LARGE AMOUNT OF VITAMIN A HAS NO MAJOR EFFECTS ON THYROIDAL HORMONE SYNTHESIS IN TWO-STAGE RAT THYROID CARCINOGENESIS MODEL USING N-BIS(2-HYDROXYPROPYL)NITROSAMINE AND THIOUREA

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ABSTRACT — In our previous investigation, which focused on two-stage carcinogenicity in the thyroid, rats were administered N-bis(2-hydroxypropyl)nitrosamine (DHPN), followed by thiourea (TU) over an experimental period of 19 weeks. Simultaneous treatment with a high level of vitamin A (VA) enhanced the induction of proliferative lesions that originated from the thyroidal follicular epithelium. To examine whether hormone synthesis in the thyroid could be inhibited by simultaneous treatment with a large amount of VA and TU, all of the rats were initially given a single subcutaneous injection of 2,800 mg DHPN/kg followed by a supply of 0% TU + 0% VA (DHPN only, control group), 0.2% TU in their drinking water (DHPN/TU group), 0.1% VA in their diet (DHPN/VA group), or 0.2% TU + 0.1% VA (DHPN/TU + VA group) during an experimental period of 4 weeks. Results obtained indicate that the iodine uptake and organification, namely iodination of tyrosine residue in thyroglobulin, of the thyroid, were significantly decreased in the DHPN/TU group compared to the DHPN control group. The variation in these values was attributable to the inhibitory effect of TU upon thyroid hormone synthesis. Results obtained from the DHPN/TU + VA and DHPN/TU groups were comparable. Therefore, the possibility that modification of hormone synthesis contributes to the enhancing effect of simultaneous treatment with a large amount of VA on thyroidal tumor induction by TU is considered to be very minimal.

KEY WORDS: Rat, Vitamin A, Iodine uptake, Organification

INTRODUCTION

In our previous study, the induction of proliferative lesions originating from the follicular epithelium of the thyroid was enhanced in rats that were given thiourea (TU) and a large amount of vitamin A (VA) simultaneously over an experimental period of 19 weeks, following an initial subcutaneous injection of N-bis(2-hydroxypropyl)nitrosamine (DHPN) (Mitsumori et al., 1996). The VA was mixed in the basal diet at a concentration of 0.1% and provided ad libitum to rats. The dosage was set to be enough to induce hypervitaminosis A, which is known to be induced with an oral dose of 250,000 IU/kg/day. The measured activity of T4-uridine diphosphate glucuronosyltransferase in the liver was also determined to be elevated in another study,
where rats were simultaneously treated with TU and VA. The proliferating stimulus instigated by the thyroid stimulating hormone (TSH) from the pituitary was increased, while there were decreased levels of serum thyroid hormones, and this was considered to be the primary reason for the observed enhancement of thyroid tumor development (Takegawa et al., 1997). Furthermore, the inhibition of thyroid function elicited by VA may be an additional factor for VA's enhancing effect on TU-induced thyroidal proliferative lesions. In our previous study, in which a 4-week repeated dose study was conducted in rats without DHPN treatment to focus on this point, no differences were observed for the hormone synthesis in the thyroid between the rats treated with TU alone and TU plus VA in combination (Okuno et al., 1996). However, the dosage of TU applied in this study was 0.1% in the drinking water and was only half of the dosage used in our original study (Mitsumori et al., 1996). Moreover, in our original study using a two-stage thyroid carcinogenesis model with DHPN initiation, the hormone synthesis could be influenced by the DHPN treatment. Therefore, the present examination was conducted to clarify whether a large amount of VA affects hormone synthesis in the thyroids of TU-treated rats in the case of the two-stage thyroid carcinogenesis model.

**MATERIALS AND METHODS**

Male F344/DuCrj rats, 5 weeks of age, were obtained from Charles River Japan, Inc. (Atsugi, Japan). The rats were housed, four animals in each bracket cage, in an air-conditioned animal room (room temperature, 23±2°C; relative humidity, 55±5%; lighting cycle, 12hr light / 12hr dark). CRF-1 diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were provided *ad libitum* during the 1-week acclimatization period. The experimental animals that exhibited no abnormalities or illness were all injected subcutaneously with 2,800 mg of DHPN/kg body weight, and randomly separated into 4 equal groups that consisted of 20 rats. The treatment groups received 0% TU + 0% VA (DHPN only, control group), 0.2% TU in the diet (DHPN/TU group), 0.1% VA in their drinking water (DHPN/VA group), or 0.2% TU + 0.1% VA (DHPN/TU + VA group).

The DHPN (Nacalai Tesque, Kyoto, Japan) was diluted in saline (Otsuka Pharmaceutical, Co., Ltd., Tokyo, Japan) and this mixture was administered in a single subcutaneous injection to the rats. One week after the DHPN injection, the rats were provided TU or VA over a 4-week experimental period. The TU (Nacalai Tesque, Kyoto, Japan) was dissolved in tap water twice a week to a final concentration of 0.2%, and provided to the rats *ad libitum* using bottles. The VA (DRY VITAMIN A ACETATE, TYPE 325 CWS/F, F. Hoffmann-La Roche Ltd., Basel, Switzerland) was mixed with the pulverized basal diet, once a week, to a final concentration of 0.1%, and provided to the rats *ad libitum*. The DHPN only (control group) and the DHPN/VA groups were provided pure tap water, and the DHPN only and DHPN/TU groups were provided basal diet *ad libitum*. We utilized the same dosage levels for TU and VA as set in our previous study (Mitsumori et al., 1996).

For all of our experimental rat groups, individual body weights, food and water consumption were determined and recorded weekly during their TU or VA treatment period. After the four-week TU or VA treatment period, 10 rats from each of the treatment groups were sacrificed and processed for histopathological examination. The remaining 10 rats from each of the 4 experimental groups were examined to determine iodine uptake and organification, namely iodination of tyrosine residue in thyroglobulin, in the thyroid following the method of Okuno et al. (Okuno et al., 1996). These rats were relocated to a facility for examination using radioisotopes and maintained at a temperature of 23±3°C, relative humidity of 55±15%, and a lighting cycle of 12hr light / 12hr dark. On the following day, they were intraperitoneally injected with a solution of NaI (NEN Research Products, Boston, U. S. A.) diluted with saline (Otsuka Pharmaceutical, Co., Ltd., Tokyo, Japan) at 80 nCi/rat by a volume of 0.4 mL/rat. Twenty-four hr after the injection, these rats were sacrificed utilizing deep ether anesthesia. The rat thyroids were excised, weighed, and recorded. The rat thyroid radioactivity was measured using a gamma counter (1480 WIZARD3, Wallac, Turku, Finland) and recorded. After homogenization of the rat thyroid in 1.0 mL of 0.15 M NaCl-1 mM KI and the addition of 1.0 mL of 10% trichloroacetic acid, each specimen was centrifuged at 1,300 × g for 10 min and the supernatants were discarded. To each of these specimens, 0.5 mL of 5% trichloroacetic acid was added. They were stirred and again centrifuged at 1,300 × g for 10 min. Following the removal of the supernatants, the radioactivity of each specimen was measured with the gamma counter and recorded. The protein concentration of the sediments from each specimen was determined utilizing the methods of Lowry et al. (Lowry et al., 1951). The radioactivity of the whole thyroids and from 1 mg of
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thyroid tissue were used as parameters for the determination of iodine uptake. The radioactivity of total protein obtained from the whole thyroid, and of 1 mg protein were used as parameters for protein-bound iodine. These parameters were calculated as percentages of the radioactivity from the injected Na$^{125}$I.

The other 10 rats from each treatment group were intraperitoneally injected with bromodeoxyuridine (BrDU, Sigma Chemical Co., St. Louis, MO, U. S. A.) at a dosage of 40 mg/kg, 1 hr before necropsy. These animals were first anesthetized with ether, and serum samples were collected for serum analysis for thyroxine (T4), 3,5,3'-triiodothyronine (T3), and TSH conducted with radioimmunoassay using Amalex-M T4, Amalex-M T3, and AmalexTSH (Ortho-Clinical Diagnostics, Co., Ltd, Tokyo, Japan). The animals were then necropsied, and the livers, thyroids, and pituitaries weighed. The thyroids were next fixed with 10% neutral buffered formalin, embedded in paraffin, stained with HE, and examined histopathologically. To facilitate screening for proliferative lesions originating from the follicular epithelium, the detected focal hyperplasias were divided into small cell and hypertrophic cell types, following the pattern developed in our previous studies (Mitsumori et al., 1996; Takegawa et al., 1997). The neoplasias observed were all of the adenomatous type, and we did not observe any lesions with solid growth patterns as were found in our previous studies (Mitsumori et al., 1996; Takegawa et al., 1997). Sections from the thyroid specimens were immunohistochemically stained with anti-BrDU mouse antibody (DAKO, Glostrup, Denmark), anti-mouse rabbit antibody (DAKO, Glostrup, Denmark), and using the avidin-biotin peroxidase complex method (StreptoAB Complex/HRP, DAKO, Glostrup, Denmark). The BrDU labeling indices were calculated as the number of positive cells per 1,000 cells for focal hyperplasias, neoplasias and surrounding follicular epithelia. Transmission electron microscopic (TEM) examination was also performed to detect changes in follicular epithelia.

**Statistical analysis**

For thyroidal iodine uptake, protein binding iodine, serum hormone levels and organ weights, the homogeneity of variance was tested using Bartlett's method (Bartlett, 1937). When homogeneity was recognized, these values from the DHPN only group (control group) and the other treatment groups were compared using the Dunnett's multiple comparison test (Dunnett, 1955). In cases homogeneity was not recognized, the Kruskal-Wallis test followed by non-parametric Dunnett's multiple comparison test was used to compare the differences (Hollander and Wolfe, 1973). Moreover, for these parameters, as well as multiplicity and the BrdU labeling indices of the thyroid lesions in the DHPN/TU and DHPN/TU + VA groups, homogeneity of variance was tested using Bartlett's method. When the variance was homogenous, the variation between the DHPN/TU group and the DHPN/TU + VA group was evaluated using the Student's t-test (Snedecor, 1959). However, in cases in which homogeneity was not recognized, the Wilcoxon test was applied (Ichihara, 1996). These comparisons of mean values between groups were evaluated by two-tailed test at a statistical significance level of 5%.

**RESULTS**

The body weight values from the DHPN/TU group and the DHPN/TU + VA group were significantly lower than those for the DHPN control group, while both the food and water intakes for these two treatment groups were decreased, when compared to the control group, throughout the experimental treatment period. The daily TU intakes for the DHPN/TU group and the DHPN/TU + VA group were 715 and 695 mg/kg/day, respectively. On the other hand, the VA intake for the DHPN/TU + VA group was 791 mg/kg/day and higher than the 660 mg/kg/day intake for the DHPN/VA group, which was probably attributable to their lower body weights.

Four weeks after the initiation of TU or VA administration, the iodine uptake per 1 mg thyroid and the amount of iodine bound to 1 mg protein were decreased for both the DHPN/TU and the DHPN/TU + VA groups compared to the DHPN control group (Table 1). The magnitude of this detected decrease was much larger for the DHPN/TU than for the DHPN/TU + VA treatment group. In contrast, when the iodine uptake and protein binding were calculated for the whole thyroids, no statistically significant decrease was detected in values from either the DHPN/TU and the DHPN/TU + VA groups, when compared to the DHPN only, control group, which was a reflection of the enlargement of the thyroid for these treatment groups.

The serum T4 values were significantly decreased for the DHPN/TU and the DHPN/TU + VA groups, when compared to the DHPN control group. The magnitude of decrease in the T4 values for the combined treatment group (DHPN/TU + VA) was much larger than for the single treatment group (DHPN/TU) as detailed in Table 2. Very similar patterns were obtained.

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for the TSH, in response to the fluctuation of the T4 level, as the TSH level for the DHPN/TU + VA group was higher than for the DHPN/TU group, although the detected difference was determined not to be statistically significant. The serum T3 levels for the DHPN/TU + VA group were also lower than for the DHPN/TU group, although the value in the latter was higher than for the DHPN control group. For the DHPN/VA treatment group, the T4, T3 and TSH levels were very comparable to the values for the DHPN control group.

The parameter of thyroid weight for the DHPN/TU and the DHPN/TU +VA groups was similarly increased when compared to the DHPN control group (Table 3). Also, values for the liver weight were found to be higher for the DHPN/TU + VA group than for the DHPN/TU group.

Histopathological examination of the rat thyroid, uncovered focal hyperplasias and neoplasias derived from the follicular epithelium from both the DHPN/TU and DHPN/TU + VA treatment groups (Photo 1, 2), but there was no statistically significant difference in the incidence or multiplicity of these parameters between the two treatment groups (Table 4). The BrdU labeling indices for the focal hyperplasias, neoplasias and surrounding follicular epithelium indicating diffuse hyperthyroidy were higher for the DHPN/TU + VA group than for the DHPN/TU group, although only hyperplasias of a small cell type were significant (Table 5, Photo 3, 4).

Under the electron microscope, thyroid follicular epithelial cells demonstrated hyperthyroidy, with increased numbers of colloid droplets and elongated microvilli at the apical surfaces in the case of the DHPN/TU and the DHPN/TU + VA treatment groups. However, there were no differences between these two groups (Photo 5, 6).

Table 1. Thyroidal iodine uptake and organification in rats given no treatment, thiourea (TU), vitamin A (VA) or both over a 4-week period, following injection with N-bis(2-hydroxypropyl)nitrosamine (DHPN).

<table>
<thead>
<tr>
<th>Groupsa)</th>
<th>Uptake (%)125I</th>
<th>Uptake per tissue (%)125I/mg thyroid</th>
<th>PBI (%)125I</th>
<th>PBI per protein (%)125I/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHPN alone</td>
<td>6.24 ± 0.89c)</td>
<td>0.579 ± 0.104</td>
<td>5.06 ± 0.90</td>
<td>2.993 ± 0.572</td>
</tr>
<tr>
<td>DHPN/TU</td>
<td>5.66 ± 3.44</td>
<td>0.106 ± 0.067**</td>
<td>4.53 ± 2.86</td>
<td>0.638 ± 0.414**</td>
</tr>
<tr>
<td>DHPN/VA</td>
<td>6.92 ± 0.93</td>
<td>0.651 ± 0.096</td>
<td>5.46 ± 0.78</td>
<td>3.380 ± 0.531</td>
</tr>
<tr>
<td>DHPN/TU + VA</td>
<td>9.89 ± 5.29#</td>
<td>0.213 ± 0.103**#</td>
<td>7.89 ± 4.61</td>
<td>1.156 ± 0.597##</td>
</tr>
</tbody>
</table>

a): Each treatment group was comprised of ten rats.
b): Protein binding iodine.
c): Mean ± S. D.
*: Statistically significant difference from the DHPN control group at p<0.05.
**: Statistically significant difference from the DHPN control group at p<0.01.
#: Statistically significant difference from the DHPN/TU group at p<0.05.

Table 2. Serum T3, T4 and TSH levels from rats given no treatment, thiourea (TU), vitamin A (VA) or both over a 4-week period following injection with N-bis(2-hydroxypropyl)nitrosamine (DHPN).

<table>
<thead>
<tr>
<th>Groupsa)</th>
<th>T3 (ng/mL)</th>
<th>T4 (ng/mL)</th>
<th>TSH (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHPN alone</td>
<td>0.60 ± 0.08b)</td>
<td>29.3 ± 2.0</td>
<td>6.14 ± 1.47</td>
</tr>
<tr>
<td>DHPN/TU</td>
<td>0.83 ± 0.23**</td>
<td>25.1 ± 3.9**</td>
<td>14.58 ± 6.01*</td>
</tr>
<tr>
<td>DHPN/VA</td>
<td>0.58 ± 0.07</td>
<td>28.2 ± 2.0</td>
<td>7.42 ± 1.37</td>
</tr>
<tr>
<td>DHPN/TU + VA</td>
<td>0.57 ± 0.08##</td>
<td>21.4 ± 1.7**#</td>
<td>20.48 ± 12.42**</td>
</tr>
</tbody>
</table>

a): Each treatment group was comprised of ten rats.
b): Mean ± S. D.
*: Statistically significant difference from the DHPN control group at p<0.05.
**: Statistically significant difference from the DHPN control group at p<0.01.
#: Statistically significant difference from the DHPN/TU group at p<0.05.
##: Statistically significant difference from the DHPN/TU group at p<0.01.
### Table 3. Thyroid, liver and pituitary weight comparison for rat groups given no treatment, thiourea (TU), vitamin A (VA) or both over a 4-week period, following injection with N-bis(2-hydroxypropyl)nitrosamine (DHPN).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Final body weight (g)</th>
<th>Absolute weights</th>
<th>Relative weights</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Thyroid (mg)</td>
<td>Pituitary (mg)</td>
</tr>
<tr>
<td>DHPN alone</td>
<td>234.3 ± 11.8^{b)}</td>
<td>10.2 ± 0.6^{c)}</td>
<td>6.54 ± 0.89</td>
</tr>
<tr>
<td>DHPN/TU</td>
<td>184.6 ± 12.6^{**}</td>
<td>44.2 ± 11.9^{*+d)}</td>
<td>6.11 ± 0.85</td>
</tr>
<tr>
<td>DHPN/VA</td>
<td>238.3 ± 11.2</td>
<td>12.1 ± 2.7^{d)}</td>
<td>5.97 ± 0.42</td>
</tr>
<tr>
<td>DHPN/TU + VA</td>
<td>192.4 ± 6.1^{**}</td>
<td>46.4 ± 9.6^{**+d)}</td>
<td>5.78 ± 0.88</td>
</tr>
</tbody>
</table>

^{a)}: There were ten rats in all of the treatment groups.
^{b)}: Mean ± S.D.
^{c)}: The number of animals examined was 7.
^{d)}: The number of animals examined was 8.
^{*}: Statistically significant difference from the DHPN control group at p<0.05.
^{**:} Statistically significant difference from the DHPN control group at p<0.01.
^{#}: Statistically significant difference from the DHPN/TU group at p<0.05.

**Photo 1.** Focal hyperplasia, small cell type, in a rat treated with thiourea (TU) and vitamin A (VA) over a 4-week period, following injection with N-bis(2-hydroxypropyl) nitrosamine (DHPN). HE staining. (×272)

**Photo 2.** Neoplasia with an adenomatous growth pattern in a rat treated with both thiourea (TU) and vitamin A (VA) over a 4-week period, following injection with N-bis(2-hydroxypropyl) nitrosamine (DHPN). HE staining. (×272)
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DISCUSSION

The results obtained from this study, specifically the decreased serum T4 levels, elevated serum TSH levels and increased cell proliferation within lesions derived from the follicular epithelium, were consistent with data collected from our previous studies (Mitsumori et al., 1996; Takegawa et al., 1997). The iodine uptake and protein binding determinations for the thyroid were suppressed for the DHPN/TU treatment group, which is consistent with the earlier published report that TU inhibits thyroidal hormone synthesis (Hill et al., 1989). The values for these parameters from the DHPN/TU + VA group, however, were not significantly decreased when compared to those in the DHPN/TU group. Accordingly, no major alteration of thyroidal hormone synthesis appears to be involved in the documented enhancement effect due to a large amount of VA on thyroidal tumor promotion elicited by TU. These results suggest that the observed elevation of T4-UDP-GT activity in the liver, as previously reported (Takegawa et al., 1997), may be the primary reason for the enhancement of thyroid tumor development.

The effect of DHPN on the hormone synthesis of the thyroid should be taken into account, although the untreated control group was not set in the present experiment for the purpose to clarify the mechanism on the enhancement of thyroid carcinogenesis of a large amount of VA in our previous study (Mitsumori et al., 1996). When the parameters indicating thyroid hormone synthetic activities in the DHPN alone group in this study were compared with those of the untreated group in the study by Okuno et al. (1996), all of the parameters were low. Therefore, there may be a possi-

| Table 4. Histopathological findings from the thyroids of rats given no treatment, thiourea (TU), vitamin A (VA) or both over a 4-week period, following injection with N-bis(2-hydroxypropyl)nitrosamine (DHPN). |
|---|---|---|
| Groups | Incidence (%) | Multiplicity |
| | Hyperplasia | Neoplasia (adenomatous growth pattern) | Hyperplasia | Neoplasia (Adenomatous growth pattern) |
| | Hyper- | Small | | Hyper- | Small | |
| | trophic | cell type | type | trophic | cell type | type |
| DHPN alone | 0 | 0 | 0 | NL | NL | NL |
| DHPN/TU | 30.0 | 90.0 | 30.0 | 0.60 ± 1.26b) | 3.80 ± 2.70** | 0.30 ± 0.48 |
| DHPN/VA | 0 | 0 | 0 | NL | NL | NL |
| DHPN/TU + VA | 30.0 | 100.0 | 30.0 | 0.60 ± 1.26 | 2.20 ± 1.55** | 0.40 ± 0.70 |

a): Each treatment group was comprised of ten rats.
b): Mean ± S. D.
NL: No lesions were detected.
**: Statistically significant difference from the DHPN control group at p<0.01.
No statistically significant difference was recognized between the DHPN/TU and DHPN/TU + VA groups for this parameter.

| Table 5. Bromodeoxyuridine (BrdU) labeling indices for the thyroids from rats treated with thiourea (TU) and vitamin A (VA) over a period of 4 weeks, following injection with N-bis(2-hydroxypropyl)nitrosamine (DHPN). |
|---|---|---|---|
| Groups | Hyperplasia | Neoplasia (adenomatous growth pattern) | Surrounding hypertrophic follicular epithelium |
| | Hypertrophic cell type | Small cell type | |
| DHPN/TU | 9.40 ± 6.14a) (5) | 1.88 ± 4.64 (37) | 1.95 ± 3.37 (3) | 0.39 ± 0.83 (10) |
| DHPN/TU + VA | 13.77 ± 21.65 (6) | 11.49 ± 23.30 (18) # | 29.56 ± 53.20 (4) | 0.98 ± 1.91 (10) |

a): Mean ± S. D.
Figures in parenthesis represent the numbers of lesions examined and labeled.
#: Statistically significant difference from the DHPN/TU group at p<0.05.
Effects of vitamin A on thyroidal hormone synthesis in rats.

The BrdU labeling indices in proliferative lesions in the thyroid were not statistically significant except for the hyperplasia of the small cell type, although the mean values of each type of lesion in the DHPN/TU + VA group were higher than those in the DHPN/TU group. Thus, the enhancing effect of a large amount of VA for the induction of proliferative lesions in the thyroid was slightly lower than that in our previous report (Mitsumori et al., 1996). This difference is possibly due to the treatment period of 4 weeks, which is extremely shorter than 19 weeks in the previous study. However, the enhancement of the decrease in serum T4 levels and increase in serum TSH levels, which was apparent in the present experiment, indicates that the same influences as those in the previous study (Mitsumori et al., 1996) were induced in these rats. In the present study, serum T3 levels in the DHPN/TU group were elevated in contrast to the result in the previous report (Mitsumori et al., 1996), in which T3 levels were decreased in the rats treated with TU after DHPN treatment. The reason for this difference is not clear. However, significant increases of TSH levels in the DHPN/TU group in comparison with the DHPN alone group clearly suggested suppressed thyroid hormone levels by the effect of TU.

Numerous factors are involved in the explanation of the mechanism for the observed enhancing effect attributable to a large amount of VA on thyroidal tumor induction due to TU. One factor is the suppression of the secretion of thyroid hormones from the thyroid, through several processes from colloid droplet formation to specific hormone release into the circulation.
which are known to be initiated by excess iodine (Collins and Capen, 1980). The increase of the number of colloid droplets observed in the DHPN/TU and DHPN/TU + VA groups is considered to suggest increased ingestion of colloid containing thyroglobulin from the lumen of the follicle in compensation for the decreased thyroid hormone levels in the circulation. However, the number and the volume of colloid droplets did not appear to be affected by the consumption of a large amount of VA, during the present study. Therefore, it seems that colloid droplet formation is not directly involved in the enhancement of thyroidal tumor induction. The significance of the elongation of the microvilli at the apical surface observed in the DHPN/TU and DHPN/TU + VA groups was unclear. However, since the microvilli are considered to be one of the important sites which play a role in the synthetic process of the thyroid hormones such as organification or coupling of iodine (Stein and Gross, 1964), this morphological change possibly relates to the inhibiting effects of TU on the thyroid peroxidase. Growth factors and their receptors may be also involved in the mechanism of enhanced thyroid tumor induction, because they have been reported as being involved in the growth of follicular cells (Polychronakos et al., 1986; Smith et al., 1986; Westermark et al., 1983). In our previous immunohistochemical study (Takegawa et al., 1998), however, no significant variation in the expression of

![Photo 5. Epithelial cells from a normal-appearing follicle in a rat given 0% TU+0% VA (control group) over a 4-week treatment period, following injection with N-bis(2-hydroxypropyl)nitrosamine (DHPN). Transmission electron micrograph (TEM). (×5,360).](image1)

![Photo 6. Epithelial cells from a background follicle in a rat treated with both thiourea (TU) and vitamin A (VA) over a 4-week period, following injection with N-bis(2-hydroxypropyl)nitrosamine (DHPN). The height of the cells as well as the number of colloid droplets are significantly increased, and the microvilli are elongated. Transmission electron micrograph (TEM). (×5,360).](image2)
transforming growth factor α (TGF-α) or for its receptor, epidermal growth factor receptor (EGF_R), was observed, even with the ingestion of a large amount of VA. It remains to be determined whether other growth factors or 5'-deiodinase, which converts T4 to T3 and is inhibited by FD&C Red No.3 (erythrosine) (Capen, 1995), may be affected by treatment with a large amount of VA.

REFERENCES


