Vitamin D Conversion by Sarcoid Lymph Node Homogenate

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Recent studies suggest that hypercalcemia of sarcoidosis is due to high blood concentrations of calcitriol and that this compound may be synthesized at an extra-renal site. We report that sarcoid lymph node homogenate metabolized calcifediol to a substance indistinguishable from calcitriol, whereas six nonsarcoid lymph nodes failed to produce this compound.

THE ASSOCIATION BETWEEN sarcoidosis and hypercalcemia has been known for many years (1, 2). More recently, high serum concentrations of calcitriol have been found in some patients with sarcoidosis and hypercalcemia (3-5). This abnormal elevation does not appear to be mediated by parathyroid hormone (6, 7) and, because it has been found in one anephric patient (8), the suggestion has been made that in sarcoidosis calcitriol may come from an extra-renal source, possibly sarcoid tissue itself (7, 8). We report the in-vitro synthesis of dihydroxylated vitamin D metabolites by sarcoid tissue.

Case Report

A 49-year-old male factory supervisor presented with a mass in the neck. Three years earlier, pulmonary opacities had been found on a routine chest roentgenogram and a tentative diagnosis of sarcoidosis had been made at another institution. Because his lung function test results were normal, no treatment was given.

Physical examination showed a firm submental mass 3 cm in diameter and bilateral supraclavicular masses 2 cm in diameter. The splenic tip was palpable 2 cm below the left costal margin. Other examination findings were normal.

Serum calcium was 9.5 mg/dL; urine calcium 160 mg/24 h; serum calcifediol, 49 nmol/L (normal, 30 to 188 nmol/L) (9); serum 24, 25-dihydroxycholecalciferol, 9 nmol/L (normal, 2.9 to 16 nmol/L) (10); serum calcitriol, 123 pmol/L (normal, 29 to 168 pmol/L) (10); and serum 25, 26 dihydroxyvitamin D 1.6 nmol/L (normal, 0.6 to 2.1 nmol/L) (11). All other biochemical values including serum angiotensin converting enzyme activity were within normal limits.

Biopsy specimens of the left supraclavicular mass showed confluent, non-caseating giant-cell granulomas consistent with sarcoidosis. Acid-fast bacilli were not found.

Lymph nodes were obtained from six persons who did not have sarcoidosis during surgery involving axillary or neck dissections. None of these nodes showed evidence of malignant disease.

Materials and Methods

Biopsy samples of the lymph nodes were homogenised and incubated with calcifediol according to the method of Tanaka and DeLuca (12) with minor modifications: A 10% weight per volume homogenate was made. To 4 mL of this homogenate were added 0.5 mL of 0.025 *M* sodium succinate, 100 nmol calcifediol (Upjohn Pty. Ltd., Sydney, Australia, and Roche Products Pty. Ltd., Sydney, Australia) in 100 μL ethanol and 0.3 μCi tritiated calcifediol (Radiochemical Centre, Amersham, United Kingdom) in 10 μL ethanol (12). The flasks were gassed with 95% oxygen and 5% carbon dioxide and incubated at 25 °C in a shaking water bath at 100 oscillations per minute for 30 minutes. At the end of this time the reaction was stopped by the addition of 12 mL of methanol and chloroform (2 to 1, volume in volume). In each case methanol and chloroform was added to one aliquot of homogenate before the addition of calcifediol.

Lipid extraction of the mixture was done according to the method of Bligh and Dyer (13). After ultrafiltration the material was dried and tested using high-performance liquid chromatography on a "Zorbax-Sil" column (Dupont Instruments, Wilmington, Delaware) in isopropanol and hexane (7 to 93, volume in volume). The elution pattern was compared to that of authentic standards. Protein content was determined by the method of Lowry and associates (14).

Material comigrating with calcitriol standard in this high-performance liquid chromatography system was collected and aliquots were again tested in two other systems: Zorbax-Sil with methanol and methylene chloride (2:98, percent volume in volume) and octadecyl-bonded silica with methanol and water (85:15, percent volume in volume). Material comigrating with 25, 26 dihydroxyvitamin D standard on silica high-performance liquid chromatography with isopropanol and hexane was also tested using the two additional chromatographic systems.

Aliquots of each 3-mL fraction obtained after high-performance liquid chromatography with isopropanol and hexane were assayed for calcitriol-like activity in a specific competitive protein binding assay system (10, 15) and displacement activities of serial dilutions were compared with those of chemical standard. The concentrations of calcitriol-like material in two fractions were determined from the specific activity and competitive protein binding assay.

Results

After incubation of tritiated calcifediol with sarcoid homogenate, material was made that comigrated with authentic calcitriol on silica high-performance liquid chromatography with isopropanol and hexane. The production rate was 83 fmol/min·mg protein (mean of three determinations). No such material was produced during incubation of six nonsarcoid lymph node homogenates or inactivated sarcoid tissue. When the calcitriol-like material produced by the sarcoid node was tested using the two other systems, the material also comigrated with authentic chemical standards (Figure 1).

When a competitive protein binding system was used to analyse sequential high-performance liquid chromatographic fractions of the sarcoid extract, two of these fractions, which comigrated with chemical calcitriol, produced displacement curves indistinguishable from that of

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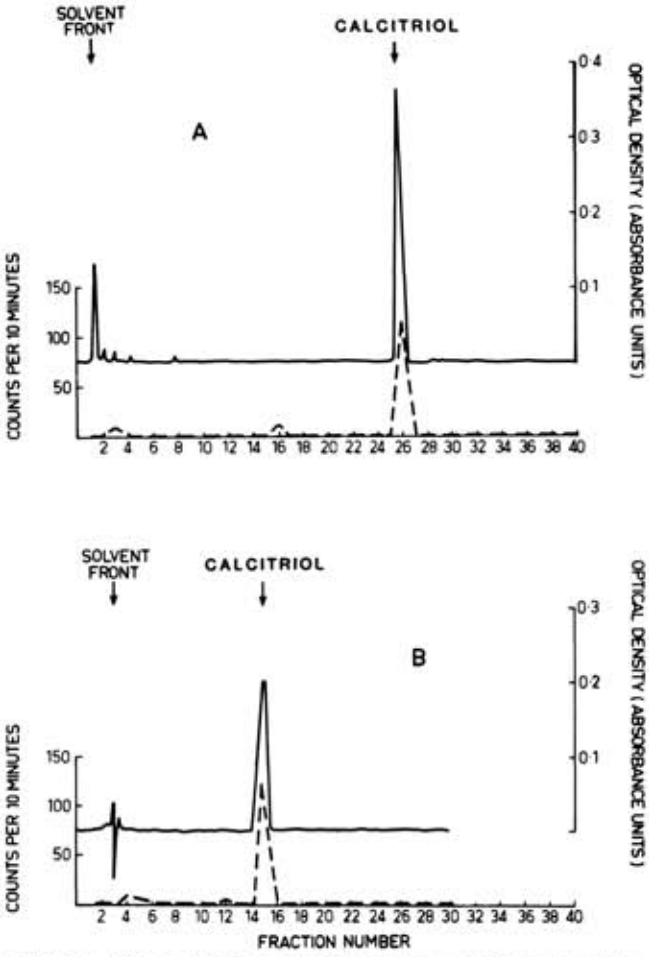

Figure 1. Elution patterns of calcitriol-like material produced by sarcoid tissue (– – –) compared with those of authentic calcitriol (——). In two chromatography systems Zorbax-Sil with methanol and methylene chloride (*A*) and octadecyl-bonded silica with methanol and water (*B*). Solvent flow rate was 1 mL/min and 1 mL fractions were collected. The peaks comigrating with calcitriol contained 85% (system A) and 86% (system B) of added counts.

authentic calcitriol (Figure 2). No such displacement was seen with the relevant fractions from either normal lymph nodes or inactivated material. Furthermore, when the concentrations of calcitriol-like material in two fractions were determined from the specific activity and from displacement in the competitive protein binding assay system, the results were substantially in agreement with one another (14.3 and 13.8 nmol/L respectively for one sample, and 1.8 and 1.6 nmol/L respectively for another sample).

Both sarcoid and nonsarcoid homogenates produced relatively large quantities of material comigrating with 25, 26 dihydroxyvitamin D (Radiochemical Centre) on straight-phase high-performance liquid chromatography with isopropanol and hexane. When aliquots of this material were examined in other systems, they comigrated with chemical 25, 26 dihydroxyvitamin D. Production rates were 523 fmol/min·mg protein for sarcoid lymph nodes (mean of three determinations) and 1675 fmol/min·mg protein for nonsarcoid lymph nodes (mean of six nodes, each assayed in duplicate).

Discussion

It is currently believed that serum concentrations of calcitriol are elevated in some patients with sarcoidosis (3-5), that sarcoid-associated hypercalcemia is caused by abnormalities of vitamin D metabolism (3-5), and, on the basis of circumstantial evidence, that significant extra-renal synthesis of calcitriol may occur in these patients (7, 8, 16). This study shows the ability of sarcoid tissue to synthesize a compound indistinguishable from calcitriol. Adams and associates (17) have recently demonstrated the synthesis of calcitriol by pulmonary alveolar macrophages from patients with sarcoidosis.

Because of the difficulty in determining the exact nature of vitamin D metabolites produced in vitro by tissue homogenates, a considerable part of this study was devoted to that question. The ideal method of identification, mass spectroscopy, requires relatively large quantities of metabolites that are unlikely to be available from human biopsy material. On the other hand, the calcitriol-like material produced by sarcoid tissue comigrated with authentic chemical standards in three different high-performance liquid chromatography systems. The calcitriol-like material also behaved like authentic standards in a biological system, and results obtained by chemical and biological methods were virtually identical. It is therefore likely that this material corresponds to the compound called calcitriol that is measured in the serum of patients with sarcoidosis (3-5, 7, 8, 17).

The rate of 1-hydroxylase activity in this sarcoid mate-

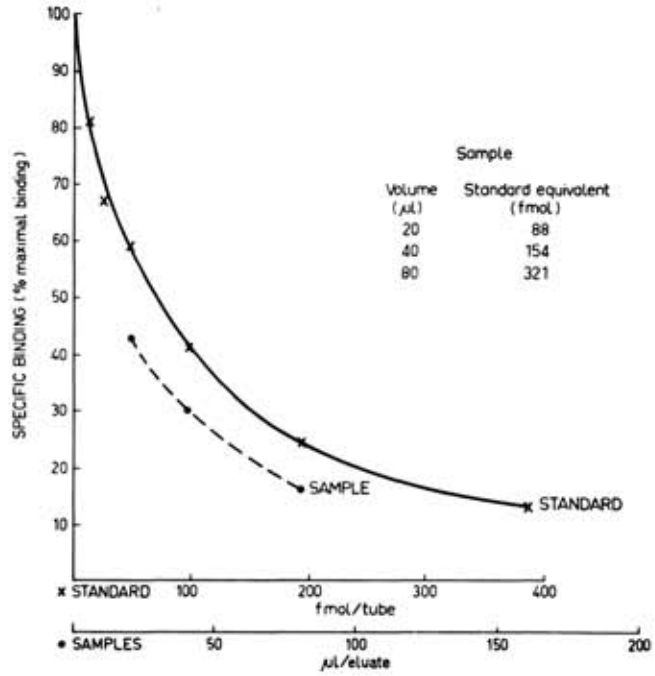

Figure 2. Displacement curve (●– – –●) showing the effect of incubating different volumes of calcitriol-like material produced by sarcoid tissue in a competitive protein binding assay system. A displacement curve obtained with authentic calcitriol is shown for comparision (x——x). Maximal specific binding in the absence of sample or standard was 32%. When the displacement activities of the serially diluted samples were converted to calcitriol values (fmol/tube) using the standard curve, the results showed a linear relationship with volume (*see* inset).

rial was similar to that of kidneys from rats with vitamin D deficiency (12) and mitochondria from human kidneys (18). The rate of activity was somewhat higher than that reported for most other extra-renal tissues (19-21).

The presence of 1-α-hydroxylase in sarcoid tissue does not necessarily mean that calcitriol was being synthesized in this tissue in vivo at the time of biopsy. Synthesis depends on the availability of substrate (calcifediol) and there is evidence that as long as substrate concentrations are low or normal, serum levels of calcitriol are not elevated in this disorder (3, 5). Not surprisingly, blood levels of calcitriol in our patient were normal and he was neither hypercalcemic nor hypercalciuric. Even in the presence of high serum calcitriol concentrations, which might result from diminished degradation (22) as well as enhanced synthesis, the development of hypercalcemia depends on many factors such as dietary calcium, intestinal function, and renal function (4). Sarcoid tissue in bone may also cause hypercalcemia by local mechanisms that may or may not be related to vitamin D metabolism (23).

Although there is some evidence to suggest that calcitriol production is abnormally regulated in patients with sarcoidosis (3, 5), it was impossible to determine in this study whether 1-hydroxylase activity in sarcoid tissue is subject to the control mechanisms that regulate this enzyme in the kidney (12) or at some extra-renal sites (19, 20). As yet, there are no data available relating to the control mechanisms for the other two enzymes that show greater activity in sarcoid than in nonsarcoid lymph nodes—angiotensin converting enzyme and lysozyme (24).

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