The effect of ammonium chloride on calcium metabolism in sheep

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1. A combination of balance and isotope techniques has been used to study the effects of dietary supplements of NH₄Cl, given for 1 and 10 weeks, on calcium metabolism in 1- and 2-year-old wethers.

2. The main effects of the NH₄Cl supplements were to decrease urinary pH, increase urinary Ca excretion and to increase Ca absorption and the percentage of dietary Ca absorbed.

3. The results were different from those obtained by other workers in man and rat, and possible explanations of this difference are discussed.

4. A difference was also found between 1- and 2-year-old animals in their long-term response to the NH₄Cl supplement. This difference may have been due to a lack of available Ca in the diet of the younger animals, and prolonged ingestion of NH₄Cl may increase the amounts of Ca absorbed and retained.

Metabolic acidosis induced by the ingestion of ammonium chloride has been shown to increase urinary excretion of calcium in man (Farquaharson, Salter, Tibbetts & Aub, 1931; Sartorius, Roemmelt & Pitts, 1949; Lemann, Litzow & Lennon, 1966). Although the mechanism by which this increase is brought about has not been fully elucidated, it has generally been assumed that the Ca originates from bone. Payne (1967) and Vagg & Payne (1970) found that NH₄Cl increased the size of the exchangeable Ca pool of cows and goats, and they suggested that this also was a result of loss of Ca from bone induced by acidosis. Recently, Barzel (1969) and Barzel & Jowsey (1969) have obtained direct evidence that NH₄Cl can cause osteoporosis and increased bone resorption in the rat. On the other hand, Hart, Steenbock, Kline & Humphrey (1931) found that feeding acidic substances to cattle increased urinary Ca excretion but had no effect on the state of Ca balance, which suggests that the extra Ca came from increased absorption from the gut rather than from increased resorption from bone. Furthermore, Ender & Dishington (1970) found a reduced incidence of milk-fever at parturition in dairy cows given a diet of mineral acid silage, and their results indicated that this was also due to an increased absorption of Ca. Vagg & Payne (1970), however, found a marked increase in Ca absorption in some animals but not in others and concluded that the mean effect of NH₄Cl in the goat was not significant. The purpose of the present work was to determine the exact effect of NH₄Cl on Ca metabolism in sheep, with particular reference to the effects on Ca absorption and skeletal metabolism.
EXPERIMENTAL

Animals, housing and diet. Six 1-year-old and six 2-year-old Dorset Horn wethers (castrated males) were used. They were placed in metabolism cages designed for the separate collection of urine and faeces and were given a diet of hay and concentrates, the composition and Ca content of which are shown in Table 1. The animals were allowed 1 month to become accustomed to the experimental conditions, and NH₄Cl (0.2 g/d per kg body-weight) was then added to the concentrate ration of three from each age group, the remaining animals acting as controls. The concentrate mixture was completely eaten by all animals. Occasionally, however, some of the hay was left, and then the daily hay consumption was determined by collecting and weighing the residues. Animals had free access to distilled water.

Table 1. Composition and calcium content of the diet given daily

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g/kg body-wt)</th>
<th>Ca content (mg/g)</th>
<th>Total Ca (mg/kg body-wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay</td>
<td>16</td>
<td>4.15</td>
<td>66.90</td>
</tr>
<tr>
<td>Barley</td>
<td>4</td>
<td>0.77</td>
<td>3.08</td>
</tr>
<tr>
<td>Flaked maize</td>
<td>2</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Bran</td>
<td>1</td>
<td>0.72</td>
<td>0.72</td>
</tr>
<tr>
<td>Linseed oil cake</td>
<td>0.5</td>
<td>3.31</td>
<td>1.65</td>
</tr>
<tr>
<td>Mineral mixture*</td>
<td>0.17</td>
<td>187.39</td>
<td>31.85</td>
</tr>
<tr>
<td>Vitamin mixture†</td>
<td>0.028</td>
<td>3.78</td>
<td>0.11</td>
</tr>
</tbody>
</table>

* Super Mindif (Boots Pure Drug Co., Nottingham).
† Drivite (Boots Pure Drug Co., Nottingham), to supply 125 i.u. vitamin A and 31 i.u. cholecalciferol daily per kg body-weight.

Experimental procedure. Two experiments lasting for a week were carried out on each of the twelve animals, starting 1 week and 10 weeks after the addition of the NH₄Cl to the diet. A known amount (5 μCi/kg body-weight) of an aqueous solution of ⁴⁶CaCl₂ (Radiochemical Centre, Amersham, Bucks) was injected into the jugular vein and samples of blood, urine and faeces were collected as previously described (Braithwaite, Glascock & Riazuddin, 1969). In addition, pH measurements were made each morning on samples of freshly passed urine. During these weekly periods, Ca balance measurements were made.

Determination of Ca and measurement of radioactivity. Samples of blood, faeces and urine were prepared and analysed by the methods previously described (Braithwaite et al. 1969).

Calculation of values for the various processes of Ca metabolism. The method of Aubert & Milhaud (1960) modified for use with the sheep (Braithwaite et al. 1969; Braithwaite & Raizuddin, 1971) was used.

RESULTS

The effects of the NH₄Cl supplement on the various measures of Ca metabolism in 1-year-old wether sheep are shown in Table 2.

After only 1–2 weeks on the NH₄Cl-supplemented diet, several differences in Ca
metabolism between the experimental and control animals were apparent. The urinary Ca excretion of the NH₄Cl-treated animals increased to between two and three times the value for the controls, and there was also a slight but significant increase in their faecal endogenous Ca loss. These increases in excretion were reflected in a significant increase in the rate of total loss (V₇) of Ca from the rapidly exchangeable Ca pool [P]. The amount of Ca absorbed from the intestine was also significantly greater

Table 2. Effect of dietary NH₄Cl on calcium metabolism in 1-year-old wether sheep

(Mean values for three animals/group with the standard errors of the differences and results of tests of significance, as determined by the t test)

<table>
<thead>
<tr>
<th></th>
<th>After 1 week on the NH₄Cl diet</th>
<th>After 10 weeks on the NH₄Cl diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control + NH₄Cl</td>
<td>Control + NH₄Cl</td>
</tr>
<tr>
<td>Rapidly exchangeable pool of Ca (P)</td>
<td>52.5</td>
<td>44.2</td>
</tr>
<tr>
<td>(mg/kg body-wt)</td>
<td>+ 5.8</td>
<td>+ 4.6</td>
</tr>
<tr>
<td>Slowly exchangeable pool of Ca in bone (E) (mg/kg body-wt)</td>
<td>87.2</td>
<td>72.1</td>
</tr>
<tr>
<td></td>
<td>+ 10.5</td>
<td>+ 7.6</td>
</tr>
<tr>
<td>Rate of ingestion of Ca (V₅) (mg/d kg body-wt)</td>
<td>101.6</td>
<td>97.2</td>
</tr>
<tr>
<td></td>
<td>+ 104.8</td>
<td>+ 101.0</td>
</tr>
<tr>
<td>Rate of loss of Ca in faeces (F) (mg/d kg body-wt)</td>
<td>88.1</td>
<td>73.8</td>
</tr>
<tr>
<td></td>
<td>+ 74.7</td>
<td>+ 67.8</td>
</tr>
<tr>
<td>Rate of irreversible loss of Ca from P (V₅') (mg/d kg body-wt)</td>
<td>79.1</td>
<td>60.9</td>
</tr>
<tr>
<td></td>
<td>+ 103.4</td>
<td>+ 73.2</td>
</tr>
<tr>
<td>Rate of excretion of Ca in urine (V₆) (mg/d kg body-wt)</td>
<td>11.7</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>+ 2.8</td>
<td>+ 2.1</td>
</tr>
<tr>
<td>Rate of excretion of Ca into intestine (faecal endogenous Ca) (V₆) (mg/d kg body-wt)</td>
<td>14.0</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>+ 15.4</td>
<td>+ 12.6</td>
</tr>
<tr>
<td>Rate of accretion of Ca into bone (V₆+) (mg/d kg body-wt)</td>
<td>53.4</td>
<td>40.9</td>
</tr>
<tr>
<td></td>
<td>+ 59.5</td>
<td>+ 38.9</td>
</tr>
<tr>
<td>Rate of resorption of Ca from bone (V₆- ) (mg/d kg body-wt)</td>
<td>51.6</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>+ 57.9</td>
<td>+ 27.5</td>
</tr>
<tr>
<td>Rate of absorption of Ca from intestine (V₆') (mg/d kg body-wt)</td>
<td>27.5</td>
<td>35.9</td>
</tr>
<tr>
<td></td>
<td>+ 45.5</td>
<td>+ 45.8</td>
</tr>
<tr>
<td>Ca absorption as % of Ca ingested</td>
<td>27.1</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>+ 43.5</td>
<td>+ 17.6</td>
</tr>
<tr>
<td>Ca balance (mg/d kg body-wt)</td>
<td>+ 13.7</td>
<td>+ 11.4</td>
</tr>
<tr>
<td></td>
<td>+ 16.6</td>
<td>+ 2.3</td>
</tr>
<tr>
<td>pH of urine</td>
<td>9.0</td>
<td>8.2</td>
</tr>
<tr>
<td>Body-wt (kg)</td>
<td>51.1</td>
<td>55.3</td>
</tr>
<tr>
<td></td>
<td>+ 52.6</td>
<td>+ 56.5</td>
</tr>
</tbody>
</table>

NS, not significant; * 0.05 > P > 0.01; ** 0.01 > P > 0.001; *** 0.001 > P.
† Sum of faecal endogenous Ca and unabsorbed Ca lost/d.
than in the controls and the increase was approximately equal to the total increase in excretion. This increase in absorption was accompanied by an increase in the percentage of dietary Ca absorbed and a decrease in the total Ca lost in the faeces. Urinary pH was also significantly lower in the treated animals. None of the other values measured, including the state of Ca balance and the rates of Ca accretion into and loss from bone, were significantly affected by administration of NH4Cl.

The differences in Ca metabolism between experimental and control animals after

### Table 3. Effect of dietary NH4Cl on calcium metabolism in 2-year-old wether sheep

(Mean values for three animals/group with the standard errors of the differences and results of tests of significance, as determined by the t test)

<table>
<thead>
<tr>
<th></th>
<th>After 1 week on the NH4Cl diet</th>
<th>After 10 weeks on the NH4Cl diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control +NH4Cl</td>
<td>Control +NH4Cl</td>
</tr>
<tr>
<td></td>
<td>SE of difference</td>
<td>SE of difference</td>
</tr>
<tr>
<td></td>
<td>between means</td>
<td>between means</td>
</tr>
<tr>
<td>Rapidly exchangeable pool of Ca (P) (mg/kg body-wt)</td>
<td>39.7 ± 41.4</td>
<td>2.7 NS</td>
</tr>
<tr>
<td>Slowly exchangeable pool of Ca in bone (E) (mg/kg body-wt)</td>
<td>57.9 ± 62.7</td>
<td>8.2 NS</td>
</tr>
<tr>
<td>Rate of ingestion of Ca (V1) (mg/d kg body-wt)</td>
<td>100.0 ± 98.6</td>
<td>2.0 NS</td>
</tr>
<tr>
<td>Rate of loss of Ca in faeces (F) (mg/d kg body-wt)</td>
<td>99.2 ± 85.2</td>
<td>2.0 **</td>
</tr>
<tr>
<td>Rate of irreversible loss of Ca from P (Vp') (mg/d kg body-wt)</td>
<td>51.3 ± 66.0</td>
<td>3.9 *</td>
</tr>
<tr>
<td>Rate of excretion of Ca in urine (V2) (mg/d kg body-wt)</td>
<td>6.8 ± 23.9</td>
<td>2.2 **</td>
</tr>
<tr>
<td>Rate of excretion of Ca into intestine (faecal endogenous Ca) (V3) (mg/d kg body-wt)</td>
<td>15.1 ± 13.9</td>
<td>1.7 NS</td>
</tr>
<tr>
<td>Rate of accretion of Ca into bone (V6+) (mg/d kg body-wt)</td>
<td>29.3 ± 28.2</td>
<td>2.6 NS</td>
</tr>
<tr>
<td>Rate of resorption of Ca from bone (V6-) (mg/d kg body-wt)</td>
<td>35.4 ± 38.7</td>
<td>3.0 NS</td>
</tr>
<tr>
<td>Rate of absorption of Ca from intestine (V5) (mg/d kg body-wt)</td>
<td>15.8 ± 27.3</td>
<td>2.2 **</td>
</tr>
<tr>
<td>Ca absorption as % of Ca ingested</td>
<td>15.8 ± 27.7</td>
<td>1.9 **</td>
</tr>
<tr>
<td>Ca balance (mg/d kg body-wt)</td>
<td>-6.0 ± -16.5</td>
<td>4.1 NS</td>
</tr>
<tr>
<td>pH of urine</td>
<td>8.3 ± 6.6</td>
<td>0.2 **</td>
</tr>
<tr>
<td>Body-wt (kg)</td>
<td>64.3 ± 62.8</td>
<td>1.5 NS</td>
</tr>
</tbody>
</table>

NS, not significant; * 0.05 > P > 0.01; ** 0.01 > P > 0.001; *** 0.001 > P.
† Sum of faecal endogenous Ca and unabsorbed Ca lost/d.
10 weeks on the diet were similar to those found after 1 week. Again the rates of urinary Ca excretion and Ca absorption, and the percentage of dietary Ca absorbed, were significantly higher in the experimental animals. The rates of irreversible loss \( V_f \) of Ca from pool P and of the loss of Ca in the faeces were also slightly higher, but not significantly so. Faecal endogenous loss of Ca, however, was now virtually the same in both groups of animals.

Table 4. Changes in the values of the various processes of Ca metabolism that occurred between 1 week and 10 weeks from the start of supplementing the diet with NH\(_4\)Cl

(Mean values for three animals/group with the standard errors of the differences and results of tests of significance, as determined by the \( t \) test)

<table>
<thead>
<tr>
<th>Process</th>
<th>1-year-old animals</th>
<th>2-year-old animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control +NH(_4)Cl</td>
<td>Control +NH(_4)Cl</td>
<td></td>
</tr>
<tr>
<td>1-year-old animals</td>
<td>2-year-old animals</td>
<td></td>
</tr>
<tr>
<td>SE of difference between means</td>
<td>SE of difference between means</td>
<td></td>
</tr>
<tr>
<td>Significance of difference between means</td>
<td>Significance of difference between means</td>
<td></td>
</tr>
<tr>
<td>Rapidly exchangeable pool of Ca(P) (mg/kg body-wt)</td>
<td>-8.3</td>
<td>-12.2</td>
</tr>
<tr>
<td>Slowly exchangeable pool of Ca in bone (E) (mg/kg body-wt)</td>
<td>-15.2</td>
<td>-28.9</td>
</tr>
<tr>
<td>Rate of ingestion of Ca ( (V_i) ) (mg/d kg body-wt)</td>
<td>-4.4</td>
<td>-3.8</td>
</tr>
<tr>
<td>Rate of loss of Ca in faeces ( (F_f) ) (mg/d kg body-wt)</td>
<td>-14.3</td>
<td>-6.9</td>
</tr>
<tr>
<td>Rate of irreversible loss of Ca from P ( (V_f) ) (mg/d kg body-wt)</td>
<td>-18.2</td>
<td>-30.2</td>
</tr>
<tr>
<td>Rate of excretion of Ca in urine ( (V_u) ) (mg/d kg body-wt)</td>
<td>-4.2</td>
<td>-6.8</td>
</tr>
<tr>
<td>Rate of excretion of Ca into intestine (faecal endogenous Ca) ( (V_e) ) (mg/d kg body-wt)</td>
<td>-1.5</td>
<td>-2.8</td>
</tr>
<tr>
<td>Rate of accretion of Ca into bone ( (V_+o) ) (mg/d kg body-wt)</td>
<td>-12.5</td>
<td>-20.6</td>
</tr>
<tr>
<td>Rate of resorption of Ca from bone ( (V_-o) ) (mg/d kg body-wt)</td>
<td>-26.6</td>
<td>-30.4</td>
</tr>
<tr>
<td>Rate of absorption of Ca from intestine ( (V_a) ) (mg/d kg body-wt)</td>
<td>+8.4</td>
<td>+0.3</td>
</tr>
<tr>
<td>Ca absorption as % of Ca ingested</td>
<td>+9.8</td>
<td>+1.8</td>
</tr>
<tr>
<td>Ca balance (mg/d kg body-wt)</td>
<td>+14.1</td>
<td>+9.8</td>
</tr>
<tr>
<td>pH of urine</td>
<td>+0.2</td>
<td>-0.8</td>
</tr>
<tr>
<td>Body-wt (kg)</td>
<td>+4.3</td>
<td>+4.0</td>
</tr>
</tbody>
</table>

NS, not significant; * 0.05 > P > 0.01; ** 0.01 > P > 0.001.

† Sum of faecal endogenous Ca and unabsorbed Ca lost/d.
The amount of dietary Ca absorbed from the intestine increased considerably in the control animals during the experimental period, but altered very little in the NH$_4$Cl-treated group. Since Ca excretion decreased slightly in the same period, the animals were all in fairly large positive Ca balance compared with the previous small positive balances.

Table 3 shows the effect of administering NH$_4$Cl on the Ca metabolism of the 2-year-old wethers. Many of the values measured were much lower than those for the younger animals, and for this reason it was not possible to combine the two sets of results. Thus, the sizes of pools P and E, and the rates of Ca accretion into bone, Ca resorption from bone and Ca absorption were all much higher in the 1-year-old (Table 2) than in the 2-year-old (Table 3) animals. Braithwaite & Riazuddin (1971) have previously reported similar decreases in metabolic activity with age in the sheep.

The differences in Ca metabolism between the experimental and control animals were nearly identical to those found in the younger animals. After only 1 week on the diet containing NH$_4$Cl, urinary Ca excretion was three times the value for the controls and Ca absorption was also significantly higher. All the animals, however, were in a state of negative Ca balance.

Ten weeks after the start of the experiment, the effects on Ca metabolism of adding NH$_4$Cl to the diet were again similar to those observed earlier. The amount of Ca absorbed increased between the two experiments in both groups of animals, but the increase was greatest in the treated group, in which it resulted in fairly high Ca retentions.

The effect of prolonged treatment with NH$_4$Cl on Ca metabolism was determined by subtracting the value for each of the various processes for each animal, measured after 1 week on the diet, from the corresponding value at 10 weeks, and then comparing the mean differences for the experimental animals with those for the controls (Table 4).

Only the amount of Ca absorbed, the percentage of dietary Ca absorbed and the retention of Ca were significantly altered. The results for animals of the two age groups were, however, quite different. In the younger animals, absorption increased considerably in the controls but remained fairly constant in the experimental group. The apparent effect of prolonged treatment with NH$_4$Cl in the diet therefore was a significant decrease, relative to the control group, in the amount and percentage of dietary Ca absorbed and a slight but non-significant decrease in Ca retention. In the older animals, the reverse occurred. Absorption increased more in the experimental animals than in the controls, and the effect of duration of treatment with NH$_4$Cl was to significantly increase absorption, percentage absorption and Ca retention in this group relative to the controls.

**DISCUSSION**

The results show that when sheep were given NH$_4$Cl there was an increase in urinary Ca excretion and a similar increase in intestinal absorption of Ca, whereas bone resorption was unchanged. These changes in metabolism were independent of age (i.e. in animals 1 or 2 years old) and length of time on the NH$_4$Cl-supplemented diet (1 or 10 weeks). Barzel (1969) and Barzel & Jowsey (1969), however, found that
the increased urinary excretion of Ca in rats given diets supplemented with NH₄Cl was derived not from increased Ca absorption, but rather from increased bone resorption.

In the non-ruminant, ingestion of NH₄Cl is equivalent to the ingestion of hydrochloric acid (Robinson, 1967) because the ammonium ion is converted into urea in the liver, leaving hydrogen and chloride ions. However, the mechanism by which the ensuing metabolic acidosis increases urinary Ca excretion is not understood. One possibility is that the acidosis stimulates the slow dissolution of alkaline bone salts in order to increase the buffering capacity of the extracellular fluid and that the Ca resorbed from bone is excreted in the urine (Lemann, Lennon, Goodman, Litzow & Relman, 1965; Lemann et al., 1966; Barzel, 1969; Barzel & Jowsey, 1969). Another more likely explanation, however, is that urinary Ca excretion is under the control of some renal mechanism which is affected by pH. In the acidotic dog, it is the filtered load of Ca that appears to determine its rate of renal excretion (Williamson & Freeman, 1957), but in man, and probably also in the rat and sheep, metabolic acidosis acts directly on the renal tubular cells causing a decreased renal tubular reabsorption of Ca (Lemann, Litzow & Lennon, 1967; Reidenberg & Sevy, 1967; Stacy, 1969; Stacy & Wilson, 1970). This would then result in hypocalcaemia.

Braithwaite, Glascock & Riazuddin (1970) and Braithwaite & Riazuddin (1971) have shown that changes in the rate of resorption of Ca from bone is the main process of Ca homoeostasis in the sheep and that increased Ca absorption takes place in older animals only after a fairly prolonged period of negative Ca balance. It is difficult to explain therefore why, in the sheep, the increased urinary Ca excretion associated with NH₄Cl treatment should result in increased intestinal absorption rather than increased bone resorption, unless Ca absorption is also directly influenced by the treatment. Certainly, NH₄Cl might be assimilated by rumen bacteria leaving HCl, and increased Ca absorption has been reported with increased intestinal pH (Thomas & Howard, 1964; Ali & Evans, 1967; Ender & Dishington, 1970). However, the contents of the upper intestine are well buffered, so it would seem unlikely that there would be any direct measurable effect on intestinal pH.

Recently, Payne, Dew, Manston & Vagg (1970) and Vagg & Payne (1970) have shown that giving NH₄Cl-supplemented diets to goats and cows, increased the size of the exchangeable Ca pool. The mean values for both the rapidly exchangeable Ca pool [P] and the slowly exchangeable pool of Ca in bone [E] were also higher in the treated animals than in the controls in the present work, but owing to animal variation, these differences were not significant. Payne et al. (1970) also suggested that the increased pool size was due to alterations in bone metabolism. The present results, however, suggest that the increased pool size might result from an increase in the rate of pool turnover caused not by a change in the rate of bone metabolism but rather by increased absorption and excretion.

These results do not oppose the theory that the reduced incidence of milk-fever in cows following the addition of mineral acid to the diet (Ender & Dishington, 1970) may be due to a higher rate of Ca absorption resulting in more Ca being available for secretion into the milk. However, unless in the parturient cow, mineral acid treatment
results in a greater increase in Ca absorption than in urinary Ca excretion or in Ca being diverted from urinary excretion to milk secretion, it is difficult to explain this beneficial effect of mineral acid.

The different effects of the duration of treatment with NH₄Cl on the absorption and retention of Ca by the two age groups are difficult to explain. The increase in Ca retention that occurred between 1 and 10 weeks from the start of the experiment suggests, however, that the animals (both experimental and control) moved into a more active state of growth during this period, and there were in fact small increases in body-weight.

It is possible to account for the differences in Ca absorption on the basis of recently published work. Braithwaite & Riazuddin (1971) have shown that young growing sheep absorb 42% of the Ca from a diet of hay and concentrates, and results indicated that this was the total amount of Ca present in the diet that was available for absorption. Furthermore, there is some evidence that absorption is regulated by an endogenous factor which is secreted during periods of bone growth or after periods of negative Ca balance (Wasserman & Taylor, 1969). A diet of composition similar to that used by Braithwaite & Riazuddin (1971) was given in the present work and it seems probable that the 1-year-old animals receiving the acid diet were already absorbing all the Ca from the diet (45%) that was available for absorption after only 1 week of treatment. In these animals therefore any extra demands for Ca brought about by the more active state of growth could not be met by increased absorption and, in fact, absorption in these animals remained fairly constant during the experimental period. Absorption in the control animals, however, which was only 27% of the total dietary Ca at 1 week, increased markedly during the experimental period, presumably in response to increased growth, but was still lower at 10 weeks than that of the animals given NH₄Cl. Thus the apparent decrease in absorption and retention of Ca with length of time on the NH₄Cl-supplemented diet by these younger animals, relative to the controls, was probably really a result of a combination of increased growth and a lack of available Ca in the diet, rather than a true effect of the diet.

Braithwaite & Riazuddin (1971) have further shown that, unlike young growing animals, older mature animals given adequate dietary Ca absorbed only enough to meet their maintenance requirements, and absorption of Ca was independent of dietary intake. In the present work, dietary Ca was present in amounts well in excess of the maintenance requirements. The greater increases with time on the NH₄Cl-supplemented diet in the amount of Ca absorbed, the percentage absorption and the amount of Ca retained by the older animals given the acid diet were therefore most probably a true effect of the diet.

I thank Dr R. F. Glascock for his advice and encouragement while this work was being performed. I also thank Miss D. Pearson and Mr B. Woods for technical assistance and Mr R. Ellis and Mr A. Wilim for their care of the experimental animals.
REFERENCES


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