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PRESENCE OF CALCIUM OXALATE CRYSTALS IN THE MAMMALIAN THYROID GLAND

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Abstract

Birefringent crystals of calcium oxalate have been previously identified in the colloid of human thyroid glands. We found such crystals in 19/20 adult thyroids at autopsy, in 4/20 infants at autopsy, and, using frozen sections, in 19/20 thyroids partially or totally removed at surgery. These crystals were soluble in hydrochloric acid, insoluble in acetic acid, and contained only calcium by energy dispersive X-ray microanalysis, confirming their calcium oxalate character. Similar crystals were found in equine and ovine thyroids.

Key Words: Thyroid, Calcium Oxalate, Crystals, Colloid, Birefringence, Ovine, Canine, Bovine, Equine, X-Ray microanalysis.

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Introduction

Richter, in 1940, published an abstract describing the presence of anisotropic crystals in the follicular colloid of thyroid glands (8). In 1954, he published a more complete account of his work (9), identifying the crystals as calcium oxalate monohydrate. Recently Reid et. al. (9) revisited the subject in order to call attention to the phenomenon. We have also been interested in the topic and present our findings including examination of human and animal autopsies as well as human surgical specimens.

Materials and Methods

Thyroid glands from twenty consecutive adult autopsies, and twenty autopsies from newborns to three year old children (Table 1) were examined by polarized light microscopy. One to two sections of formalin fixed and hematoxylin and eosin stained tissue were available from each autopsy. In addition, frozen sections of thyroid from twenty consecutive surgical thyroidectomies were studied (Table 2). The thyroids were partially or totally removed for primary thyroid disease, or, coincidentally, as a part of laryngectomy for cancer. Frozen sections were examined unfixed, or after fixation, and hematoxylin and eosin staining. Freshly cut surfaces of thyroids were scraped and the scrapings were examined unfixed. Selected, deparaffinized sections were exposed to dilute hydrochloric and acetic acids and examined for crystal dissolution. Separately, frozen sections were placed on plastic cover slips, sputter coated with silver or gold-palladium and examined with a scanning electron microscope equipped for energy dispersive X-ray microanalysis. Finally, sections of formalin fixed thyroid from adults of several species of mammals including two dogs, five cows, five sheep and five horses were also examined.

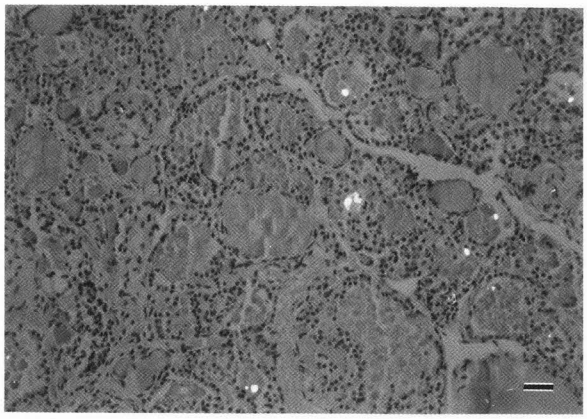


Fig. 1a. An example of thyroid graded as 1+. Bar = 100μm.

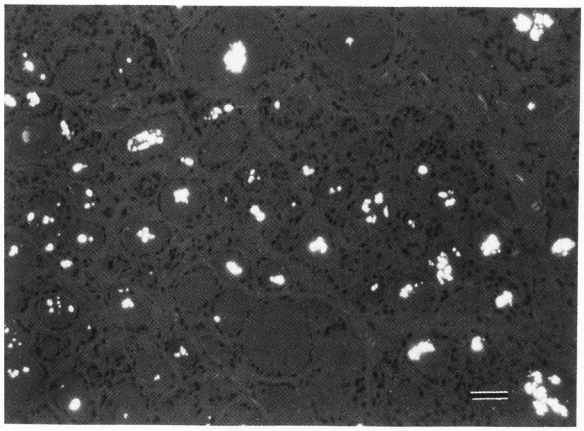


Fig. 1b. An example of thyroid graded as 3+. Bar = 100μm.

TABLE 1

AUTOPSIES: NEWBORNS/INFANTS

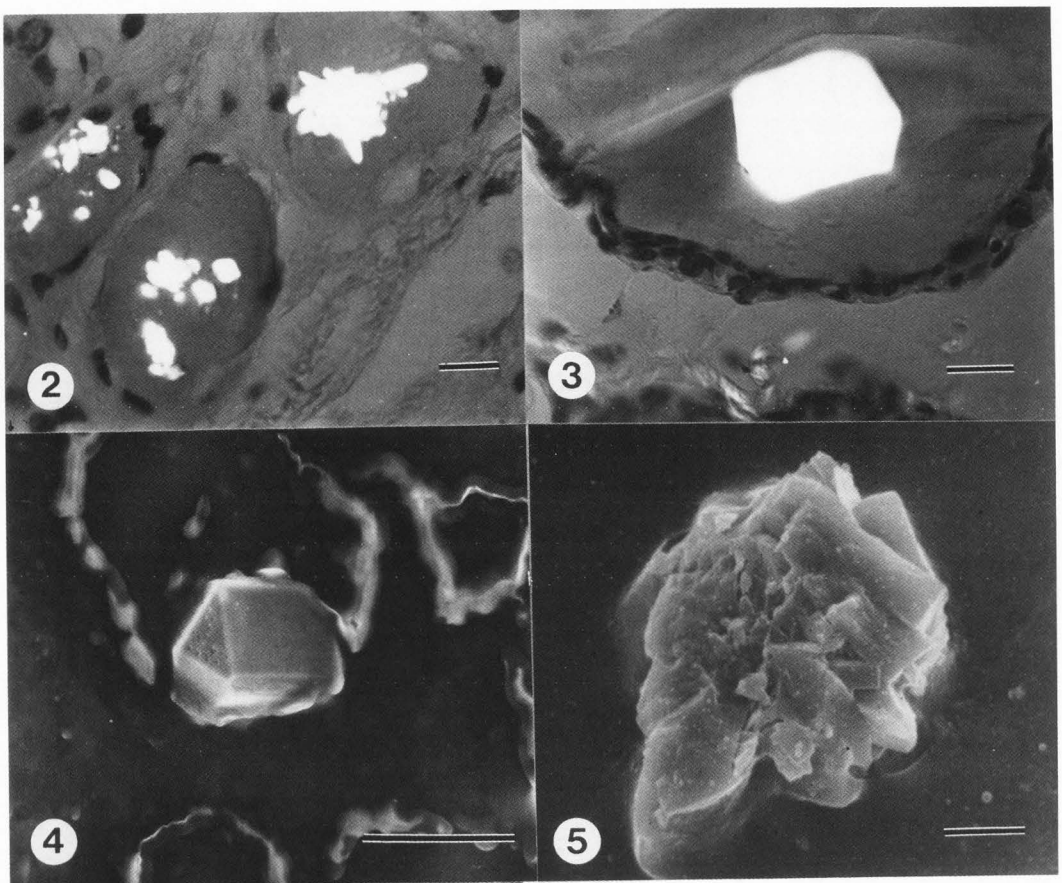
Diagnosis (# cases)	Birefringent Crystals		Sex M	Sex F	Age Range	Race W	Race B
Congenital Anomalies (9)	1/9	1+	6	3	1 day-2 1/2yrs	7	2
Diseases due to Pre/Immaturity (7)	0/7		6	1	1 day-21 mo.	4	3
Infection (3)	2/3	1+	3	0	3 days-2 yrs	3	0
Tumor (1)	1/1	1+	1	0	16 mo.	1	0

To determine the crystal density, paraffin sections of the formalin fixed thyroids were routinely prepared, stained with H & E, and examined under the low powers of a light microscope using polarized light. Sections from all series were graded as follows: no crystals present - 0, crystals present in up to 1/3 of the section -1+, crystals present in more than 1/3 but less than 2/3 of the section - 2+, greater than 2/3 involvement - 3+.

Results

Examination of adult and newborn autopsies confirmed previous reports. With one exception, a sixty year old female with rheumatic valve disease and acute endocarditis, all adult autopsies had birefringent crystals present within the colloid of the follicles. No interstitial crystals were identified. The presence of crystals was unrelated to disease state, sex, or race, and they were distributed diffusely throughout the gland. About half were graded as 1+ (Fig. 1a) and the remainder equally distributed between 2+ and 3+ (Fig. 1b). In the newborn and infant series four positive thyroids were found, all graded as 1+. In two of these, only scattered individual crystals were identified whereas the other two were more strongly involved.

In the surgical series 19/20 thyroids contained crystals. They could be identified in fresh scrapings, unfixed, and fixed and stained frozen sections. Again, the crystals were present independent of disease process, sex, or race (Table 2). In the three patients with adenoma, the one with papillary carcinoma, crystals were limited to non-neoplastic parts of the gland with the exception of one adenoma which contained crystals within the tumor. In general, the crystal grade was similar to that of the autopsy series (Table 3). Crystals in the autopsy thyroids were typical of thyroids from all series.



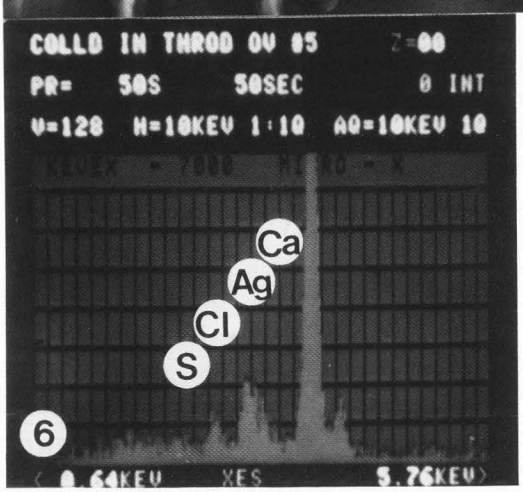


Fig. 2. Typical clusters of birefringent calcium oxalate crystals in a human thyroid. Bar = 50 μm.

Fig. 3. Highly birefringent single crystal from a human thyroid. Bar = 50μm.

Fig. 4. Single rhombic crystal from a human thyroid. Bar = 50μm.

Fig. 5. Aggregated tubular crystals from an ovine thyroid. Bar = 5μm.

Fig. 6. X-ray microanalysis spectrum of crystals from an ovine thyroid. (Ca-calcium, Ag-silver from coating, Cl-chloride from plastic cover slip, S-sulfur).

In the animals examined, crystals were present in equines and ovines (Table 3); all ovines had crystals to some degree, and in one the thyroid was heavily involved.

Highly birefringent, the crystals were present in aggregates of blunt and pointed rods and needles (Fig. 2) and there appeared to be no difference in crystal morphologies between humans and other mammals. Individual crystals often lacked the usual calcium oxalate appearance and were more rhomboid in habit (Figs. 3,4). In frozen sections the crystals were scattered within the colloid (Fig. 1b), but in paraffin embedded tissue, tended to concentrate in the periphery adjacent to follicular cells. Under scanning electron microscopy, occasional crystals had the plate like shape of calcium oxalate monohydrate (Fig. 5) while still some

TABLE 2

SURGICAL SPECIMENS

Diagnosis (# cases)	Birefringent Crystals	Sex M	Sex F	Age Range Years	Race B	Race W
Laryngectomy for carcinoma (9)	8/9	8	1	35-81	9	0
Nodular Goiter (6)	6/6	2	4	10-50	5	1
Adenoma (3) *	3/3	0	3	12-72	2	1
Lymphocytic Thyroiditis (1)	1/1	0	1	51	1	0
Papillary Carcinoma (1)**	1/1	0	1	68	1	0

*Crystals present within one adenoma
** No crystals present within the carcinoma

others had bipyramidal appearance of calcium oxalate dihydrate, but the crisp definition of crystal structure, as seen in urine (5), was lacking in thyroid. Elemental analysis of the crystals revealed only calcium (Fig. 6); magnesium and phosphorus were absent. Crystals dissolved in hydrochloric acid, but not in acetic acid.

Discussion

Richter (9), reporting his results from 928 autopsies, concluded that anisotropic crystals are found in thyroid colloid but rarely in other organs, that crystals occurred more often in older than younger individuals, that they rarely occurred in exophthalmic goiter, that the crystals, as identified by X-ray diffraction, were calcium oxalate monohydrate, and that they did not occur in "lower animals". In 1968, MacMahon (6) described essentially similar findings after examining thyroids from 500 autopsies. He paid special attention to younger age groups and determined that crystals could be found even in premature infants although the number of infants involved and the number of crystals per gland were considerably fewer as compared to adults. Furuta (1) identified similar crystals in the thyroids of a group of lepers and Schaefer (11) found crystals in 110 of 212 autopsies. Reid et. al. (7) recently reported the presence of crystals in thyroids of 79 of the one hundred consecutive autopsy specimens, from infants to 99 year olds, and suggested that the crystals appeared during childhood and increased in number with age.

In fact, the presence of intra-thyroidal crystals was described in 1877 by Zeiss, and by subsequent authors in the German and Italian literature (c.f. reference #9). In our more limited study, we have confirmed the results of previous reports. The crystals can occur as early as three days after birth and are found in increasing numbers with age, regardless of sex, race, or disease process. We were concerned that, because most previous investigations were limited to autopsy series, the finding might be due to pre-terminal or post-mortem conditions but in examining surgically removed thyroids, we found that crystals were present. Of interest, though, is that crystals were present within one of the adenomas suggesting that pathologic processes are not necessarily prohibitive to crystal production. Schaefer (11) also identified crystals in non-functioning adenomas, but not in "hyperactive adenomas". These data, along with Richter's inability to find crystals in exophthalmic goiter, suggest that

TABLE 3

CRYSTAL GRADE

Specimen Type (n)	0	1+	2+	3+
Adult Autopsies (20)	1	11	4	4
Infant Autopsies (20)	16	4	0	0
Surgical Specimens (20)	1	9	5	5
Equine (5)	2	1	2	0
Ovine (5)	0	2	2	1
Bovine (5)	5	0	0	0
Canine (2)	2	0	0	0

crystals do not form in functional, hyperplastic states. None of our patients had primary hyperplasia, thus, we were unable to investigate this point.

We found, as did others, that the shape of the thyroid calcium oxalate crystals is somewhat different from that seen elsewhere in biological systems (4,5). The sharp plates and defined rosettes of calcium oxalate monohydrate or dipyramids of calcium oxalate dihydrate are not clearly apparent. We attribute this to the colloid which appears to form a thick, viscid, and adherent layer over the crystals. Are they then truly calcium oxalate? Richter (9) found that the crystals dissolved in hydrochloric and sulfuric acids but were relatively insoluble in glacial acetic acid, a fact which we confirmed. He also showed that the crystals had identity with calcium oxalate monohydrate by X-ray diffraction and Schaefer (11) subsequently demonstrated the same finding. Reid et. al (7) isolated the crystals by Clorox digestion of the thyroid and identified them as calcium oxalate dihydrate by infrared spectroscopy and x-ray diffraction. In our studies using scanning electron microscopy and energy dispersive X-ray microanalysis, only calcium peaks were found. In our experience (5), the only biological crystals with this finding can be calcium oxalate or calcium carbonate. But the crystals were insoluble in acetic acid and soluble in hydrochloric acid indicating that the crystals indeed were calcium oxalate.

Of importance, the phenomenon appears to be a general one in that it can be found in newborns with progressive involvement in the adult human approaching 50% (9) and increasing with age, and is present in other mammals as well. In our limited series, 19/20 adults in both the autopsy and surgical series had intra-follicular crystals. Furthermore, the process, in contradiction to Richter (9), is not limited to primates. Actually Reid et. al. (7) did not find calcium oxalate crystals in thyroids of 22 primates that included an orangutan, two gibbons and various other species. Chimpanzee or gorilla thyroids were not available to them. Although we examined few animals, equines and ovines, especially ovines, had easily identifiable thyroidal crystals. Whether this reflects a division between carnivores and herbivores or one between the different herbivores requires a more extensive examination. Our point is that mammalian thyroids have an innate capacity to accumulate calcium oxalate crystals. Excluding the kidney and thyroid, calcium oxalate crystals are not found in other tissues in the absence of systemic oxalosis, (however, c.f. ref. #4). In our study, no crystals were present in the sub-maxillary glands or tracheal sub-mucosal glands of radical neck dissections, the viscera from autopsies, or parathyroid glands from parathyroidectomies. We examined no eyes.

As far as is known, oxalate is an end product of metabolism and has no known metabolic value in man. Because of the absence of oxalate degradative enzymes in man, elevated levels of oxalate result in the formation of calcium oxalate crystals, especially in kidneys, and, in the face of a severe challenge, in other tissues as well. Why then does calcium oxalate accumulate in colloid in the absence of an oxalate challenge? No specific research or evidence has been directed to this question and suggested mechanisms are speculative. Oxalate could be transported across the follicular lining, a process that would probably entail an active transport process similar to iodine as the interior of the follicular cell carries a negative charge. Because there is no known metabolic use for oxalate, this seems unlikely. Alternatively, oxalate could be produced locally within the follicle. One candidate for an oxalate precursor would be tyrosine. Even though less than 3% of oxalic acid is derived from tryptophan/tyrosine side chains (2), the presence of tyrosine in colloid represents a potential source of oxalate for the formation of crystals. In partial support of this concept, localized calcium oxalate deposits have been described in long standing retinal detachments, another tissue rich in tyrosine (4). This mechanism presumes that tyrosine side chains eliminated during the formation of the iodinated hormones would be available in colloid for the formation of oxalate. Because these reactions are generally accepted as occurring at the apical surface of the follicular cell (3), this idea has some attraction. On the other hand, both the thyroid and the eye contain ascorbic acid, the most common precursor for oxalate, and oxalate for crystal formation could be derived from that source. As far as calcium is concerned, both colloid and the epithelial cells lining the follicles of the human thyroid, are rich in calcium (11). This calcium may be involved in the formation of calcium oxalate crystals. Obviously, the topic awaits further analysis.

Acknowledgements

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Discussion with Reviewers

K.P.H. Pritzker: Is there any correlation of colloid area/volume or texture to the presence of crystals?
Authors: We noted no such correlation. Crystals were observed in small, fetal sized follicles as well as in large, distended follicles.

K.P.H. Pritzker: Is there any correlation to height of follicular epithelium, colloid scalloping or other markers of cellular activity to crystal deposition?
Authors: We found no such relationship in our material. However, none of our patients had diffuse hyperplasia and previous studies are conflicting as concerns oxalate deposition in hyperplasia. Most reports indicate absence of crystals in hyperplasia. Reid (text ref. 7), by contrast, found crystals in 28 of 30 hyperplastic thyroids although most of these had been treated, and all demonstrated "some degree of reversion to the resting colloidal state".

G.M. Roomans: Did you compare fixed and unfixed material quantitatively? Are crystals lost during fixation?
Authors: We did not do any quantitative studies. But Reid et. al. (text ref.#7) reported that frozen sections stored in buffered formalin or water began to lose visible oxalate by six hours and within 24 hours all of the oxalate was lost. They also reported that prolonged storage of bulk tissue in buffered or acid formalin did not effect crystal numbers as compared with quickly processed blocks.