This project work was submitted in partial fulfilment for the award of a degree in Bachelor of Pharmacy of the University of Nairobi.

June, 1979
ACKNOWLEDGMENT

I am greatly indebted to my supervisors Prof. C.K., Maitai and Dr. G. Bariuki, without whose guidance this work would not have been possible. I am particularly grateful to them for reading through the script and guiding me in any alterations and corrections that had to be made.

I am also grateful to the technical staff of the Pharmacology section for their unfailing assistance during the course of this project.

I am thankful to Mrs. Nkungu for typing the manuscript.
This project was carried out to investigate the influence of urine pH changes on the renal excretion of drugs that are weakly acidic. In choosing the drugs to be used in the project, the practicability of their assay in a common laboratory was considered. Hence four drugs were chosen: Aspirin, Chlorothiazide, Sulphathiazole, and Sulphafurazole. Rats were used in all the experiments that were carried out. In each experiment, the urine was collected over a period of 24 hours and analysed for drug in question. In this way, the amount of drug excreted in urine under varying urine pH conditions were determined. Sodium bicarbonate and Ammonium chloride were used to make the urine alkaline and acidic respectively.

The system essentially consists of a filter called the Bowman's capsule, with a long tube called a renal tubule. The collecting duct is also functionally a part of the system. The blood vessels that supply the capillaries in the tubules are also an essential part of the system. The Bowman's capsule is coated with a layer of branching inner connecting capillaries (glomerular net) which provide a large surface area of capillary circulation through which fluid and small molecules may filter into the capillary and pass down the nephron. The glomerular net together with the Bowman's capsule constitutes the glomerulus. The glomerular capillary and the inner and the supporting layers of Bowman's capsule constituting the glomerulus.
This project was designed to determine the effects of oral administration of alkali and acid upon the renal excretion of drugs that are weakly acidic.

The kidney is the most important organ of excretion and as such most substances are excreted in urine. However, some other substances are excreted in bile, sweat, saliva, gastric juice, or from the lungs. The excretory unit of the kidney is the Nephron. In the human kidney there are millions of these units. The nephron essentially consists of a filter called the Bowman's Capsule, with a long stem called a renal tubule. The collecting duct is also functionally a part of the nephron. The blood vessels that supply the capsule and the tubule are also an essential part of the nephron. The Bowman's Capsule is packed with a mass of branching inter connecting capillaries (glomerular tuft) which provides a large surface area of capillary endothelium through which fluid and small molecules may filter into the capsule and pass down the tubule. The glomerular tuft together with the Bowman's capsule constitute the glomerule. The glomerular capillary endothelium and the supporting layer of Bowman's capsule form an active transport of organic solutes and ions into the lumen (tubular secretion), each by a
have pores ranging upwards to 40° A. Hence, unbound solutes (drugs) and even a little amount of albumin pass into the glomerular filtrate.

The post-gloernalr vessels which lie close to the tubules are critically important to renal function in the sense that substances re-absorbed from the filtrate by the tubules are returned to the blood along these vessels. Functionally the tubule can be divided into three major parts, the proximal convoluted tubule, the loop of Henle and the distal convoluted tubule.

After filtration the glomerular filtrate passes through the proximal tubule, where some solutes may be re-absorbed through the tubular epithelium and returned to the blood stream. Re-absorption occurs partly by passive diffusion and partly by active transport especially with sodium and glucose. Consequently, by these processes the filtrate becomes diminished in volume by approximately 80% in the proximal tubule, although it is not concentrated. In the proximal tubule some acidification occurs as a result of carbonic anhydrase activity in the tubule cells and the diffusion of hydronium ion reacts with the bicarbonate ion, which is converted to re-absorbable non-ionic carbon dioxide.

There is also active transport of organic cations and ions into the lumen (tubular secretion), each by a
a separate system. These active transport systems are extremely important in the excretion of a number of drugs. For example, penicillin G is rapidly secreted by the anion transport system and tetraethylammonium by the cation transport system.

As the filtrate travels down the tubule through the loop of Henle, it becomes concentrated, especially at the bottom, as a result of active reabsorption and the countercurrent distribution effect of the renal apparatus. In the distal tubule sodium re-absorption occurs partly in exchange for potassium and hydrogen ions. Ammonia secretion may be either acidified or alkalinised according to the acid-base and electrolyte requirement.

Drugs are also re-absorbed in the distal tubule and the pH of the urine here is extremely important in determining the rate of re-absorption and amount re-absorbed. The pH of the tubular fluid also affects the tubular secretion of drugs. When the drug in the tubule is highly ionised only a little of the drug can be reabsorbed. Hence most of the drug is rapidly excreted in urine. The urine pH and hence drug excretion may fluctuate widely according to the diet, exercise, drugs, time of day and other factors.
Obviously, the excretion of weak acids and bases can be partly controlled with acidifying or alkalining salts for example Ammonium Chloride or sodium bicarbonate respectively.

Comparative studies on potency and efficacy of drugs in man have demonstrated the importance of controlling urinary pH. Urine pH is important only when the drug in question is a weak acid or base of which a significant fraction is excreted through the renal route. The plasma levels of the drug will be affected due to the change in the excretory rate. This may have far reaching effects on the therapeutic effect of the drug.

The importance of urine pH in the excretion of drugs has been illustrated by several workers. 

Himo et al (1957) found that mecamylamine (basic) is excreted more than four times faster when the urine pH is less than 5.5 than when it is above 7.5. 

Haag and Larsson (1942) demonstrated that in the case of nicotine the extent of urinary excretion of the chemical may be related to the pH of the urine.

They emphasized the importance of taking into consideration the dissociation constant of a drug and the relative reabsorbility of the free and dissociated base. Extending these studies to the urinary excretion of quinine in man, Haag, Larson and Schwartz (1943) found that the urinary excretion could be doubled by passing from an alkaline to an acidic urine and they ascribed the difference to greater
the latter i. converted to quinine chloride. Thus, the chloride to the kidney is increased and reversible, since re-absorption re-absorption of quinine from the urinary tract when the urine is alkaline. Pearson and Bolo (1943) found that renal clearance of quinine was subject to 100-fold variations due principally to two variables, the urinary pH and the renal plasma flow. The army malaria research unit at Oxford (1945) emphasised the striking parallelism between excretion of quinacline and Ammonia.

### The effect of Ammonium Chloride and Sodium bicarbonate on the urine

After ingestion and absorption, the NH\(_4^+\) ion is converted by the liver to Urea, during which process hydrogen ions are liberated.

\[
\begin{align*}
NH_4^+ &\rightarrow NH_3 + H^+ + 3^- \\
NH_3 &\rightarrow Urea
\end{align*}
\]

The hydrogen ion so formed reacts with bicarbonate and other buffers in the extra-cellular fluid. Reduction in the bicarbonate concentration causes an increase in

\[
\frac{[H_3CO_3^-]}{[HCO_3^-]^+}
\]

Thus causing an increase in the concentration of hydrogen ions in the extra-cellular fluid. Consequently there is a fall in the pH, resulting in the formation of acid urine. The end result is that chloride ion displaces the bicarbonate ion.
the latter is converted to carbon dioxide. Thus, the chloride to the kidneys is increased and appreciable escapes resorption along with an equivalent amount of cation (mainly Na\(^+\)) and i.e. 0.05% quantity of water.

The purpose of these set of experiments was to determine after injection of sodium bicarbonate, the excess of the accumulate amount of either lanolin chloride or carbonate load presented to the distal tubule is not completely sodium bicarbonate required to achieve acidic and alkaline neutralised by the available hydrogen ions. Consequently the presence of excess bicarbonates in the extra-cellular fluid result in the production of alkaline urine.

bicarbonate were weekly fed to the rats and the urine collected over a period of 24 hours. The urine obtained was determined using a pH meter. The results was reported until the minimum amount of either lanolin chloride or sodium bicarbonate required to produce expected alkaline or acidic bicarbonate was obtained. (In this case the expected urine pH was obtained, (In this case the expected urine pH was obtained) was observed until the minimum amount of either lanolin chloride or sodium bicarbonate required to produce expected alkaline or acidic bicarbonate was obtained. (In this case the expected urine pH was obtained, (In this case the expected urine pH was obtained).

(i) Alkalining agent - sodium bicarbonate - (DIM)
(ii) Acidifying agent - Ammonium chloride - Analytical reagent
(iii) Drugs used-

Aspirin Tablets, 300mg B.P (Jawa Pharmaceuticals Ltd)
Sulphathiazole Tablets, 500mg. B.P (Lough England)
Sulphadiazole Tablets, 500mg. B.P (Lough England)
Chlorothiazide Tablets, 500mg B.P (A.T. Monk and Co, Ltd)

(iv) Animal - Rats
(v) Flasks and burette - each consisting of 3 animals
(vi) Metabolic cages.

**MATERIAL AND APPARATUS:**

- Ultraviolet spectrophotometer - Unicorn P 800
- pH meter - Pye Unicam

To one of the groups sodium bicarbonate was given orally and to the second group lanolin chloride was given. The next day the urine was collected.
Experimental

The purpose of these set of experiments was to determine the approximate amount of either Ammonium chloride or Sodium bicarbonate required to achieve acidic and alkaline urine pH values respectively in rats. In each case increasing amounts of either Ammonium Chloride or Sodium bicarbonate were orally fed to the rats and the urine collected over a period of 24 hours. The pH of the urine obtained was determined using a pH meter. This exercise was repeated until the minimum amount of either Ammonium chloride or Sodium bicarbonate required to produce expected urine pH was obtained. (In this case the expected urine pH was either acidic or alkaline) The results are shown on Table 1.

Quantitative Estimation of Drug in Urine under Varying Urine pH

Three groups of rats each consisting of 3 animals were chosen and starved for at least 24 hours. Three rats were used for each experiment to ensure enough urine was obtained. The rats were hydrated to ensure reasonable amount of urine was collected within the experimental period. To one of the groups Sodium bicarbonate was given orally and to the second group Ammonium Chloride was given. The
The third group was used as a control. Then equal amounts of the drug were given orally to the three groups of rats. The three groups of rats were then placed in three different metabolic cages and the urine collected in measuring cylinders over a period of 24 hours. The pH of the urine was noted to ensure it was within the expected range. The urine was filtered and decolourised with charcoal. The urine was then quantitatively analysed for the drug under test. This was done by obtaining an absorption spectra for the drug. 0.1M sulphuric acid and 0.1M sodium hydroxide were used as the solvents to obtain the absorption spectra of Aspirin and Chlorothiazide respectively. Typical absorption spectra are shown on figures 1 and 2. The results obtained are shown on Tables II and III.

Quantitative estimation of sulphadiazine and sulphathiazole in urine

The experimental set up was the same as for Aspirin and Chlorothiazide just described except that the method of analysis was titrimetric. A titration was carried out with 0.1M standard sodium nitrite solution at a temperature below 15°C until a drop of solution immediately gives a blue colour on starch-iodide paper. The end point was complete when the end point could be reproduced after the titrated solution was allowed to stand for one minute. The titre was noted. The results obtained are shown on Tables IV and V.
RESULTS

The spectrophotometric method was used to analyse the urine for

Table I: Determination of amount of acidifying and alkalining agents

Aspirin and Chlorotheiside, since the extinction coefficients

for both drugs at their Amax is known, it is possible to

calculate their excretion in urine using the Beer-Lambert

<table>
<thead>
<tr>
<th>Amount given to each rat</th>
<th>Sodium Bicarbonate</th>
<th>Ammonium Chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>70 mg/0.25 kg.</td>
<td>140 mg/0.25 kg.</td>
<td>210 mg/0.25 kg.</td>
</tr>
<tr>
<td>300 mg/0.25 kg.</td>
<td>60 mg/0.25 kg.</td>
<td>90 mg/0.25 kg.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Volume of urine collected</th>
<th>Sodium Bicarbonate</th>
<th>Ammonium Chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 ml.</td>
<td>23 ml.</td>
<td>21 ml.</td>
</tr>
<tr>
<td>21 ml.</td>
<td>22 ml.</td>
<td>20 ml.</td>
</tr>
</tbody>
</table>

Jailer, J.W., Rosenfeld, J. and Shannon, J.A

in their work on renal excretion of quinaorine,

Chloroquine and Santequine used Sodium bicarbonate

and Ammonium Chloride to make urine alkaline and

acidic respectively.

Similarly, the amount of Chlorotheiside excreted in alkaline

urine can be calculated.
Calculation of Drug in urine from absorbance:

The spectrophotometric method was used to analyse the urine for Aspirin and Chlorothiazide. Since the extinction Coefficients for both drugs at their \( \lambda_{max} \) is known, it is possible to calculate their concentration in urine using the Beer–Lambert relationship.

\[
A = \varepsilon_{\lambda_{max}} \, C \, l
\]

Where

- \( A \) = Absorbance at \( \lambda_{max} \)
- \( \varepsilon_{\lambda_{max}} \) = Extinction Coefficient at \( \lambda_{max} \)
- \( C \) = Concentration in g/100 ml
- \( l \) = Path length of the cell in Centimetres.

For example, the amount of Aspirin excreted in urine under alkaline conditions was calculated as shown below:

\[
0.63 = 65.5 \times 10^{-2} \times 1
\]

<table>
<thead>
<tr>
<th>( C )</th>
<th>( g/100 \text{ml} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>65.5</td>
<td>( 0.00962 )</td>
</tr>
</tbody>
</table>

\[
C = 9.62 \text{mg of aspirin}
\]

Similarly, the amount of Chlorothiazide excreted in alkaline urine can be calculated as:

\[
A = \varepsilon_{\lambda_{max}} \, C \, l
\]

\[
0.63 = \varepsilon_{\lambda_{max}} \times \text{other values}
\]

\[
C = \text{other values calculated as shown above}
\]

\[
C = \text{other values}
\]
$$A = \frac{e_{1/2} \text{ loa.}}{g/l.}$$

$$0.92 = 700 \times C \times 1$$

$$C = \frac{0.92}{700} \text{ g/100ml}$$

<table>
<thead>
<tr>
<th>Ph</th>
<th>Amount of Chlorothiazide</th>
<th>Amount of Chlorothiazide in urine</th>
<th>Amount of Chlorothiazide in urine</th>
<th>Amount of Chlorothiazide in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13.144 µg</td>
<td>13.144 µg</td>
<td>13.144 µg</td>
<td>13.144 µg</td>
</tr>
</tbody>
</table>

But since a dilution of ten-fold was made during the analysis, then concentration of Chlorothiazide in urine

The other values were similarly calculated as shown above.

The results are shown in tables II and III.

<table>
<thead>
<tr>
<th>Control</th>
<th>3000 µg</th>
<th>0.29</th>
<th>4.43 µg</th>
<th>7.39 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>(no agent)</td>
<td>3000 µg</td>
<td>0.395</td>
<td>6.03 µg</td>
<td>9.73 µg</td>
</tr>
</tbody>
</table>
### Table II

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Amount of Drug given</th>
<th>Absorbance at λmax (276 nm)</th>
<th>Amount of drug excreted in urine</th>
<th>Average amount of drug excreted in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>8.7</td>
<td>300mg</td>
<td>0.70</td>
<td>10.69mg</td>
<td>9.62mg</td>
</tr>
<tr>
<td>bicarbonate</td>
<td>0.56</td>
<td></td>
<td></td>
<td>0.55mg</td>
<td></td>
</tr>
<tr>
<td>Ammonium</td>
<td>5.6</td>
<td>300mg</td>
<td>0.15</td>
<td>2.23mg</td>
<td>2.18mg</td>
</tr>
<tr>
<td>Chloride</td>
<td></td>
<td>0.135</td>
<td></td>
<td>2.069mg</td>
<td></td>
</tr>
<tr>
<td>Control (no agent)</td>
<td>7.1</td>
<td>300mg</td>
<td>0.29</td>
<td>4.43mg</td>
<td>5.23mg</td>
</tr>
<tr>
<td></td>
<td>0.395</td>
<td></td>
<td></td>
<td>6.03mg</td>
<td></td>
</tr>
</tbody>
</table>
Table III

<table>
<thead>
<tr>
<th>Drug</th>
<th>pH</th>
<th>Amount of drug given</th>
<th>Amount of drug excreted in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium bicarbonate</td>
<td>8.5</td>
<td>40mg</td>
<td>0.92</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>5.7</td>
<td>40mg</td>
<td>0.80</td>
</tr>
<tr>
<td>Control (no agent)</td>
<td>7.2</td>
<td>40mg</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Table continued...

* A ten-fold dilution was made

\[ \text{Molecular weight of aliphathiazole} \]

\[ 0.0256g \text{ of } 21.71_{11} \text{H}_{13} \text{F}_{0.92} \]
In calculating the amount of sulphathiazole or sulphafurazole excreted, the following relationship was derived. From the titration reaction, 1 mole of sodium nitrite is required for every mole of either sulphathiazole or sulphafurazole.

\[
\text{Ar-\text{NH}_2} + \text{NaNO}_2 \rightarrow \text{Ar-H} + \text{NCL} + 2\text{H}_2\text{O}
\]

N\(\text{H}_4\)

Therefore

1 molar solution of Na\(\text{SO}_4\) \(=\) 255g of \(\text{C}_9\text{H}_9\text{N}_3\text{O}_2\) (sulphathiazole)

1000ml of 1N Na\(\text{SO}_4\) \(=\) 255g of \(\text{C}_9\text{H}_9\text{N}_3\text{O}_2\)

1 ml of 1N Na\(\text{SO}_4\) \(=\) 0.255g of \(\text{C}_9\text{H}_9\text{N}_3\text{O}_2\)

1 ml of 0.1N Na\(\text{SO}_4\) \(=\) 0.0255g of \(\text{C}_9\text{H}_9\text{N}_3\text{O}_2\)

255g = Molecular weight of sulphathiazole

Similarly, a similar relationship can be derived for sulphafurazole:

1 molar solution of Na\(\text{SO}_4\) \(=\) 267.3g of \(\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}_3\)

1000ml of 1N Na\(\text{SO}_4\) \(=\) 267.3g of \(\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}_3\)

1 ml of 1N Na\(\text{SO}_4\) \(=\) 0.2673g of \(\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}_3\)

1 ml of 0.1N Na\(\text{SO}_4\) \(=\) 0.02673g of \(\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}_3\)
The above relationship were used to calculate the amount of drug excreted in urine as follows:

For example in, alkaline conditions, the amount of *Sulphathiazole*

<table>
<thead>
<tr>
<th>Passed in urine</th>
<th>Amount</th>
<th>Sulphathiazole</th>
<th>Amount excreted in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>5.9 g</td>
<td>0.0255 * 5.9 g</td>
<td>150.5 mg</td>
</tr>
<tr>
<td>Guinea</td>
<td>14 mg</td>
<td>0.1505 g</td>
<td>22.5 mg</td>
</tr>
<tr>
<td>Guinea</td>
<td>150 mg</td>
<td>150.5 mg of <em>Sulphathiazole</em></td>
<td></td>
</tr>
</tbody>
</table>

Similarly, the amount of *Sulphafurazol* excreted in alkaline conditions can be calculated:

<table>
<thead>
<tr>
<th>Passed in urine</th>
<th>Amount</th>
<th>Sulphathiazole</th>
<th>Amount excreted in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>6.8 g</td>
<td>0.02673 * 6.8 g</td>
<td>181.16 mg</td>
</tr>
</tbody>
</table>

The other values were similarly calculated as shown above. The results are shown in Tables IV and V.

### Table IV

<table>
<thead>
<tr>
<th>Species</th>
<th>Drug taken</th>
<th>Amount (mg)</th>
<th>Amount excreted (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea</td>
<td>Bacteria</td>
<td>210 mg</td>
<td>3.5 mg</td>
</tr>
<tr>
<td>Guinea</td>
<td><em>Sulphathiazole</em></td>
<td>300 mg</td>
<td>113.56 mg</td>
</tr>
<tr>
<td>Guinea</td>
<td><em>Sulphathiazole</em></td>
<td>200 mg</td>
<td>113.56 mg</td>
</tr>
<tr>
<td>Control</td>
<td>(no agent)</td>
<td>210 mg</td>
<td>3.48 mg</td>
</tr>
<tr>
<td>Control</td>
<td>(no agent)</td>
<td>210 mg</td>
<td>144.34 mg</td>
</tr>
</tbody>
</table>
### Table IV

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Amount of drug given</th>
<th>Titre</th>
<th>Amount of drug excreted in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>6.4</td>
<td>100mg</td>
<td>5.9ml</td>
<td>156.5mg</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium</td>
<td>5.7</td>
<td>100mg</td>
<td>2.8ml</td>
<td>71.5mg</td>
</tr>
<tr>
<td>Chloride</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.0</td>
<td>100mg</td>
<td>4.1ml</td>
<td>104.55mg</td>
</tr>
<tr>
<td>(No agent)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This table contains data on the excretion of sodium and ammonium ions in urine following the administration of drug. The results show that the amount of drug excreted in urine is higher when the urine is slightly alkaline.

### Table V

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Amount of drug given</th>
<th>Titre</th>
<th>Amount of drug excreted in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>8.7</td>
<td>210mg</td>
<td>6.8ml</td>
<td>183.76mg</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium</td>
<td>5.5</td>
<td>210mg</td>
<td>3.5ml</td>
<td>113.56</td>
</tr>
<tr>
<td>Chloride</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.1</td>
<td>210mg</td>
<td>5.4ml</td>
<td>144.34mg</td>
</tr>
<tr>
<td>(No agent)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This table contains data on the excretion of sodium and ammonium ions in urine following the administration of drug under alkaline conditions. The results show that the amount of drug excreted in urine is higher when the urine is alkaline.
DISCUSSION

Aspirin

In the body Aspirin (acetylsalicylic acid) is hydrolysed to salicylic acid. Hence, due to the presence of this metabolite in urine the estimation of aspirin in urine could not be done using a direct titration method. Consequently a spectrophotometric method was adopted to discriminate between the two. Aspirin and Salicylic acid do not absorb at the same wavelength, aspirin absorbs at a max 276nm, 0.1M sulphuric acid used as the solvent.

From the results obtained, shown on table II, it is apparent that more Aspirin is excreted in urine under alkaline condition compared to the amount excreted under acidic or normal conditions. This is in keeping with what would be expected - the least amount being excreted under acidic conditions.

This result can be explained by considering the Chemistry of the acetyl salicylic acid molecule and the mechanisms of excretion in the kidney. Aspirin being an acidic drug will readily ionize under alkaline conditions. In this state, the aspirin is less lipid soluble and hence not readily absorbed through biological membranes. Therefore, alkalizing the tubular fluid by ingesting sodium bicarbonate the aspirin will be ionized to some extent. Consequently, in this form, very little of the aspirin in the tubules will be reabsorbed back into the blood stream. As such more aspirin will appear in urine.
Conversely, under acidic conditions very little of the drug will be in the ionized form, resulting in more drug being reabsorbed back into the blood stream from the renal tubules. Hence there will be a decrease in the amount of drug appearing in urine.

By alkalinising the urine the amount of aspirin excreted in urine increases to about 3 - 4%, whereas the amount excreted in acidic conditions is about 1%.

Aspirin and other salicylates are rapidly distributed to all body tissues. This may explain why only a small amount is excreted in urine. The rate of excretion of aspirin varies with the pH of the urine, increasing as the pH rises and being greatest at pH 7.5 and above.

*Ionisation Equilibria of Aspirin*
Chlorothiazide was also spectrophotometrically analysed at λ max 288 (A25 cm 700) using 0.1N sodium hydroxide as the solvent.

The sulphamido group in chlorothiazide makes the molecule slightly acidic. Consequently, under very alkaline conditions it will ionize to some extent by losing a proton. This ionization takes place only to a limited extent. Hence very little of the drug will be in the ionized form. This explains why there was only a small variation in the amounts of the drug excreted in urine by varying urine pH.

By alkalising the urine there is only a small increase in the amount of the drug excreted in urine compared to the amount excreted in acidic conditions. It has been shown that after an oral dose, the average recovery of the drug in urine over 24 hours in men is about 15-20%. In this experiment the amount recovered in urine within a period of 24 hours was found to be higher than this, increased to about 30%.
Ionization equilibrium of chlorothiazide

The quantitative estimation of these drugs involves a diazotization reaction which involves the reaction of a primary aromatic amine with nitrous acid to form a diazonium salt.

In the body these drugs are readily acetylated, to form conjugated derivatives. Hence in analysing the urine for these drugs, the drugs have to be subjected to an hydrolytic reaction to unmask the primary amine which is made use of in the analysis. The hydrolysis was done by boiling with dilute sodium hydrosyde.

These drugs are readily excreted in urine with about 90 - 95% of the drug appearing in urine after 24 hours in man. This is exemplified by the results that were
obtained in this experiment. By alkalinizing the urine there was an appreciable increase in the amount of the drugs excreted in urine. This can be explained by the fact that under these conditions the drugs are highly ionized. As such there is reduced re-absorption of the drugs from the renal tubules resulting in more drug appearing in urine. In acidic conditions the reverse is true.

Ionization Equilibrium for Sulphathiazole and Sulphafurazole

SULPHAFURAZOLE

\[ \text{Base} \rightarrow \text{Acid} \]

SULPHATHIAZOLE

\[ \text{Base} \rightarrow \text{Acid} \]

It must be noted, that explanations advanced for the results obtained only apply for the drug that has been absorbed from the gastro-intestinal tract and has been filtered in the kidney into the renal tubule.
The pH of urine is normally maintained within fairly strict limits, usually pH 6.3 - 7.4. The acidification or alkalization of urine, which takes place in the distal tubules and collecting ducts, may have a profound effect upon the rate of drug excreted. Acidification of urine increases the re-absorption (and thus diminishes the excretion) of weak acids. Conversely, alkalization decreases the re-absorption (and thus increases excretion) of such weak acids. Hence, the results obtained agree with the observation, that the excretion of weak acids is promoted in alkaline urine and retarded in acid urine.

However, it must be pointed out that there are limitations to the results obtained. The use of charcoal which is a powerful adsorbent, as the colouring agent could be a source of appreciable loss of the drug. Individual rats do not necessarily respond in the same way to a particular drug. Hence, the use of a group of rats for the same experiment could result to biased results. Consequently, the results obtained should be assessed in the light of these factors.
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