Precursor Cytogenetic Changes of Transplantable Thyroid Carcinoma in Iodine-deficient Goiters

Abdul Al-Saadi

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SUMMARY

The cytogenetic changes in the thyroid cells of 199 Fischer rats are reported here. Fifty-eight rats were maintained on a normal iodine diet and 141 rats were maintained on an iodine-deficient diet for 7-25 months. Rats were sacrificed in groups of two to ten at two-week to one-month intervals; their thyroid cells were grown in cell culture and prepared for chromosomal analyses. An average of 4.5 ± 0.5% aneuploidy was found in the thyroid cells of control rats and no structural abnormalities. In the iodine-deficient rat, the percent of aneuploid cells increased with time of iodine deficiency from that of the control to 46.4 ± 5.1% at 21-25 months of iodine deficiency.

The percent cells of 41 chromosomes increased from 1.1 ± 0.05 in the control to 21.3 ± 6.1 at 21-25 months of iodine deficiency. Similarly, the percent of cells with 43 chromosomes changed from 0.15 ± 0.1 to 29.3 ± 3.9%. The number of marker chromosomes was also significantly increased with prolonged time of iodine deficiency. Ninety to 100 percent of the cells with 41 chromosomes have the same chromosome missing: "one of pair #15." After one year of iodine deficiency, 5% of the rats acquired a new modal number in their thyroid glands. The weight of the thyroid gland increased from an average of 0.7 ± 0.3 in the control to 18.2 ± 7.4 mg/100 gm body weight at 21-25 months of iodine deficiency. Similarly, the weight of the pituitary was about more than twice that of the control after more than 14 months of iodine deficiency.

The implication of these chromosomal changes to the production of neoplastic thyroid tumors from iodine-deficient goiters is discussed.

INTRODUCTION

The role of chromosomal changes in the causation of cancer has been a controversial matter for over half a century. Nevertheless, the concept of malfunctioning DNA as a direct and obligatory factor in the causation of cancer is well accepted (5). Chromosomal changes could result from induced changes on the gene level.

The primary genetic changes may not be sufficient to induce a cancerous state, but they may lead to such a state through somatic mutation(s).

One of the major difficulties in assessing the role of chromosomal changes in carcinogenesis is the finding of an acceptable and reliable system for a thorough study of the chromosomes of the preneoplastic stages. Such a system is essential to the understanding of the cause-effect relationship between the chromosomes and neoplasia. The induction of thyroid tumors in Fischer rats by feeding the animals an iodine-deficient diet, and the production of neoplastic tumors by transplanting bits of these thyroids into other rats, offer an excellent system for the study of the chromosomes of the progression stages of neoplasm from normal to hyperplastic to preneoplastic. In a previous publication (1), we described the chromosomal changes in the thyroid cells of Fischer rats maintained on an iodine-deficient diet for 0.5-6.0 months. We have also studied (2) the chromosomal changes in thyroid tumors produced by transplanting bits of thyroid glands from rats iodine deficient for 16-18 months.

In this report, we present (a) a cytogenetic study of the developing goiters from six months of iodine deficiency to the time at which neoplasms may be produced from the transplanting of "preneoplastic" iodine-deficient thyroids, and (b) a study of the chromosomes of thyroid cells from rats iodine deficient for prolonged periods beyond the time at which neoplastic thyroid tumors may be produced when transplanted. An attempt will be made to correlate the histopathologic changes with the cytogenetic changes. The comparison of the chromosomes and histopathology of the preneoplastic thyroids with those of the produced tumors will, hopefully, throw more light on the problem of cytogenetic change and carcinogenesis.

MATERIALS AND METHODS

Rats. One hundred and ninety-nine inbred Fischer rats were used in this study. Generally, the rats were received at 5-6 weeks of age weighing 40-50 gm. Fifty-eight rats, 27 males and 31 females, were maintained on a normal iodine intake of 2.5 μg of iodine per gram of food. These animals were sacrificed and processed in groups of two at 2- to 4-week intervals at the same time iodine-deficient rats were sacrificed. One hundred and forty-one rats, 63 males and 78 females, were maintained for 7-25 months on a modified Remington low-iodine diet (8 lb. of Brewer’s yeast is added to each 100 lb of the Remington test diet) containing <0.05 μg of iodine per gram of food and distilled water. These animals were sacrificed in groups of 1-10 at intervals of 2-4 weeks.

All rats were sacrificed in 1-2 min by carbon dioxide in-
Table 1

<table>
<thead>
<tr>
<th>Months iodine deficient</th>
<th>No. of rate</th>
<th>Mean thyroid wt. (mg/100 gm B.W.)</th>
<th>Mean pituitary wt. (mg/100 gm B.W.)</th>
<th>Mean PBI (µg %)</th>
<th>No. of rate</th>
<th>Mean % aneuploid</th>
<th>Chromosomal analysis</th>
<th>Distribution</th>
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<td>6.7 ± 0.3</td>
<td>3.8 ± 0.36</td>
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<td>39</td>
<td>4.5 ± 0.56</td>
<td>&lt;39</td>
<td>32</td>
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<td></td>
<td>(Control rate on normal iodine intake)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>39</td>
<td></td>
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<tr>
<td>0.5-3</td>
<td>19</td>
<td>16.0 ± 1.7</td>
<td>18.2 ± 2.4</td>
<td>2.4 ± 0.49</td>
<td>19</td>
<td>16.2 ± 2.4</td>
<td>1.68 ± 0.57</td>
<td>2.95 ± 1.1</td>
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<td>3-6</td>
<td>17</td>
<td>45.2 ± 5.6</td>
<td>27.4 ± 1.8</td>
<td>12.8 ± 5.7</td>
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<td>20.5 ± 2.1</td>
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<td>6-9</td>
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<td>11</td>
<td>20.4 ± 1.9</td>
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<td>15-18</td>
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<td>128.4 ± 4.8</td>
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<td>183.2 ± 7.4</td>
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<td>46.4 ± 5.1</td>
<td>3.8 ± 1.8</td>
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<td>3.8 ± 1.8</td>
<td>21.3 ± 6.1</td>
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PBI, protein bound iodine; B.W., body weight.

Table 1—Continued

<table>
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<th>Months iodine deficient</th>
<th>No. of rate</th>
<th>Mean thyroid wt. (mg/100 gm B.W.)</th>
<th>Mean pituitary wt. (mg/100 gm B.W.)</th>
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<td>1.8 ± 0.42</td>
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<td>45.2 ± 5.6</td>
<td>0.94 ± 0.42</td>
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<td>0.94 ± 0.42</td>
<td>0.71 ± 0.34</td>
<td>0.59 ± 0.40</td>
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<td>0.82 ± 0.42</td>
<td>11</td>
<td>0.77 ± 0.27</td>
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<tr>
<td>9-12</td>
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<td>19.0 ± 1.1</td>
<td>0.063</td>
<td>0</td>
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<td>37.3 ± 3.4</td>
<td>12.0 ± 7.76</td>
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<td>12.0 ± 7.76</td>
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<td>80.1 ± 8.0</td>
<td>1.7</td>
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<td>7</td>
<td>2.0 ± 1.0</td>
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Mean % cells with markers

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halation. Blood samples were usually collected for serum protein bound iodine (PBI) determinations. Thyroid glands were removed quickly under aseptic conditions, weighed, and processed for cell culture and chromosome study. A small piece of thyroid was sent to our Pathology Department along with the pituitary gland for histopathologic determination.

**Cell Culture and Chromosomal Preparation and Scoring.**

Cell culture and chromosomal metaphase plate preparation and scoring procedures were carried out essentially as before (1). Fifty to 150 metaphase chromosome complements were analyzed from each culture. The results of each 3-month period of iodine deficiency were pooled and compared statistically.

**RESULTS**

Charts 1–5, which summarize data on iodine-deficient cells, include the result of 1–6 months of iodine deficiency from a previous publication (1) for the purpose of comparison.

**Controls**

Satisfactory chromosomal analysis was obtained on 39 animals, 19 males, and 20 females, from the original 58 control rats. An average of 4.5 ± 0.5% (Table 1) aneuploidy, a range of 0–8%, and no structural chromosomal changes were observed in the thyroid cells of these rats. An average of 1.38 ± 0.07% of tetraploidy and a range of 0–4% was found in the control rats. The average weight of the thyroid gland of the control rats was 6.7 ± 0.3 mg/100 gm of body weight; the average pituitary weight was 3.8 ± 0.3 mg/100 gm of body weight. The average serum PBI was 3.1 ± 0.3 μg %. The thyroid and pituitary histopathologies were normal. The thyroid gland follicles were of variable sizes and somewhat hyperplastic in the old controls (over one year of age).

**Iodine-Deficient Animals**

Chromosomes. In our previous publication (1) we reported that 19–30% of the thyroid cells were aneuploids after 1–3 months of iodine deficiency. The aneuploidy was increased to 44% at 6 months of iodine deficiency. When these results and the results of this study were analyzed after grouping the animals into 3-month periods of iodine deficiency, a significant increase in the percent of aneuploidy was observed beyond 6 months of iodine deficiency (Chart 1). Between the 6th and 11th months of iodine deficiency, the percent of aneuploidy, weight of thyroid, and the other cytogenetic parameters used (see below) were lower than those of the preceding groups. It was established beyond doubt that these unexpected results were due to iodine contamination of the diet. Total iodine was high in the diet used during that period; it was 2-2.4 μg/gm of food, compared to <0.05 μg/gm of food in the noncontaminated, iodine-deficient diet. The serum PBI in the rats which were sacrificed within the “contamination” period was 2.0–2.7 μg %; it was 0.8–0.5 μg % in the iodine-deficient rats.

A modal shift was observed in the thyroidal cells of five rats (4.3%) iodine deficient for a year or more, but not in any of the controls. In three animals, at 15, 18.5, and 21 months of iodine deficiency, the new modal number was 41 chromosomes and a karyotype having one chromosome of pair #15 missing with no other changes (Fig. 1). In the other two rats which were iodine deficient for 12.5 and 19 months, the new modal number was 44 chromosomes. The karyotype (Fig. 2) was characterized by two additional acrocentric chromosomes, one rather large chromosome resembling pair #4 and the other smaller chromosome resembling the 10th pair. These additional chromosomes were found in all cells with 44 chromosomes. In 18 rats (16%) iodine deficient for a year or more, stem line cells with 41 or 43 chromosomes were found in more than 30% of the cells, but not in any of the controls.
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The percent of aneuploidy changed from 4.5 ± 0.56% in the controls to 46.4 ± 5.1% in the group iodine deficient for 21-25 months.

There was a tendency for the number of cells with 41 chromosomes to increase with time on iodine deficiency (Chart 2). Such a tendency was especially evident at after more than 15 months of iodine deficiency ($P < 0.1$ compared to the preceding group). Ninety to 100% of the karyotypes of the cells with 41 chromosomes showed the same abnormality, the dropping of one of the small metacentric chromosomes. It is certain from our analyses that the same chromosome was always missing, and it is strongly believed to be one of pair #15 (Fig. 1). The percent of cells with 41 chromosomes changed from 1.1 ± 0.5% in the control to 21.3 ± 6.1% at 21-25 months of iodine deficiency ($P < 0.001$).

The number of cells with 43 chromosomes increased from 0.15 ± 0.1% in the controls to 2.3 ± 0.7% ($P < 0.001$) at 0.5-3 months of iodine deficiency (Chart 3) to 12.9 ± 3.9% at 21-25 months of iodine deficiency ($P < 0.001$). The cells with 43 chromosomes did not always have the same extra chromosomes. In some cases the extra chromosome was a marker chromosome; in others it was similar to one of the chromosomes in the set. In 83% of the cells with 43 chromosomes of a rat 18 months iodine deficient, the same extra chromosome was found.

There was a significant tendency toward a higher percent of cells with marker chromosomes with prolonged iodine deficiency (Chart 4). The percent of cells with marker chromosomes has increased from 0% in the controls to 11.6 ± 2.0 at 21-25 months of iodine deficiency ($P < 0.001$). The cells with marker chromosomes were scored if one or more marker chromosomes were found. Such a cell was photographed and then karyotyped to verify the microscopic observation. The marker chromosomes were then identified. Several types of marker chromosomes were found, small to large metacentric, submetacentric, or acrocentric chromosomes. Minute marker chromosomes were also found. Sixty percent of the marker chromosomes were of the large metacentric or submetacentric kinds, 20% were small metacentric and submetacentric, and the remainder were of the minute and acrocentric chromosomes.

**Thyroid Weight and Histology.** In addition to the cytogenetic changes, some significant changes in the weight and the histology of the thyroid and pituitary glands were observed. The weight of the thyroid was more than 27 times that of the control (6.7 ± 0.3 mg/100 gm body weight) in the rats iodine deficient for 21-25 months (183.2 ± 7.4 mg/100 gm body weight). The thyroid gland weight increased steadily with duration of iodine deficiency except for the contamination period, where the weight of the thyroid gland dropped (Chart 5) below the preceding period. The histology of the thyroid also changed from mild hyperplasia at 0.5-6 months of iodine deficiency to hyperplastic with papillary infoldings and the appearance of nodules after 7-9 months of iodine deficiency. After

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**Chart 3.** A histobar showing the increase of % thyroid cells with 43 chromosomes with time on iodine deficiency.

**Chart 4.** A histobar showing the increase of % thyroid cells which have acquired one or more marker chromosomes.

**Chart 5.** A histobar showing the increase in the weight of the thyroid glands of iodine-deficient rats for different intervals. The unexpected results between 6 and 12 months of iodine deficiency were due to the iodine-contaminated diet.
one year or more of iodine deficiency, the thyroid became more nodular and more hyperplastic, and calciospherites were commonly found. Beyond 15 months of iodine deficiency, some neoplastic changes were described in five out of 99 thyroid glands (this total represents the number of thyroid glands studied from 15–25 months of iodine deficiency) or in about 5% of the thyroid glands studied after more than 15 months of iodine deficiency.

**Pituitary.** The weight of the pituitary remained practically unchanged up to one year of iodine deficiency. The average weight of the pituitary in the control rats was 3.8 ± 0.35 mg/100 gm body weight; it was 4.4 ± 0.5 mg/100 gm body weight at 9–12 months of iodine deficiency.

After more than 14 months of iodine deficiency, the weight of the pituitary almost doubled, changing from 4.4 ± 0.5 mg/100 gm body weight to 8.5 ± 1.1 mg/100 gm body weight. The histology of the pituitary gland seemed to remain unremarkable, at least at the level of the light microscope, up to about one year of iodine deficiency. After a year or more of iodine deficiency, chromophobe hyperplasia was observed. Between 12 and 15 months of iodine deficiency, 35% of the pituitaries were hyperplastic (or showed chromophobe adenoma); after 15 months of iodine deficiency, more than 90% of the pituitaries had hyperplasia or chromophobe adenoma. Chromophobe adenomas were described in six (7.2%) of the pituitary glands from rats more than 15 months iodine deficient.

**DISCUSSION**

When bits of thyroid gland from rats iodine deficient for more than 15 months were transplanted into the flanks of other rats by Matovinovic (3) and by us (unpublished data), they grew into thyroid tumors. Recently we were able to harvest thyroid tumors as a result of transplanting bits of thyroid gland from rats iodine deficient for only 4 months (unpublished data). The development of neoplasms from hyperplastic thyroid glands with no apparent neoplasm led us to analyze the chromosomes of the thyroid cells from iodine-deficient rats. The increase of aneuploid cells, i.e., cells with 41 and 43 chromosomes, and the increase of aneuploid cells are transplanted.

The gradual increase in aneuploid cells (cells with 41 chromosomes which have one chromosome of pair #15 missing) and cells with 43 chromosomes, and the increase of aneuploid or quasidiploid cells with marker chromosomes after prolonged periods of iodine deficiency, could be explained on the assumption that some cells are selectively favored to flourish under these unusual environmental conditions of high TSH and low iodine after they have undergone some genetic changes. This view is supported by the fact that when a heterogeneous population of thyroid cells with a portion of the cells possessing some of the genetic changes mentioned above are transplanted into the flank of another inbred rat, a tumor commonly is produced with a more homogeneous cell population (2) characteristic of the “dependent” type tumor.

It is of significance to note that the majority of cells found with 41 chromosomes were always missing the same chromosome. In recent experiments we have observed a higher percent of tumor take, faster growth rate, and tumors becoming anaplastic, autonomous carcinomas within two generations when the thyroid cells used for transplantation have a higher percent of cells with 41 chromosomes (unpublished data).

The data presented demonstrate the change from a cytogenetically homogeneous cell population in the controls to a heterogeneous cell population which contains neoplastic and preneoplastic cells in the iodine-deficient rats. Such a change could be recognized at early stages (0.5–3 months) of iodine deficiency. However, the cellular heterogeneity increases with increasing time of iodine deficiency. These cells seem to have acquired neoplastic characteristics, for when such a cell population is transplanted, a neoplastic tumor, rather homogeneous, commonly is produced.

**ACKNOWLEDGMENTS**

The author is indebted to Dr. William H. Beierwaltes for criticism and review of the manuscript and to Dr. Ronald H. Nishiya and Dr. Beierwaltes for their review of the histopathology of the thyroid and pituitary glands.

**REFERENCES**

3. Matovinovic, J., Hilbert, R. D., Armstrong, W. F., and Helgen-
Fig. 1. A representative karyotype of a cell with 41 chromosomes from the thyroid gland of an iodine-deficient rat. Arrow points to the missing chromosome (one of pair #15). Such a karyotype was found in 90–100% of the 41 chromosome cells produced by iodine deficiency.

Fig. 2. A representative karyotype of a thyroid cell from a rat iodine deficient for 18.5 months. The thyroid gland in this rat acquired a new modal number of 44 (normal, 42 chromosomes). The arrows point to the two additional chromosomes which characterize the new karyotype after the modal-shift.
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