Experimental Studies on Struma

On the Effect of Imidazol Derivatives on Thyroid Gland

By

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PREFACE

Researches on struma are contributing much to the advance of study of the thyroid gland itself, but the problem of the cause of endemic goiter is as yet unsolved.

Many researches have been indeed carried out on its cause and many theories have been proposed, of which that attributing the pathogenesis to deficiency in iodine is the most widely accepted. It is well known that iodine plays an important rôle in the process of synthesis of thyroid hormone, but since struma is found prevalent in some regions where deficiency of iodine seems highly improbable, it is difficult to attribute the pathogenesis of struma to iodine insufficiency alone.

Accordingly, the probability of some substance causing tumefaction of the thyroid gland being contained in food has come to be considered and the study of the so-called food goiter is gaining in general attention. In particular, based on researches on food goiter, studies on thioureic compounds and such antithyroid substances have come to be rapidly promoted recently, and it has come to be known that many substances impair the function of the thyroid gland and upon continued administration cause struma. Especially, 5-5-dimethyl-2-2-thioxazolidone and similar compounds have been isolated from many vegetables and have come into limelight as pathogenic factors of endemic goiter.

In Japan ARAI reported that by persistent administration of histidine, an amino acid, to rabbits, frank struma parenchymatosa is induced in them and infers the pathogenic mechanism to be as follows: Histidine is transformed into urocanic acid, and thence into glutamic acid; this urocanic acid contains an unsaturated double bond which is apt to take up the iodine in blood into combination and the compound is thus excreted out of the body, so that a relative deficiency of iodine is caused in the vital body and then struma is induced. On this viewpoint, he has proposed his blood iodine bond theory (body iodine combination theory).

ARAII proceeds thence to the general proposition that the pathogenesis of struma of any type can be interpreted unitarily by this theory. In particular, he gives the same mechanism for thiouracil struma as that for histidine struma and opines that the direct chemical combination of thiouracil and iodine induces struma or lowering of the thyroid function.
The opinion admitting chemical combination of thiouracil with iodine has been already voiced by Pitt-Rivers, but it is biologically a more surprising fact that histidine—one of the indispensable amino acids—is pathogenic and its persistent administration leads to occurrence of struma. Therefore, the present author has attempted a closer study of the effect of histidine of the thyroid gland and have arrived at a new conclusion on the relation between histidine and struma. At the same, author has succeeded in causing tumefaction of the thyroid gland by experimental continued administration of creatine, another imidazol derivative as is histidine, but not containing an unsaturated double bond in its constitution.

Next, similar experiments were made with methyl-thiouracil for rather highly fundamental morphological and biochemical comparative studies of the effects of thiouracil and histidine on the thyroid gland and some new informations were obtained, as reported in the following.

**EXPERIMENTAL METHODS**

Mature rabbit, guinea-pigs and rats were used as experimental animals. The rabbits and the guinea-pigs were fed with soy-bean curd refuse, bran and vegetables and the rats with cereals in the main and a small quantity of vegetables.

Of the reagents, histidine hydrochloride and creatine were prepared into 2% physiological saline solutions, and 2% solution of Metiocil made by Chugai Chemical Works was used in testing methyl-thiouracil.

All these preparations were intraperitoneally injected in the dosis of 4cc every other day in rabbits, 2cc every other day in guinea-pigs and 2cc every day in rats, continuously for 1-12 months.

The methods of morphological and biochemical experiments were as follows.

1) Histological methods.

The experimental animals were sacrificed by causing air embolism, their thyroid glands were weighed immediately and fixed in Bouin’s solution together with their pituitary glands. The other visceral organs were fixed in 10% formalin solution.

After fixation, the sampled organs were cut into sections upon paraffin imbedding and hematoxylin-eosin stained for microscopic examination.

2) Tracting with I<sup>131</sup>.

a) Radioautographing of the thyroid gland

To some rats and guinea-pigs injected with the reagents as above, physiological saline solution of NaI containing 10-30μc of I<sup>131</sup> (received from the Japan Isotope Association) was administered 24 hours before sacrificing. Their thyroid glands were prepared as above into paraffin imbeded sections, which were affixed on object glasses in juxtaposition with similar sections of the thyroid gland of control animals (administered with I<sup>131</sup> of the same dosis at the same time but not injected with the reagents) and stained with hematoxylin-eosin; then a 1% celloidin film is placed on them, the emulsion membrane of stripping
plates is affixed thereupon, exposed for 3 days and developed by fine-grain development method.

b) Measurement of rate of $^{131}$I uptake by the thyroid gland of rabbits

The $^{131}$I uptake rate of 1 or 2 rabbits chosen from each experimental group was measured 41, 60, 63 and 98 days after the commencement of the experiments.

On these days, 0.5cc of $^{131}$I dissolved in distilled water and containing 10μc of the isotope was intraperitoneally injected and the resultant count (A) was measured with a scintillation counter 1, 3, 6, 12, 24 and 48 hours thereafter. The same dosis of $^{131}$I solution was placed in a metal saucer and its count (B) was taken as the standard for the respective hour, and the uptake rate of the thyroid gland was computed by the formula $\frac{A - C}{B - C} \times 100$, where C stands for the background radioactivity in the atmosphere.

c) Rate of $^{131}$I uptake by the thyroid gland of rats

After daily administration of the tested reagents for 21 consecutive days, 30μc of $^{131}$I was intraperitoneally injected to the rats and the rates of $^{131}$I uptake and of its conversion into organic $^{131}$I were measured by HAMOLSKY and MIYAKE et al.'s methods.

Besides, for determining the possible difference between the effect on the thyroid gland of histidine hydrochloride and that of Methiocil, two normal rats were intraperitoneally injected with a mixture of 4cc of 2% histidine hydrochloride solution and 10μc of $^{131}$I, and a mixture of 1cc of 2% Methiocil solution and 10μc of $^{131}$I respectively, and their thyroid $^{131}$I uptake rate, rate of conversion into organic $^{131}$I and $^{131}$I content in total blood were measured.

3) Measurement Protein-bound Iodine in Blood

One or two rabbits were chosen from each experimental group, blood was sampled from them before and 10, 25, 95 and 115 days after the commencement of the experiment, serum was extracted from the blood samples and the content of protein-bound iodine in 1cc of it was measured using an electrophotometer with a 450μm filter by a modified distillation method based on BARKER and CONNOR's methods.

4) Measurement of Cholesterol content

Using 0.2cc of serum prepared as in the preceding paragraph, the subject value was determined photometrically by a modified BLOOR's method, using a 634μm filter.

EXPERIMENTAL RESULTS

I Findings on the Thyroid Glands of Various Animals upon Continued Administration of Small Daily Doses of Histidine Hydrochloride.

I. In Rabbits

The change in the weight of the thyroid gland of rabbits intraperitoneally injected with 4cc each of 2% physiological saline solution of histidine hydrochloride every other day was as shown in Tab. 1.
The results show that the weight of the thyroid gland within the first 3 months of the experiment ranges between 85 and 210 mg, its weight relative to the body weight standing at 0.06-0.14. These values were not much different from those in normal animals and remained within the normal range in general. Around 6 months after the beginning of the experiment, the thyroid gland begins gradually to gain in weight, the value attaining the high of 310 mg in HK 12 subjected to 12 month's administration of the reagent. In HK 8, HK 10, HK 11 and HK 12, the thyroid gland was always macroscopically hyperemic and medullary, a little lobulated on its surface and swollen, indicating occurrence of struma.

Under a microscope, in the rabbits given histidine for 1-3 months (HK 1, 2, 3, 4 and 5), the thyroid gland was found little different from that in normal rabbits (Fig. 1), only a slight reduction of the follicles in size being noted in HK 4 and 5, but in the animals subjected to longer histidine treatment, especially in HK 12, the follicles were found very perceptibly reduced in size, their lumen narrowed down and devoid of colloid and the epithelium was being cuboidal—in short, the picture of the so-called struma diffusa parenchymatosa microfollicularis was apparent (Fig. 2). The blood vessels in the stroma were somewhat proliferated and a considerable hyperemia was seen in some parts.

Thus when rabbits are administered with histidine continuously, no perceptible change occurred in their thyroid gland within the first 3 months of the treatment, at least 6 months of continued histidine administration being required before the swelling of the

<table>
<thead>
<tr>
<th>No. of Rabbits</th>
<th>Sex</th>
<th>Months</th>
<th>Total Volume of Histidine gm.</th>
<th>Final Body Weight gm.</th>
<th>Thyroid Weight mg.</th>
<th>Thyroid Weight mg. per l gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HK 1</td>
<td>♂</td>
<td>1</td>
<td>1.2</td>
<td>1500</td>
<td>85</td>
<td>0.06</td>
</tr>
<tr>
<td>HK 2</td>
<td>♀</td>
<td>1</td>
<td>1.2</td>
<td>1500</td>
<td>210</td>
<td>0.14</td>
</tr>
<tr>
<td>HK 3</td>
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<td>210</td>
<td>0.12</td>
</tr>
<tr>
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<td>2.4</td>
<td>1800</td>
<td>180</td>
<td>0.10</td>
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<td>HK 5</td>
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<td>3.6</td>
<td>2000</td>
<td>100</td>
<td>0.05</td>
</tr>
<tr>
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<td>2400</td>
<td>190</td>
<td>0.08</td>
</tr>
<tr>
<td>HK 7</td>
<td>♂</td>
<td>5</td>
<td>6.0</td>
<td>2000</td>
<td>220</td>
<td>0.11</td>
</tr>
<tr>
<td>HK 8</td>
<td>♂</td>
<td>6</td>
<td>7.2</td>
<td>2100</td>
<td>270</td>
<td>0.13</td>
</tr>
<tr>
<td>HK 9</td>
<td>♂</td>
<td>9</td>
<td>10.8</td>
<td>2000</td>
<td>260</td>
<td>0.13</td>
</tr>
<tr>
<td>HK 10</td>
<td>♂</td>
<td>9</td>
<td>10.8</td>
<td>2100</td>
<td>270</td>
<td>0.13</td>
</tr>
<tr>
<td>HK 11</td>
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<td>12</td>
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<td>2100</td>
<td>290</td>
<td>0.14</td>
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<tr>
<td>HK 12</td>
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<td>14.4</td>
<td>2250</td>
<td>310</td>
<td>0.14</td>
</tr>
</tbody>
</table>

The results show that the weight of the thyroid gland within the first 3 months of the experiment ranges between 85 and 210 mg, its weight relative to the body weight standing at 0.06-0.14. These values were not much different from those in normal animals and remained within the normal range in general. Around 6 months after the beginning of the experiment, the thyroid gland begins gradually to gain in weight, the value attaining the high of 310 mg in HK 12 subjected to 12 month's administration of the reagent. In HK 8, HK 10, HK 11 and HK 12, the thyroid gland was always macroscopically hyperemic and medullary, a little lobulated on its surface and swollen, indicating occurrence of struma.
gland becomes evident.

It is a noteworthy finding that even in the control rabbits fed for 9 months or longer without such treatment the thyroid gland tends to swell, and microscopically observed, its follicles tend to be atrophic and its epithelial cells to turn cubic, the gland turning parenchymatous as a whole.

2. In Guinea Pigs

In guinea pigs intraperitoneally injected with 2cc each of 2% physiological saline solution of histidine every other day, the weight of the thyroid gland changed as shown in Tab. 2.

Table 2. Effect of Histidine on Thyroid Weight in Guinea pigs.

<table>
<thead>
<tr>
<th>No. of Guinea Pigs</th>
<th>Sex</th>
<th>Months</th>
<th>Total Volume of Histidine gm.</th>
<th>Final Body Weight gm.</th>
<th>Thyroid Weight mg.</th>
<th>Thyroid Weight mg. per gm.</th>
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</thead>
<tbody>
<tr>
<td>HM 1</td>
<td>♂</td>
<td>1</td>
<td>0.6</td>
<td>350</td>
<td>30</td>
<td>0.09</td>
</tr>
<tr>
<td>HM 2</td>
<td>♀</td>
<td>1</td>
<td>0.6</td>
<td>300</td>
<td>35</td>
<td>0.12</td>
</tr>
<tr>
<td>HM 3</td>
<td>♂</td>
<td>2</td>
<td>1.2</td>
<td>300</td>
<td>70</td>
<td>0.23</td>
</tr>
<tr>
<td>HM 4</td>
<td>♀</td>
<td>2</td>
<td>1.2</td>
<td>280</td>
<td>90</td>
<td>0.32</td>
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<tr>
<td>HM 5</td>
<td>♂</td>
<td>3</td>
<td>1.8</td>
<td>250</td>
<td>60</td>
<td>0.24</td>
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<tr>
<td>HM 6</td>
<td>♀</td>
<td>3</td>
<td>1.8</td>
<td>350</td>
<td>118</td>
<td>0.34</td>
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<tr>
<td>HM 7</td>
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<td>4</td>
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<td>550</td>
<td>200</td>
<td>0.36</td>
</tr>
<tr>
<td>HM 8</td>
<td>♀</td>
<td>6</td>
<td>3.6</td>
<td>540</td>
<td>190</td>
<td>0.35</td>
</tr>
<tr>
<td>HM 9</td>
<td>♂</td>
<td>8</td>
<td>4.8</td>
<td>720</td>
<td>270</td>
<td>0.38</td>
</tr>
<tr>
<td>HM 10</td>
<td>♀</td>
<td>8</td>
<td>4.8</td>
<td>670</td>
<td>290</td>
<td>0.43</td>
</tr>
<tr>
<td>HM 11</td>
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<td>9</td>
<td>5.4</td>
<td>600</td>
<td>245</td>
<td>0.41</td>
</tr>
<tr>
<td>HM 12</td>
<td>♀</td>
<td>9</td>
<td>5.4</td>
<td>600</td>
<td>160</td>
<td>0.27</td>
</tr>
<tr>
<td>M 1</td>
<td>♂</td>
<td>1</td>
<td>200</td>
<td>30</td>
<td>0.15</td>
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</tr>
<tr>
<td>M 2</td>
<td>♀</td>
<td>2</td>
<td>480</td>
<td>50</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>M 3</td>
<td>♂</td>
<td>3</td>
<td>480</td>
<td>50</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>M 4</td>
<td>♀</td>
<td>4</td>
<td>600</td>
<td>95</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>M 5</td>
<td>♂</td>
<td>6</td>
<td>600</td>
<td>110</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>M 6</td>
<td>♀</td>
<td>9</td>
<td>660</td>
<td>150</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>

In the first 3 months of the experiment, only a slight swelling is observable, but later, the tendency for the gland to swell became rather conspicuous, its weight rising to twice-thrice of the normal around 8 months after the beginning of the experiment, showing a stronger tendency of thyroid swelling than in rabbits.

Upon microscopic examination, it was found that no mentionworthy anomaly appeared in the cases sacrificed comparatively early (Fig. 3), but in HM 8, 9 and 10, some points were noted. These cases, all sacrificed during summer, showed frank picture of struma colloid in their thyroid gland, the follicles showing vehement inequality in size, the follicular lumina being generally enlarged and filled up with darkly eosin-staining colloid; the follicular epithelial cells were severely flattened, their nucleus was also flattened and the chromatin was perceptibly augmented (Fig. 4).

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The long-fed but untreated control guinea-pigs also showed some tendency of enlargement in their thyroid gland, but no particularly noteworthy finding was obtained under a microscope.

3. In Rats

The change in weight of the thyroid gland of rats following intraperitoneal injection of 2cc each daily of 2% solution of histidine in physiological saline was as shown in Tab. 3.

Table 3. Effect of Histidine on Thyroid Weight in Rats.

<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>Sex</th>
<th>Months</th>
<th>Total Volume of Histidine gm.</th>
<th>Final Body Weight gm.</th>
<th>Thyroid Weight mg.</th>
<th>Thyroid Weight mg. per 1 gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR 1</td>
<td>♂</td>
<td>1</td>
<td>1.2</td>
<td>180</td>
<td>19</td>
<td>0.11</td>
</tr>
<tr>
<td>HR 2</td>
<td>♀</td>
<td>1</td>
<td>1.2</td>
<td>150</td>
<td>15</td>
<td>0.10</td>
</tr>
<tr>
<td>HR 3</td>
<td>♂</td>
<td>2</td>
<td>2.4</td>
<td>180</td>
<td>20</td>
<td>0.11</td>
</tr>
<tr>
<td>HR 4</td>
<td>♀</td>
<td>2</td>
<td>2.4</td>
<td>160</td>
<td>18</td>
<td>0.11</td>
</tr>
<tr>
<td>HR 5</td>
<td>♂</td>
<td>3</td>
<td>3.6</td>
<td>235</td>
<td>20</td>
<td>0.09</td>
</tr>
<tr>
<td>HR 6</td>
<td>♀</td>
<td>3</td>
<td>3.6</td>
<td>185</td>
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<td>0.11</td>
</tr>
<tr>
<td>HR 7</td>
<td>♂</td>
<td>6</td>
<td>7.2</td>
<td>150</td>
<td>20</td>
<td>0.13</td>
</tr>
<tr>
<td>HR 8</td>
<td>♀</td>
<td>6</td>
<td>7.2</td>
<td>200</td>
<td>22</td>
<td>0.11</td>
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<tr>
<td>HK 9</td>
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<td>12</td>
<td>14.4</td>
<td>230</td>
<td>30</td>
<td>0.13</td>
</tr>
<tr>
<td>HR 10</td>
<td>♀</td>
<td>12</td>
<td>14.4</td>
<td>200</td>
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<td>0.15</td>
</tr>
<tr>
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<td>0.10</td>
</tr>
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<td>0.13</td>
</tr>
<tr>
<td>R 3</td>
<td>♂</td>
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<td></td>
<td>130</td>
<td>18</td>
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<tr>
<td>R 4</td>
<td>♀</td>
<td>6</td>
<td></td>
<td>130</td>
<td>12</td>
<td>0.09</td>
</tr>
<tr>
<td>R 5</td>
<td>♂</td>
<td>12</td>
<td></td>
<td>150</td>
<td>10</td>
<td>0.07</td>
</tr>
<tr>
<td>R 6</td>
<td>♀</td>
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<td></td>
<td>170</td>
<td>10</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Nearly no perceptible change was here observed and even in the case HR 12 subjected to the administration for 12 months in continuance, the increased weight of the thyroid gland was not significantly different from the weight of the gland of the control. Microscopic observation also failed in revealing mentionable anomaly in the thyroid gland of the histidine-administered rats (Fig. 5), only the finding of the so-called metaplasia into flat epithelium was visible accompanied by a slight parenchymatous alteration in HR 12 (Fig. 6).

II. Occurrence of Struma Following Persisting Administration of Creatine in Small Doses.

1. In Rabbits

Following intraperitoneal injection of 4cc each of 2% physiological saline solution of creatine, the weight of the thyroid gland changed as shown in Tab. 4. Here, swelling of the thyroid gland appeared in about 3 months after the beginning of the experiment, and in KK 9 given creatine for 12 months, the weight rose to 545 mg or about twice of the normal value. The ratio of the weight of the gland to the body weight was 0.19, a ratio
still larger than that in rabbits given histidine hydrochloride. Macroscopically observed, the gland in KK 5, 7, 8, 9 and 10 was highly hyperemic and strongly medullar in appearance, showing a picture of struma diffusa parenchymatosa microfollicularis severer than in the cases given histidine, especially in the cases administered creatine for many months (Fig. 7). But no mentionable histological change could be seen in the thyroid gland of the cases given creatine for only 1-3 months, quite as in the histidine-administered rabbits.

2. In Guinea Pigs

The change in weight of the thyroid gland of guinea pigs following intraperitoneal injection of 2cc each of 2% physiological saline solution of creatine was shown in Tab. 5. Here too, the thyroid gland began to show swelling in 3 months, and in KM 8 subjected to 8 months of creatine administration, the weight rose to about 450% of the normal (360 mg).

The histological picture of the thyroid gland of the guinea pigs given creatine was quite identical with that of histidine-administered guinea pigs, no marked anomaly being seen in the gland of animals sacrificed in the first few months, but in KM 7 and 8 given creatine for 8 months, a picture of severe struma colloides was observable, the follicles showing marked inequality in size, the follicular lumina enlarged and filled up with darkly eosin-staining colloid, small vacuoles seen in the periphery of a part of the colloid and the follicular epithelium being markedly flattened (Fig. 8).
3. In Rats

The weight of the thyroid gland of rats intraperitoneally injected with 2cc each of 2% solution of creatine in physiological saline changed as shown in Tab. 6.

Table 6. Effect of Creatine on Thyroid Weight in Rats.

<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>Sex</th>
<th>Months</th>
<th>Total Volume of Creatine gm.</th>
<th>Final Body Weight gm.</th>
<th>Thyroid Weight mg.</th>
<th>Thyroid Weight mg. per 1 gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>KR 1</td>
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<td>1</td>
<td>1.2</td>
<td>180</td>
<td>18</td>
<td>0.10</td>
</tr>
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<td>1.2</td>
<td>160</td>
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</tr>
<tr>
<td>KR 3</td>
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<td>160</td>
<td>17</td>
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</tr>
<tr>
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<td>0.10</td>
</tr>
<tr>
<td>KR 5</td>
<td>♂</td>
<td>3</td>
<td>3.6</td>
<td>150</td>
<td>19</td>
<td>0.12</td>
</tr>
<tr>
<td>KR 6</td>
<td>♂</td>
<td>3</td>
<td>3.6</td>
<td>150</td>
<td>20</td>
<td>0.13</td>
</tr>
<tr>
<td>KR 7</td>
<td>♂</td>
<td>6</td>
<td>7.2</td>
<td>170</td>
<td>30</td>
<td>0.17</td>
</tr>
<tr>
<td>KR 8</td>
<td>♂</td>
<td>6</td>
<td>7.2</td>
<td>170</td>
<td>25</td>
<td>0.15</td>
</tr>
<tr>
<td>KR 9</td>
<td>♂</td>
<td>12</td>
<td>14.4</td>
<td>190</td>
<td>28</td>
<td>0.15</td>
</tr>
<tr>
<td>KR 10</td>
<td>♂</td>
<td>12</td>
<td>14.4</td>
<td>200</td>
<td>30</td>
<td>0.15</td>
</tr>
<tr>
<td>R 1</td>
<td>♂</td>
<td>1</td>
<td></td>
<td>100</td>
<td>10</td>
<td>0.10</td>
</tr>
<tr>
<td>R 2</td>
<td>♂</td>
<td>2</td>
<td></td>
<td>120</td>
<td>15</td>
<td>0.13</td>
</tr>
<tr>
<td>R 3</td>
<td>♂</td>
<td>3</td>
<td></td>
<td>130</td>
<td>18</td>
<td>0.14</td>
</tr>
<tr>
<td>R 4</td>
<td>♂</td>
<td>6</td>
<td></td>
<td>130</td>
<td>12</td>
<td>0.09</td>
</tr>
<tr>
<td>R 5</td>
<td>♂</td>
<td>12</td>
<td></td>
<td>150</td>
<td>10</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Here also, the thyroid gland did not change perceptible in weight, as in the group given histidine above, a weak tendency to swelling to supernormal weight (about 30 mg) in the case being barely perceptible in the animals given creatine for 6–12 months.

Histologically speaking, no perceptible change was found in the early stage of creatine administration, but in KR 8 and 10, the follicles were generally contracted, the follicular epithelium was thickened and turned cuboidal, the follicular lumina were narrowed down and the colloid content was drastically lowered (Fig. 9). The hyperemia of the stroma was rather remarkable, but no strong degenerative change was ever observed in any case.

III The Effect of Methyl-thiouracil on the Thyroid Gland

The change in weight of the thyroid gland during long-continued injection with 2% methiocil solution was as shown in Tab. 7, 8, 9.

In this case, rabbits, guinea pigs as well as rats showed marked increase in weight of the thyroid gland already at the end of the first month and with the advance of the experiment, the swelling of the thyroid gland became still more obvious. The gland of the animals subjected to more than one month of methiocil administration was considerably hyperemic, dark red-brown and lustrous, its surface was apparently smooth, its cut surface medullar and the isthmus frankly swollen (Fig. 10). This tendency of producing thyroid swelling was most pronounced in rats, followed by rabbits and guinea pigs in the order named, but was always stronger than after application of histidine or creatine. The histological findings were very similar in all these animals, revealing frank existence of struma parenchymatosa in 2–3 months after beginning of the experiment. Thus, the findings in rat's thyroid gland will be described as representative instances hereunder.

Table 7. Effect of Methiocil on Thyroid Weight in Rabbits.

<table>
<thead>
<tr>
<th>No. of Rabbits</th>
<th>Sex</th>
<th>Months</th>
<th>Total Volume of Methiocil gm.</th>
<th>Final Body Weight gm.</th>
<th>Thyroid Weight mg.</th>
<th>Thyroid Weight mg. per 1 gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK 1</td>
<td>♂</td>
<td>1</td>
<td>1.2</td>
<td>1650</td>
<td>136</td>
<td>0.08</td>
</tr>
<tr>
<td>MK 2</td>
<td>♀</td>
<td>1</td>
<td>1.2</td>
<td>1580</td>
<td>120</td>
<td>0.08</td>
</tr>
<tr>
<td>MK 3</td>
<td>♂</td>
<td>2</td>
<td>2.4</td>
<td>1760</td>
<td>185</td>
<td>0.11</td>
</tr>
<tr>
<td>MK 4</td>
<td>♀</td>
<td>2</td>
<td>2.4</td>
<td>1720</td>
<td>170</td>
<td>0.10</td>
</tr>
<tr>
<td>MK 5</td>
<td>♂</td>
<td>3</td>
<td>3.6</td>
<td>2110</td>
<td>380</td>
<td>0.18</td>
</tr>
<tr>
<td>MK 6</td>
<td>♀</td>
<td>3</td>
<td>3.6</td>
<td>2070</td>
<td>395</td>
<td>0.19</td>
</tr>
<tr>
<td>MK 7</td>
<td>♂</td>
<td>6</td>
<td>7.2</td>
<td>2570</td>
<td>1350</td>
<td>0.53</td>
</tr>
<tr>
<td>MK 8</td>
<td>♀</td>
<td>6</td>
<td>7.2</td>
<td>2520</td>
<td>1210</td>
<td>0.48</td>
</tr>
<tr>
<td>MK 9</td>
<td>♂</td>
<td>12</td>
<td>14.4</td>
<td>2810</td>
<td>3550</td>
<td>1.26</td>
</tr>
<tr>
<td>MK 10</td>
<td>♀</td>
<td>12</td>
<td>14.4</td>
<td>2730</td>
<td>2480</td>
<td>0.91</td>
</tr>
</tbody>
</table>

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After methiocil, obvious changes were already observable in the thyroid gland immediately upon its first application. In the case sacrificed in 24 hours, the follicular epithelial cells were heightened and cuboidal, the colloid was thinned down, numerous small vesicles were found in the peripheral parts of the follicular lumina and the stroma was evidently

### Table 8. Effect of Methiocil on Thyroid Weight in Guinea pigs.

<table>
<thead>
<tr>
<th>No. of Guinea pigs</th>
<th>Sex</th>
<th>Months</th>
<th>Total Volume of Methiocil gm.</th>
<th>Final Body Weight gm.</th>
<th>Thyroid Weight mg.</th>
<th>Thyroid Weight mg. per 1 gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM 1</td>
<td>♂</td>
<td>1</td>
<td>0.6</td>
<td>280</td>
<td>95</td>
<td>0.34</td>
</tr>
<tr>
<td>MM 2</td>
<td>♀</td>
<td>1</td>
<td>0.6</td>
<td>250</td>
<td>97</td>
<td>0.39</td>
</tr>
<tr>
<td>MM 3</td>
<td>♂</td>
<td>2</td>
<td>1.2</td>
<td>310</td>
<td>179</td>
<td>0.58</td>
</tr>
<tr>
<td>MM 4</td>
<td>♀</td>
<td>2</td>
<td>1.2</td>
<td>300</td>
<td>165</td>
<td>0.56</td>
</tr>
<tr>
<td>MM 5</td>
<td>♂</td>
<td>3</td>
<td>1.8</td>
<td>480</td>
<td>270</td>
<td>0.56</td>
</tr>
<tr>
<td>MM 6</td>
<td>♀</td>
<td>3</td>
<td>1.8</td>
<td>455</td>
<td>315</td>
<td>0.69</td>
</tr>
<tr>
<td>MM 7</td>
<td>♂</td>
<td>6</td>
<td>3.6</td>
<td>570</td>
<td>490</td>
<td>0.86</td>
</tr>
<tr>
<td>MM 8</td>
<td>♀</td>
<td>6</td>
<td>3.6</td>
<td>570</td>
<td>475</td>
<td>0.87</td>
</tr>
<tr>
<td>MM 9</td>
<td>♂</td>
<td>12</td>
<td>7.2</td>
<td>780</td>
<td>845</td>
<td>1.08</td>
</tr>
<tr>
<td>MM 10</td>
<td>♀</td>
<td>12</td>
<td>7.2</td>
<td>730</td>
<td>790</td>
<td>1.08</td>
</tr>
</tbody>
</table>

### Table 9. Effect of Methiocil on Thyroid Weight in Rats.

<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>Sex</th>
<th>Months</th>
<th>Total Volume of Methiocil gm.</th>
<th>Final Body Weight gm.</th>
<th>Thyroid Weight mg.</th>
<th>Thyroid Weight mg. per 1 gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR 1</td>
<td>♂</td>
<td>1</td>
<td>1.2</td>
<td>130</td>
<td>65</td>
<td>0.50</td>
</tr>
<tr>
<td>MR 2</td>
<td>♀</td>
<td>1</td>
<td>1.2</td>
<td>140</td>
<td>68</td>
<td>0.48</td>
</tr>
<tr>
<td>MR 3</td>
<td>♂</td>
<td>2</td>
<td>2.4</td>
<td>170</td>
<td>125</td>
<td>0.74</td>
</tr>
<tr>
<td>MR 4</td>
<td>♀</td>
<td>2</td>
<td>2.4</td>
<td>140</td>
<td>70</td>
<td>0.50</td>
</tr>
<tr>
<td>MR 5</td>
<td>♂</td>
<td>3</td>
<td>3.6</td>
<td>180</td>
<td>120</td>
<td>0.67</td>
</tr>
<tr>
<td>MR 6</td>
<td>♀</td>
<td>3</td>
<td>3.6</td>
<td>130</td>
<td>75</td>
<td>0.58</td>
</tr>
<tr>
<td>MR 7</td>
<td>♂</td>
<td>6</td>
<td>7.2</td>
<td>180</td>
<td>90</td>
<td>0.50</td>
</tr>
<tr>
<td>MR 8</td>
<td>♀</td>
<td>6</td>
<td>7.2</td>
<td>240</td>
<td>120</td>
<td>0.50</td>
</tr>
<tr>
<td>MR 9</td>
<td>♂</td>
<td>12</td>
<td>14.4</td>
<td>310</td>
<td>150</td>
<td>0.48</td>
</tr>
<tr>
<td>MR 10</td>
<td>♀</td>
<td>12</td>
<td>14.4</td>
<td>190</td>
<td>130</td>
<td>0.79</td>
</tr>
</tbody>
</table>

After methiocil, obvious changes were already observable in the thyroid gland immediately upon its first application. In the case sacrificed in 24 hours, the follicular epithelial cells were heightened and cuboidal, the colloid was thinned down, numerous small vesicles were found in the peripheral parts of the follicular lumina and the stroma was evidently
When the methiocil administration was continued from one week to one month, the hyperplasia of the follicles became further remarkable, while the epithelial cells were markedly proliferated, hypertrophied and prominently grown out in papillary form into the lumen (Fig. 11). The follicular epithelial cells were further heightened, turned cuboid or cylindrical, the cellular nuclei became vesicular and were sporadically in mitosis, and the basophilia of the protoplasm was considerably strengthened. The colloid was extremely thinned down and nearly invisible and the hyperemia in the stroma was evermore obvious.

In the cases of methiocil administration for 3-6 months, the thyroid gland was found completely solid, no follicular lumina being visible, the colloid being extinct, the follicular epithelial cells turned further cuboidal and the protoplasm being greatly swollen and hyperplased, the stromatic blood vessels being highly hyperemic; a complete picture of parenchymatous struma was present here (Fig. 12). Such findings remained present as long as the methiocil injection was continued.

Especially, in one case given methiocil for 12 months in continuance, a finding suggestive of incipient thyroid cancer was apparent. Namely, the atypia of the cells proliferated in the parts considered as “Krebsknospe” (cancer buds) was considerably advanced, the basophilia of their protoplasm was strengthened and the atypia of their nuclei was also heightened, while rather many of them were found in mitosis, the proliferated cells strongly oppressing the surrounding stromatic tissue—showing the apparent finding of cylindrical epithelial cancer of papillary adenocarcinoma type (Fig. 13).

IV Experimental Study on the Effect of Imidazol Derivatives and its Difference from that of Thiouracil Derivatives

1) On the Difference in Morphological Findinings

A) Radioautography of the Thyroid Gland

The radioautographs taken 24 hours after injection of 30μc of 131I in guinea pigs administered with histidine for one month showed no difference from those of normal animals, strong deposition of 131I being observable on all the follicles. The deposited quantity of 131I showed considerable difference from follicle to follicle, but generally speaking, it was large on the minor follicles but small on the major follicles (Fig. 14).

The radioautograph of the thyroid gland of the guinea pig HM 8 treated with histidine for 8 months showed positive reaction uniformly over the entire gland, in the follicular lumina as well as the epithelial cells, but generally speaking, it was large on the minor follicles but small on the major follicles (Fig. 14).

The radioautograph of the thyroid gland of the guinea pig HM 8 treated with histidine for 8 months showed positive reaction uniformly over the entire gland, in the follicular lumina as well as the epithelial cells, but somewhat different from the normal findings, the blackening due to 131I was generally weaker, but considerably stronger in the follicular epithelium than in the lumina (Fig. 15).

In the case of guinea pigs administered with Methiocil, the radioautograph taken 24 hours after 131I injection was quite different from that pretreated with histidine, no black spots due to 131I being ever found in any part of the thyroid gland (Fig. 16), suggesting that in animals treated with Methiocil, no elaboration of thyroglobulin takes place.
The radioautographs of rats given histidine (Fig. 17) or Methiocil (Fig. 18) gave the same findings in guinea pig, respectively, confirming that Methiocil totally inhibits the synthesis of thyroid hormones.

B) Findings of the Pituitary Gland

A very prominent morphological change was observed in the pituitary gland of rabbits, guinea pigs and rats given Methiocil. Their anterior and middle lobes were hypertrophied and seemingly fairly hyperemic, and microscopically too, around one month after the first application of Methiocil, the decrease of acidophil cells and the hypertrophy of basophil cells became evident, the protoplasmic granules were nearly extinguished so that the protoplasm was clarified and vacuoles began to appear in it; thus the appearance of the so-called thyroidectomy cells was distinctly observed.

On the other hand, no specially mentionable finding was ever detected in the rabbits, the guinea pigs and the rats given histidine, throughout the whole periods of experiment, except that a slight hypertrophy of the basophil cells in the anterior lobe was observed in the rabbit and the guinea pig treated with histidine for 12 months and 8 months, respectively.

2) Biochemical Findings

A) Protein-bound Iodine (PBI) in Serum

The results of measurement of the serum PBI content in rabbits given histidine, creatine and Methiocil are given in Tab. 10.

<table>
<thead>
<tr>
<th>Name of Reagents and Dose of Administration (intraperitoneally, daily)</th>
<th>No. of Animals</th>
<th>Sex</th>
<th>Before</th>
<th>After Administration in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine hydrochloride (2% 4cc)</td>
<td>7</td>
<td>♂</td>
<td>4.80</td>
<td>4.40 4.20 5.08 4.80</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>♀</td>
<td>3.92</td>
<td>3.72 3.32 4.20 4.40</td>
</tr>
<tr>
<td>Creatine (2% 4cc)</td>
<td>4</td>
<td>♂</td>
<td>3.72</td>
<td>3.39 3.20 — —</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>♀</td>
<td>3.72</td>
<td>3.28 3.20 3.86 3.72</td>
</tr>
<tr>
<td>Methiocil (2% 4cc)</td>
<td>3</td>
<td>♂</td>
<td>5.08</td>
<td>3.20 3.32 — —</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>♀</td>
<td>4.20</td>
<td>2.96 3.20 — —</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>3.72—6.40 (Mean Average of 19 Cases)</td>
<td></td>
</tr>
</tbody>
</table>

In Methiocil-administered animals, the PBI value ran down rapidly after the first administration, by around 40% in all the cases. The low value persists as long as the Methiocil treatment is kept up, but in the cases given histidine, little change is observed in their PBI value, except that in 1–3 weeks after the beginning of the experiment, the PBI value tended to drop a little, but upon further continuance of the experiment, the value rose to a level higher than the pre-experimental level in 3rd or 4th month. Creatine showed a similar effect to histidine in this respect.
B) Serum Cholesterol Value

The measured values of cholesterol content in serum of histidine-, creatine- and Methiocil-administered rabbits were as shown in Tab. 11.

The value tended to rise with the first injection and rose to 2-3 folds of the normal on the 10th day already. On the other hand, no change in the value was observable in male rabbits administered with histidine or creatine, but in female rabbits treated similarly, the serum cholesterol value was found to rise, to around twice the pre-experimental value.

C) I"Uptake in Rabbits

Rabbits administered with histidine, creatine and Methiocil continuously for 41-98 days were intraperitoneally injected with I" in physiological saline solution; the rates of I" uptake of their thyroid gland 1, 3, 6, 12, 24, 48 and 72 hours after the injection were as shown in Tab. 12.
In all the cases, the largest accumulation of $^{131}I$ in the thyroid gland occurred between 3 and 6 hours, the collected quantity decreasing gradually thereafter, quite as in normal cases. In the rabbits administered with histidine or creatine, the uptake rate was always higher than normal, but in all the cases given Methiocil, the rate was very subnormal, dropping to about half of the normal or lower, evidently indicative of impaired function of the thyroid gland. It is also to be noted that the rate was higher in the rabbits treated with histidine or creatine for 41 days only than in those treated for 98 days.

D) Ditto in Rats

In Tab. 13 are shown the rates of $^{131}I$ uptake of the thyroid gland in rats given histidine, creatine or Methiocil every day for 21 days, 24 hours after intraperitoneal injection of $36\mu g$ each of $^{131}I$. The rate was always nearly normal in the rats given histidine or creatine, but in the cases administered with Methiocil, the rate was markedly subnormal and proved the existence of pronounced hypofunction of the thyroid gland.

E) Experiments of Simultaneous Administration of $^{131}I$ with Histidine and with Methiocil

In the last place, the findings on the thyroid glands in normal rats, injected with 10 $\mu g$ of $^{131}I$ mixed with 4cc of 2% histidine or 1cc of 2% Methiocil and sacrificed 24 hours thereafter, were studied in comparison, using normal rats injected with the same doses of $^{131}I$ alone as controls. As shown in Tab. 14, the uptake rate of $^{131}I$ (14.10% on the average) and the conversion rate into organic $^{131}I$ (97.52% on the average) in the rats given histidine in admixture with $^{131}I$ were not much different from the rates in the controls, but in the cases injected with Methiocil in admixture with $^{131}I$, the uptake rate was found drastically reduced to 1.93% and the conversion rate to 3.25%.

The total content of $^{131}I$ in total blood at the time of sacrificing was 0.766% in the cases given histidine+$^{131}I$, not much different from that in the controls (0.913%), but in
the cases injected with Methiocil + I\(^{131}\), the content was considerably higher than normal (2.450%). These results also show that Methiocil has the effect of drastically inhibiting the accumulation of I\(^{131}\) and conversion into organic iodine of I\(^{131}\) in the thyroid gland, i.e., the elaboration of thyroid hormones, but that histidine has no such an effect at all.

**DISCUSSION**

Very many antithyroid substances have been reported to date; these can be classified into the 3 major groups of 1) KSCN or organic cyanides, 2) aniline derivatives, especially, sulfonamide derivatives and 3) thiourea derivatives\(^{67}\).

Of these, KSCN is said to lead to hypofunction of the thyroid gland through inhibiting the production of thyroid hormone by hampering the accumulation of iodine\(^ {68, 69}\) and the other two by hampering the synthesis of hormone in the gland. Now, there have been many studies on the mechanism of the effect of these substances, but yet, no final conclusion has been arrived at. In particular, the thiourea derivatives are said to interfere with the enzyme activity in the process of once oxidizing the iodides taken up by the thyroid cells into free\(^ {59, 60, 57, 60, 49}\) iodines, and oxydase\(^ {63, 60}\) or peroxydase\(^ {67, 60, 49}\) are pointed to as the inactivated enzymes.

Some other authors assume the direct chemical combination of the antithyroid compounds with iodine; for example, CAMPBELL\(^ {59}\) and MILLER\(^ {59}\) opined that the thiourea derivatives directly combine with the free iodine produced in the thyroid cells, causing difficulty in iodization of tyrosine and consequently, inhibiting the production of thyroid hormone.

ARAII also has been one of this school; he has tried to attribute the cause of all kinds of struma due to chemical substances to their chemical reaction with iodine in the vital
organism and proposed his blood-iodine capturing theory or vital-iodine combination theory. He reports that, when small dosis of histidine is given to animals persistently, parenycymatous struma frankly occurs in them, and interprets the mechanism as follows:

Histidine passes over in the body into glutamic acid through urocanic acid which has a double bond -C= C- in its constitutions, so that it readily reacts with iodine in blood, forming the chain -CHI-CHI- or -CHI- HCH-; this iodized compound is excreted in urine, so that a relative deficiency of iodine is induced and parenchymatous struma follows. He says that oleic acid, fumalic acid, maleic acid, aconitic acid and similar compounds having unsaturated double bonds in their constitution have the effect of causing tumefaction of the thyroid gland, and infers that the antithyroid effect of the thiourea derivaties and other antithyroid compounds is due to their chemical combination with iodine, as in the case of histidine.

On the other hand, however, some one year before ARAI’s vital iodine combination theory was propounded, attempts have been made to induce thyroid diseases experimentally with iodine reagents and such chemical compounds very sensitive in reaction with iodine. OKAMOTO, NISHIZUKA, MIDORIKAWA and their co-workers, starting from the fact that the thyroid gland is very rich in iodine, anticipated that when a compound very strongly iodine-affine is administered and the compounds is toxic to the thyroid cells, injury to the gland would naturally follow. Acting on this assumption, they have conducted animal experiments with the very strongly iodine affine reagent PdCl₂.

More recently, MIDORIKAWA and the present author have made the experiment of administering PdCl₂ and α-naphthoflavone to rats for more than a month, for studying the possibility of causing struma, but we were disappointed, for no typical experimental struma could be observed in occurrence. It was inferred that PdCl₂ is not an ideal iodine reagent, being affine not only with iodine but also with protein etc., and is too toxic for use in any large enough quantity, so that it cannot penetrate the thyroid cells; at the same time, it shows that if the reagent is ever so effective in vitro, it may not react quite similarly in vivo. That is to say, an attempt to interpret biological processes in living organism by sheer reliance of chemical reaction may be very fascinating, but is dangerous unless taken up with utmost circumspection. From such a viewpoint too, struma due to histidine must be also studied with utmost caution.

The relation between histidine was reported for the first time by LAUSON and RIMINGSTONE. They discovered the goitrogenic factor of ergothioneine always contained in erythrocytes in a very small quantity. This is a histidine derivatie and has an imidazol radical produced by S-substitution. It is of course an interesting phenomenon that a substance in unfailing existence in a vital body should have a strong antithyroid activity, but it is not yet clarified whether histidine changes into ergothioneine in a living body. LAUSON et al. assign the goitrogenic activity of ergothioneine to HSC in it, but
fail to touch on the effect of histidine.

Now, my experimental results show that the effect of histidine is fairly different by animals. In rabbits and guinea pigs, histidine evidently causes enlargement of the thyroid gland, but no such an effect is observed in rats and mice. By closer analysis, we are led to infer that such a difference is rather closely related with the difference in the main food of the animals. Rat and mice are usually fed with rice and wheat, while the main feeds of rabbits and guinea pigs are soy-bean cuad refuse and weeds, in Japan.

It is known that soy-beans contain a considerable quantity of antithyroid substances and also, weeds and vegetables are fairly effective in causing hypertrophy of the thyroid gland. Thus, laboratory rabbits and guinea pigs are constantly under the effect of various antithyroid substances in small doses and this fact may be understood from the increase of the weigh of the thyroid gland of our control rabbits and guinea pigs in the long run. This phenomenon, we believe, is a point especially to be attended to, in making experiments on struma using herbivorous animals.

Now, if it is assumed that the mechanism of histidine causing struma consists in capturing iodine, the following conditions must be satisfied:

1) The blood PBI value in animals given histidine should be found lowered, due to deficiency in iodine.
2) This iodine deficiency should cause hypofunction of the thyroid gland in such animals, the serum cholesterol content being raised and some anomaly should be observable in the uptake rate or conversion rate into protein iodine in the function tests of the thyroid gland with I$^{131}$, in histidine-treated animals.
3) When histidine is injected simultaneously with a small dose of I$^{131}$, all iodine should combine chemically with urocanic acid produced by change of histidine, so that no I$^{131}$ would remain for use in producing thyroid hormone.

But in my experiments the serum PBI value remained nearly normal, the serum cholesterol level was not increased and rates of I$^{131}$ uptake and conversion remained within the normal range, too.

The contents of inorganic and organic I$^{131}$ in the thyroid gland 24 hours after injection of histidine and I$^{131}$ in combination were also equal to those in the control animals not given the dose of histidine.

As another ground, and a decisive one, for denying that the effect of histidine in causing struma is due to its chemical combination with iodine, author wishes to point forcibly to the success of experiments on causing struma with creatine. As is generally known, creatine is one of the final decomposition products of protein and has an H-CN radical as histidine has. But being a final metabolate, it is chemically inactive, has of course no unsaturated double carbon bond and cannot be expected to combine with iodine in a living body. Yet, this compound, upon long continued administration, was found to cause parenchymatous struma in rabbits and guinea pigs, as effectively as histidine. The
same can be said of arginine struma as well. Namely, arginine is also devoid of unsaturated double bond and its chemical combination with iodine is unthinkable. Especially, the possibility for arginine to pass over into histidine has been already utterly denied, and we are forced to assume that the enlargement of the thyroid gland is due to the \( \text{H-C} \) \( \text{N} \) radical also present in argine.

This antithyroid effect of imidazol derivatives has the characteristic of promoting the tendency of hypertrophy of the thyroid gland due to vegetable feed in herbivorous animals. Their effect is not attributable to their chemical combining power with iodine, but to the \( \text{H-C} \) \( \text{N} \) radical in them. This specific action of imidazol derivatives, being observable only in herbivorous animals and not lowering the function of the thyroid gland, should be called a goitrogen-accelerating action and strictly distinguished from the goitrogenic activity of thiourea derivatives which obviously lowers the function of the thyroid gland and increases the secretion of TSH from the pituitary gland of all the experimental animals.

Guanidine, one of the imidazol derivatives, is said to lack in antithyroid activity as manifested by thiourea derivatives, but on its possible goitrogen-accelerating action, experiments with herbivorous animals as subjects must be carried out before decision is taken.

Now, for clarifying the notion of goitrogen-accelerating action as distinguished from the goitrogenic action proper, author will discuss the difference of thiouracil and imidazol derivatives in the following.

1) The imidazol derivatives are not effective in causing enlargement of the thyroid gland of all animals, but only in expediting the swelling of the gland in rabbits, guinea pigs and such herbivorous animals. With thiourea derivatives, however, the effect is manifested in all the experimental animals, and in a much higher intensity than with imidazol derivatives. By administration of thiourea derivatives, the struma parenchymatosa microfollicularis are induced, while with imidazol derivatives, the feature were those of the same type of struma in rabbits, but in guinea pigs, pictures suggestive more of struma colloides were obtained.

2) Imidazol derivatives, even in large doses, do little affect the thyroid gland morphologically, unless the administration is continued long enough, while for definite observation of the histological changes in the gland administration of the reagents continued for more than three months is required. On the other hand, considerably marked changes occur in the thyroid following only a single injection of thiouracil and its derivatives, a tendency to parenchymatous struma becoming apparent already in several days after the first administration.

3) Imidazol derivatives little affect the function of the thyroid gland, the blood PBI, the serum cholesterol content and the uptake rate of \( ^{131} \) remaining normal, whereas, following administration of thiourea derivatives, the PBI is lowered, the serum cholesterol level
is raised, the uptake rate of \( I^{131} \) substantially reduced, the thyroid gland being in manifest hypofunction. Such a state of hypofunction was clearly evidenced by radioautography of the thyroid gland following \( I^{131} \) injection.

4) Administration of imidazol derivatives causes no morphological change in the pituitary gland, whereas following administration of thiourea derivatives, the basophil cells in the anterior lobe of the gland are swollen and hypertrophied and at last the so-called thyroidectomy cells make appearance.

Accordingly, the goitrogenic activity of thiouracil and its derivatives is seemingly very closely related with TSH of the anterior pituitary lobe, the hyperplasia of the thyroid cells being due to the action of TSH, while the goitrogen-accelerating activity is not so closely related with the pituitary gland.

From the above, it may be easily understood that the actions of thiouracil and imidazol derivatives are essentially dissimilar.

In the last place, author will cursorily touch upon the meaning of the goitrogen-accelerating factor of imidazol derivatives and the clinical significance of creatine struma.

In considering the outbreak of endemic struma in regions where deficiency in iodine is utterly unthinkable, as in the case with the so-called seaside struma, it is reasonable to seek the pathogenesis in a disturbance of protein metabolism. The significance of imidazol derivatives is great in this connection. In particular, creatine is a final metabolate of protein and in vital organisms is nearly all contained in striated muscles\(^9\). Therefore, when subjects with predisposition to struma in maritime regions are inclined to heavy consumption of fish meat, it is easy to infer that struma may be induced in them.

Besides, the thyroid function of the patients with simple struma is found usually not much impaired\(^{99, 69, 61}\). This also leads us to conclude that it is more reasonable to take up, as causative factors accounting for the etiology of endemic struma, goitrogen-accelerating substances, in particular, imidazol derivatives that do not largely affect the function but yet expedites the enlargement of the thyroid gland, than to seek the cause in the direct goitrogenic effect of thiourea derivatives that obviously cause atrocious devastation in the function of the thyroid gland.

**SUMMARY**

Rabbits, guinea pigs and rats were administered with imidazol derivatives and methylthiouracil for months in continuance and subjected to morphological and biochemical examinations. The results obtained may be summarized as follows;

1. When histidine or creatine was administered for 3 months and longer, the thyroid gland of rabbits and guinea pigs was found swollen, to a higher degree after creatine.
2. When histidine or creatine was administered for long duration to rabbits, their thyroid gland showed the microscopic figure of struma parenchymatosa diffusa microfollicularis.
3. When histidine or creatine was administered to guinea pigs for long time, their
thyroid gland microscopically showed the figure of struma colloides.

4. When histidine or creatine was administered to rats for long time, no symptom of struma was forthcoming.

5. Administration of methyl-thiouracil to rabbits, guinea pigs and rats caused severe struma parenchymatosa diffusa microfollicularis.

6. Histidine or creatine administration, unless continued long enough, induced neither macroscopic nor microscopic changes in the thyroid gland of any of the experimental animals.

7. Administration of methyl-thiouracil, even of short duration, caused evident morphological changes in the thyroid gland.

8. Following administration of histidine and creatine the I\textsuperscript{131} uptake rate and the I\textsuperscript{131} conversion rate into organic iodine were found remaining within the normal ranges.

9. Following administration of methyl-thiouracil, the above rates were found fallen frankly below normal.

10. The PBI value of blood and the serum cholesterol level following histidine and creatine administration were generally within the normal range.

11. The blood PBI value was found markedly reduced and the serum cholesterol content risen after administration of methyl-thiouracil.

12. No marked change was observed in the pituitary gland after histidine or creatine administration.

13. Following methyl-thiouracil administration, however, basophil cells were proliferated and hypertrophied and thyroidectomy cells began to appear in the anterior lobe of the pituitary gland.

14. From the above findings, it is inferred that the effect of histidine and creatine of the thyroid gland is utterly different from that of thiouracil derivatives, and may be called a goitrogen-accelerating action.

This action has been confirmed as being due to the imidazol radical but not to a direct chemical combination of the imidazol derivatives with iodine.

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EXPLANATION OF FIGURES

Fig. 1. The thyroid gland of rabbit HK 2 given histidine for 1 month.
Histologically, the thyroid gland is found little different from that in normal rabbits.
Hematoxylin-eosin.

Fig. 2. The thyroid gland of rabbit HK 12 injected histidine for 12 months.
In this thyroid gland, the feature of struma parenchymatosa microfollicularis is apparent.
Hematoxylin-eosin.

Fig. 3. The thyroid gland of guinea pig HM 3 injected histidine for 2 months.
No mentionworthy anomaly is seen in the histological picture.

Fig. 4. The thyroid of guinea pig HM 10 treated with histidine for 8 months.
This case shows frank picture of struma colloides showing vehement inequality in size of
follicles.
Hematoxylin-eosin.

Fig. 5. The thyroid of the histidine-administered rat HR 5 for 3 months.
Microscopic observation fails in revealing mentionable anomaly in the thyroid gland.

Fig. 6. The thyroid of the histidine for 12 months administered rat HR 10. The so-called metaplasia in epithelial cells is obviously visible accompanied by slight parenchymatous alteration in other parts.
   Hematoxylin-eosin.

Fig. 7. The thyroid of the rabbit KK 9 administered creatine for 12 months.
   This thyroid gland shows a picture of parenchymatous struma.
   Hematoxylin-eosin.

Fig. 8. The thyroid gland of the guinea pig KM 8 given creatine for 8 months.
   A picture of severe struma colloides is observable.
   Hematoxylin-eosin.

Fig. 9. The thyroid gland of the rat KR 10, administered creatine for 12 months.
   This thyroid gland shows the tendency of parenchymatous struma.
   Hematoxylin-eosin.

Fig. 10. The thyroid gland of the rat MR 10 given Methiocil for 12 months.
   The enlargement of the thyroid gland becomes more obvious and the istmus is frankly swollen.

Fig. 11. The thyroid gland of the rat MR 2 administered Methiocil for 1 month.
   The epithelial cells were markedly proliferated, hypertrophied and prominently grown out in papillary form into the lumen.
   Hematoxylin-eosin.

Fig. 12. The thyroid gland of the rat MR 7 administered Methiocil for 6 months.
   Complete picture of parenchymatous struma is present.
   Hematoxylin-eosin.

Fig. 13. The thyroid gland of the rat MR 9 administered Methiocil for 12 months.
   The proliferated cells are strongly oppressing the surrounding adenomatous tissue, showing the apparent picture of cylindrical cancer.
   Hematoxylin-eosin.

Fig. 14. The radioautograph of thyroid gland taken 24 hours after injection of 30 μc of I¹³¹. Guinea pig administered with histidine for 1 month.
   It shows no difference from those of normal animals.

Fig. 15. The radioautograph of thyroid gland of the guinea pig HM 8 treated with histidine for 8 months.
   It shows positive reaction uniformly over the entire gland. The blacken due to I¹³¹ was generally weaker.

Fig. 16. The radioautograph of thyroid gland of the guinea pig administered with Methiocil for 1 month.
   No black spots due to I¹³¹ is ever found in any part of the thyroid gland.

Fig. 17. The radioautograph of the rat administered with histidine.
   Positive black spots due to I¹³¹ is seen all over the gland.

Fig. 18. The radioautograph of thyroid gland of Methiocil treated rat.
   There is no blackening spot in all the area of thyroid.

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