RESULTS AND DISCUSSION
RESULTS

A severe state of pyridoxine deficiency was produced by the calculegenic diet, in experimental animals (Fig. 2). The animals showed retarded growth, falling of hair, asthenyria etc., which are morphological features characteristic of the deficiency. Table 3 gives the urinary oxalate excretion of rats maintained on normal and calculegenic diets. It may be seen from the table that the normal oxalate excretion is 5.69 ± 0.38 and that of pyridoxine deficient rats were 13.61± 2.78 mg/100 ml urine (p 0.001).

At the time of sacrifice the kidney sections appeared to contain milky patches, possibly due to embryonic calculi.

The degree of crystallisation in the renal tissue was followed by estimating the crystal constituents (Table 4). It may be observed that rats on the calculegenic diet show statistically significant elevations in calcium, inorganic phosphorus and oxalic acid levels.
Fig. 2. Control rat.

Fig. 2. Pyridoxine deficient rat.
Fig. 2A. Section of Kidney from Control Rat.
Haeatocytin - Resina x 120.

Section of Kidney from pyridoxine-deficient rat.
Haeatocytin - Resina x 120.
### Table 3

**Urinary Oxalate Excretion**

<table>
<thead>
<tr>
<th></th>
<th>Oxalate excreted in 100 ml urine</th>
<th>Oxalate excreted/100 mg creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td>5.69±0.58</td>
<td>3.76±0.76</td>
</tr>
<tr>
<td>Rats on calculogenic diet</td>
<td>13.01±2.75</td>
<td>18.73±2.36</td>
</tr>
<tr>
<td><strong>Statistical significance:</strong></td>
<td><strong>P &lt; 0.001</strong></td>
<td><strong>P &lt; 0.001</strong></td>
</tr>
</tbody>
</table>

In each set of analysis the urine of 4 animals were pooled, and 3 such sets were made for the controls, and 5 sets of experimental animals.
Table 4

The effect of calciferol diet on the calcium, magnesium, phosphorus and oxalic acid content in kidney tissue. Control group consisted of 12 rats and experimental group consisted 20 rats.

<table>
<thead>
<tr>
<th></th>
<th>Total calcium mg/100 gm of wet tissue</th>
<th>Magnesium mg/100 gm wet tissue</th>
<th>Inorganic phosphorus mg/100 gm wet tissue</th>
<th>Oxalic acid mg/100 gm wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td>81.61±3.33</td>
<td>1.71±0.80</td>
<td>98.32±5.7</td>
<td>257.59±40.32</td>
</tr>
<tr>
<td>Rats on calciferol</td>
<td>192.70±23.3</td>
<td>0.97±0.38</td>
<td>155.02±27.00</td>
<td>393.20±65.00</td>
</tr>
<tr>
<td>Statistical</td>
<td>p &lt; 0.02</td>
<td>p = not significant</td>
<td>p &lt; 0.02</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>significance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Increase in calcium, inorganic phosphorus and oxalic acid levels and decrease in magnesium have been analysed to assess statistical significance.
The elevated levels of calcium in test animals (192.70 ± 23.00 mgs/100 gms of tissue as compared to 81.61 ± 9.33 mgs/100 gms in controls, \( p < 0.02 \)) along with oxalate acid (295.20 ± 65 mgs/100 gms of tissue for test rats and 287.99 ± 40.32 mgs/100 gms of tissue for controls, \( p < 0.01 \)) revealed the increased deposition of these constituents in the kidney tissue.

Phosphorus level in the tissue was also elevated (155.03 ± 27 mgs in the case of tests and 98.38 ± 3.70 mgs in the case of controls, \( p < 0.02 \)). Suggesting that some amount of oxalite may also get precipitated with calcium oxalate. The level of magnesium in the renal tissue did not show any striking change in experimental animals. The values were 0.97 ± 0.38 mgs in calceinogenic diet fed rats and 1.71 ± 0.89 mgs in the case of controls respectively.

The results of the lipid constituents in the rat kidney tissues are given in table 5. The total lipid content showed elevations in the case of test animals 96.20 ± 1.20 mgs/gm as compared to the controls 93.60 ± 6.73 mgs/gm.
Table 5

The effect of calculeogenic diet on total lipids, total cholesterol, free cholesterol phospholipid and triglycerides in kidney tissue. Control group consisted of 12 rats and test consisted of 22 rats.

<table>
<thead>
<tr>
<th></th>
<th>Total lipid</th>
<th>Total cholesterol</th>
<th>Free cholesterol</th>
<th>Phospholipid</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/gm wet tissue</td>
<td>mg/gm wet tissue</td>
<td>mg/100 gm wet tissue</td>
<td>mg/100 gm wet tissue</td>
<td>mg/100 gm wet tissue</td>
</tr>
<tr>
<td>Control</td>
<td>92.08±6.75</td>
<td>7.56±2.23</td>
<td>3.97±0.65</td>
<td>68.08±20.62</td>
<td>1.87±6.35</td>
</tr>
<tr>
<td>Rats on calculeogenic diet</td>
<td>98.20±2.30</td>
<td>10.26±1.78</td>
<td>12.00±2.39</td>
<td>72.57±26.46</td>
<td>2.07±1.71</td>
</tr>
<tr>
<td>Statistical significance</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.001</td>
<td>p is not significant</td>
<td>p is not significant</td>
</tr>
</tbody>
</table>

Total lipid, total cholesterol, free cholesterol and phospholipid and triglycerides were analysed in kidney tissue of both groups to assess the statistical significance.
The total cholesterol level showed a significant elevation though it was moderate (19.36 ± 1.79 mg in the case of test and 7.36 ± 2.53 mg/gm of wet tissue in the case of controls). On the other hand free cholesterol showed a statistically striking elevations in test animals (13.00 ± 2.39 mg/gm wet tissue and 3.97 ± 0.66 mg/gm test and controls respectively with a p value less than 0.001.

Alterations with respect to the phospholipids and the triglyceride fractions were not significant as seen in table 5. Table 6 represents the serum mucoprotein levels in experimental rats. The stone forming rats showed a statistically significant increased level (161.97 ± 23.6 mg/100 ml) as compared to the control rats (216.71 ± 27.7; p < 0.01). The electrophoretic separation of mucoprotein (Fig. 28) shows interesting alterations in pyridoxine deficient serum.

The histopathological section shows alteration in tubules of kidney tissue of pyridoxine deficient test rats as compared to control rat section. The eosin-haematoxylin stained kidney section of pyridoxine deficient rat shows also slight accumulation of some materials in one part, when compared to controls.
<table>
<thead>
<tr>
<th></th>
<th>Serum mucoproteins (mg/100 ml serum)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>216.71 ± 27.7</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Rats on calculogenic diet</td>
<td>301.97 ± 25.6</td>
<td></td>
</tr>
</tbody>
</table>

In each set of analysis the serum of 4 animals were pooled, and 3 such sets were made for the controls, and 5 sets of experimental animals.
Fig. 21. Polyacrylamide gel electrophoretic pattern of serum Musoproteins from control rats.

Polyacrylamide gel electrophoretic pattern of Serum Musoproteins from Pyridoxine deficient rats.
DISCUSSION

The increased excretion of oxalic acid in P6 deficient state has long been recognised in man and experimental animals (Gershoff, 1964). Certainly Vit. P6 intake is an important parameter contributory to the urinary excretion of endogenously synthesised oxalic acid.

The endogenous precursors of oxalic acid in pyridoxine deficient condition are Glycine, serine, ascorbic acid, Glycollic acid, Glyoxylic acid and various other compounds which can form Glyoxylate, since it is the most immediate precursor (Weinhouse and Friedmann, 1951). The biochemical conversions of some of these compounds are shown in Fig. (3). Glycine is the major source and its conversion to Glyoxylate can occur either by oxidative degradation catalyzed by a flavoprotein, Glycine oxidase (E.C. (Rotner et al., 1946) or by transamination with α-conglutenate brought by specific transaminase (Gomara and Cohen, 1950).
FIG. 1 BIOCHEMICAL REACTIONS LEADING TO THE FORMATION OF OXALIC ACID FROM GLYCINE, SERINE AND ASCORBIC ACID

\[
\begin{align*}
\text{CH}_2\text{OH} \quad \text{CH}_2\text{OH} & \quad \text{CH}_2\text{OH} \\
\text{HC} - \text{NH}_2 & \quad \text{HC} - \text{NH}_2 & \quad \text{CHO} \\
\text{COOH} & \quad \text{ETANOL} & \quad \text{AMINE} \\
\text{SERINE} & & \Downarrow \\
\text{CH}_2\text{OH} & \quad \text{CHO} & \quad \text{COOH} \\
\text{CH}_2\text{OH} & \quad \text{GLYCINELALDEHYDE} & \\
\text{COOH} & & \\
\text{GLYCOLIC ACID} & \quad \text{CO}_2 \text{H}_2 \text{O} & \quad \text{HCOOH} + \text{CO} \\
\text{HCOOH} & \quad \text{FORMIC ACID} & \quad \text{COOH} & \quad \text{COOH} \\
\text{COOH} & \quad \text{GLYOXYLIC ACID} & \quad \text{GLYOXYLIC ACID} & \quad \text{OXALIC ACID} \\
\text{GLYCINE} & \quad \text{Flavoprotein} & \quad \text{Glycine oxidase} & \quad \text{B}_6 \text{P} \quad \text{Transaminase} \\
\text{CH}_2\text{NH}_2 & \quad \text{CHO} & \quad \text{COOH} & \quad \text{COOH} \\
\text{COOH} & \quad \text{GLYOXYLIC ACID} & \quad \text{OXALIC ACID} & \\
\text{L-ASCORBIC ACID} & \quad \text{DEHYDRO} & \quad \text{L-ASCORBIC ACID} & \\
\text{HO-CH} & \quad \text{HO-CH} & \quad \text{HO-CH} & \\
\text{CH}_2\text{OH} & \quad \text{2,3 DIKETO} & \quad \text{GULONIC ACID} & \quad \text{L-TERONIC ACID} \\
\text{HO-CH} & \quad \text{HO-CH} & \quad \text{CH}_2\text{OH} & \\
\text{CH}_2\text{OH} & \quad \text{H}_2 \text{O} & \quad \text{Cleavage} & \\
\text{HO-CH} & \quad \text{HO-CH} & \quad \text{HO-CH} & \\
\text{CH}_2\text{OH} & \quad \text{CH}_2\text{OH} & \quad \text{CH}_2\text{OH} &
This transaminase reaction like all other transaminases in the system is D6P (Pyrudomal phosphate) dependent.

Serine by way of interconversion to Glycine can form glyxynate. Glyxynate can also be formed by decarboxylation of serine, through glycoaldehyde and glycolate acid. Further oxidation of Glycolate acid to glyxynlic acid (Kun et al., 1986) and subsequently to oxalate acid (Richardson et al., 1961) are catalyzed by Glycolate acid oxidase (E.C. 1.1.1.1), a flavoprotein.

Once glyxynlic acid is formed under the usual course of events, the compound gets metabolized in a manner which does not result in significant oxalate formation. Hahnmanse and his associates (1939) have shown that glyxynlic acid is rapidly oxidized to formic acid and carbon dioxide. The further oxidation of formic acid also proceeds fast. In pyridoxine deficiency the usual reactions requiring D6P are impaired, thus resulting in enhanced oxalate production which very easily gets deposited in the renal tissue and forms one of the essential prerequisites for calcium oxalate stone formation.
Holman and Barnes (1958) have shown that only a smaller portion of ingested vitamin C is excreted as oxalic acid in man and a major portion is excreted unchanged. Species variations in the nature of the excretory products have been recognized.

Thus the induction of pyridoxine deficiency is undoubtedly an excellent tool to increase the urinary levels of oxalate as well as renal tissue levels of calcium and oxalate as revealed in the present study. The renal papilla is the site of early stone growth (Knight and Hodgkinson, 1972) and they have also observed elevation in calcium and oxalate concentration in this region. Administration of the vitamin is found to restore ammonia and prevent recurrences of stones (Cawthoofd and Andrew, 1963; Lyon et al, 1968). These workers also observed that the addition of magnesium oxide along with vitamin B6 is found to have a greater advantage in preventing stone formation. Magnesium certainly has a significant influence on many of the B6P requiring enzymes in intermediary metabolism. Secondly magnesium oxalate is more soluble than calcium oxalate and hence a high urinary magnesium by way of excreting with urinary oxalate can reduce the level
of calcium oxalate. Male rats are normally employed for such studies because evidence has been obtained from experimental studies (Cawthoff, 1970) that these are highly susceptible to oxalate lithiasis than castrated male or female rats. He has pointed out that the vitamin requirement of the males may be greater than the females and this may be the reason for their increased susceptibility to oxalate stone formation.

It was also observed by Richardson et al. (1961) and Syson and Cawthoff (1966) that the oxidation of glycollic to glyoxylic acid and then to oxalic acid proceeded very rapidly in males than in females.

Other compounds like ethylene glycol and ethanalamine etc. can also form oxalate (Lyea, Borden and Vermaelen, 1966). These too are converted to glycollic acid via glyoxaldehyde. Vitamin B6 deficiency results in an altered metabolism of these compounds.

The electron microscopic studies by Neume and Cawthoff (1972) wherein they observed accumulation of osmiophilic lipid bodies in the renal tissue of stone forming rats, prompted us to study their biochemical nature. Total lipid content was elevated in stone forming rats in the kidney tissue as observed by us.
As regards to the different lipid fractions, striking elevations with respect to total cholesterol and in particular free cholesterol were observed. An elevation in triglyceride and phospholipid levels were also seen but they were not significant.

Vitamin \( B_6 \) is known to be implicated in lipid metabolism (Vitten and Salzman, 1963; Rahill, 1968 etc.). Alterations in blood lipid patterns have been observed by several workers in \( B_6 \) deficiency (Gershoni and Sadik, 1960; Greenberg and Noon, 1961). It was also observed that a state of hypercholesterolaemia, lowered essential fatty acids etc. Similar observations were made by us in our laboratory in stone forming men (Gourishankar et al., 1977).

The alterations observed with respect to lipids may have a bearing in stone genesis, by way of relating them to certain physical forces. The lipids are present as a complex with proteins and carry a number of negative and positive charges. The lipoprotein complex exists as an emulsion in the aqueous phase. Since the fatty acids have a water soluble carboxylic end connected to a lipid soluble chain, the emulsion is stabilized by the proteins carrying immersible charged portions in the molecule.
and they produce a great surface area, where the cations and anions (necessary for stone formation) can be held wide apart from each other. In other words the physical force may be responsible for keeping the two types of ions separated and thereby prevent crystal aggregation. In this connection the acid mucopolysaccharides may also have a similar role to play because they also have a highly charged surface and they are polymenoids in nature. The nature of the acid mucopolysaccharides in the kidney tissue has also been worked out simultaneously (Vasalabram et al, 1970 unpublished data) and interesting alterations have been recorded.