure (the osmotic pressure created by the plasma proteins within the vasculature), which tends to hold or prevent the filtration of water and solutes across the glomerular capillaries into Bowman’s space;¹ and (3) governed by the intrarenal signals that allow each nephron to adjust the filtration rate through its own glomerular capillary network ². So, the cardiovascular and renal functional status of an individual will also affect the rate of filtration of plasma components through the glomeruli. In addition, neonates and the elderly usually have a reduced GFR.³,⁴

The fraction of the total renal plasma flow that is filtered collectively by the glomeruli per unit time (i.e., the filtration fraction) is about one-fifth². This means that only one-fifth (or 20%) of the plasma presented to the kidneys in a given period undergoes filtration at the glomeruli (i.e., about 650 mL of plasma flow through the kidneys each minute, approximately 125 mL/min of which are filtered through the glomerular capillaries). The remaining four-fifths (or 80%) of the renal plasma flow is directed into the peritubular capillaries (Fig. 1.1). Each minute only 1 mL of urine is formed from the 125 mL of glomerular filtrate⁵. Thus, approximately 99% of the glomerular filtrate is normally reabsorbed.

There are four major anatomic sites along the nephron that are responsible for the bulk of sodium reabsorption ⁵, ⁶ (Fig. 1.1): **site 1**, the convoluted and straight portions of the proximal
tubule; **site 2**, the thick ascending limb of Henle's loop; **site 3**, the distal convoluted tubule; and **site 4**, the connecting tubule (i.e., the terminal portion of the distal convoluted tubule) and the cortical collecting tubule. The actual transport processes involved in sodium reabsorption at each of these sites are highlighted in (Fig. 1.3) through (Fig. 1.6).

**Site 1**

The sodium transport systems that is responsible for the reabsorption of sodium and associated solutes in the proximal tubule. In response to the action of the Na⁺ K⁺-ATPase, sodium ions in the luminal fluid move down the concentration gradient into proximal tubule cells by a combination of at least three distinct processes, which are labeled A, B and C in Fig. 1.3. (A) Transcellular reabsorption of sodium/bicarbonate, which is controlled by carbonic anhydrase (CA). Acetazolamide and other CA inhibitors block sodium reabsorption by this route. (B) Transcellular reabsorption of sodium coupled to glucose, amino acids, and phosphate. (C) Para cellular transport of sodium/chloride. There are no commercially available agents that inhibit sodium reabsorption by routes B or C. The Na⁺,K⁺-ATPase is indicated by filled circles on the antiluminal membrane ⁶⁻¹².
Fig 1.3 Site 1: The sodium transport systems that are responsible for the reabsorption of sodium and associated solutes in the proximal tubule.
Site 2

The sodium-transport systems that are responsible for the reabsorption of sodium and associated solutes in the water-impermeable cortical and medullary portions of the thick ascending limb of Henle’s loop (Fig. 1.4). The collective actions of the antiluminal membrane-bound Na⁺,K⁺-ATPase and the luminal membrane-bound 1Na⁺/1K⁺/2Cl⁻ cotransport system account for transcellular reabsorption of sodium chloride, in a ratio of 3 sodium/6 chloride, and the generation of a lumen-positive potential that drives the reabsorption of sodium and other cations via the paracellular pathway (dashed line). Diuretic agents that block sodium reabsorption in the thick ascending limb by inhibition of the luminal membrane-bound 1Na⁺/1K⁺/2Cl⁻-cotransport system include Furosemide, bumetanide, torasemide, ethacrynic acid, and a number of miscellaneous agents.¹²-¹⁵

Site 3

The sodium-transport systems that are responsible for the reabsorption of sodium and chloride in the water-impermeable distal convoluted tubule (Fig. 1.5). Inhibitors of the luminal membrane-bound Na⁺/Cl⁻ cotransport system include the thiazides and thiazide-like diuretics.⁶,¹¹.
Fig 1.4 Site2: The sodium-transport systems that are responsible for the reabsorption of sodium and associated solutes in the water-impermeable cortical and medullary portions of the thick ascending limb of Henle’s loop.
**Fig 1.5 Site 3:** The sodium-transport systems that are responsible for the reabsorption of sodium and chloride in the water-impermeable distal convoluted tubule.
Site 4

The connecting tubule (i.e., late portion of the distal convoluted tubule) and the cortical collecting tubule house the fourth and final major site for the reabsorption of sodium from the luminal fluid\(^6\) (Fig. 1.6). This portion of the nephron is composed of two distinct cell types, the principal cells and the intercalated cells. The principal cells are most important for sodium reabsorption and potassium secretion, whereas the intercalated cells are most important for the generation and secretion of hydrogen ions. The intercalated cells do not possess Na\(^+\),K\(^+\)-ATPase on their antiluminal membranes, but they do contain intracellular CA, which catalyzes the formation of carbonic acid from CO\(_2\) and water. The carbonic acid ionized to hydrogen ions and bicarbonate ions. The hydrogen ions can be pumped actively into the luminal fluid by the luminal membrane-bound H\(^+\)-ATPase. The driving force for the reabsorption of sodium in the principal cells is once again the deficit of intracellular sodium created by the Na\(^+\),K\(^+\)-ATPase on the antiluminal membrane, which pumps three sodium ions uphill from the principal cells into the interstitium and two potassium ions uphill from the interstitium into the principal cells. In response to the deficit of sodium in the principal cells, the sodium in the luminal fluid moves downhill into the principal cells through sodium channels in the luminal membrane and subsequently is pumped actively into the interstitium by the antiluminal membrane-bound Na\(^+\),K\(^+\)-ATPase. This creates a lumen-negative transepithelial voltage. In response to this voltage difference, some combination of the following three processes occur: (1) chloride ions move paracellularly from the
Fig 1.6 Site 4: The sodium-transport systems that are responsible for the reabsorption of sodium in the connecting and cortical collecting tubules.
lumen into the interstitium, (2) potassium in the principal cells moves downhill into the luminal fluid through potassium channels in the luminal membrane, and (3) hydrogen ions generated in the intercalated cells move into the luminal fluid by way of the $H^+$-ATPase. $^{10}$ Because the latter two processes dominate, one may view the activities at site 4 as an exchange of luminal fluid sodium ions. The exchange of luminal fluid sodium ions for principle cell potassium ions and intercalated cell hydrogen ions. The exchange of luminal fluid sodium for intracellular hydrogen or potassium ions normally is associated with the reabsorption of only 2% to 3% of the filtered load of sodium, $^6$ and the distal location of this exchange system dictates the final acidity and potassium content of the urine.

The amount of sodium reabsorbed at site 4, and therefore the amount of hydrogen ions and potassium ions present in the final urine, is modulated by the (1) plasma and renal levels of mineralocorticoids like aldosterone – the higher the levels of circulating aldosterone, the greater the degree of sodium reabsorption and potassium and hydrogen ion excretion – (2) luminal fluid flow rate and the percentage of the filtered load of sodium presented to the exchange sites—the greater the flow rate and the load of sodium, the greater the amount of exchange—and (3) acid-base status of the individual-acidosis favors exchange of sodium and hydrogen ions, whereas alkalosis favors exchange of sodium and potassium ions.$^{6,16}$ The classes of diuretics that inhibit the reabsorption of sodium at sites 1, 2, or 3 (i.e., sites proximal to site 4) ultimately increase the luminal fluid flow rate and the percentage of the filtered load of sodium delivered to site 4. Thus, many diuretics acutely enhance the urinary loss of
potassium ions and may be associated with the induction of hypokalemic.

In short, sodium reabsorption and potassium secretion take place in the principal cells, whereas hydrogen ion formation and secretion occur in the intercalated cells. Spironolactone inhibits sodium reabsorption by competitively antagonizing the effects of aldosterone on the principal cells. Triamterene and Amiloride "plug" the sodium channels in the luminal membrane of the principal cells, thereby preventing sodium reabsorption and potassium and hydrogen ion secretion. Hence, while producing a modest natriuresis, these drugs prevent potassium loss and are commonly referred to as potassium-sparing diuretics (Fig. 1.7).

1.1C FUNCTION OF THE NEPHRON DURING REDUCED PLASMA VOLUME (HYPOVolemIA)

An individual's plasma volume may be reduced by hemorrhage, diarrhea, vomiting, excessive sweating, or the overzealous use of diuretic agents. When this occurs, the renal processes previously discussed shift into a "conserve" mode to prevent further loss of vital body fluids and solutes. The reduction in arterial pressure that accompanies a reduction in plasma volume triggers several crucial events. First, there is an increase in sympathetic tone that stimulates the release of catecholamines, which in turn stimulate the intra renal release of renin, an enzyme that catalyzes the formation of angiotensin I. Angiotensin I is then converted to angiotensin II, a potent renovasoconstrictor. The catecholamines and angiotensin II work in concert to constrict the renal vasculature and to reduce renal
Fig 1.7 The Site of action of potassium sparing Diuretics
blood flow and the GFR. Angiotensin II also stimulates the production of aldosterone, which ultimately enhances reabsorption of sodium at site 4 and, together with other factors, reabsorption of all substances normally reabsorbed in the proximal tubule.\textsuperscript{14,17} Second, antidiuretic hormone (ADH) is released into the bloodstream from the posterior pituitary gland in response to the reduced arterial blood pressure and elevated plasma osmolality. ADH conserves water by increasing the permeability of the cortical and medullary collecting ducts to water. In the presence of ADH, the high osmolality of the medullary interstitium (created in part by the collective actions of the luminal membrane-bound \( \text{INa}^+/IP^+ /2\text{Cl}^- \) transport system and the antiluminal membrane-bound \( \text{Na}^+,\text{K}^+-\text{ATPase} \) of the thick ascending limb cells) draws the water out of the lumens of the collecting tubules by osmosis. Water is conserved and vascular volume is restored.

1.1D FUNCTION OF THE NEPHRON DURING DISEASE STATES ASSOCIATED WITH RETENTION OF BODY FLUIDS (EDEMA'TOUS STATES)

Frequently, the kidneys of individuals with congestive heart failure, cirrhosis of the liver with ascites, or the nephritic syndrome receive messages that are interpreted to mean that they are being hypoperfused, this may occur whether or not there is an actual plasma volume reduction. The kidneys attempt to retain body fluids and solutes by any one or combination of the processes discussed above, ultimately edema ensues\textsuperscript{18}. 
1.2 STATUS OF DIURETICS

A diuretic is any substance, which increases urine and solute excretion. This wide definition, however, includes substances not commonly thought of as diuretics, e.g. Water. To be therapeutically useful, a diuretic should increase the output of sodium as well as of water. The diuretics are normally required to remove oedema fluid, which is composed of water and solutes, of which sodium is the most important\textsuperscript{19}.

Diuretics, though a slightly neglected part of pharmaceutical research, have found important applications in the life threatening diseases either alone or in combination with other drugs, for e.g. diuretics are recommended for first step treatment of hypertension. In fact they may be more effective than β-adrenergic blocking agents in controlling blood pressure. They are not only powerful antihypertensive drugs but also enhance the antihypertensive activity of other drugs\textsuperscript{20}. 
### 1.3 Classes of Diuretics

<table>
<thead>
<tr>
<th>Class</th>
<th>Drugs</th>
<th>Mode of action</th>
</tr>
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<tbody>
<tr>
<td>Osmotic diuretics.</td>
<td>e.g. Ammonium chloride, Mannitol, Isosorbide.</td>
<td>Prevents reabsorption of water and impairs Na⁺ reabsorption in proximal tubules by osmotic effect.</td>
</tr>
<tr>
<td>Carbonic anhydrase inhibitors.</td>
<td>e.g. Acetzolamide, Methazolamide etc.</td>
<td>Inhibition of carbonic anhydrase.</td>
</tr>
<tr>
<td>Thiazide diuretics</td>
<td>e.g. Chlorothiazide, Hydrochlorothiazide etc.</td>
<td>Depletion of sodium and subsequent reduction in plasma volume in cortical portion or ascending loop of Henley.</td>
</tr>
<tr>
<td>Potassium sparing diuretics</td>
<td>e.g. Triamterene, Amiloride, and Spironolactone.</td>
<td>Interfere with sodium absorption in the late distal tubules and cortical collecting ducts thereby promoting sodium excretion while conserving potassium.</td>
</tr>
<tr>
<td>Loop diuretics</td>
<td>e.g. Furosemide, Ethacrynic acid, etc.</td>
<td>Inhibit active chloride ions and possibly sodium transport in the ascending thick limb of Henley.</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>e.g. Probenecid.</td>
<td>Inhibits tubular reabsorption of urate at proximal convoluted tubules.</td>
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1.4 CLINICAL SIGNIFICANCE OF DIURETICS

1.4A CARDIAC FAILURE

The underlying abnormality in cardiac failure is a cardiac output that is inadequate to meet the metabolic demands of the body during exercise [and ultimately also at rest]. This leads secondarily to an increased central venous pressure [increased pre-load] and to peripheral vasoconstriction [increased after load]. It may be caused by disease of the myocardium itself [most commonly ischaemic heart disease], or by circulatory factors such as volume overload [valvular incompetence, arteriovenous shunts], etc. or pressure overload [hypertension, valvular narrowing], which require greater cardiac work to be performed to achieve a sufficient output. When the cardiac output decreases, an increase in body fluid volume occurs, partly because the increased venous pressure causes increased formation of tissue fluid, and partly because the reduced renal blood flow leads to activation of the rennin-angiotensin-aldosterone system. A highly simplified diagram of sequence of events is shown in Fig 1.8.

There are four main points at which drugs can act-

- **Increased force of contraction** – Cardiac glycosides were once the mainstay of treatment of cardiac failure, but their use is now largely restricted to patients who also have supraventricular dysrhythmias. Their adverse effects out weigh their benefits with long-term treatment in other situations. Dobutamine [selective adrenoreceptor
Fig 1.8 Simplified scheme showing the pathogenesis of heart failure, and the sites of action of some of the drugs to treat it. The symptoms of heart failure are produced by reduced tissue perfusion, oedema and increased central venous pressure.
agonist] is used intravenously when a rapid treatment is needed in the short term, for example following open-heart surgery.

➢ **Increased fluid excretion** – Diuretics are routinely used to treat cardiac failure. A diuretic may suffice its own effect and is increasingly often combined with ACE inhibitors.

➢ **Vasodilatation** – The use of rapidly acting vasodilators such as glycercyl trinitrate is well established for treating acute episodes cardiac failure. The vasodilator effect of these drugs is helpful in reducing venous pressure, and their effect in increasing the compliance of the arterial system is useful in reducing cardiac work. The use of arteriolar vasodilators, such as hydralazine or prazocin, for longer-term treatment has largely been replaced by ACE inhibitors.

➢ **ACE Inhibition** – ACE inhibitors (ACEi) are now commonly used, in combination with diuretics. By blocking the formation of angiotensin II they reduce both vascular resistance [thus improving tissue perfusion and reducing cardiac after load] and the secretion aldosterone [thus reducing Na⁺ retention]. They have a beneficial effect on longevity, which is, greater than that provided by vasodilator therapy with a combination of organic nitrates and hydralazine. Neither digoxin nor diuretics have been shown to improve survival.
1.4B MECHANISM OF DIURETIC INDUCED POTASSIUM DEPLETION

Conventional diuretics

Urine sodium excretion

Contraction of Extracellular fluid volume

Renin-angiotensin-Aldosterone system

Distal tubular Sodium-Potassium Hydrogen ion exchange.

Potassium depletion

Uric acid Excretion

Proximal tubular HCO$_3^-$ absorption

Metabolic alkalosis.

Distal tubular HCO$_3^-$ delivery
Any diuretic which increases the delivery of sodium out of the ascending limb of Henle’s and increased urinary flow rate within this tubular segment will cause significant potassium secretion.

Since aldosterone increases the intracellular potassium concentration via the active Na⁺/K⁺ pump at the basolateral membrane and also modifies the sodium channel at the luminal membrane of the distal convoluted tubular cell, this hormone modifies distal tubular potassium excretion.

Loss of volume will stimulate renin secretion, thereby causing secondary hyperaldosteronism, which then provokes an additional kaliuresis by facilitating distal tubular potassium and hydrogen ion secretion in exchange for sodium reabsorption.

**ECFV** depletion also causes enhanced proximal bicarbonate reabsorption. This in addition to the distal loss of H⁺ as titrable acid and ammonium will result in metabolic alkalosis. Subsequently the increased delivery of the poorly reabsorbable bicarbonate to the distal tubule will further accelerate potassium and hydrogen secretion in this nephron segment.

**1.4C NEED FOR POTASSIUM SUPPLEMENTATION**

The sodium ion is the most important ion in the body if one considers the composition of only plasma, extracellular fluid, and ultra filtrates. Nevertheless, the body as a whole contains approximately 140 g potassium (K⁺) and only 105 g sodium \([ \text{Na}^+ \])]. A calculation shows that 98% of the K⁺ is intracellular and only 2% is in the extracellular compartment.
Therefore removal or addition of a small amount of K⁺ to this extracellular pool will be very evident³³.

Hypokalemia is the risk factor in hypertension. Patients with base line electrocardiographic abnormalities, a pre-existing abnormal rhythm, left ventricular hypertrophy, cardiac failure, preexisting coronary artery disease, acute myocardial infarction, or those on digitalis therapy³⁴ are more susceptible to the life-threatening arrhythmias or sudden death due to Hypokalemia than are other subjects³⁵.

Severe Hypokalemia is often accompanied by loss of magnesium³⁶ which may not only contribute to the sudden risk of cardiac arrhythmias and sudden death, but magnesium replacement may be required to reverse Hypokalemia and/or associated cardiac risks³⁷,³⁸. Prevention or correction of hypomagnesia is therefore needed in these patients. Also potassium supplementation has been shown to blunt the rise in blood pressure or to decrease blood pressure in patients with diuretic induced Hypokalemia.

Clinical Hypokalemia is frequently associated with leg cramps, Cardiac abnormalities, and generalized weakness. It can be treated with potassium supplements, including dietary supplementation, or adjunctive use of potassium sparing agents³⁹,⁴⁰.

Potassium Chloride supplements are still widely used to prevent or treat diuretic induced Hypokalemia, although they are relatively ineffective even in high doses⁴¹. Low doses (8-16 mmol/d) are often combined with diuretics in a single tablet.
Combined tablets of this type may cause esophageal injury if the esophagus is obstructed.\textsuperscript{42}

Barium swallow and endoscopy showed a bendrofluazide – potassium chloride tablet embedded in the esophageal wall 30 cm from the incisor teeth. The tablet was removed to reveal a deep ulcer with ragged edges.

The potassium-sparing drugs Amiloride, Spironolactone and Triamterene correct diuretic-induced Hypokalemia more reliably but also have hazards. Severe hypokalemia is the most important adverse reaction, and this may develop if the patients taking potassium-sparing drugs also use commercially available “salt substitutes” which contain potassium salts.\textsuperscript{43}

1.4D AMILORIDE

\textit{Relationship between cardiac effects and inhibition of Na\textsuperscript{+}/Ca\textsuperscript{++} exchange} :-

Amiloride is a well-known potassium sparing diuretic\textsuperscript{44} whose natriuretic action is due to the block of the Na\textsuperscript{+} entry pathway in distal tubules. The capacity of drug to inhibit passive Na\textsuperscript{+} entrance has been studied in different tissues. It has been shown that low Amiloride concentration [< 1 \(\mu\text{m}\)] blocks Na\textsuperscript{+} entry in epithelium of toad bladder\textsuperscript{45}, while concentration higher than 1 \(\mu\text{M}\) inhibit the Na\textsuperscript{+}/H\textsuperscript{+} exchange in Necturus gallbladder\textsuperscript{46} and in mouse soleus muscle\textsuperscript{47}. Besides these effects, Amiloride has been shown to be active on the cardiovascular system also: it produces a positive inotropic effect when added to isolated guinea pig atria\textsuperscript{48} and when administered to man\textsuperscript{49}. 
More recently attention has been focused on Amiloride because of its capacity to inhibit the Na+/Ca++ exchange in erythroleukemic cells\textsuperscript{50} and in rat brain synaptosomes\textsuperscript{51}. Na+/Ca++ exchange is one of the key system together with slow Ca++ channels and Ca++ ATPase regulating Ca++ entrance and exit across cardiac sarcolemma and thus the free intracellular Ca++ concentration during the contraction – relaxation cycle of cardiac cells. Ca++ movements mediated by the exchanger are vectorially oriented by the trans-membrane Na+ gradient. It is generally accepted that Na+/Ca++ exchange normally acts as a system for extruding Ca++ from the cells but when [Na]O is reduced or [Na]I becomes very high, Ca++ accumulates within the cell through partial reversal of the Na+/Ca++ exchange. However, the exact role of the Na+/Ca++ exchange in allowing Ca++ in allowing Ca++ extrusion or Ca++ uptake in cardiac contraction is still under debate. In order to clarify this point a specific inhibitor and Na+/Ca++ exchange could be very useful, however to date such an inhibitor remains to be found. Two drugs have been shown to interfere with this exchange activity: the antitumor anthranylocycline doxorubicin as inhibitor of Na+/Ca++ exchange activity in cardiac sarcolemma\textsuperscript{52} and Amiloride in erythroleukemia cells\textsuperscript{53} and in rat brain synaptosomes\textsuperscript{54}.

Maura F.\textsuperscript{55} reported that, in an isolated preparation of cardiac sarcolemmal vesicles; Amiloride inhibited Na+/Ca++ exchange activity at the same concentration that induced a positive ionotropic effect in isolated guinea pig atria. In addition, it has been observed that ouabain toxicity in isolated atria was reduced by pretreatment with Amiloride. The possibility is
considered that the inhibition of Na+/Ca++ exchange activity by Amiloride can be responsible for the positive ionotropic effect and protective action against digitalis toxicity.

1.4E SIDE EFFECTS OF TRIAMTERENE AND AMILORIDE

Triamterene

Two further cases of pancytopenia and megaloblastic bone marrow developing acutely in patients with alcoholic cirrhosis during treatment with Triamterene have been reported. 

All the 7 patients had alcoholic cirrhosis. The doses of Triamterene ranged from 200-400 mg/d, and the cumulative dosage from 1.2 to 6.9 gm. The mechanism is thought to be inhibition of dihydrofolate reductase by the Triamterene in patients rendered susceptible by alcohol abuse, dietary deficiency and chronic liver disease56.

Amiloride -

Amiloride itself may have side effects when used long-term, it is expensive, and the evidence that glucose intolerance would be avoided is unsatisfactory. However the main concern is the fact that Amiloride is inferior to the thiazides in lowering blood pressure even when it is used at high doses. It should not be used as the only diuretic for the treatment of essential hypertension57,58.

Hypokalemia and glucose intolerance, renal function:

There is evidence to suggest that the glucose intolerance that complicates long term diuretic treatment may be a direct consequence of altered potassium homeostasis, but it remains to
be shown that diuretic regimens, which maintain a normal potassium status, are superior in this respect during long-term treatment. Prolonged severe depletion can cause hypokalemic nephropathy\textsuperscript{59-61}.

1.4F ADVANTAGES AND DISADVANTAGES OF POTASSIUM SPARING DIURETICS

Combination of a potassium-sparing diuretic with other diuretics produces a desirable balance of properties. These diuretic combinations have become preferred agents for initial antihypertensive therapy because they avoid the risk of hypokalemia or the need for potassium supplementation. They encourage patient compliance with one daily dosing\textsuperscript{62}.

The concomitant use of these diuretics with other hypotensive drugs synergises the hypotensive response. Thus it makes possible the achievement of an adequate hypotensive response with lower dosage of the hypotensive agent and enables the patient to remain free from the untoward side-effects which would have been encountered at high dosage levels \textsuperscript{63,64}.

1.5 METHODS OF EVALUATION OF DIURETIC ACTIVITY

Three types of assays are used to characterize the activity,

1. The orally dosed saline loaded rat was employed as first screen. Base line data obtained in this protocol with standard diuretic agents indicated that urine volume was a reasonably reliable indicator of natriuresis.
2. The orally dosed sodium deficient rat has measured the natriuretic potency of the compounds in animals in a state of avid sodium retention due, at least in part, to high levels of endogenous mineralocorticoids.

3. Renal clearance experiments in saline loaded 9-α-flurohydrocortisone treated dogs. [Saline-9-α-FHC dog] provides more detailed data on the effects of the compounds on Na⁺, K⁺, Cl⁻ and water excretion, urinary pH, and glomerular filtration rate.

Since these three tests measure diuresis in different systems, correlation between these tests was not always good, although in general the compounds highly active in rat were also active in the dog and vice-versa.
1.6 NEWLY INTRODUCED DIURETICS

1. Torasemide (1993)\textsuperscript{23} -

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TORASEMIDE (1993)

Torasemide is the novel loop diuretic launched in 1993 after a 12 years gap from the last diuretic introduction. It is indicated for the treatment of hypertension and edema associated with chronic congestive heart failure, renal disease and hepatic cirrhosis; exerts major diuretic activity on the thick ascending loop of Henley to promote marked excretion of water, Na\textsuperscript{+}, and to a lesser extent, K\textsuperscript{+}, and Ca\textsuperscript{2+}.

2. Azosemide (1986)\textsuperscript{24} -

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\end{center}

AZOSEMIDE (1986)
It is a high ceiling diuretic similar in structure and profile to furosemide; somewhat less potent and less bioavailable. Useful in congestive heart failure and similar edematous conditions.

3. **Muzolimine** *(1984)*

![Muzolimine Structure]

**MUZOLIMINE (1984)**

It is a pyrazolone diuretic with high ceiling profile, effective in edema of cardiac, hepatic and renal origin. Also appears to be effective as an antihypertensive agent.

4. **Tizolimine** *(1984)* - It is a sulphonamide type of diuretic.

![Tizolimine Structure]

**TIZOLIMINE**
5. **Bemitradine**\textsuperscript{27,28} - It is a triazolopyrimidine found to be 5.5 times more potent than hydrochlorothiazide.

\[
\text{BEMITRADINE}
\]

6. **DS 210**\textsuperscript{29} - It was found to produce a maximal natriuretic effect similar to hydrochlorothiazide without affecting potassium excretion.

\[
\text{DS 210}
\]

7. **Benzbromarone**\textsuperscript{30} - It is a benzofuran derivative. It is known to be a uricosuric agent.