STUDIES OF $^{47}$CA METABOLISM IN SARCOIDOSIS: EVIDENCE FOR INCREASED SENSITIVITY OF BONE TO VITAMIN D

By

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ABSTRACT

Calcium metabolism was studied in two patients with sarcoidosis and hypercalcaemia before and again after treatment with prednisone. Calcium balance, miscible calcium pool (P), calcium »accretion« rate (vo+) and »bone resorption« rate (vo−) were measured.

In both patients before treatment, faecal calcium was low and serum and urinary calcium were elevated, P was normal and vo+ and vo− were elevated.

In both patients after treatment with prednisone, faecal calcium had increased and serum and urinary calcium, P, vo+ and vo− had decreased. The results are interpreted to mean that in sarcoidosis, the bones as well as the gastrointestinal tract are sensitive to vitamin D.

The defect in calcium metabolism in patients with sarcoidosis has been characterized as excessive absorption of calcium (Anderson et al. 1954; Henneman et al. 1956; Mather 1957; Bell et al. 1961; McSwiney & Mills 1956) which may be further enhanced by doses of vitamin D (Anderson et al. 1954; Bell et al. 1961, 1964; Mather 1957) that have little, if any, effect in normal subjects (Bell et al. 1964). Both the spontaneous hyperabsorption of calcium (Anderson

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et al. 1954; Henneman et al. 1956; Bell et al. 1961, 1964; Mather 1957; Mc
Swiney & Mills 1956; Scholz 1959) and that inducible with vitamin D (Anderson et al. 1954; Bell et al. 1961, 1964; Mather 1957) can be diminished with carbohydrate-active steroids. Further, serum anti-rachitic activity in such patients has been shown to be within the range observed in normal subjects (Bell et al. 1961, 1964). These findings have led to the conclusion that the defect results from an abnormal sensitivity to vitamin D (Anderson et al. 1954; Bell et al. 1961, 1964) and not from hypervitaminosis D (Henneman et al. 1956).

Henneman et al. (1956) have described negative balances of calcium and phosphorus, comparable to those which have been produced with large doses of vitamin D (Albright et al. 1938), in patients with sarcoidosis and hypercalciuria. These authors postulated an additional abnormality of bone metabolism produced by »endogenous hypervitaminosis D«.

Results of the present study indicate that there are spontaneous abnormalities in $^{47}$Ca metabolism in patients with sarcoidosis and hypercalcaemia similar to those produced by vitamin D in normal subjects and in patients with hypoparathyroidism (Bell & Bartter 1963), which may persist despite correction of hypercalcaemia with carbohydrate-active steroids. It is suggested that these abnormalities are caused by an abnormal sensitivity of bone to vitamin D.

METHODS

Two patients, in whom the diagnosis was confirmed by biopsy, were 41 and 47 years of age. The seven normal subjects were volunteers ranging in age from 18 to 57 years. They were maintained on an air-conditioned metabolic ward and given constant dietary and fluid intakes. Patients were maintained on this regimen throughout the study. Stools were collected in four-day pools, and urine in one- or two-day pools. Stools, diets and urine were analyzed for calcium (Fiske & Logan 1931). Prednisone, 12.5 mg every six hours by mouth, was given to the patients during part of the study.

$^{47}$CaCl$_2$ (Oak Ridge National Laboratory), 5 or 10 μc, was given as a single intravenous injection in total volumes varying from 2.5 to 5.0 ml. Four-ml aliquots of serum were obtained at intervals of 12 hours or less for 4 to 8 days afterwards and were analyzed for radioactivity with a well-type scintillation detector (Nuclear Chicago Corp.). The samples in the earlier portion of the curve which were used for calculations (see below) were counted until one standard deviation of each value was two per cent or less of the counts per minute corrected for background. The standard deviation for later samples which were not used for calculation was 5 per cent. Stools were collected in quart-sized paint cans, diluted to 500 ml with distilled water, homogenized by agitation for at least three hours and analyzed for radioactivity. 500 ml aliquots of urine were also analyzed.

Calculations derived by Aubert & Milhaud (1960) and Aubert et al. (1963) were used.

The miscible calcium pool (P) is determined by dividing the intercept ($R_0$) derived by extrapolating the exponential fall in specific activity of serum $^{47}$Ca (expressed as fraction of dose administered per gram of calcium) into the dose injected ($R_f$):
\[ P = \frac{R_i}{R_{so}} \]  

(1)

The constant \( a \) is determined from the experimentally obtained half-time \( (T_\frac{1}{2}) \) of the exponential:

\[ a = \frac{0.693}{T_\frac{1}{2}} \]  

(2)

The pool turnover \( (vT) \) is calculated:

\[ vT = aP \]  

(3)

Urinary calcium \( (vu) \) is measured directly and endogenous faecal calcium \( (vf) \) is determined with the assumption that the specific activity of \( vf \) is the same as \( vu \):

\[ vf = \frac{Rs \int_{t_1}^{t_2} vu}{Ru \int_{t_1}^{t_2}} \]  

(4)

where \( Rs \int_{t_1}^{t_2} \) and \( Ru \int_{t_1}^{t_2} \) are faecal and urinary radioactivity excreted between times \( t_1 \) and \( t_2 \) (when the slope of radioactivity in the serum is linear on a semilog plot). A lag period of one day was used for faecal radioactivity.

Calcium »accretion« rate \( (vo+) \) is estimated:

\[ vo+ = vT - vu - vf \]  

(5)

»Bone resorption« rate \( (vo-) \) is calculated:

\[ vo- = vo+ + \Delta s \]  

(6)

where \( \Delta s \) is calcium balance, determined by subtracting urinary calcium and faecal calcium \( (vF) \) from calcium intake \( (vi) \).

**RESULTS**

Result are shown in Table 1 and in Figs. 1 and 2.

In M. C., with no treatment (Fig. 1, days 1 through 8), faecal calcium averaged 156 mg a day, serum calcium ranged from 11.4 to 12.2 mg per 100 ml and urinary calcium averaged 361 mg a day. After treatment with prednisone, 50 mg a day for two weeks, mean faecal calcium had increased to 436 mg a day, serum calcium had fallen to a range of from 10.3 to 10.8 mg per 100 ml and mean urinary calcium had decreased to 216 mg a day (days 27 through 34).

In J. T., with no treatment (Fig. 2 days 1 through 8), faecal calcium averaged 60 mg a day, serum calcium ranged from 10.7 to 13.2 mg per 100 ml and urinary calcium averaged 212 mg a day. After treatment with prednisone, 50 mg a day for 12 days, mean faecal calcium had increased to 221 mg a day, serum calcium had decreased to a range of from 9.6 to 10.2 mg per 100 ml and urinary calcium had fallen to an average of 135 mg a day (days 21 through 28). Thus, in both patients, prednisone increased faecal calcium an decreased serum and urinary calcium.

In Figs. 1 and 2 is shown the specific activity of serum calcium, plotted semilogarithmically, in each of the studies. The results of the studies with \(^{47}\)Ca are summarized in Table 1.
The effects of prednisone on miscible calcium pool (P), turnover of calcium (vT), urinary calcium (vu), endogenous faecal calcium (vf), faecal calcium (vF), calcium »accretion« rate (vo+), »bone resorption« rate (vo–), calcium balance (Δ s), and body weight in two patients with sarcoidosis. Values for seven normal subjects are also shown.

<table>
<thead>
<tr>
<th>Pt.</th>
<th>P mg Ca</th>
<th>vT</th>
<th>vu</th>
<th>vf</th>
<th>vF</th>
<th>vo+ mg Ca/day</th>
<th>vo–</th>
<th>vi</th>
<th>Δs</th>
<th>Weight kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. C.</td>
<td>4263</td>
<td>1687</td>
<td>361</td>
<td>52</td>
<td>156</td>
<td>1274</td>
<td>1519</td>
<td>272</td>
<td>-245</td>
<td>47.18</td>
</tr>
<tr>
<td>M. C.*</td>
<td>2494</td>
<td>1040</td>
<td>216</td>
<td>26</td>
<td>436</td>
<td>798</td>
<td>1178</td>
<td>272</td>
<td>-380</td>
<td>46.18</td>
</tr>
<tr>
<td>J. T.</td>
<td>4503</td>
<td>2495</td>
<td>212</td>
<td>20</td>
<td>60</td>
<td>2263</td>
<td>2283</td>
<td>230</td>
<td>-20</td>
<td>68.44</td>
</tr>
<tr>
<td>J. T.*</td>
<td>3685</td>
<td>2362</td>
<td>135</td>
<td>97</td>
<td>221</td>
<td>2130</td>
<td>2256</td>
<td>230</td>
<td>-126</td>
<td>66.15</td>
</tr>
</tbody>
</table>

Normal subjects

| E. H. | 4410 | 825 | 44 | 217 | 330 | 564 | 314 | 624 | +250 | 64.7 |
| C. H. | 3891 | 626 | 21 | 89 | 348 | 516 | 403 | 482 | +113 | 76.3 |
| J. R. | 4902 | 1029 | 116 | 65 | 303 | 848 | 830 | 437 | +18 | 64.5 |
| C. D. | 4673 | 851 | 67 | 56 | 254 | 726 | 867 | 182 | -139 | 62.4 |
| M. S. | 3484 | 854 | 42 | 26 | 76 | 786 | 756 | 148 | +30 | 41.9 |
| P. W. | 4525 | 738 | 57 | 0 | 129 | 681 | 679 | 182 | +3 | 62.1 |
| J. W. | 4630 | 787 | 147 | 50 | 144 | 590 | 602 | 279 | -12 | 43.7 |

* Prednisolone, 50 mg/day, for 12 or 16 days.

With no treatment (Table 1), P was 4263 mg in M. C. and 4503 mg in J. T., vo+ was 1274 mg/day in M. C. and 2263 mg/day in J. T. and vo– was 1519 mg/day in M. C. and 2283 mg/day in J. T.

After treatment with prednisone, 50 mg a day for 12 or 16 days (Table 1), P was 2494 mg in M. C. and 3685 mg in J. T., vo+ was 798 mg/day in M. C. and 2130 mg/day in J. T., vo– was 1178 mg/day in M. C. and 2256 mg/day in J. T. Thus, P was within the normal range and vo+ and vo– were abnormally increased in both patients before treatment and P, vo+ and vo– had all decreased after treatment with prednisone.

**DISCUSSION**

In both patients in the present study with no treatment, P was normal and vo+ and vo– were abnormally increased. Faecal calcium was low and serum and urinary calcium were elevated. Similar changes in 47Ca metabolism have been produced in normal subjects and in patients with hypoparathyroidism with vitamin D, 100 000 units a day for 4 days (Bell & Bartter 1963). Whereas prednisone corrected the abnormal calcium absorption and the hypercalcaemia...
The effects of prednisone on serum calcium, specific activity of the serum calcium, calcium balance and body weight in M. C.

In this and in the subsequent chart, balance data are plotted as follows: intake is plotted from the zero line downward, and urinary and faecal values are plotted upwards from the intake line. A positive balance is shown as a clear area below the zero line, a negative one by faecal or urinary values above the line.

in both of the patients, it lowered vo+ to normal only in M. C. It is possible that the fall in serum calcium with prednisone resulted from an effect on bone as well as from a decrease in calcium absorption.

In studies with 45Ca, it has been shown that cortisone lowers P, vo+ and vo− and increases faecal calcium in the rat (Milhaud et al. 1960). These findings are quite similar to those produced in the patients as described above.

Henneman et al. (1956) reported metabolic balance studies in three patients with sarcoidosis and hypercalcaemia and found negative calcium and phosphor-
The effects of prednisone on serum calcium, specific activity of serum calcium, calcium balance and body weight in J. T.

us balances in each of them. They noted the similarity between these findings and those produced with large doses of vitamin D in a patient with hypoparathyroidism (Albright et al. 1938) and proposed that these abnormalities, as well as the excessive intestinal absorption of calcium also present, resulted from "endogenous hypervitaminosis D".

In both patients in the present study, it has been previously shown that serum anti-rachitic activity (as determined by bioassay in rachitic rats) was within the range observed in normal subjects, and that the abnormality in calcium absorption could be augmented with vitamin D in doses (10 000 units a day for 12 days) which have virtually no effect in normal subjects and do not increase serum anti-rachitic activity to abnormal values (Bell et al. 1961, 1964). Further, negative balances of calcium and phosphorus occurred spontaneously.
in M. C. and were induced in J. T. with the same dose of vitamin D (10 000 units a day for 12 days). It was concluded that these abnormalities in calcium metabolism resulted from an abnormal sensitivity to vitamin D (Anderson et al. 1954; Bell et al. 1961, 1964). An increase in the incidence of hypercalcaemia in patients with sarcoidosis in the summer has been demonstrated; this presumably is related to an increased synthesis of vitamin D₃ from 7-dehydrocholesterol in the skin by ultraviolet radiation with exposure to sunlight (Taylor et al. 1963). Conversely, it has been demonstrated that hypercalcaemia and the abnormal calcium absorption in sarcoidosis can be reversed by treatment with a vitamin D-deficient diet (Hendrix 1963).

X-ray findings of generalized radiolucency of bone similar to that described in adult subjects with vitamin D intoxication (Howard & Mayer 1948; Chaplin et al. 1951; Verner et al. 1958) have been reported in patients with sarcoidosis and hypercalcaemia (Van Creveld 1941; Klatskin & Gordon 1953). Radiographic lesions suggestive of the metaphyseal sclerosis and resorption (Harris & Innes 1931; Thomas & Morgan 1958), produced by overtreatment of growing animals with vitamin D, have been demonstrated in some children with vitamin D intoxication (Ross 1952) and in some with hypercalcaemia of infancy (Lowe et al. 1954; Creary & Neill 1954), a syndrome whose pathogenesis has been attributed to vitamin D (Lightwood 1953; Fellers & Schwartz 1958).

As mentioned above, vitamin D, in normal subjects and in patients with hypoparathyroidism, produced as much as a two-fold increase in vo⁺ and vo⁻, but very little change in serum calcium and in calcium absorption (Bell & Bartter 1963). Vitamin D has also been shown to increase the serum calcium (Carlsson 1954) and the rate at which ⁴⁵Ca and ⁳²P are deposited in and removed from the long bones of rachitic rats (Cohn & Greenberg 1939; Greenberg 1945; Underwood et al. 1951; Bauer et al. 1955). These findings suggest that vitamin D increased the rate of exchange of calcium and phosphorus between bone and extracellular fluid. The results of the present studies demonstrate that this process occurs spontaneously in patients with sarcoidosis and hypercalcaemia. They provide evidence that in sarcoidosis the bones, like the gastrointestinal tract, show hypersensitivity to vitamin D.

REFERENCES


Received on September 15th, 1966.