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Nature and significance of calcium oxalate crystals in normal human thyroid gland

A clinicopathological and immunohistochemical study

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Abstract. To elucidate the significance and nature of calcium oxalate crystals in the thyroid, we studied these crystals clinicopathologically and immunohistochemically in 182 normal thyroids from patients autopsied within 5 h of death. Under polarized light, calcium oxalate crystals showed brilliant birefringence and were invariably found within the colloid. The crystals were found in 73.1% of all cases but were more prevalent and denser in older individuals, with the highest prevalence (85.2%) being observed in those over 70 years of age. No crystals were seen in those under 10 years of age. Although underlying diseases seemed to have little influence, post-mortem delay apparently affected the prevalence and density of occurrence since the crystals tended to disappear with hours after death. An immunohistochemical study using anti-thyroid hormone antibodies revealed that the crystals were within negatively or weakly stained colloid and were not common in strongly stained colloid. These findings support the hypothesis that the occurrence of calcium oxalate crystals in normal human thyroid is associated with a low functional state of the thyroid follicles.

Key words: Calcium oxalate crystal – Thyroid gland – Immunohistochemistry – Clinicopathological study

Introduction

Oxalic acid is consumed daily with impunity in small amounts of food, yet causes serious illness or death when ingested in large amounts in pure form (Jeghers and Murphy 1945). Calcium oxalate crystals may be found in different organs as a result of either hereditary or acquired oxalosis. In the thyroid gland, these crystals have been seen in normal and diseased tissue without apparent disturbance of oxalate metabolism (Richter and McCarty 1954; MacMahon et al. 1968; Schaefer and Rentzschke 1975; Reid et al. 1987; Hackett and Khan 1988) and have been regarded as "dystrophic" oxalosis.

The prevalence of calcium oxalate crystals reported in various autopsy series varies between 41% and 79% (Richter and McCarty 1954; MacMahon et al. 1968; Schaefer and Rentzschke 1975; Reid et al. 1987; Hackett and Khan 1988). Reid and colleagues (1987) suggested that this difference may reflect variables such as age, fixation, or even the size of blocks. In addition to these factors, post-mortem changes or effects of underlying disease may be significant but no previous observations have been made regarding these problems. Some investigators (Richter and McCarty 1954; Schaefer and Rentzschke 1975) have speculated that the occurrence of the crystals is influenced by the functional state of the thyroid, but further evidence supporting this hypothesis has not been reported.

We have examined the morphological and clinicopathological features of calcium oxalate crystals in normal human thyroid glands. We studied the influences of underlying disease and post-mortem change in their prevalence and density, and the immunohistochemical relationship between the occurrence of the crystals and the functional state of the thyroid follicles.

Materials and methods

Thyroid tissues from 278 routine autopsies performed at Yamanashi Medical University between 1985 and 1991 were examined. The series was almost consecutive, and did not include patients with thyroid tumours, severe thyroiditis, or chronic renal failure with oxalosis. The specimens were all fixed in 10% buffered formalin, embedded in paraffin, and routinely stained with haematoxylin and eosin (H&E). The slides were studied by normal light microscopy and under polarized light to determine the number of thyroids with crystals and to estimate the density of the crystals. Crystal density was graded as: neg. (negative) when none were found, 1+ (mild deposits) when crystals were present in some follicles, 2+ (moderate deposits) when crystals were present in many follicles, and 3+ (severe deposits) when crystals were abundant in many follicles. One slide was examined per patient. The histological

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findings were unremarkable. The Student's *t*-test was used for statistical analysis of the pathological data.

Special stains specific for different calcium salts were employed on some of the specimens containing crystals. These included von Kossa's, alizalin red, and silver nitrate/rubeanic acid with and without acetic acid pre-treatment (Yasue 1969). Silver nitrate/rubeanic acid with 5% acetic acid pre-treatment is fairly specific for calcium oxalate, because the acetic acid dissolves calcium phosphate and calcium carbonate. A kidney with multiple calcium oxalate crystals and a thyroid with papillary carcinoma containing numerous psammoma bodies were used as positive controls for calcium oxalate and calcium phosphate, respectively.

Immunohistochemistry was performed on 20 thyroids; 10 showed marked deposition of crystals and 10 were crystal negative. Antibodies against the following markers were used: monoclonal triiodothyronine (T_3) (1:1,000; BioPacific, USA), monoclonal thyroxine (T_4) (1:1,000; BioPacific, USA), and polyclonal thyroglobulin (Tg) (1:1,000; produced in our laboratory) (Kawaoi and Tuneda 1986).

The indirect immunoperoxidase method was used for immunohistochemical studies (Nakane and Pierce 1966). Endogenous peroxidase activity was blocked by incubation with hydrogen peroxide. Then, the deparaffinized sections were incubated with the primary anti-bodies for 30 min at room temperature, the secondary antibodies were applied to the sections for 30 min at room temperature, and the peroxidase reaction was performed using 3,3′-diaminobenzidine tetrahydrochloride. The immunohistochemical preparations were counterstained with haematoxylin or methyl green.

Results

It was difficult to identify thyroid crystals by conventional light microscopy. However, under polarized light, they showed brilliant birefringence and clearly appeared as true crystals with varied shapes and sizes; They looked like plates, rosettes of rods, diamond-shaped and were occasionally loosely clustered in a ring-like fashion (Fig. 1). Nomarski interference image was also useful in identifing the crystals (Fig. 1 E) which were characteristically located within the lumina of follicles. No associated inflammatory reaction was noted. No clear morphological difference was seen between the follicles containing crystals and those without. Although other birefringent materials such as glove powder, haematoxylin crystals, and minute dust motes were occasionally seen, the morphology and distribution of these materials were different from those of the birefringent thyroid crystals.

Silver nitrate/rubeanic acid with or without pre-treatment with 5% acetic acid (Yasue's method) stained the thyroid birefringent crystals intensely to a black-brown colour (Fig. 1 F); they were not stained by von Kossa's method or alizarin red. The crystals showed the same pattern of staining as was seen in the case of renal oxalosis (Table 1). The psammoma bodies in papillary carcinoma, however, were positive for von Kossa's stain, alizalin red at pH 4.2, and silver nitrate/rubeanic acid without acetic acid pre-treatment, but were not stained after pre-treatment with 5% acetic acid.

In immunohistochemical staining overnight incubation with the primary antibodies led to an apparent decrease in the density of the crystals. Therefore, we employed a short incubation period (under 30 min) which had little influence on the number of crystals when compared with that in an H&E slide from the same block. Thyroid follicles were reacted with all three antibodies against thyroid hormones in a similar fashion, but T_3 and T_4 were more heterogeneous than Tg in their positive patterns of follicles. In T_3 and T_4 staining, the follicular cells and/or colloid substances were immunostained in a wide variety of staining patterns and intensities (Fig. 2A). We failed to recognize the apparent differences in staining between the tissues with severe deposits of crystals and the tissues with no crystals. However, in the tissue with severe deposits of crystals, polarized light microscopy revealed that birefringent crystals were more commonly located in the negatively or weakly stained colloid than in the strongly immunoreactive colloid (Fig. 2B).

The influence of post-mortem delay was examined in 140 patients over 60 years of age. Delay before au-

Table 1. Staining of birefringent thyroid crystals with a series of special stains for different calcium salts

	No. of cases	SN/RA with acetic acid	SN/RA without acetic acid	von Kossa	Alizarin red
Thyroid crystals	10	+	+	−	−
Psammoma bodies	10	−	+	+	+
Renal oxalosis	3	+	+	−	−

SN/RA, Silver nitrate/rubeanic acid

Table 2. Age distribution and incidence of calcium oxalate crystals

Age (years)	No. of glands	Incidence of crystals (%)			Crystal density			
		Total	Male	Female	Neg.	1+	2+	3+
<10	13	0.0	0.0	0.0	13	0	0	0
10–29	11	36.4	33.3	40.0	7	4	0	0
30–49	30	66.7	73.7	58.3	10	18	1	1
50–69	67	85.1	83.7	87.5	10	38	14	5
>70	61	85.2	88.2	81.5	9	36	8	8
Total	182	73.1	75.2	69.9	45	96	23	14

Crystal density was graded as: neg., absent; 1+, present in some follicles; 2+, present in many follicles; 3+, abundant in many follicles

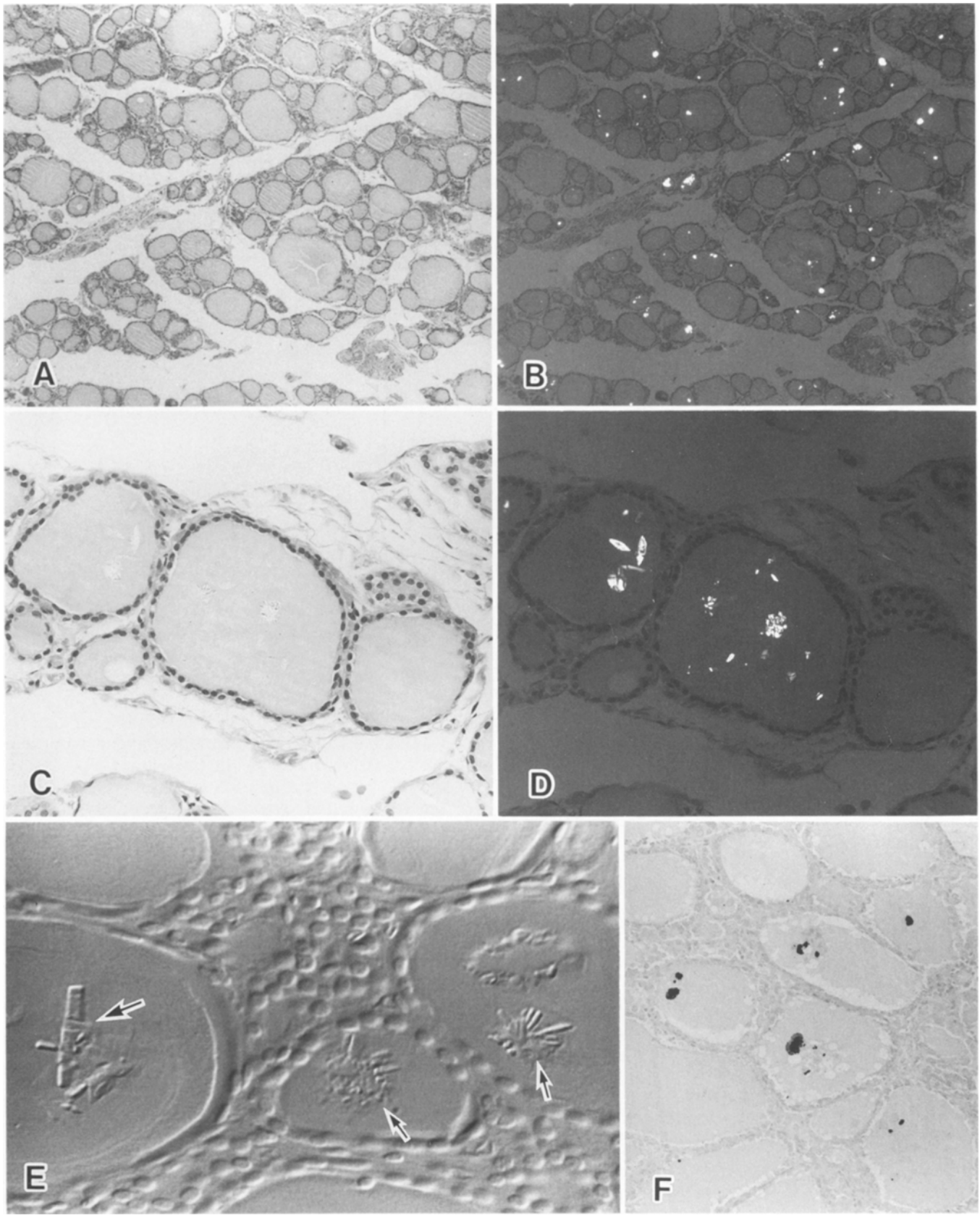

Fig. 1. **A** Light microscopic appearance of thyroid tissue. H&E, ×40. **B** Under polarized light, the same field as **A** shows many birefringent crystals within the colloid. ×40. **C** Light microscopic appearance of follicles with inconspicuous intraluminal crystals. H&E, ×200. **D** Under polarized light, the crystals are clearly identified in the same field as irregular birefringent granular or plate-like fragments. ×200. **E** Nomarski interference image shows the crystals as plates (*large arrow*) and aggregation of rods (*small arrows*) within the colloid. ×400. **F** The crystals are stained black by silver nitrate/rubeanic acid after acetic acid pre-treatment. Yasue's method, ×200

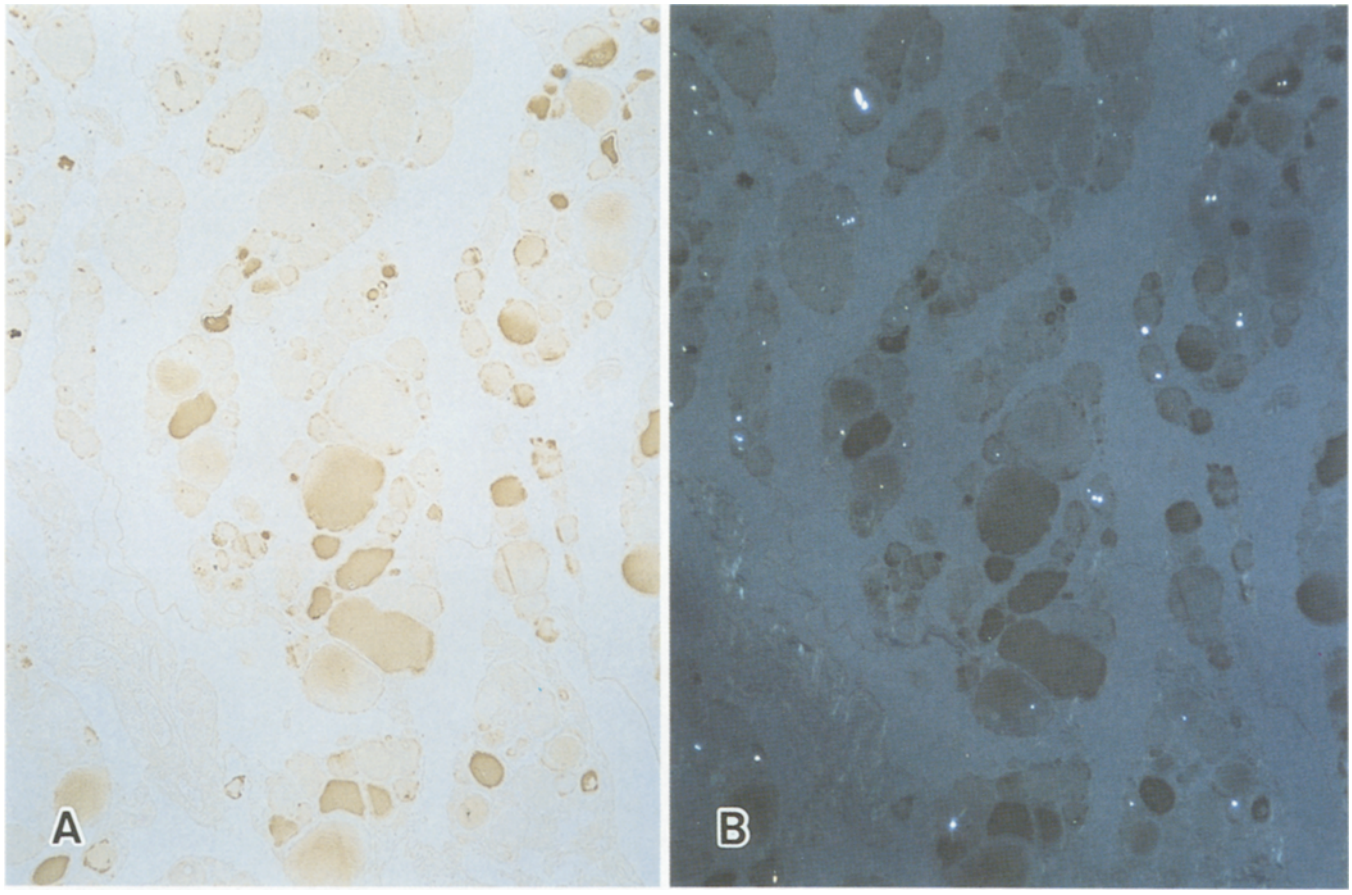

Fig. 2. **A** Immunoperoxidase staining with an anti-triiodothyronine (T_3) antibody. Follicular cells and colloid were immunostained in a wide variety of positive patterns and intensities. × 100. **B** Under polarized light in the same field as **A**, the crystals are mainly localized in the negatively or weakly stained colloid of follicles. × 100

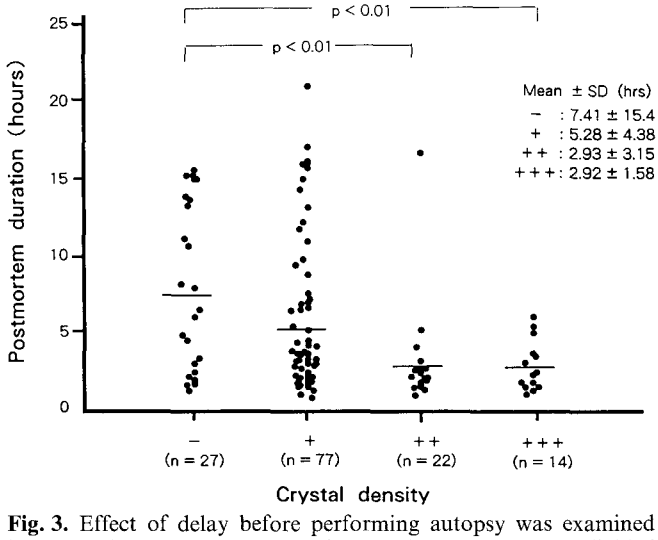

Fig. 3. Effect of delay before performing autopsy was examined in 140 patients over 60 years of age. The patients were divided into four groups according to their crystal density (*n*, number of patients, −, absent; +, present in some follicles; ++, present in many follicles; +++, abundant in many follicles)

topsy was significantly ($P<0.01$) longer in the crystal-negative group than in the moderate or severe deposit group (Fig. 3). Almost all patients in the moderate or severe deposit group were autopsied within 5 h of death.

We selected 182 thyroids from the patients who were autopsied within 5 h of death (to avoid the influence of post-mortem delay) and found that in autopsy slides of the thyroid, the overall incidence of crystals was 73.1%. No differences in the prevalence rates were evident between males and females, with crystals appearing in 75.2% of the males and in 69.9% of the females. Regarding the density of the crystals, a severe deposit was identified in 8% of all the glands we examined.

There was a tendency for an increase in the frequency of crystals with age (Table 2). A high prevalence was observed in the subjects aged 50 to 69 years and in those over 70 years (85.1% and 85.2%, respectively). In contrast, only 36.4% of the patients from 10 to 29 years had thyroid crystals, and no crystals were seen in those under 10 years of age. As for crystal density, a severe deposit was noted in the patients over 40 years of age and it was most frequent (13.1%) in the patients over 70 years of age.

We examined the influence of underlying disease in the patients over 60 years of age who were autopsied within 5 h of death. We divided them into two groups according to underlying disease: one group was composed of patients who died of malignant neoplasms including cancer, sarcoma and leukaemia and the other was composed of patients who died of other diseases including myocardial infarction, pneumonia, or rupture

Table 3. Relationship between the underlying disease and the incidence of calcium oxalate crystals

	No. of glands	Incidence of crystals (%)	Crystal density			
			Neg.	1+	2+	3+
Neoplasia	83	90.4	8	48	16	11
Others	22	77.3	5	12	3	2

Crystal density was graded as in Table 2

of an aneurysm. The prevalence and density of the crystals were slightly higher in the patients who died of malignant neoplasms than in those who died of other diseases (Table 3).

Discussion

Calcium oxalate crystals can be identified from their appearance in H&E sections under polarized light and from their patterns of staining with a series of special stains for different calcium salts (Johnson and Pani 1962; Chaplin 1972, 1977; Evans et al. 1973). In agreement with most observers, we found that the morphology and the characteristic intracolloidal localization of the crystals permitted their reliable identification despite the occasional presence of other birefringent materials such as glove powder, haematoxylin crystals, and dust motes. In addition, special stains for different salts also indicated that the birefringent crystals were calcium oxalate.

We found calcium oxalate crystals in 73.1% of normal human thyroids. This figure is very high, but not surprising, because Reid and colleagues (1987) reported a similar prevalence (79.0%). In this study, an increase in the frequency and density of crystals with age was also observed regardless of sex. Therefore, it is reasonable to regard the occurrence of thyroid crystals as a normal age-related phenomenon.

MacMahon et al. (1968) reported that crystals were present even in premature infants although the incidence and the number of crystals per gland were considerably lower when compared with those in adults. However, Reid and colleagues (1987) found no crystals in 20 subjects from 20 to 44 weeks of gestation and from birth to 90 days of age. In this study, we did not find any crystals in the thyroids of 13 patients under 10 years of age. Thus, we agree that thyroid crystals appear in childhood and increase slowly in number with age.

The reported prevalence of calcium oxalate crystals in the thyroid varies from series to series (Richter and McCarty 1954; MacMahon et al. 1968; Schaefer and Rentzschke 1975; Reid et al. 1987; Hackett and Khan 1988). It is suggested that differences in its prevalence may reflect variables such as age, fixation, or even the size of blocks (Reid et al. 1987). In addition to these factors, agonal or post-mortem changes must be considered in studies using autopsy material. Post-mortem delay was significantly longer in the crystal negative group than in the moderate or severe deposit group in our investigation leading to the suggestion that it may be one of the most important factors affecting thyroid crystals.

Underlying disease seemed to have a little influence in crystal prevalence; they were present in 90.4% of the patients who died of malignant neoplasms and in 77.3% of the patients who died of other diseases. The reason for this difference was not clear.

Calcium oxalate crystals are insoluble in water, alkali, alcohol, and other organic solvents, that they are poorly soluble in periodic acid, and are highly soluble in mineral acids (Yasue 1969; Chaplin 1972; Evans et al. 1973). However, Reid and associates (1987) noted a marked loss of crystals from deparaffinized sections that had been immersed in water for 24 h. A similar finding was obtained in our present study; overnight incubation of deparaffinized sections for immunohistochemistry led to a marked loss of crystals.

Oxalate is a metabolic end-product and has no known value to humans (Hackett and Khan 1988). Because of the absence of appropriate degradative enzymes, elevated levels of oxalate may result in the formation of calcium oxalate crystals in humans. The intracolloidal localization of the thyroid crystals indicates that their formation was initiated extracellularly, perhaps reflecting the concentration of oxalate within the colloid.

Localization to the thyroid suggests some peculiarly thyroidal mechanism, as proposed by MacMahon and colleagues (1968). Schaefer and Rentzschke (1975) identified such crystals in non-functioning adenomas, but not in "hyperactive adenomas". Richter and McCarty (1954) also failed to find these crystals in exophthalmic goitres. These investigators speculated that the occurrence of the crystals is influenced by the functional state of the thyroid gland. Therefore, we performed an immunohistochemical study to assess the relationship between the occurrence of crystals and the functional state of the thyroid follicles using anti-T_3, anti-T_4, and anti-Tg antibodies. We found that the crystals were more frequently seen in follicles with negatively or weakly immunoreactive colloid than in those with strongly immunoreactive colloid. These findings suggest that calcium oxalate crystals form within inactive thyroid follicles, and that they reflect a low functional state.

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