ABSTRACT
Changes in function of the parathyroid gland were studied periodically for 36 days in two groups of calves fed high and low calcium diets. After changes to the experimental diets, parathyroid function was altered in some calves at 9 days of high or low calcium feeding. Parathyroid function was reduced in high calcium feeding and increased in low calcium feeding. These changes increased progressively in intensity with the lapse of time during experimental feeding periods.

INTRODUCTION
Several workers observed a functional acceleration of the calcium-regulating system in animals fed a low calcium diet. Boda and Cole (1) prevented parturient paresis by low calcium feeding before parturition and observed frequent parturient paresis among animals fed a high calcium diet in the prepartum period. Other workers (3, 10, 12) also indicated that low calcium feeding prior to parturition was effective in preventing parturient paresis in cows. Generally these responses have been attributed to alteration in parathyroid function. However, no reports have shown directly that parathyroid function changes in response to experimental feeding of high and low calcium diets.

It was suggested that a functional test of the calf parathyroid gland may be comprised of parathyroid hormone secretion as a response after an intravenous infusion of ethylenediamine tetraacetic acid (EDTA). Function of the parathyroid gland may be higher in animals fed a diet low in calcium than in those fed a diet rich in this element (8).

The present experiment was to evaluate the relationship between duration of high or low control calcium feeding and alteration in function of the parathyroid gland in castrated bull calves.

MATERIALS AND METHODS
Six castrated Holstein bullocks, weighing 182 to 284 kg at the start of experiment, were housed in a barn and fed hay and concentrate to meet dietary requirements. Mean intake of calcium and phosphorus were 48.0 and 17.9 g, and the ratio of Ca/P was 2.7 before experimental feeding. All calves were subjected to a functional test of the parathyroid gland in response to infusion of EDTA. Calves then were divided equally into two feeding groups. High calcium fed calves (I, II, and III) were fed hay, calcium-added concentrate, and additive calcium salt in addition to sufficient dietary nutrients normally required. Amounts of calcium and phosphorus received per day and ratio of Ca/P were: 117.5 g, 20.2 g, and 5.82 in calf I; 113.9 g, 25.1 g, and 4.54 in calf II, 115.7 g, 24.0 g, and 4.82 in calf III. The other group of calves (IV, V, and VI) received the following amounts of calcium and phosphorus per day and ratio of Ca/P: 27.8 g, 25.1 g, and 4.54 in calf II, 115.7 g, 24.0 g, and 4.82 in calf III. The other group of calves (IV, V, and VI) of the low calcium content received the following amounts of calcium and phosphorus per day and ratio of Ca/P: 27.8 g, 25.1 g, and 1.08 in calf IV; 32.4 g, 32.2 g, and 1.01 in calf V; 27.2 g, 25.0 g, and 1.09 in calf VI. All animals were infused with .25 M neutralized EDTA at .15 ml/kg of body weight per minute during 2 min into the jugular vein on days 9, 22, and 36 after the start of experimental feeding of high or low calcium diets. Calves were examined individually for functional alteration in parathyroid gland.
Heparinized jugular blood samples were collected twice within 20 min before EDTA infusion for a baseline measure and at 5, 10, 30, and 60 min after end of infusion. Blood samples were collected from the jugular vein opposite the side of EDTA infusion. Immunoreactive parathyroid hormone was measured by radioimmunoassay method (7). Plasma calcium was measured by a titration method (11). Magnesium, sodium, and potassium of plasma were determined by atomic absorption spectrophotometry. Inorganic phosphorus in plasma was determined by a colorimetric method (4). Total calcium and phosphorus in the diet were measured by atomic absorption spectrophotometry and a colorimetric method, respectively, after complete digestion with nitric acid and perchloric acid. All samples were analyzed at least in duplicate.

Changes in immunoreactive parathyroid hormone of plasma were analyzed by least squares analysis of variance to evaluate effects of diet, calves, days of experimental feeding, minutes past infusion and high order interactions. Mean changes in plasma calcium, inorganic phosphorus, magnesium, sodium, and potassium were compared by the multiple conventional t test between, before, and after 36 days of experimental feeding.

**RESULTS**

Figure 1 shows changes in plasma concentration of immunoreactive parathyroid hormone in three calves (I, II, and III) of high calcium diet after infusion of EDTA with its mean in each experiment, before and 9, 22, and 36 days after experimental feeding. In calves I and III, a decrease in response of immunoreactive parathyroid hormone secretion was observed at 9 days of high calcium feeding. The decrements became more pronounced at 22 and 36 days of high calcium feeding. Calf II showed a slightly high immunoreactive parathyroid hormone secretion at 5 min after infusion of EDTA at 9 days of high calcium feeding. The response of the parathyroid gland 36 days after the end of experimental feeding was more distinct than before experimental feeding.

![Figure 1](image-url)

**Figure 1.** Immunoreactive parathyroid hormone in plasma of bullocks I, II, and III with its mean following EDTA infusion before and 9, 22, and 36 days after starting of high calcium feeding.
Figure 2 shows changes in plasma concentration of immunoreactive parathyroid hormone in three calves (IV, V, and VI) of control low calcium diet in each experiment with its mean. In calf V, an increase in response of the parathyroid gland was observed at 9 days of experimental feeding. It was particularly distinct at 22 and 36 days of experimental feeding. In calf VI, the response was almost the same at 9 days of low calcium feeding and before experimental feeding. The mean response was more marked at 22 days of experimental feeding than before experimental feeding, and was even more responsive at 36 days of experimental period. However, in calf IV, the response of the parathyroid gland remained mostly unchanged for 36 days of the experimental period.

Results of least squares analysis of immunoreactive parathyroid hormone in plasma of bullocks fed high and low calcium diets are in Table 1. Calves differed statistically in their concentrations of immunoreactive parathyroid hormone within the same feeding group \([C(T), P<.01]\). Differences among calves changed from day to day \([C(T) \times (D)]\) within same treatment. For example, calf I was intermediate in response on days 0 and 36 but lowest on days 9 and 22. Calf III was intermediate in response on days 9 and 22 but lowest on days 0 and 36.

Concentrations of immunoreactive parathyroid hormone differed \((P<.01)\) between high and low calcium feeding from day 0 to day 36 \([T \times (D)]\). Immunoreactive parathyroid hormone concentration increased from day 0 to 36 in the low calcium treatment group whereas it decreased from day 0 to day 36 in the high calcium group.

Figure 3 and 4 depict changes in plasma calcium and inorganic phosphorus, magnesium, and potassium before and after EDTA infusion, 36 days of high (Figure 3) or low calcium (Figure 4) feeding. Calcium in plasma was higher on only 36 days of high calcium feeding than before experimental feeding. There were no significant differences in plasma inorganic phosphorus, magnesium, or sodium. However, potassium was higher at 36 days of high calcium
TABLE 1. Least squares analysis of parathyroid hormone in plasma of bullocks fed high and low calcium diets.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>Test denominator</th>
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<tbody>
<tr>
<td>Treatment (T)</td>
<td>1</td>
<td>.71</td>
<td>C(T)</td>
</tr>
<tr>
<td>Calves [C(T)]**</td>
<td>4</td>
<td>53.74</td>
<td>E</td>
</tr>
<tr>
<td>Day (D)</td>
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<td>C(T) X D</td>
</tr>
<tr>
<td>T X D**</td>
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<td>85.11</td>
<td>C(T) X D</td>
</tr>
<tr>
<td>C(T) X D*</td>
<td>12</td>
<td>10.82</td>
<td>E</td>
</tr>
<tr>
<td>Minutes (M)**</td>
<td>5</td>
<td>113.25</td>
<td>C(T) X M</td>
</tr>
<tr>
<td>T X M</td>
<td>5</td>
<td>5.72</td>
<td>C(T) X M</td>
</tr>
<tr>
<td>C(T) X M***</td>
<td>20</td>
<td>8.15</td>
<td>E</td>
</tr>
<tr>
<td>D X M</td>
<td>15</td>
<td>5.71</td>
<td>E</td>
</tr>
<tr>
<td>T X D X M</td>
<td>15</td>
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<td>E</td>
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<tr>
<td>Residual</td>
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</table>

* P<.05.
** P<.01.
*** P<.10.

Figure 3. Changes in mean calcium, inorganic phosphorus, magnesium, and potassium of plasma following EDTA infusion in three bullocks before ○——○ and 36 days after (••••••) starting of high calcium feeding (mean ± SD, *P<.05, **P<.02, ***P<.01).

Feeding than before experimental feeding. In the control calves (Figure 4), there was no significant difference in plasma calcium between before experimental feeding and 36 days of experimental feeding. The inorganic phosphorus before EDTA infusion was higher on 36 days of experimental feeding than before experimental feeding. There was no significant difference in either magnesium or sodium concentration in calves of the low calcium diet. When examined 30 and 60 min after EDTA infusion, the potassium of plasma was higher at 36 days of experimental feeding than before experimental feeding.

**DISCUSSION**

A decrease in dietary calcium intake has little influence on serum calcium in animals. This generally has been attributed to bone resorption by parathyroid hormone. Rate of bone resorption changes inversely in relation to dietary calcium intake (2, 5, 6, 9). It is assumed that an alteration in parathyroid function may cause changes in bone resorption.

In this experiment, high calcium diet reduced and low calcium diet increased secretion of parathyroid hormone within weeks. This homeostatic adaptation may be affected by function of the parathyroid gland but also by other calcium-regulating systems such as vitamin D activation and intestinal calcium-
Changes in mean calcium, inorganic phosphorus, magnesium, and potassium in plasma following EDTA infusion in three bullocks before (○-○) and 36 days after (●-●) starting of control low calcium feeding (mean ± SD, *P<.05, **P<.02, ***P<.01).

Figure 4. Changes in mean calcium, inorganic phosphorus, magnesium, and potassium in plasma following EDTA infusion in three bullocks before (○-○) and 36 days after (●-●) starting of control low calcium feeding (mean ± SD, *P<.05, **P<.02, ***P<.01).

binding protein. Sammon and coworkers (9) indicated that adaptation could occur in absence of parathyroid gland function. In our experiment, one calf (IV) seemed to have been adapted preferentially to some other calcium-regulating system, as no clear changes were detected in response to EDTA infusions. Calcium concentration in plasma in calves on the high calcium diet was higher than before this diet was fed. Calcium concentration of control calves did not differ before or after the start of experimental feeding for 36 days. This latter result indicates that perhaps the parathyroid gland may have adapted to the feeding condition within a month.

Generally it is believed that 1 to 1.5 h are needed for parathyroid hormone to increase the calcium concentration in plasma. Changes in calcium recovery pattern were not different in this experiment, since the sampling time was only for 1 h. If calves had been given continuous hypocalcemic stimuli in the same manner in this experiment as in parturient paresis, their recovery of calcium in plasma may have been better in control calves than in calves fed a high calcium diet. Results of this experiment support the endocrinological views of workers (1, 3, 10, 12) who reported that low calcium feeding prior to parturition caused alteration of parathyroid gland function effectively that would prevent parturient paresis.

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REFERENCES

Calcium and magnesium determination in bovine blood by EDTA titration. J. Dairy Sci. 43:1014.