Scanning Electron Microscopic Studies on Parafollicular Cells of Puppies with Particular Reference to the Effect of Fixation and Cracking Methods upon Micrographs

By

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Summary. It has been known from transmission electron microscopic studies that cytoplasmic granules of parafollicular cells in the thyroid gland show different micrographic images depending on the types of fixation method used. The authors have made scanning electron microscopic observations of parafollicular cells by using various cracking methods to see the differences in micrographic images affected by different fixation methods. 1. In scanning electron microscopic observation, the arrangement of parafollicular cells showed the same distribution and orderliness as in transmission electron microscopic observation. Cytoplasm of parafollicular cells looked more coarse than that of follicular cells and suggested the translucency seen on transmission electron microscope micrographs. 2. When method (1) was used for fixation, on transmission electron microscope micrographs, cytoplasmic granules of parafollicular cells appeared as a number of well-defined black circular images. On scanning electron microscope micrographs they were observed as clearly protruded spherical granules. 3. When method (2) was used for fixation, on transmission electron microscope micrographs, cytoplasmic granules of parafollicular cells were observed as vesicles having only demarcation membranes left and its contents missing. On scanning electron microscope micrographs they were seen as flat and circular figures. 4. For an overall observation of parafollicular cells the double-fixation method employed in method (1) produced the best results. Fixation method (2) was suitable for the study of various types of membranes such as cell membrane. And fixation method (3) produced favorable results in observing granules. 5. As for cracking methods, the frozen resin cracking method using Epon 812 produced the best result. But some utilities were found in the alcohol cryofracture method and acetone cryofracture method.

The existence of parafollicular cells within the thyroid gland of mammalian animals has been confirmed by Baber (1876), Takagi (1922/23), Nonidez (1932a, b; 1933), and Raymond (1932). Embryologically speaking, these para-
follicular cells have ultimobranchial origin, and there have been many reports concerning the mergence of the ultimobranchial body into the thyroid gland and the appearance of parafollicular cells (Godwin, 1937; van Dyke, 1945; Kroon, 1958; Dumont, 1959; Aoi, 1966; Sato et al., 1966; Sugiyama, 1969, 1971; Sugiyama et al., 1969; Takagi et al., 1974).

In other instances, calcitonin, which possesses a hypocalcaemic function by antagonizing the function of parathyroid hormone, was discovered in the thyroid gland by Copp and others (1961, 1962) and Hirsch and others (1963, 1964). Ever since Foster and others (1964) advocated close relationship between parafollicular cells and calcitonin, many experimental studies have been conducted on the relationship of the two, and today the theory that calcitonin is secreted from parafollicular cells of the mammalian animals is strongly supported (Hargis et al., 1966; Matsuzawa, 1966; Pearse, 1966a, b; Matsuzawa et al., 1967; Pearse et al., 1967; Bussolati et al., 1967).

Parafollicular cells can be distinguished from follicular cells from the fact that, while follicular cells are orderly arranged along the follicular lumen, parafollicular cells exist singly or in groups along the basal border of follicular cells and never face the follicular lumen. Furthermore, when observed with a light microscope, parafollicular cells contain granules which can be stained by the silver impregnation method (Grimelius, 1968; Kameda, 1968; Solcia et al., 1969; Vassallo et al., 1971) and lead-hematoxylin method (Solcia et al., 1969). These granules are recognized on transmission electron microscope micrographs as dark spherical granules of high electron density with their diameters varying from 200 to 400 nm when double-fixed specimens by glutaraldehyde and osmium tetroxide are used. It is reported that when osmium tetroxide alone is used for fixation, these granules are seen as tubules or vesicles of low electron density suggesting the absence of their contents (Matsuzawa, 1966; Matsuzawa et al., 1967). A number of light microscopic studies (Bauer et al., 1966) and transmission electron microscopic studies on parafollicular cells (Wissig, 1962; Young, 1963; Luciano et al., 1964; Matsuzawa, 1966; Ekholm et al., 1968) have been reported by now.

As for the studies done with scanning electron microscopy, some can be found on follicular cells (Kobayashi, 1973; Nunez, 1976), but very few reports are found concerning parafollicular cells. The authors have studied varying images of parafollicular cells on micrographs apparently caused by different methods of fixation and cracking (Haggis, 1970; Lim, 1971; Nemanic, 1972; Tanaka, 1972a, b; Tanaka et al., 1973; 1974a; Humphreys et al., 1973; 1974; 1975a, b; Hama et al., 1973a, b; Tokunaga et al., 1974).

Materials and Methods

The total of 43 mongrel puppies of 1 to 2 months old were used for this study. The reason for using puppies was that parafollicular cells could be found more abundantly in them than in other kinds of animals (Stux et al., 1961; Thompson et al., 1962; Kameda, 1968; Rohr et al., 1968).

The puppies were first chloroformed and thyroid glands were extracted from the pharynx. In preparing specimens for light microscopic observation, either Bouin's fixation method or GPA fixation method was employed (Solcia et al., 1968a, b). Then, the specimens were dehydrated with alcohol, embedded in paraffin, cut into thin sections, and stained according to Grimelius' silver impregnation method (Grimelius, 1968; Solcia et al., 1969; Vassallo et al., 1971) or lead-hematoxylin method (MacConaill, 1974; Solcia
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Specimens for transmission electron microscopic observation were fixed by either one of the following two methods: (1) After an hour of prefixation in a mixture of 2.5% glutaraldehyde and 2.0% paraformaldehyde adjusted by phosphate buffer of pH 7.4, the specimens were fixed for an hour in 2.0% osmium tetroxide adjusted by Millonig’s phosphate buffer of pH 7.4. (2) Other specimens were fixed in 2.0% osmium tetroxide alone adjusted by Millonig’s phosphate buffer. The specimens fixed by one of the methods were further dehydrated with alcohol, substituted with n-BGE, and embedded in Epon 812 (Luft, 1961). Then, ultrathin sections were cut out by Porter-Blum MT-II and LKB 8800 ultramicrotomes, and they were observed with HITACHI HU-12A electron microscope.

Specimens for scanning electron microscopic observation were fixed according to one of the following three methods: The same methods as (1) and (2) for transmission electron microscopic observation and (3) a single fixation with a 2.5% glutaraldehyde-2.0% paraformaldehyde fixative adjusted by phosphate buffer. Then each group of the fixed specimens were cracked in accordance with one of the three different methods; i.e., the frozen resin cracking method (Tanaka, 1972a, b; Tanaka et al., 1973; Humphreys et al., 1973), the alcohol cryofracture method (Hamano et al., 1973a, b; Humphreys et al., 1974; 1975a, b; Lim, 1971; Tokunaga et al., 1974), and the acetone cryofracture method.

Procedures for the frozen resin cracking method were as follows: After the fixation, the specimens were dehydrated with alcohol just as the preparation for transmission electron microscopic observations, substituted with propylene oxide, embedded in Epon 812, and frozen and solidified with liquid nitrogen. After cracking the specimens were deprived of the resin with propylene oxide and critical point dried (Anderson, 1951; Horrige et al., 1969; Boyle, 1969; Tanaka, 1972c; Tanaka et al., 1974b). Then, they were either ion-etched with EIKO IB-I Ion Coater or spatter-coated with carbon, gold, and palladium (Tanaka et al., 1974b; 1976; Fujita et al., 1974) in AKASHI vacuum evaporator, and observed with AKASHI-HITACHI MINI-SEM-4 and JEOL JSM-F7 scanning electron microscopies.

The materials for alcohol cryofracturing and acetone cryofracturing were dehydrated with alcohol and acetone respectively, placed in gelatin capsules with alcohol or acetone, solidified with liquid nitrogen, and cracked. The alcohol cryofractured specimens and acetone cryofractured specimens were melted with alcohol and acetone respectively. Then the specimens were observed in the same methods as that for the frozen resin cracking specimens.

Observations

Cytoplasmic granules of parafollicular cells stained by Grimelius’ silver impregnation method were extraordinarily well-stained dark brown, and the nuclei showed Kernechtrot’s red (Fig. 1). In lead-hematoxylin stained cells cytoplasmic granules were also excellently stained dark purple, but the nuclei seemed to be missing by leaving blank spaces (Fig. 2). In either instances of staining, parafollicular cells were found among follicular cells singly or in groups but never directly bordering the follicular lumen, and were penetrating into the spaces between follicular cells (Figs. 1, 2).

Observations of parafollicular cells with transmission electron microscopy were as follows: In the cytoplasm of the specimen which had been double-fixed by method (1), a fixation method now widely used, a
variety of granules of high electron density with diameter from 200 to 400 \( \mu \)m were found. There were also well-developed mitochondria and rough-surfaced endoplasmic reticulum. Just as in light microscopic observations, parafollicular cells did not directly face the follicular lumen, they were larger than follicular cells, and the cytoplasm was translucent and light (Fig. 3). On the other hand, in the specimens prepared by fixation method (2), what was supposed to be cytoplasmic granules were found as a number of vacuolated vesicles. Nevertheless, a few relatively large granules still remained. No special features about other organelles were noted (Fig. 4).

Observations of parafollicular cells with scanning electron microscopy were as follows: In the frozen resin cracking samples which had been fixed by method (1), granules in parafollicular cells appeared to be spheres of 200 to 400 \( \mu \)m in diameter and cristae mitochondriales were also seen. But the shapes of rough-surfaced endoplasmic reticulum were hardly recognizable except for an assumption of their being there, and cytoplasm appeared rather coarse. On the other hand, in follicular cells no granules were found, cytoplasm was dense, and very few organelles were recognizable. Hence, it was easy to distinguish the two types of cells, parafollicular cells and follicular cells. Although both cells had nuclei of similar structure, the nucleus of parafollicular cells had a nucleolus and the karyoplasm consisted of fine granules (Figs. 5–8). In the case of fixation method (2), the cell membrane of parafollicular cells appeared to be elevated and boundaries with other cells were clearly recognized. A few desmosomes were also seen. Cytoplasm of parafollicular cells was coarse and could be distinguished from that of follicular cells which appeared to be refined and dense. The images of the cytoplasmic structure were very similar to those in the specimens of fixation method (1). Cytoplasmic granules of parafollicular cells were very rarely found, but they appeared to be flat and circular with little protrusion, and only a trace of demarcation membrane could be recognized. The granules in this case could be assumed to be identical with the vesicles seen on transmission electron microscope micrographs in the case of fixation methods (2). Mitochondria also looked flat. The structure of the nuclei in parafollicular cells could be clearly examined since the nuclei were protruded. But the distinction between the nuclei and cytoplasm was very clear in follicular cells (Figs. 9, 10). In the case of fixation method (3) parafollicular cells appeared to be the same as that seen in method (1). There were many spherical granules in the cytoplasm which appeared to be coarse, and no organella could be recognized. Follicular cells also appeared to be similar to those in method (1) (Figs. 11, 12).

As for the alcohol cryofracture method, in fixation method (2), the results were quite similar to those of frozen resin cracking method and neither method seemed to be any more superior to the other. Some images resembling rough-surfaced endoplasmic reticulum were seen, but their identities were not certain. Karyoplasm appeared rather coarse compared with that in the case of the frozen resin cracking method (Fig. 13). In the specimens fixed by method (3), distinctions between follicular cells and parafollicular cells were clear as in the case of the frozen resin cracking method since there were many spherical cytoplasmic granules in parafollicular cells. But neither in this case, no organella could be recognized. On magnified micrographs the granule surface appeared protruding and spherical. In follicular cells both
cytoplasm and nuclei looked flat and were not very obvious just as in the frozen resin cracking specimens (Figs. 14, 15).

In the acetone cryofracture method, sectional surfaces presented by fixation methods (1) and (3) appeared rather coarse. Of the three methods, method (1) produced the best results. Cytoplasmic granules of parafollicular cells were spherical and cells cracked along the nuclear membrane were often observed and some nuclear pores were also seen (Fig. 16). In fixation method (2), the sectional surface looked rather flat lacking in tridimensionality.

**Discussion**

The earlist report on cytoplasmic granules in parafollicular cells was made by Nonidez (1932a, b) who had employed Cajal's silver impregnation method of nerves. He then noted a number of argyrophilic granules throughout the cytoplasm. Later, Azzali (1964), Pearse (1966a), Matsuzawa (1966), Matsuzawa and others (1967), and Stoeckel and others (1967a, b) have also made reports on the granules. However, hematoxylin eosin staining, Heidenhain's iron-hematoxylin staining, periodic acid-schiff staining, and Cajal's silver impregnation, all of which are widely used for making distinctions between parafollicular cells and follicular cells today, present many problems. Thus, Kameda (1968), for example, made some improvements on Davenport's silver impregnation method (1930) and made detailed reports on the numbers and other features of parafollicular cells today, present many problems. Later it was reported that Grimelius' silver impregnation method (1968) and MacConaill's lead-hematoxylin staining (1947) were useful for detecting cytoplasmic granules; i.e., for distinguishing parafollicular cells (Solcia et al., 1969; Kameda, 1970; 1971a, b; 1974; Vassallo et al., 1971). When parafollicular cells are stained by Grimelius' silver impregnation method, cytoplasmic granules turn into black. When they are stained with lead-hematoxylin, they turn into dark purple. In this way, parafollicular cells can be distinguished from follicular cells. The reactions of parafollicular cells to staining also depend on solutions used for fixation; furthermore, it is reported that different result can be obtained by adding a special treatment in the staining process (Solcia et al., 1968a, b). This is considered to be attributable to the chemical composition of the cytoplasmic granules.

There have been many reports on transmission electron microscopic observations of parafollicular cells. Like in light microscopic observations, follicular cells are reported to be arranged orderly along the follicular lumen while parafollicular cells are found on the basal border of follicular cells never facing the lumen. Parafollicular cells possess relatively larger nuclei and cytoplasm than follicular cells, and their cytoplasm is translucent and extremely light containing a number of spherical granules of 200 to 400 nm in diameter. These granules appear dark and black when method (1) is used for fixation (Matsuzawa, 1966; Aoi, 1966; Bauer et al., 1966; Matsuzawa et al., 1967; Stoeckel et al., 1967a, b; Kameda, 1968, 1970; Ericson, 1968; Young et al., 1968; Murakami, 1970) and they present vesicular shapes when method (2) is used for fixation (Matsuzawa, 1966). Thus, Sato and others (1966) report these granules as vesicles. It has also been reported that, as in the case of light microscopic observations, the granules show different reactions to staining when different methods of fixation are employed (Vassallo et al., 1971).

The authors made both light microscopic observations and transmission
electron microscopic observations of parafollicular cells in the thyroid gland of puppies and were able to obtain the same result as what had already been reported on the methodological questions of fixation and staining. By referring to the result of those observation, comparisons were made through scanning electron microscope on the appearances of parafollicular cells affected by different methods fixation and cracking.

Although some reports have already been made on the scanning electron microscopic observations of the thyroid gland, for example, the ones by Kobayashi (1973) with the focus on follicules and by Nunez (1976) on cilia, nothing is noted on parafollicular cells. They used dog just as the authors did and report as follows: Follicles exist independently in the thyroid gland and are either spherical or oval. When seen from the angle of the follicular lumen follicular cells presents a hexagonal column-like shape. At the cellular surface bordering the lumen some microvilli, central fibrils, and spherical protuberances can be observed. Although the authors' study was focused on parafollicular cells, follicular cells were also examined and quite similar results as the previous reports could be obtained even though the preparation methods were not the same.

The observation results of the differences in fixation methods are as follows: Since the solution for fixation method (2) is that of a heavy metal, it has an advantage of increasing the conductibility of the tissue as a whole and preventing the building-up of charges. But it lacks in permeability and a uniform fixation is difficult unless specimens are small. Murakami (1973, 1974) devised a technique of metal impregnation (electrified staining) to remove the weaknesses, and Tanaka (1972a, b), and Tanaka and others (1973) report it is also possible to obtain favorable results by selecting appropriate cracking methods. In our observation, in the case of fixation method (2), very few cytoplasmic granules were seen regardless of the cracking method. Only relatively large granules were found. They were flat and only recognizable as granules from the fact that demarcation membranes were slightly raised. This agrees with the comparative transmission electron microscopic observations of method (2) specimens to the fact the granules are very rare and they look like hollow vesicles. Mitochondria also looked flat, but they were distinguishable from the granules from their length. Cytoplasm generally showed a meshy pattern as seen on transmission electron microscope micrographs. Follicular cells possessed uniform and plain nuclei and cytoplasm, thus making it easy to distinguish from parafollicular cells. Cellular membranes were clearly raised and desmosomes were also observed. The nuclei looked elevated like plateaux, but no differences were found between parafollicular cells and follicular cells. On the three, this fixation method revealed the most detailed picture of karyoplasm. Generally speaking, the characteristic of method (2) is that it presents membranes very clearly.

Fixation method (3) is most widely used today in the preparation of scanning electron microscope specimens since it is less expensive and easier to handle than methods (2). Although this method tends to build up charges easily, it can be prevented by a process of coating. In method (3) cytoplasmic granules of parafollicular cells were seen as spheres presenting a clear tridimensionality. A number of minute vacant spaces were seen in the cytoplasm and presented a meshy and coarse appearance suggesting the translucent appearance observed with
transmission electron microscopy. Neither in this method nor in method (2) no distinctions were found between the nuclei of parafollicular cells and follicular cells. Follicular cells could be easily distinguished from parafollicular cells since their nuclei and cytoplasm were uniform and flat.

The significance and importance of the double-fixation method employed in method (1) reveals a lack of explanation. This practice seems to be followed simply because it is usually done for transmission electron microscope specimens. However, method (1) produced favorable result since it presented a clear contrast and osmium tetroxide helped to reduce the building-up of charges. The specimens seemed to be more fragile than those of method (3) and required a careful handling until the completion of solidification. But cracking was easy and no irregularity in fixation was found. In fixation method (1) cytoplasmic granules appeared as well-defined tridimensional spherical protuberances, and mitochondrial cresta (Kurahashi, 1969; Tanaka, 1972a, b; Tanaka et al., 1973) and figures resembling rough-surfaced endoplasmic reticulum were also observed. Like in other fixation methods cytoplasm of follicular cells appeared dense and showed uniformity just as the nuclei did, and it was easy to distinguish follicular cells from parafollicular cells. The total picture of parafollicular cells suggested the translucency of the cells observed with transmission electron microscopy. Just as in other methods, no differences were found between the nuclei of follicular cells and parafollicular cells. But, while in method (2) the nucleus as a whole was raised like a plateau, in method (1) only the nuclear membrane was raised and the karyoplasm itself was depressed. This depressed portion looks meshy resembling the picture of cytoplasm in method (2). What appeared to be nucleoli were also observed. Although nucleus and cytoplasm of follicular cells were more easily recognized than in other methods because nuclear membranes were raised, it was as difficult to make detailed examinations of cellular structures as in other methods. Although in method (2) nuclear membranes and cell membranes had well-defined outlines and cellular granules appeared flat, in method (1) the membranes appeared rather ambiguous and the granules had tridimensionality.

Based on the above observations, it can be concluded that fixation method (1) used for the preparation of specimens for transmission electron microscopic observation is most suitable for the preparation of parafollicular cells specimens to be cracked for scanning electron microscopic observations. In method (1), when viewed from the angle of follicular lumen, hexagonally-shaped follicular cells, microvilli on the cell surface, and central fibrils were recognized just as Kobayashi (1973) observed when he used the cracking method using Cemedine (Tanaka, 1972a). But no cracked section of follicular cells suitable for the observation of organella was obtained in this case, though that of parafollicular cells was obtained.

The following comparisons can be made on various methods of cracking: According to Tanaka (1973), and Tanaka and others (1976) even an inexperienced researcher can easily succeed in frozen resin cracking method. It will provide him with good specimens having rough sectional surfaces. However, he reports that it takes relatively a long time for resin to penetrate into the tissue and to be deprived, and handling of apparatus gets to be cumbersome since they become soiled easily. As for the alcohol cryofracture method, it is reported that the permeability and the rate of deliquescence of the substance are high, the process is quick, and apparatus do not become soiled.
too badly. But the chances of succeeding in cracking are relatively small. Reporting on the acetone cryofracture method, Hotta and others (1976) notes that there is a tendency for cells to be cracked at their boundaries, thus making it suitable for the observation of vascular systems.

Our study produced the following result: The chances of getting good cracking were extremely high in frozen resin cracking method as had been reported by Tanaka and others (1973, 1976). The entire handling should not be cumbersome if researchers are well-experienced in preparing transmission electron microscope specimens. For studying parafollicular cells the frozen resin cracking method can be considered the best. In the alcohol cryofracture method images which could possibly be those of artificially produced crystals were often see (Tanaka et al., 1973). But the results were overall favorable. The acetone cryofracture method showed fragileness in the process of solidication, and there was a tendency of cracking in large units (Tanaka et al., 1973). Although sectional surfaces were coarse, relatively good results were obtained when fixation method (1) was used. Since there is also a tendency to crack along the nuclear membrane revealing some nuclear pores, this method could be considered beneficial for the observation of nuclei.

In sum, images of parafollicular cells on light microscope and transmission electron microscope micrographs changed along with substances used for fixation. Images on scanning electron microscope micrographs also changed in the same fashion. Especially the images of cytoplasm and cytoplasmic granules on transmission electron microscope and scanning electron microscope micrographs suggested certain common regularities. Differences in cracking method also produced some variation on micrographs. Specimens from the frozen resin cracking method which had been double-fixed [method (1)] and embedded in Epon 812 produced the most excellent scanning electron microscope micrographs. But depending on the portions to be observed and the purposes of the observation, it would be necessary to select the method of cracking. According to previous reports on light microscopic and transmission electron microscopic studies, cytoplasmic granules, to which we have paid our most attention, can be considered to contain calcitonin and 5-hydroxytryptamine (Copp et al., 1962; Hirsch et al., 1963; Pearse, 1966a, b; Ericson et al., 1970). It can be considered that the granules observed with scanning electron microscopy also have the same contents and functions as those observed with transmission electron microscopy since they showed the same changes in images on scanning electron microscope micrographs as transmission electron microscope micrographs.

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References

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PLATES
Explanation of figures

Key to Abbreviations used in Figures

FC: Follicular cell
FL: Follicular lumen
N: Nucleus
PC: Parafollicular cell
⇒: Cytoplasmic granule of parafollicular cell
⇒: Tube or vesicle like cytoplasmic granule of parafollicular cell

Plate I

Fig. 1. Light microscope micrograph of parafollicular cells in the puppy thyroid gland stained with Grimelius' silver impregnation method by Bouin's fixation. Parafollicular cells are filled with stained argentophil granules. ×200

Fig. 2. Light microscope micrograph of parafollicular cells in the puppy thyroid gland stained with lead-hematoxylin method by GPA fixation. Only parafollicular cells are blackened. ×200

Fig. 3. Transmission electron microscope micrograph of the puppy thyroid gland. Notice numerous small dense secretory granules in the cytoplasm of parafollicular cells double-fixed with 2.5% glutaraldehyde-2.0% paraformaldehyde and 2.0% osmium tetroxide. Staining: uranyl acetate and lead. ×4,000

Fig. 4. Transmission electron microscope micrograph of the puppy thyroid gland. Notice numerous clear vacuolated secretory vesicles in the cytoplasm of parafollicular cells fixed with 2.0% osmium tetroxide alone. Staining: uranyl acetate and lead. ×12,500
Plate II

Figs. 5, 7. Scanning electron microscope micrograph of the puppy thyroid gland cracked with frozen resin cracking method after 2.5% glutaraldehyde-2.0% paraformaldehyde and 2.0% osmium tetroxide fixations. Fig. 5. ×4,800, Fig. 7. ×7,000.

Figs. 6, 8. High magnification of the parafollicular cells in Figures 5 and 7. A view of many clearly protruded spherical secretory granules. Fig. 6. ×9,600, Fig. 8. ×14,700.
Plate II

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Plate III

Fig. 9. Scanning electron microscope micrograph of the puppy thyroid gland cracked with frozen resin cracking method after 2.0% osmium tetroxide fixation. ×5,500

Fig. 10. High magnification of the parafollicular cells in Figure 9. A view of many flat and circular secretory granules. ×7,000

Fig. 11. Scanning electron microscope micrograph of the puppy thyroid gland cracked with frozen resin cracking method after 2.5% glutaraldehyde-2.0% paraformaldehyde fixation. ×5,000

Fig. 12. High magnification of the parafollicular cells in Figure 11. A view of cytoplasm containing a number of spherical secretory granules appears coarse. ×7,400
Plate IV

Fig. 13. Scanning electron microscope micrograph of the puppy thyroid gland cracked with alcohol cryofracture method after 2.0% osmium tetroxide fixation. All the cells appeared rather coarse compared with those in the case of the frozen resin cracking method. 
×9,000

Fig. 14. Scanning electron microscope micrograph of the puppy thyroid gland cracked with alcohol cryofracture method after 2.5% glutaraldehyde-2.0% paraformaldehyde fixation. 
×6,000

Fig. 15. High magnification of a parafollicular cell. A view of many protruded spherical secretory granules.

Fig. 16. Scanning electron microscope micrograph of the puppy thyroid gland cracked with acetone cryofracture method after 2.5% glutaraldehyde-2.0% paraformaldehyde and 2.0% osmium tetroxide fixation. A view of many spherical granules and nuclear pores. 
×16,000