ANISOTROPIC CRYSTALS IN THE HUMAN THYROID GLAND *

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Anisotropic crystals have been reported in the thyroid gland by several observers. However, only occasional reports of such material have appeared, and little is known about the nature of the crystals or their significance.

The earliest report of crystals in the thyroid gland appears to be that of Zeiss,[1] who mentioned, without further description or comment, the presence of octahedral crystals of calcium oxalate in the gland. Podack[2] mentioned various crystalline forms, among them calcium oxalate, and calcium or magnesium salts of a higher fatty acid.

Günther[3] described "crystalloids" as protein-like substances that are different from the crystals under consideration, but he also noted inorganic birefringent crystals of complicated form, soluble in hydrochloric acid. The latter, presumably, were of the type now under discussion.

Sanderson-Damberg[4] studied the thyroid glands of persons from 15 to 25 years of age, from different localities. She made the interesting observation that in those from Bern, in which there were small, uniform vesicles with vacuolated colloid, crystals of calcium oxalate were commonly found, while in thyroid glands from Kiel, Berlin, and Königsberg, in which the vesicles were large, no crystals were found.

Buscaino[5,6] studied the solubility of the crystals in various agents. He noted that they were dissolved by hydrochloric and nitric acids, and that sulfuric acid transformed them into an opaque mass. In acetic acid the crystals remained unaltered. Sodium hydroxide (40 per cent) and potassium hydroxide (50 per cent) also transformed them into granular masses that retained low-grade birefringence. The crystals were blackened by silver nitrate in the von Kossa reaction. On the basis of these reactions, Buscaino concluded that the crystals were probably calcium oxalate. He noted, however, that they differed in form from the ordinary (octahedral) crystals of that material. Buscaino's only statement regarding the relation of the crystals to disease was the suggestion that an increase occurred in cases of dementia praecox with epilepsy.

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One of us[7] has studied the occurrence of the crystals in 547 glands, and noted that they occurred in a greater proportion of the older than of the younger individuals, and in a greater proportion of normal than of abnormal glands in the same age group. It was suggested that the occurrence of the crystals is related to the functional state of the gland. Solubility studies gave results similar to those of Buscaino, except that solution occurred in dilute acetic acid. The crystals were not found in the thyroid glands of lower animals.

Martino[8] found the crystals in 22 of 30 cases of various ages. He observed that under the age of 30 years (7 cases) no crystals were found, while over that age they were present in 23 of 26 cases. They occurred in all (14) cases over the age of 50 years.

The crystals described by Popoff[9] and by Lichtman and McDonald[10] are of different nature.

Material and Methods

The preliminary studies of the senior author have been supplemented by observations of thyroid glands from 485 necropsies and a smaller number of surgically removed glands. Tissues were fixed routinely in Zenker's stock solution without addition of acetic acid, or in Zenker's-formol (Helly). The tissues were dehydrated in alcohol, embedded in paraffin, and the sections stained with hematoxylin and eosin.

Sections were examined with transmitted light, both diffuse and polarized. The presence or absence of crystals was noted by scanning the sections (often only one section of a gland) under a magnification of about 100 diameters, with crossed polarizer and analyzer. Inasmuch as crystals lying in certain directions to the plane of polarized light are invisible, as will be noted, this method may not disclose all of the crystals. Because of this fact, as well as the uneven distribution sometimes found, division of the glands into two groups, those in which crystals were found and those in which they were not, is somewhat arbitrary.

Crystals were isolated from fresh glands by placing scrapings or small fragments of tissue in physiologic saline solution, and teasing the tissue out under low magnification, with polarized light. This is most conveniently done with a binocular dissecting microscope. The crystals were removed from the colloid with dissecting needles, and placed on slides for further studies. For identification by x-ray diffraction techniques, crystals were placed in a thin-walled glass capillary tube, as will be described.

Observations

Description of the Anisotropic Material

Examination of histologic sections between crossed polarizer and analyzer revealed, in certain instances and under the conditions to be described, minute particles of material that were brilliantly lighted against a dark ground (Fig. 2). The particles were of different sizes and of various forms: some were elongated polyhedrons; others were roughly spindle-shaped; still others were in the form of large irregular plaques. The most minute particles were barely visible with a magnification of 500 diameters. Small particles often were closely grouped but ordinarily had no special mutual orientation. Occasionally, however, radial arrangement of elongated particles was observed.

Considerably larger plaques often were cracked during sectioning. There also were many particles of irregular shape and of various sizes. As the object stage was rotated, extinction was observed at points 90° from the angle of maximum transmission.

The material was unstained and practically colorless, and thus was invisible or nearly so by ordinary examination in diffuse light (Figs. 1, 3, and 5). It was visible by dark-field examination which, however, illuminated only the edges of the particles. Occasionally they might be seen, but indistinctly, by partially closing the substage diaphragm. Once the particles were located in polarized light, their presence might sometimes be detected by diffuse light. Such examination, however, did not show the particles as clearly, nor in as large numbers, as with crossed prisms. Often the particles could not be seen in diffuse light even when their presence and position had been demonstrated with polarized light.

In both fresh preparations and in sections the particles might be moved, and the larger ones fragmented, by manipulation with a probe, thus indicating that they were material substances rather than refraction effects caused by varied molecular structure of the colloid.

With crossed prisms the particles usually were white, but suggestions of interference colors sometimes were seen, the color being faintly yellowish or purplish depending on the orientation of the particle with respect to the axis of polarization. On insertion of a retardation plate (first order red) these colors changed to deep yellow or red and blue, and were reversible on rotation of the object stage through 90°.

The foregoing description applies to the particles as seen in histologic sections of fixed thyroid glands, in which they occur only within the vesicles. They may also be seen in fresh teased glands, but the presence of other anisotropic material renders observation difficult. The crystals do not occur in other organs.

Influence of Age

The influence of age was observed in the preliminary studies of one of us[7] on glands from 443 necropsies from the Presbyterian Hospital and Babies Hospital. The increase was not uniform when studied by decades, but when the age groups were made larger, the increase of incidence with advancing age became apparent. This is shown in Table I, in which the 443 cases are divided into three groups according to age. In the group up to and including the age of 30 years (85 cases), 15, or 17.6 per cent, contained crystals; in the age group from 31 to 60 years (232 cases), 93, or 40.0 per cent, had crystals; and over the age of 60 years (126 cases), crystals were found in 79, or 62.7 per cent. The probability that this result is due to chance distribution is less than 1 in 1000 (Chi-square = 85.9, N = 2, P = < .001).

TABLE I

Incidence of Crystals in Thyroid Glands in Different Age Groups

Age groups	0–30 years			31–60 years			61–90 years			
	Positive	Negative	Positive	Positive	Negative	Positive	Positive	Negative	Positive	
			%			%			%	
Presbyterian Hospital	15	70	17.6	93	139	40	79	47	62.7	443
University Hospital	8	92	8.0	106	121	46.7	86	72	54.4	485
Totals	23	162	12.4	199	260	43.3	165	119	58.1	928

A study of 485 glands from University Hospital (Table I) shows essentially the same distribution. In the cases from 0 to 30 years (100 cases), 8, or 8 per cent, contained crystals; from 31 to 60 years (227 cases), 106, or 46.7 per cent; and over 60 years (158 cases), 86, or 54.4 per cent. Again, the possibility of this being a chance distribution is less than 1 in 1000 (Chi-square = 176.8, N = 2, P = < .001). The combined Chi-square of the two series is thus 262.7, N = 4, P = < .001.

Influence of Functional State of the Thyroid Gland

Two observations clearly indicate that the occurrence of the crystals is related to the state of the gland. First, there usually was disappearance of the crystals in the diffuse hyperplasia of exophthalmic goiter, and in focal areas of hyperplasia. Second, in nodular goiters, there often were striking differences in the crystal content of different nodules. It was not unusual for some nodules to contain no crystals, while in adjacent nodules, or in the surrounding zones, crystals were present in abundance (Figs. 4 and 6).

Forty-five additional thyroid glands, removed surgically for hyperthyroidism and showing diffuse hyperplasia, were examined. Crystals were found in only five glands, in four of which there was partial involution. The crystals found were in involuted areas and were few in number. In the fifth case, only a single small crystal was observed. Forty glands had no crystals. Several sections from each of the surgically removed glands were examined, so the results are not comparable to those from glands of necropsied cases. They do show, however, the comparative rarity of crystals in hyperplasia.

Nature of the Anisotropic Crystals

By immersing slides in various solvents, the following facts were observed. The material was readily soluble in dilute acids (hydrochloric, sulfuric, acetic). It was relatively insoluble in water (cold or hot), ethyl alcohol, acetone, chloroform, xylol, diethyl ether, petroleum ether, benzene, normal butyl alcohol, and glacial acetic acid. Crystals were still present after fixation in formalin or Zenker's solution without addition of acetic acid, but fixatives containing acid dissolved them. They remained unstained by hematoxylin and eosin.

The crystals could be dissolved from the fresh glands or from sections by dilute acids, but on re-crystallization by evaporation the minute crystals obtained were not readily recognizable as of the same nature as those originally present. Attempts to remove the colloid without dissolving the crystals usually resulted in a viscid mass that prevented purification of the crystals even on long centrifugation.

A number of single, large crystals were dissected from sections and placed in a drop of distilled water on a coverslip. The water was removed by allowing the coverslip to stand in a $CaCl_2$ desiccator, after which the coverslip containing the material was placed in an incinerator. As the temperature was raised to 300° C. in 30 minutes, the crystals showed reduced birefringence. No change in shape of the crystals occurred on heating to 700° C. in 50 minutes. Similar observations were previously made by the senior author[7] by incinerating mounted sections.

Because of the presence of colloid around the crystals, it was believed that determination of their optical properties and histochemical reactions would be unreliable. Instead, the identification of isolated crystals by x-ray diffraction was attempted.* Considerable difficulty

* The x-ray diffraction studies were made by Dr. I. Fankuchen at the Polytechnic Institute of Brooklyn. We are indebted to him for his collaboration and for this description.

was encountered due to the fact that the crystals were embedded in colloid. At first, attempts were made to study the diffraction pattern of single perfect crystals, removed from sections by means of a metal dissecting needle and attached to the end of a glass fiber by using shellac. Photographs so made were unsatisfactory, as a conclusive pattern was not obtained. Therefore, thirty crystals were isolated and packed, without grinding, into one end of a thin-walled glass capillary tube. Grinding was avoided because of the danger of destroying the crystals. The capillary tube was mounted in the usual way on the arcs of a goniometer, and Debye-Scherer (powder) diagrams were obtained. The radius of the cylindric camera was 5 cm. Filtered Cu radiation was used. The spacings and intensities of the lines on the x-ray patterns were then compared with those listed in the index of the American Society of Testing Materials. The compound was readily identified as calcium oxalate monohydrate.

Discussion

Calcium is a normal constituent of cells and tissues, and also occurs in the form of pathologic calcifications and concretions. Crystals of calcium oxalate are commonly found in urinary sediments, but otherwise calcium compounds do not normally occur in crystalline form, with the exception of those in the colloid of the thyroid gland, as has been described. Oxalates, on the other hand, are toxic. They are eliminated as calcium oxalate in the urine, and are found in the renal tubules as calcium salts in oxalic acid poisoning. It is surprising, therefore, to find crystals of calcium oxalate occurring in the thyroid gland in a high proportion of all adults.

The significance of this finding will not be clear until the factors determining the formation of the crystals are known. An obvious factor in crystallization is the concentration of the substance in solution. Since histologic examination discloses only that portion of the calcium content that has crystallized, it cannot be used to indicate the total amount present. As the solubility product of calcium oxalate is small, crystallization may be expected in even very low concentrations of oxalate ion. There is no immediate explanation, however, for its occurrence in the thyroid gland.

Another factor is introduced by the occurrence of crystallization in a colloid rather than an aqueous medium. The presence of colloid not only may influence the formation of crystals, but may also alter their morphology. The possibility that the observations made are due to changes in thyroid colloid with age and disease, as well as to variations in the oxalate and calcium content of the gland, must be considered.

Summary

Anisotropic crystals occur in the colloid of the thyroid gland, but are not normally found in other organs.

There appears to be a tendency for the crystals to occur more often in older than in younger individuals.

The crystals are rarely found in exophthalmic goiter, and their occurrence in nodular goiters varies considerably. It is not uncommon to find nodules without crystals closely associated with other nodules in which crystals are abundant, or surrounded by crystal-containing vesicles of the more normal thyroid tissue. In other cases crystals appear within nodules, but not in the surrounding tissue. These findings indicate that the occurrence of the crystals is influenced by the state of the gland.

The anisotropic crystals have been identified by x-ray diffraction methods as calcium oxalate monohydrate.

It is suggested that the development and morphology of the crystals is influenced by the colloid, as well as by the concentration of calcium and oxalate ions in the secretion.

The photomicrographs were prepared by the staff of the Armed Forces Institute of Pathology.

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LEGENDS FOR FIGURES

FIG. 1. Section of thyroid gland, stained with hematoxylin and eosin, and photographed by diffuse light. The outlines of some of the crystals are seen in the acini. × 300.

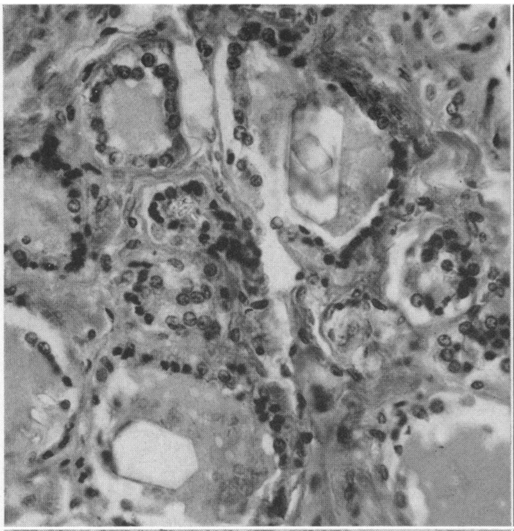
FIG. 2. Same field as shown in Figure 1, but photographed by polarized light with crossed polarizer and analyzer. The crystals are more clearly seen than in diffuse light. × 300.

FIG. 3. Edge of nodule in thyroid gland, photographed by diffuse light. × 100.

FIG. 4. Same field as seen in Figure 3, but photographed by polarized light. The polarizer and analyzer are only partially crossed, so that details of the gland remain visible. Crystals are seen only in the zone around the nodule. × 100.

FIG. 5. Edge of nodule in thyroid gland. Photographed by diffuse light. × 100.

FIG. 6. Same field as shown in Figure 5, but photographed by polarized light with polarizer and analyzer partially crossed. Crystals are seen only within the nodule. × 100.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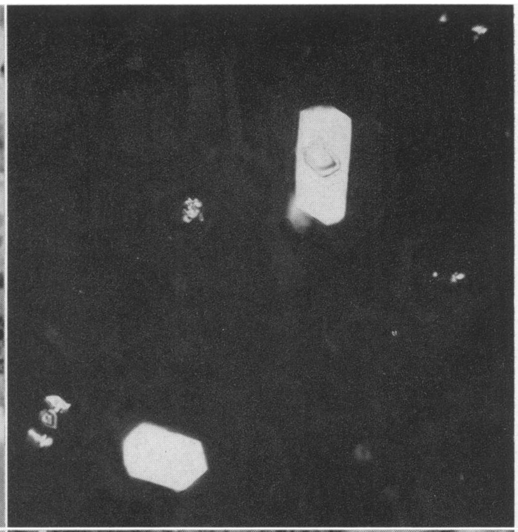
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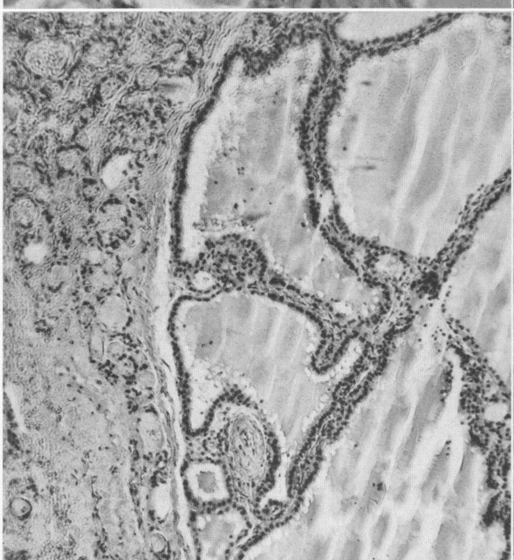
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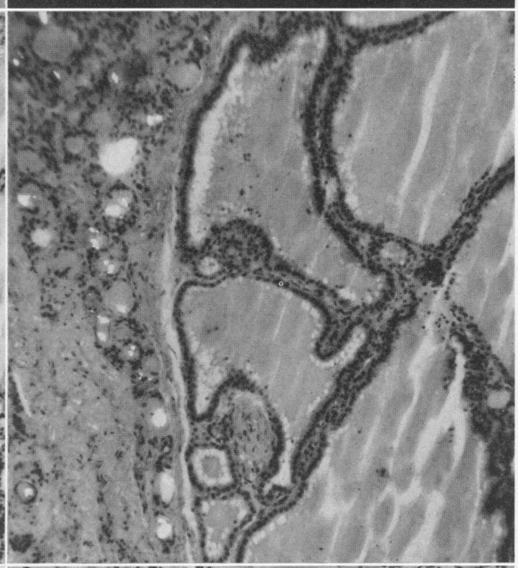
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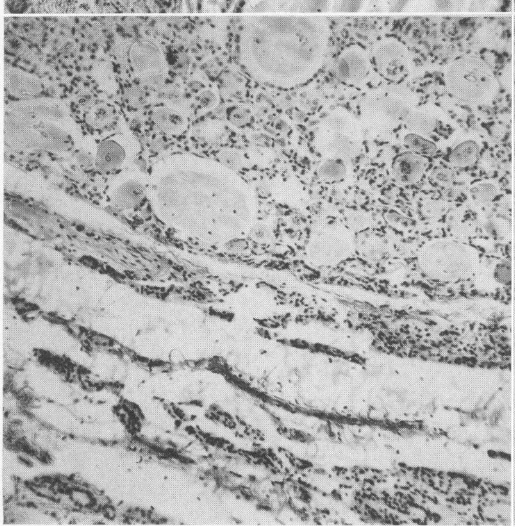
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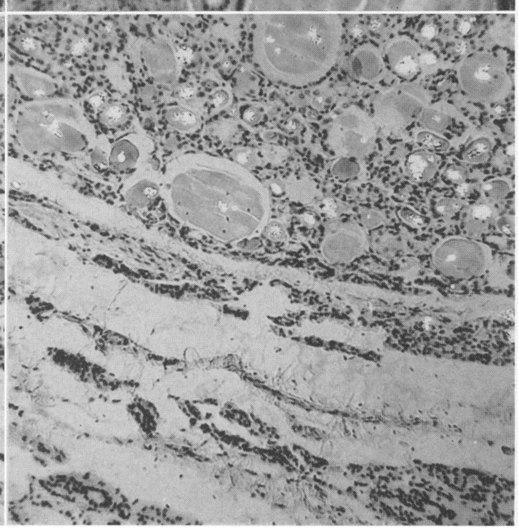
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