Calcium Oxalate in Sarcoid Granulomas

With Particular Reference to the Small Ovoid Body and a Note on the Finding of Dolomite

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The nature, prevalence, and specificity of birefringent calcific particles in granulomas of sarcoidosis have been examined, including histochemical reactions, single particle, and microchemical analyses. Particular attention was paid to small ovoid forms of which most were calcium oxalate monohydrate. Larger crystals, those within giant cells, and the birefringent component of a Schaumann complex were also calcium oxalate. Small ovoids appeared to originate in macrophages and to be precursors of other forms; they were found in 86% of lymph nodes and 73% of surgical lung specimens. They were not specific for sarcoidosis. Organisms could not be certainly identified in them. Their origin is discussed in relation to activated macrophages, calcium, and oxalate metabolism, and the role of calcium oxalate in granulomas is considered. Four particles from two cases were dolomite and two were a calcium-sulphur compound. The biologic origin of dolomite is reviewed. (Key words: Sarcoidosis; Calcium oxalate; Crystals; Schaumann bodies; Activated macrophages; Dolomite) Am J Clin Pathol 1988;90:545-558

WHILE ZIEHL-NEELSEN (Z-N) stained sections of lymph nodes were being examined from a woman with a 45-year history of sarcoidosis, numerous small refractile particles were seen under polarized light (Fig. 1), apparently within epithelioid cells. These bodies were not visible in the original hematoxylin and eosin (H and E) slides, nor in parallel sections stained by Gomori's methenamine silver (GMS) or periodic acid-Schiff (PAS) methods. Other recent biopsy specimens of lymph nodes with sarcoidosis showed similar particles in varying numbers in eight of ten specimens but only in Z-N stained slides examined by polarizing light microscopy (PLM).

Birefringent crystalline material has long been recognized in giant cells and in the Schaumann bodies of sarcoidosis and has been variously thought to be calcite\textsuperscript{40,65} (one of the crystal forms of CaCO\textsubscript{3}) or calcium oxalate.\textsuperscript{27} (CaC\textsubscript{2}O\textsubscript{4}) The small ovoid macrophage-related bodies, however, have been infrequently described\textsuperscript{27,40} and the objectives of this investigation were to establish their nature, prevalence, origin, specificity, and pathogenic significance, with the assistance of highly specialized microanalytic techniques and single particle analysis. When it was confirmed that the small bodies were composed of calcium oxalate (as indicated by Johnson\textsuperscript{27} from histochemical examination), a few typical examples of the larger and better known birefringent particles of the sarcoid granulomas were also submitted to microanalytic examinations. In the course of this work, small bodies other than oxalate were encountered and were defined by single-particle methods of analysis.

Materials and Methods

Light Microscope Evaluation

Unstained slides and/or paraffin blocks from patients with clinical and pathologic diagnoses consistent with sarcoidosis were retrieved from files at Robinson Memorial Hospital (RMH) and from other local and international sources, as indicated in the acknowledgments. Brief clinical abstracts of five cases used for illustrations are given in Appendix I. Examination was made with a standard American Optical\textsuperscript{®} microscope fitted with analyzer and polarizer with full wave-plate attachment. Because of the frequency with which extraneous birefringent material is found on coverslips and (particularly in old preparations) in mounting media, extreme care was taken in identification, and only full ovoids in varying numbers in eight of ten specimens but only in Z-N stained slides examined by polarizing light microscopy (PLM).

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FIG. 1 (upper, left). Cervical lymph node showing numerous small birefringent bodies (arrows) in epithelioid cell granulomas. Case 1, first biopsy (1984). Hematoxylin and eosin (without acid differentiation) (×200).


FIG. 3 (upper, right). Bronchial wall showing multiple bodies in a diffuse macrophage reaction. Case 3. Hematoxylin and eosin (Gill), partially polarized (×350).
Table 1. Small Oxalate Bodies in Sarcoid and other Granulomas

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Total</th>
<th>Positive</th>
<th>Negative</th>
<th>Percentage Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Sarcoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node</td>
<td>45</td>
<td>39</td>
<td>6</td>
<td>86</td>
</tr>
<tr>
<td>Lung</td>
<td>15</td>
<td>11</td>
<td>4</td>
<td>73</td>
</tr>
<tr>
<td>Autopsy material</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Surgical material</td>
<td>13</td>
<td>2</td>
<td>11</td>
<td>15</td>
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<tr>
<td>Skin</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>28</td>
</tr>
<tr>
<td>Spleen</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Heart</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Breast</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II. Other granulomas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodes, cat-scratch</td>
<td>12</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Nodes associated with tumor</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>20</td>
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<tr>
<td>Histoplasmosis—spleen, nodes, lung, liver</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>16</td>
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<td>Tuberculosis, lung</td>
<td>8</td>
<td>1</td>
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<td>12</td>
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<td>0</td>
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<td>Lepromatous</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>2</td>
<td>0</td>
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</tr>
<tr>
<td>Foreign body granulomas</td>
<td>22</td>
<td>0</td>
<td>22</td>
<td>22</td>
</tr>
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</table>

formalin–alcohol fixative had been used in some instances. The time in fixative before processing was generally not known, except that skin biopsies to the Cleveland Skin Pathology Laboratories (CSPL) and to the Armed Forces Institute of Pathology (AFIP) had been submitted by mail with an estimated delay of several days to several months; and that autopsy material had usually been held for several days to several weeks. To test directly the effects of buffered formalin over different periods of time, the number of bodies visible in unstained and in H and E–stained frozen sections from five cases were compared with those in paraffin sections from the same blocks held in fixative for 6–8 hours before routine overnight processing.

Because preliminary studies indicated that the recognition of ovoid bodies depended on the use of acetic instead of hydrochloric acid in the acid–alcohol differentiation step of the conventional staining sequences, all materials were reexamined by Putt’s modification of the Z-N stain performed at RMH. Routine H and E preparations and some in which the HCl–alcohol wash was deliberately omitted were also evaluated. Slides accompanying a few outside cases had been stained with Gill’s progressive method and could be directly compared. Three forms of birefringent particles were studied: (1) small ovoid bodies apparently within epithelioid cells, (2) free-standing crystals or crystals within giant cells, and (3) Schaumann complexes, defined here as basophilic conchoids or rings encircling or juxtaposed to unstained crystalline material.

In four cases (nodes and lung) in which ovoid bodies were sufficiently numerous to ensure that they could be found in serial sections, Gram’s, GMS, PAS, iron, and calcium stains were performed, the last including von Kossa, Yasue, and Alizarin red reactions. In eight lung specimens, Alcian blue and iron reactions were studied in consecutive sections. Except where otherwise specified, all staining methods were those detailed in the AFIP manual. To identify oxalate, tissues were incinerated on a pyroceram plate, with a commercial propane gas torch for 5 minutes, followed by Alizarin red staining. Attempts to demonstrate carbonate by the production of gas after running 2 mol/L acetic acid or concentrated H₂SO₄ under coverslips were made on frozen-section material from one case and deparaffinized material from two cases.

The prevalence of small bodies was studied in 94 specimens of sarcoidosis in different tissues, after Z-N stains and with PLM (Table 1). Seventy-five granulomas of other types were used as controls, including caseating lesions of tuberculosis (RMH and Cleveland Metropolitan General Hospital [CMGH]); lepromatous and tuberculoid leprosy (ex Africa, via AFIP); nodes with diagnoses of cat-scratch disease (RMH); histoplasmosis (RMH, CMGH); granulomas in nodes draining tumors (CMGH); and granulomas associated with a wide variety of foreign bodies (RMH). These are also listed in Table 1.

The relative frequency of different forms of birefringent particles in sarcoid granulomas was studied in 25 lymph nodes and in 8 lung lesions, taken at random (Table 2).

Presence of Acid-Fast Material. Because sarcoidosis has histologic, radiologic, and clinical similarities to tuberculosis, and because of previous reports of organisms within Schaumann bodies, an intensive oil-immersion search was made for acid-fast material within the small calcified bodies in cases 1 and 3, where they were most numerous. The sensitivity of the Putt stain was first confirmed by sectioning colonies from (a) five different my-
**Table 2.** Relative Frequency and Combinations of Oxalate Crystals of Different Types in Samples of 25 Nodes and 8 Lung Tissues

were strong birefringence and failure to stain by H and E, von Kossa, and Yasue techniques. These were not further characterized, and no material has been previously seen in tissue specimens (M.E.A., unpublished observations).

Larger birefringent extracellular crystals of ovoid or irregular shape, singly or in aggregates, were also unstained by H and E and Z-N. Similar material was found in giant cells, a few showing iron-positive rings and some having laminations stained by Alcian blue. One group of large particles dissected out of the tissue, crushed, and examined by x-ray diffraction analysis showed only calcium oxalate monohydrate. The calcium–iron–phosphorus phase was not detected, indicating that the calcium–phosphorus component was either present in a low proportion in the diffracted sample or was present as an amorphous material.

Schaumann complexes (Fig. 11) included irregular shells or bands of basophilic material, surrounding or closely associated with birefringent unstained crystalline material. A scanning electron photomicrograph of one such particle mounted on beryllium is shown in Figure 12. Its composition was in two different phases. By special analytic techniques one zone proved to be a phosphate-rich material, whereas the other larger area contained calcium, with low phosphorus, consistent with calcium oxalate. Elemental maps of the distribution of calcium and phosphorus are shown in Figures 14 and 15. Basophilic conchoids without crystals were found in relatively few cases, chiefly in hyalinized areas, where they were often faded or weakly stained.

Prevalence and Coexistence of Different Forms of Oxalate

Small ovoids were found in 86% of sarcoid nodes and in 73% of surgical lung specimens (Table 1), with lesser numbers in autopsy specimens and at other sites as indicated. The duration in fixative and type of solution used was generally not known, and these results must be taken as conservative.

The extent to which different types of particles coexisted is indicated in Table 2, based on a limited random sample of 25 nodes and 8 lung specimens. The full Schaumann complex was not encountered in the absence of ovoids or other crystals; and complexes were seen in lung tissue relatively more often than in nodes.

Disease Specificity

Ovoid forms of calcium oxalate were not unique to sarcoidosis but were also found in cases with diagnoses of tuberculosis and histoplasmosis and in nodes draining carcinomas. They were not found in other granulomas (Table 1).
Search for Mycobacteria in Ovoid Bodies

In case 1, acid-fast material was found but as small fragments and in an occasional body only. Sometimes this was linear and seemed to lie artifactually between laminations. One faintly birefringent body enclosed two well-stained clearly visible acid-fast fragments, quite consistent with juxtaposed organisms. Two linear acid-fast fragments, possibly bacilli, were also found lying free. In case 3, acid-fast material was relatively easily found within oxalate bodies. Most of this was dense and irregular, and very few rod-like or bacillary forms were seen; some were linear, between the laminations. Many ovoid bodies contained tiny black granules, and a few were darkly speckled over a central core while retaining a peripheral clear rim. Some black material was linear. This pigment was thought to be carbon, which was relatively abundant in adjacent macrophages.

Discussion

In 1917 and again in 1941, Schaumann described doubly contoured and stratified, often calcified corpuscles in the lymphatic glands and tonsils of patients who had lymphogranulomatosis benigna (now termed sarcoidosis). The smallest were the size of leukocytes; the periphery was a strongly stained double-contoured membrane, whereas the remainder made itself noticeable by a “more or less distinct refringency.” The compound nature of Schaumann bodies has been more fully described by Engle and Williams. In 1974 Mohri drew attention to small refractile bodies, 0.8–2 μm by 3–7 μm, with a central dark isotropic “kern,” also described by Johnson in sarcoid and other granulomas as ring or notched forms. To be seen, frozen sections, or (for paraffin sections) short periods of fixation, avoidance of mineral acids, and PLM are needed.

No clear descriptions have been found in electron microscope studies of sarcoid granulomas. Pickett and associates illustrated somewhat larger irregular fragments within giant cells, and Lunardelli described small probably calcified bodies, up to 65 nm diameter, in the mitochondria of epithelioid cells. To be seen, frozen sections, or (for paraffin sections) short periods of fixation, avoidance of mineral acids, and PLM are needed.

<table>
<thead>
<tr>
<th>Tissue S85-9435</th>
<th>Reference JCPDS Card 20-231 (Whewellite)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d Spacings</td>
<td>I/I₀</td>
</tr>
<tr>
<td>Reference</td>
<td>d Spacings</td>
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<td>20-231</td>
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<td>100</td>
</tr>
<tr>
<td>3.645 A²</td>
<td>50</td>
</tr>
<tr>
<td>2.966 A²</td>
<td>25</td>
</tr>
<tr>
<td>2.348 A²</td>
<td>10</td>
</tr>
</tbody>
</table>

Different sequences of spacings and relative intensities of peaks are characteristic of a particular crystalline material.
3μm Birefringent Particle Removed From Tissue
FIG. 7 (upper). Scanning electron photomicrograph of an irregular particle identified later as dolomite. Lymph node, case 1 (×10,000) (back-scattered image).

FIG. 8 (center). Raman spectrum of particle from Figure 7 with peaks at wave numbers corresponding to dolomite.

FIG. 9 (lower). Particle shown in Figure 7 remounted for electron microprobe examination after Raman spectroscopy. The energy-dispersive x-ray spectrum shows calcium and magnesium peaks. Semiquantitative analysis data: Mg 25.82 (atom %) 25.96 (wt. %) Ca 24.20 (atom %) 40.54 (wt. %). The Ca-Mg ratio is 1:1, as in dolomite.

with 28% found by Mohri in 50 nodes. Both figures are probably low, considering technical difficulties. We did not find them in sarcoid spleens (most of which had been fixed in Zenker’s fluid), although small unidentified crystals are known to be present in Kveim suspensions prepared from spleens. Extracellular or intra-giant cell crystals were found in 21 (64%) of 33 lymph nodes and lung tissues, with Schaumann complexes in 15 (45%). It

FIG. 10 (upper). Energy-dispersive x-ray spectrum of the particle from the gallbladder wall, case 4, identifying dolomite composition.

FIG. 11 (lower, left). Schaumann complex with peripheral basophilic material (arrow) and central crystals, the whole related to giant cell. Case 5. Hematoxylin and eosin (×630).

FIG. 12 (lower, right). Schaumann complex mounted on beryllium. Back-scattered SEM image (×4,000).
is difficult to relate these figures to published reports because of variations in definitions. Williams found bi-refrangent particles of all sizes in 41% and conchoid bodies in 35%, of 17 sarcoid tissues. Mohri reported Schaumann bodies (defined as crystals in giant cells) in 22% of 50 sarcoid lymph nodes.

Some indication of the frequency with which different crystal forms may coexist is given in Table 2. Small ovoids were the sole form in 10 (40%) of 25 nodes and were otherwise combined with larger crystals or with Schaumann complexes. Isolated basophilic rings without crystals were infrequent, and it seemed likely from their configuration that crystals had been previously present and had been dissolved out. This was confirmed by observations in case 3 where abundant crystalline material was present within conchoids in original blocks but had completely disappeared from new blocks obtained after three months fixation. Two cases with large area of hyaline material included massive confluent basophilic dystrophic-type aggregates. There was a strong suggestion that these had been centered around conchoids, but whether as a metathetic phenomenon (with a less soluble phosphate salt-replacing oxalate) or a superadded epitaxic phenomenon was not clear.

Origin

Because small ovoid forms of oxalate appear to develop in macrophages, particularly those of sarcoidosis, their origin is presumably related to particular biologic properties of such cells as they occur in that disease. The questions then become the cause of activation and the mechanism of calcium oxalate deposition. It was at first thought that the dark areas within bodies might represent un-stained or degenerated bacteria potentially capable of direct or indirect immunologic activation and of acting as nucleation centers. Many organisms have been seen or recovered from sarcoid tissues, most often mycobacteria and propionobacteria, but including others such as histoplasma. Organisms have been reported within Schaumann bodies experimentally produced in hamsters, although this has been questioned. The Michaelis-Guttmann bodies of the bladder have been thought to be calcific incrustations on bacteria, and dental calculi have been related to the bacterial production of amorphous calcium phosphate and apatite.

The activated macrophage known to occur in sarcoidosis has an "almost staggering" number of properties, one of which is an increase in 1,25-dihydroxy vitamin D3, thought to account for the not-infrequent hypercalcemia of sarcoidosis. Normally this vitamin acts in the intestinal epithelium to increase absorption of calcium ions; applied to the macrophage, this might lead to toxic intracellular levels and neutralization by the formation of insoluble oxalate. It has been suggested that macrophages may elaborate a calcium binding mucoglycoprotein. Impaired cell membrane barriers (possibly inflammatory) might allow calcium influx. Platelet-activating factor is reported to increase macrophage calcium ion concentration.

The hypercalcemia of hyperparathyroidism or multiple myeloma is associated with tissue phosphate deposits whereas in sarcoidosis oxalate predominates in tissues, urine, and renal deposits. A primary anomaly of oxalate metabolism is suggested. Such disturbances include congenital hyperoxalosis (related to glycolate and glyoxylate metabolism); oxalosis following increased intake or absorption of dietary oxalate or of oxalate precursors such as ethylene glycol, methoxyflurane, and xylitol; and that associated with pyridoxine deficiency. None is obviously implicated in sarcoidosis. However, about half of endogenous oxalate is derived from ascorbate, a route thought responsible for the calcium oxalate found in the normal thyroid and in cataractous lenses. Ascorbate can be converted to oxalate by peroxide and superoxide, both of which are known products of activated macrophages. Vitamin D metabolites and lymphokines have been implicated in this pathway.

Because granulomas are often of infectious nature, it is proper to ask if any microorganisms elaborate calcium oxalate or its precursor ions. Some can produce calcium carbonate in media with a high calcium content; others actively incorporate calcium or form extracellular deposits of calcium phosphate, and still others, such as M. phlei, show calcium turnover and maintain internal levels by excretion. Oxalate can be synthesized directly by fungi, particularly the Aspergilli. This property is not known to extend to lower organisms, although there is a 1954...
report that mycobacteria may be associated with increased oxalic acid in the culture media. \(^5^1\) Ascorbate is not a recognized product of prokaryotes, but glyoxylate may be produced from citrate by cell-free extracts of \textit{Pseudomonas aeruginosa}. \(^4^6\)

To summarize, no bacteria have been seen in this study and the “kerns” appear to be crystal voids. Organisms may activate macrophages but are not necessary for this function (witness silica, beryllium, zirconium, and starch granulomas). Review of microbial calcium and oxalate metabolism does not point to any particular candidate organism, and none is known to induce calcium oxalate incrustation.

\textbf{Disease Specificity and Role in Granuloma Formation}

Typical small ovoid bodies were found in lesions of tuberculosis and histoplasmosis and in nodes draining tumors, in general agreement with previous indications of nonspecificity of oxalate deposits. \(^4^0,4^6,6^5\) Johnson and Pani\(^2^4\) have proposed that oxalate is a secondary product of the inflammatory process.

It is also clear from its presence in the normal thyroid\(^4^6\) and in cataractous lenses, \(^1^0\) that sequestered oxalate is not sufficient to initiate inflammation; the intracellular bodies of sarcoidosis may be similarly inert. Although calcium oxalate on experimental injection can induce an inflammatory reaction, \(^4^6\) it is of foreign body rather than epithelioid cell type, which also applies to the oxalate deposits related to ethylene glycol, \(^1^7\) methoxyflurane exposure, \(^6\) and congenital oxalosis. \(^3^8\) Whether an intracellular submicroscopic but toxic excess of calcium or oxalate ions could modify the macrophage to its epithelioid form is an unanswered speculation.

\textbf{Dolomite}

This has not been previously recognized in mammalian tissues, apart from inhaled particles in lungs of dolomite miners. \(^3\) In urinary calculi, analysis of several million stones submitted to the Lewis C. Herring Laboratory has shown dolomite on several occasions, but in each instance consultation with the referring physician has not excluded the possibility that these were artifacts (Oldroyd NO, personal communication). The three bodies found in the cervical nodes in case 1 and the particle in the gallbladder wall in case 4 indicate an endogenous biologic origin, and there are two known examples of recurrent dolomite bladder stones in dogs \(^3^6\) (Osborne CA, personal communication). The calcareous cupules of cestodes have the form of dolomite. \(^5^1,6^0\) A recent article discussing magnesium in tooth enamel indicates that the solubility of dolomite formed by precipitation at ordinary temperatu-

\textbf{References}


Appendix: Selected Case Histories

Case 1
A black woman had sarcoidosis diagnosed at 10–11 years of age, by routine school chest x-ray and negative tuberculin skin tests; with iridocyclitis at age 30; scalene node biopsy at age 32; positive liver biopsy, age 47; interstitial pulmonary infiltrates; skin and bone lesions; biopsy-positive scalp lesions and cervical nodes, age 55; positive inguinal nodes, age 57 (RMH).

Case 2
A 32-year-old black woman with a history of pancreatitis presented with cervical, axillary, inguinal, hilar, and superior mediastinal lymphadenopathy with positive biopsies from liver and cervical nodes (Berea, OH).

Case 3
A 43-year-old asthmatic black woman with biopsy-positive sarcoid lesions in mediastinal nodes and skin presented with spontaneous recurrent pneumothoraces for which right pneumonectomy was performed with numerous sarcoid lesions in bronchial walls and parenchyma (Cincinnati, OH).

Case 4
A white woman had BCG vaccination (bacille Calmette-Guérin, an avirulent strain of M. bovis) as a student nurse; hyperthyroidism at 41, treated with radioactive iodine; hypercalcemia with slight interstitial pulmonary infiltrates; and cholelithiasis at about age 53. Biopsies with positive results for sarcoidosis were obtained from mediastinal nodes, bone marrow, and nodes accompanying the gallbladder removed when she was 57 (Chicago, IL).

Case 5
A 41-year-old white woman with a history of shortness of breath and cough and x-ray evidence of hilar and mediastinal lymphadenopathy had mediastinoscopy with node biopsy for confirmation of clinical diagnosis of sarcoidosis (Wellington, New Zealand).