DEMONSTRATION OF INTRACELLULAR CANALS IN THYROID GLAND CELLS OF RATS WITH HIGH IODIDE INTAKE

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It is the present acceptance in general that the level of dietary iodine is of paramount importance for the proper working of the thyroid gland. Remington low iodine diet is much used for studies on iodine deficiency in rats. This diet induces the hyperactive state of the thyroid gland, which is proved by the increase of $^{131}$I uptake and thyroid-serum radioiodide concentration ratio (T/S ratio) or by the change of the histological feature (Rosenberg et al., 1963), whereas the high iodine diet inhibits the release of thyroid hormone (Wolff-Chaikoff effect) and induces hypofunction of the thyroid gland (Miani, 1956). It is general consideration that hypofunction of the thyroid gland due to prolonged and excessive intake of potassium iodide takes place as the results of the decrease of hormone release, inhibition of hormone synthesis and of organic binding of iodine (Yamada et al., 1963; Frey, 1964).

Morphological changes in the thyroid gland of rats due to various kinds of diets and the variations in administration of iodine were reported by Hägmüller (1958). He pointed out that low iodine diets led to signs of cell activation and the formation of parafollicular cells and that the administration of a large amount of iodine in drinking water was unable to prevent the activation of the thyroid gland, without goitrogenic response, in rats fed with low iodine diets, whereas high iodine dosage combined with a normal diet did not lead to changes in the thyroid gland. Few reports have been presented concerning the ultrafine structures of thyroid gland cells of rats fed with high iodine diets. Feldman (1961) investigated electron microscopically the changes of thyroid gland cells during the iodine deficient period. Milcu, Lupulesen and Petrovici (1963), Lupulescu and Petrovici (1964) studied the ultrastructural changes in the goitre induced by the low iodine diet in rats.

In the present investigation, the particular big canals in the thyroid gland cells of rats given drinking water containing a large amount of iodine with a normal diet have been successfully observed in detail.

MATERIALS AND METHODS

In this investigation, 77 adult male Wistar-Imamichi rats, weighing 120 to 350 g, corresponding with 60 to 130 days of age, were used. All animals had been fed with the original compound diet* prepared in our laboratory since the weaning period. The experimental animals

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* wheat (7); corn meal (4); soybean meal (1); fish meal (1).
were divided into five groups as shown in Table 1. The animals in Groups I, II and III were fed with CE2 normal pellet diet** prepared in Central Laboratory of Experimental Animals during the experiments. The animals in Group IV and V were fed with the compound diet before and successively during the experiment. All animals had been given tap water ad libitum before the experiment. In the distilled water given to Group I and IV 200 ppm of sodium iodide was dissolved, and to Group II 1-2 ppm, but the animals in Group III and V were given just distilled water without iodine ad libitum during the experiment. The daily average amount of the drinking water consumed by each rat was 25 to 30 cc. The experiments ranged over 5, 15 and 60 days respectively. Three of rats in the respective subgroups of 5 and 15 days in Groups I, II and III were used for the measurement of thyroid/serum radioiodide concentration ratio by the method of Vanderlaan and Vanderlaan (1947).

### Table 1. Experimental conditions

<table>
<thead>
<tr>
<th>Exp. group</th>
<th>Diet</th>
<th>Water supply</th>
<th>Total number of animals in each group</th>
<th>Number of animals in each duration subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CE2</td>
<td>200 ppm l</td>
<td>31</td>
<td>16 for 5 days</td>
</tr>
<tr>
<td>II</td>
<td>CE2</td>
<td>1-2 ppm l</td>
<td>18</td>
<td>8 for 15 days</td>
</tr>
<tr>
<td>III</td>
<td>CE2</td>
<td>dist. water</td>
<td>19</td>
<td>7 for 60 days</td>
</tr>
<tr>
<td>VI</td>
<td>compound diet</td>
<td>200 ppm l</td>
<td>4</td>
<td>7 for 5 days</td>
</tr>
<tr>
<td>V</td>
<td>compound diet</td>
<td>dist. water</td>
<td>5</td>
<td>6 for 60 days</td>
</tr>
</tbody>
</table>

**Light microscopy:** After weighing of the unilateral thyroid glands, the materials were fixed with Levi's fixative, and the paraffin sections 3 μ in thickness were treated with periodic acid-Schiff's reagent (PAS) with the counterstain of iron hematoxylin (M. Heidenhain). The mean value of cell-height index of 100 follicular gland cells selected at random was measured histometrically by the procedure of Uotila and Kannas (1952). These cells were located exclusively in the central area of the gland where the small follicles were concentrated. The maximal and minimal diameter in each of 25 central follicles selected at random were measured and the average value was calculated.

**Electron microscopy:** The small pieces of the contralateral thyroid glands of rats were fixed with 2 % osmium tetroxide in phosphate buffer solution (Millonig, 1961) for 2 hrs. The tissues dehydrated with ethanol were embedded in epon resin (Luft, 1961) and sectioned with Porter-Blum microtome. The ultrathin sections were stained with lead hydroxide (Millonig, 1961; Reynolds, 1963). The specimens coated with carbon were observed by Akashi Tronscope 60 B electron microscope.

**RESULTS AND DISCUSSION**

**Changes in organ/body-weight ratio**

** protein (24.6%); fat (3.8%); fiber (5.3%); carbohydrate (50.5%); mineral (6.0%); vitamins.
No significant change was observed in the weight of the thyroid gland per 100 g body-weight throughout the experimental groups (Table 2). So far as the present experimental condition of iodine intake is concerned, changes in weight of thyroids were not consistent to evaluate any tendency.

### Table 2. T/S ratio and thyroid weight

<table>
<thead>
<tr>
<th>Duration</th>
<th>Group no.</th>
<th>Number of rats</th>
<th>Amount of added iodine (I)</th>
<th>Mean value of thyroid weight (mg)</th>
<th>T/S ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 days</td>
<td>I</td>
<td>3</td>
<td>none</td>
<td>21</td>
<td>63.3</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3</td>
<td>2 ppm</td>
<td>18</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>3</td>
<td>200 ppm</td>
<td>20</td>
<td>7.6</td>
</tr>
<tr>
<td>15 days</td>
<td>I</td>
<td>3</td>
<td>none</td>
<td>17</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3</td>
<td>2 ppm</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>3</td>
<td>200 ppm</td>
<td>18</td>
<td>10</td>
</tr>
</tbody>
</table>

2. **Thyroid/serum radioiodide concentration ratio**

As shown in Table 2, in rats in Group I which had been fed with CE2 and given the distilled water for 5 and 15 days, T/S ratio was elevated. The CE2 diet used in the present experiment is considered possibly to contain such a normal level of iodine, 1-3 ppm, as Kawada (1964; personal communication) suggested. Nevertheless T/S ratio was usually higher. It is considered that the reason might be because the thyroid cells of rats bred with the Japanese ration, which may contain relatively high level of iodine, become sensitive to iodine uptake when the ration given to the rats was changed to such “low” level iodine diet as CE2. In Group II given 2 ppm of iodine, the T/S ratio was kept in standard level (approximately 30), whereas in Group III given 200 ppm of iodine it was low. This indicates that a large amount of iodine might inhibit the uptake of iodine by the thyroid cells.

3. **Cell-height index and the size of follicles**

As Table 3 indicates, 5-day-subgroups only have a tendency that the cell-height index of the thyroid gland of rats becomes lower when they were given higher amount of iodine, but other subgroups not. The maximal and minimal diameter of follicles obviously increased in general with the additional supply of iodine. Such an increase in size of follicles was common with 5, 15 and 60 days subgroups. The size of follicular lumen was calculated by the formula, \(1 - 2 \times \text{ch/ma. d. + mi. d.}/2\). The value in the 5-day-subgroups (Table 3) exhibited an increasing gradient on luminal size; the lumen tended to be enlarged with the advancing dosage of iodine. This augmentation is compatible with the increasing gradient on the maximal diameter of follicles in the 15-day-subgroups.
Table 3. Cell-height index and follicle size

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Diet, amount of added I⁻</th>
<th>Duration (days)</th>
<th>Number of animals</th>
<th>Cell height (µ)</th>
<th>Follicle size (µ)</th>
<th>2(ch⁻¹mi.d. + ma.d.)⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CE₂</td>
<td>5</td>
<td>3</td>
<td>11.2±1.9</td>
<td>28.1±5.8</td>
<td>39.1±7.8</td>
</tr>
<tr>
<td></td>
<td>none I⁻</td>
<td>15</td>
<td>3</td>
<td>11.8±2.1</td>
<td>28.2±5.5</td>
<td>39.9±7.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>2</td>
<td>12.2±2.2</td>
<td>33.7±6.4</td>
<td>46.4±9.1</td>
</tr>
<tr>
<td>II</td>
<td>CE₂</td>
<td>5</td>
<td>3</td>
<td>9.4±1.9</td>
<td>32.3±6.3</td>
<td>42.3±6.6</td>
</tr>
<tr>
<td></td>
<td>2 ppm I⁻</td>
<td>15</td>
<td>3</td>
<td>11.1±1.8</td>
<td>36.5±6.8</td>
<td>46.2±7.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>1</td>
<td>10.1±2.0</td>
<td>29.1±6.8</td>
<td>41.3±7.8</td>
</tr>
<tr>
<td>III</td>
<td>CE₂</td>
<td>5</td>
<td>3</td>
<td>9.5±1.3</td>
<td>31.2±6.2</td>
<td>42.6±7.6</td>
</tr>
<tr>
<td></td>
<td>200 ppm I⁻</td>
<td>15</td>
<td>3</td>
<td>10.2±1.8</td>
<td>36.8±6.1</td>
<td>46.5±7.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>1</td>
<td>12.3±2.0</td>
<td>33.2±6.3</td>
<td>46.3±7.3</td>
</tr>
<tr>
<td>IV</td>
<td>Comp. diet none I⁻</td>
<td>5</td>
<td>3</td>
<td>11.4±1.7</td>
<td>30.8±7.1</td>
<td>43.9±7.7</td>
</tr>
<tr>
<td>V</td>
<td>Comp. diet 200 ppm I⁻</td>
<td>5</td>
<td>3</td>
<td>11.3±1.8</td>
<td>37.0±6.7</td>
<td>46.6±7.4</td>
</tr>
</tbody>
</table>

*ch: cell height, ma.d.: maximal diameter of follicle, mi.d.: minimal diameter of follicle
4. Light microscopic observation

In rats of Group IV, given the compound diet successively during the experiment, the epithelium of follicles became moderately high, and the cytoplasm bulged into the lumen (Fig. 1). Many follicles included irregularly shaped and uneven lumens full of colloid substance; the cytoplasmic projections suggesting the apocrine secretion were sometimes seen in the lumen, and circumference vacuoles were found frequently in adherence to the edge. Large intracellular vacuoles often arose in the apical part of the cell-body and were sometimes accumulated there. The thyroid cells usually pale, contained scanty of colloid droplets. Many parafollicular cells intervening among the epithelial cells or making a cell-sprouting outside the follicles were stained diffusively red with PAS and dark shadowy. They contain neither vacuoles nor PAS-positive granules and had not the character of “light cell” denominated by Young and Leblond (1963).

In Group I given CE₂ and distilled water, the findings were different in response to the duration days of feeding with CE₂ in subgroups. In the 5-day-subgroup, there were many follicles in the gland and their constituent cells were commonly small in size (Fig. 2).
The interfollicular connective tissues were abundant. These findings imply the proliferation of follicles, because the small immature follicles contained either pseudolumen or small cavity, and sometimes elongated themselves or bulged. In the 5-day-subgroup, the circumference vacuoles, apocrine secretory processes, intracellular vacuoles and colloid droplets failed to be detectable, but in the 15- and 60-day-subgroups the epithelium of thyroid follicles became taller and numerous vacuoles and a few colloid droplets came to arise in the cytoplasm. This pattern resembles a slightly goitrogenic response, or it is likely to be hyperfunctional (Fig. 3); the luminal colloid was less in stainability to iron hematoxylin and had a strong affinity to PAS. Parafollicular cells almost disappeared and could not be distinguished from the common gland cells. It was observed easily that there was a contrary pattern between Figure 2 and Figure 3.
In rats in the 5-day-subgroup of Group II given 2 ppm of iodine, the epitheliums of some follicles were subject to be low in height and became dark shadowy (Fig. 4). The luminal surface of the lumen was flat. Parafollicular cells characterized by light tone increased in number. It had already been reported by Yoshimura et al. (1962) that the flattening of the epithelium following some hormonal agencies such as hypophysectomy and thyroxine administration urged the marked proliferation of the parafollicular cells compensatorily. The intake of 1-2 ppm of iodine for 15 days made the epithelium tallest, and the apocrine secretion was most remarkable as shown in Figure 5. This kind of hyperfunctional picture was more significant than that following the supply of CE₂ plus distilled water for 15 days. This accounts for the apparent accelerated pattern identical with a goitrogenic response. The reason of the induction of the effective hyperactive picture caused by 1-2 ppm of iodine intake may be because such a concentration of iodine is probably appropriate to keep the thyroid in temporal active status irrespective of normal value in T/S ratio. These findings prevent the authors from accepting the view that the cytoplasmic processes performing the apocrine secretion, associated with the formation of circumference vacuoles, is concerned with the picture of reabsorption. On the contrary, the duration for 60 days tended to make the epithelium low in height to some extent. It is interesting that long-term drink-
Fig. 4. Thyroid gland of a rat, male 95 days of age, fed with CE2 and given 1 ppm of sodium iodide for 5 days. The epithelium of some follicles tend to be low in height. p: parafollicular cell

ing of iodine, even a small amount of 1-2 ppm, resulted in a slight dysfunction of thyroid cells.

In the 5-day-subgroups in Group III, given 200 ppm of iodine, the epithelium diminished in height, whereas in 15- and 60-day-subgroups the evident reduction in height was not observed.

The colloidal lumens invaginated occasionally into a deep part as a kind of intracellular canals. The cross-sections of invaginations were observed as huge granules (Fig. 6). The canals further elongated themselves to the infranuclear region. Sometimes they seem to be transformed abruptly into the narrow canal-
Fig. 5. Thyroid gland of a rat, male 105 days of age, fed with CE₂ and given 2 ppm of sodium iodide for 15 days. The epithelium of follicles is high exceedingly and the typical picture of apocrine secretion is illustrated. The cells contain numerous vacuoles (v).

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Iculi (Fig. 6); the cross-sections of them represent granular shape and their diameter is as large as the colloid droplet. Finally, the canals came in contact with the basement membrane (Fig. 6), and in fact the cross- and oblique-sections with the various dimensions were encountered at the vicinity of the basement membrane (Fig. 6). Upon a consideration of the shape and the size of the cannal, it is likely that the canal is nothing but a sort of tubular invagination, sometimes dilated, and sometimes constricted. In the 15-day-subgroup, the sections of the canals appeared most numerously in each cell (Fig. 7). The total number of canals per gland was highest in Groups I and IV among all the experimental groups. These canals were demonstrated in 29 instances among 35 rats. The infrequent existence of
canals only in 7 instances among 18 rats in Group II and barely in 2 instances among 19 rats of Group 3, was confirmed. But their total number per gland remained always extraordinarily small. The evidence also is present in Figure 7 that the huge granular elements are actually responsible for the cross-sections of the canals. Some follicles contained vacant lumen devoid of colloid substance, and their lining epithelial cells included huge granules not only at the apical part but concentratedly at the basal part. The occurrence of this peculiar canal system in thyroid gland cells has not yet been elucidated, and this is utterly different from the colloid droplets in structure. As the canals disappeared in the 60-day-subgroup in Group I, the occurrence of them is actually transient.
Fig. 7. Thyroid gland of a rat, male 105 days of age, fed with CE₂ and given 200 ppm of sodium iodide for 15 days

The intracellular big canals are present most numerously and the lumen is often vacant of the colloid substance. The small granules are accumulated, which are identical in size with the colloid droplets.

Fig. 8. Thyroid gland of a rat, male 5 days of age, fed with CE₂ and given 200 ppm of iodine for 5 days

The hollows (arrow) of the lumen invaginated slightly at the apical part of cell-body. The surface of them is equipped with the elongated microvilli.

P: parafollicular cell

Fig. 9. Thyroid gland of a rat, male 105 days of age, fed with CE₂ and given 200 ppm of iodine for 15 days

The cross-sections of the big intracellular canals are found numerously in the cell-body. They are usually equipped with the microvilli.
5. *Electron microscopy on the intracellular canal*

The electron microscopic observations on the intracellular canals in thyroid gland cells of rats given 200 ppm of iodine indicated that the canals were formed initially with the hollows of the free surface (Fig. 8) and followed by the transformation of some caves facing the lumen. The caves equipped with the densely arranged microvilli further invaginated deeply (Figs. 8 and 9) and turned into the canals, which did not always invade the cell-body perpendicularly but extended themselves obliquely or irregularly downwards, representing the complicated pathways. The sections of the canals revealed the various shapes corresponding with the respective position. The biggest one acquired the same size as the nucleus. The similar but slender structures, amounting to 0.5 to 1.0 μ in diameter, were occasionally recognized not only at the supranuclear regions but also at the infranuclear area (Figs. 10 and 11). These slender canaliculi were not bordered by the tensioned membrane but furnished the identical wall-structure characterized by the microvilli. It was not determined, however, that the canaliculi belonged to the constricted narrow part of the same canal or to their branchlets. In the present investigation the fate of the branchlets has been of ignorance.

Electron microscopy demonstrated that the sections of the canals were situated at the deepest area of the cell-body (Fig. 12). Figure 13 illustrates the presence of them near the basement membrane. This picture also suggests a possibility of discharging its content, because the canals are directly in contact with the basement membrane. This might be a morphological evidence to release the colloid substance.

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**Fig. 10.** The slender canaliculus in the thyroid cells of a rat, male 105 days of age, fed with CE₂ and given 200 ppm of iodine for 15 days.

The cells contain numerous rough surfaced endoplasmic reticulums of irregular shape. Occasionally the cross-sections of the slender canaliculus(sc) is present, in which the microvilli are interdigitated.

**Fig. 11.** The slender canaliculus and the big intracellular canal in thyroid gland cells of a rat, 105 days of age, fed with CE₂ and given 200 ppm of iodine for 15 days.

In these cells, slender canaliculus together with the big canal is located. The former may be regarded as the constricted narrow channel or the branchlets of the latter. Both of them are characterized by the microvilli.
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As the ultrastructure of the canals is quite different from some morphological expressions concerning the mechanism of both secretion and reabsorption which has been reported previously, the existence of the canals is worthy of notice. The fact that T/S ratio is low would appear to suggest the inhibition in uptake of iodine, and the thyroid gland cells containing the big canals without the apocrine secretory process were not hypertrophic. Therefore these canals may not be a pathway through which the thyroglobulin is transported into the lumen. It is tentatively anticipated that the transient reabsorption of thyroglobulin is undertaken via this canal, contradictory to Wolff-Chaikoff effect, but this assumption may take a disadvantage because the thyroxine level in serum of venous blood of thyroid gland was not examined in this investigation.

SUMMARY

Adult male Wistar-Imamichi rats were given the drinking water containing 1-2 ppm or 200 ppm of iodine ad libitum for 5, 15 and 60 days. The thyroid gland cells were investigated light and electron microscopically. The mean value of T/S ratio was lower markedly in rats with 200 ppm of iodide intake. The peculiar big canals invaginated deeply the gland cell from the colloid lumen in most of rats given 200 ppm of iodine for 5 and 15 days, whereas they were not

Fig. 12. The intracellular big canal invading the deepest area of a cell-body of a rat, 105 days of age, fed with CE2 and given 200 ppm of iodine for 15 days

The big canal sometimes invaginates deeply into the cell-body.

Fig. 13. The intracellular big canal being in contact with the basement membrane, of a rat, 105 days of age

This cannal is in contact with the basement membrane. The content is possibly eliminated through the membrane facing the lumen into the space of the basement membrane.
recognized in rats given for 60 days, therefore the occurrence of them is temporal. These canal systems extend irregularly and are complicated in shape, either dilated or constricted. The cross-sections of the canals represent huge granules and are eventually in contact with the basement membrane, invading the deepest area. The content of the canals seems to be identical with the luminal colloid and their wall is equipped with the elongated numerous microvilli. The intracellular canals in thyroid gland cells have been observed for the first time in the present electron microscopy. As to their functional significance, the possible transient reabsorption of thyroglobulin has been suggested.

REFERENCES

Kawada, J. (1964). Personal communication