Rat Mammary Gland Atypia Produced by Iodine Blockade with Perchlorate

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SUMMARY

Prior published work from our laboratory concluded that there was a need for appropriate metabolic activity of iodine in breast tissue for normal growth and development. Results from studies in rats that were made iodine deficient showed histological changes in the breasts that were atypical and dysplastic. These tissue findings were further affected by the presence of estrogen and thyroxine. These changes parallel the iodine uptake of the tissues, thus representing a difference in the utilization of iodine by the mammary glands.

Using an ion blockade agent, sodium perchlorate, breast tissues lacking iodine were evaluated by both endocrine and histological techniques. A dose-response series was completed that showed that perchlorate therapy for 8 weeks at 400 mg/100 ml produced breast blockade by a reduction in iodine uptake of greater than 52% of the control. At these levels, the histological experimentation showed atypia and some pleomorphism of the cells, particularly in the glands of the lobules. Blockade was less effective in estrogen-treated groups. It is especially notable that both histological changes and uptake reduction were greatest in those breasts that had been rendered euthyroid by thyroxine replacement, thus clearly indicating the necessity of iodine itself for maintenance of normal breast development. By this blockade the responses of iodine inadequacy in the breast were shown to cause abnormal tissue changes relative to the percentage of the block obtained.

INTRODUCTION

Estrogen given to the normal rat produces epithelial cell hyperplasia of the mammary glands, principally in the lobules (1). We have previously shown that dietary iodine deficiency enhances this change (6, 7). It had been our hypothesis that this response to dietary iodine deficiency was due to the iodine deficiency itself and not, or at least not solely, to thyroid hormone deficiency and thyroid hormone alteration secondary to the dietary iodine deficiency. Support for our hypothesis has included the findings that the breast traps iodine (12) and organifies it (13), although not as efficiently as does the thyroid gland. Because the breast does carry out these activities, we have come to feel that iodine itself may be necessary for the maintenance of normal breast function and normal breast histological architecture.

Since dietary iodine deficiency in the rat is an experimental design with multiple variables, the most important being effects on the thyroid gland, we wished to investigate our hypothesis by an alternative method. It has been known for many years that a number of small anions, among them perchlorate, effectively block the uptake of iodine by the thyroid gland (2), presumably by competing directly for transport mechanisms (3, 15, 19). Since such a blockade has been demonstrated to occur in extrathyroidal sites of iodine uptake, it seemed likely that it might occur in breasts as well (4, 20). With this idea in mind, the research reported here was designed and carried out. Rat breasts were rendered iodine deficient using perchlorate ion for chemical blockade of iodine (8). The resulting breast tissue changes were studied with sex steroid and thyroid hormone stimulation.

Prior radioactive uptake studies in rats (7) and more recent ones in humans (9–11) have shown that, in certain breast abnormalities, a remarkable increase in the percentage of uptake of radioactive iodine occurred. In order to expand these findings, and as a check on the iodine parameters used in this experiment, uptake studies of breast tissues were carried out along with the histology.

MATERIALS AND METHODS

Forty-eight Sprague-Dawley albino virgin female rats (Charles River Breeding Laboratory, Wilmington, Mass.), initially weighing 180 to 200 g, were separated into 2 major categories: normal intact and perchlorate-treated rats. These 2 major categories of rats were subdivided into 4 groups of 6 randomly selected animals, receiving: (a) no further treatment, (b) thyroxine replacement, (c) estrogen therapy, and (d) thyroxine replacement with concurrent estrogen therapy.

Thyroxine treatment consisted of 4 μg l-thyroxine sodium salt (Warner-Lambert Research Institute, Morris Plains, N. J.) daily, injected i.p. in 0.1 ml 0.9% NaCl solution. Estrogen therapy was 50 μg estradiol (Schering

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Corp., Bloomfield, N. J.) suspended in 0.1 ml sesame oil and injected daily i.m. for the last 5 days of the experiment. Vaginal smears were taken twice during the study, once before and once after estrogen therapy had begun; these verified the presence of an estrogen response.

The 4 normal groups of animals received tap water ad libitum. The 4 perchlorate groups of rats received 400 mg/100 ml NaClO₄ (Fisher Scientific Co., Fair Lawn, N. J.) in deionized distilled water ad libitum for the duration of the experiment. On this regimen, perchlorate rats grew and gained weight at the same rate as did the normal controls (Table 1).

At the end of 8 weeks of treatment, all the animals were given i.p. injections of 1 μCi of ¹²³I (Amersham/Searle, Arlington Heights, Ill.) suspended in 0.1 ml 0.9% NaCl solution 60 min before being killed with ether. The 3 inferior bilateral abdominal mammary glands of each rat were dissected free, weighed, and fixed in 10% formaldehyde solution (Table 2). One side was counted and the other side was prepared for histological study. In addition the thyroids, adrenals, ovaries, and uteri were dissected, weighed, and fixed in formaldehyde. Venous blood was removed from the inferior vena cava. The blood was centrifuged for 10 min immediately after it was drawn; 0.2 ml of the resulting serum was drawn off and counted.

Counting of all the tissues and the blood was carried out in an automated well-type scintillation counter (Searle Analytic Inc., Des Plaines, Ill.) precalibrated for ¹²³I. The counts were expressed in cpm/mg of tissue.

Microscopic slides of the breast tissue were stained with hematoxylin and eosin and examined under high and low powers.

RESULTS

Morphological Findings

Group 1a: Normal Rat Breast. The normal rat breast in the nonpregnant state, as shown in these control animals, is a compound tubuloalveolar gland that is fundamentally similar to the human breast (Fig. 1). The gland consists of abundant s.c. mammary fat in which there is a scattered arborescence of excretory ducts and small, widely dispersed lobules. The lobular units are composed of smaller divisions of the excretory ducts, with numerous side branches and distal terminal branches with enlarged end bulbs. Occasional acinar formations are clustered about the small excretory ducts. The larger ducts are invested with a circularly arranged coat of connective tissue fibers, but otherwise there is little intralobular stroma in the rat breast as compared with the human breast. The terminal ducts and acini are lined by a single layer of cuboidal epithelial cells with a well-defined basal layer of myoepithelial cells, as shown in Fig. 2, a high-power photomicrograph.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean</th>
<th>SE</th>
<th>Mean</th>
<th>SE</th>
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<td>4.861</td>
<td>285</td>
<td>5.241</td>
<td>62</td>
<td>4.151</td>
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Table 1
Comparative body weights of rats during treatment with hormones alone and with perchlorate

Table 2
Radioactive iodine uptake in rat breasts following treatment with hormones alone and with perchlorate

<table>
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<tr>
<th>Group</th>
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<th>SE</th>
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<td>691</td>
<td>114.940</td>
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<td>153</td>
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<td>71.214</td>
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<td>127</td>
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<td>618</td>
<td>91.852</td>
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**Group 1b: Thyroxine Effect.** Thyroxine had a slight stimulatory effect on mammary growth but significantly enhanced secretory activity in the previously untreated rats (Fig. 3). In the majority of animals, the pattern of mammary growth could be summarized as variable and irregular. A few well-stimulated, hyperplastic lobules with abundant secretory activity might coexist with lobules of inactive appearance or with those in which growth was only partly suppressed. In the more active lobules, the ducts and acini were often distended and lined by hyperplastic epithelial cells with abundant foamy cytoplasm in which the nuclei were irregularly suspended (Fig. 4). There was no abnormal alteration of the growth pattern of the epithelial elements of the breast with thyroxine administration.

**Group 1c: Estrogen Effect.** Stimulation of the normal rat breasts by estrogen induced marked growth and development, with the formation of greatly hypertrophied lobules and abundant alveolar and ductal secretion. There was a corresponding reduction in the amount of interstitial adipose tissue (Figs. 5 and 6). Rapid multiplication of the glandular epithelium with increased branching of blunt-end ducts and budding evaginations of alveoli accounted for the lobular enlargement. Often, acinar cystic development produced a "honeycomb" effect. Ductal hyperplasia was not as conspicuous as was lobular alveolar hyperplasia, but occasionally there was mild ductal dilatation with increased proliferation of the lining epithelial cells. Throughout the lobules there was a high degree of normal epithelial proliferation but no evidence of abnormal growth patterns.

**Group 1d: Thyroxine and Estrogen Effect.** Thyroxine and estrogen given concurrently produced a moderate to marked increase in mammary growth and development with a prominent secretory response (Figs. 7 and 8). Secretory material often filled the lumina of dilated ducts and acini. However, in most animals, thyroxine in combination with estrogen appeared partly to suppress the full stimulatory effects of estrogen on the breast tissues while enhancing secretory activity. Morphologically, with combined thyroxine and estrogen administration, the ductal and alveolar portions of the lobules showed marked epithelial hyperplasia and, occasionally, what appeared to be a loss of uniformity of cell pattern with nuclear "scrambling." This latter picture was due to the different location of the nuclei within the epithelial cells, which were in different stages of secretory activity. The changes generally conformed to a regular pattern of epithelial proliferation without atypia or disturbance of the growth pattern.

**Group 2a: Perchlorate Effect.** The breasts of the rats receiving perchlorate alone are generally of normal size or slightly hypoplastic. Perchlorate induced mild atrophy, with significant but not marked atypia of the lobular epithelium. The interlobular ducts with their terminal ramifications and lateral and end buds, together with the small alveolar formations when present, are involved in the process, as can be seen in photomicrographs (Figs. 9 and 10). Morphologically, there appears to be a general reduction in cell size in the atrophic areas, but throughout there are scattered foci of marked hyperplastic activity where the epithelial cells lose their relatively small, uniform appearance and normal abnormalities such as increased cellularity, variability of nuclear shape and size, and hyperchromatism. There is some loss of cellular polarity and orientation of cells to the ductal basement membranes, but there is no loss of cellular cohesiveness. Frequently, there is dilatation with fibrous thickening of the medium-sized ducts, with some piling up of the epithelial lining cells. In areas, the changes are highly reminiscent of atypical lobular hyperplasia seen in the human breast, but the picture in no way indicates that the process has proceeded to a malignant transformation.

**Group 2b: Perchlorate and Thyroxine Effect.** The rats that were given a combination of perchlorate and thyroxine showed abnormal breast changes corresponding to those seen in animals under the influence of perchlorate alone (Fig. 11). The limited stimulatory effect of thyroxine on mammary gland development and the beneficial effect on secretory activity corresponded to that previously described with thyroid hormone administration in the absence of perchlorate. Microscopically, the atrophic and atypical epithelial changes produced by perchlorate alone persisted in approximately the same degree, or rarely, to a slightly greater degree, throughout the lobules when thyroxine and perchlorate were given in combination (Fig. 12). No synergistic or potentiating effect could be observed in the majority of rat mammary glands when the 2 compounds were given together.

**Group 2c: Perchlorate and Estrogen Effect.** Rats treated with perchlorate and estrogen showed the activating influence of estrogen on the growth and development of the breast tissues. The degree of lobular alveolar and ductal epithelial hyperplasia, however, was moderately reduced when the 2 compounds were given concurrently (Fig. 13). In sections from various parts of the breast, the lobular stroma appeared to be increased in amount, and the long ducts with their distal terminal branches were frequently surrounded by a thick coat of fibrous connective tissue. Morphologically, when compared with the effect of perchlorate alone, the cumulative effect of perchlorate and estrogen together resulted in a significant reduction in the atypical changes in the lobular alveolar and ductal epithelium. Nonetheless, atrophic changes together with foci of recognizable atypia persisted in various parts of the lobule (Fig. 14).

**Group 2d: Perchlorate, Estrogen, and Thyroxine Effect.** The rats that were given perchlorate and a combination of thyroxine and estrogen showed irregularly distributed areas of atypical epithelial hyperplasia of the mammary epithelium, often involving isolated lobules (Fig. 15). The epithelial changes that were most prominent in the terminal ducts and alveoli strongly resembled lobular atypia or so-called atypical lobular hyperplasia of the human breast. Microscopically, the epithelium in the altered lobules frequently showed considerable overgrowth with cells partly filling the glandular lumina. There was considerable variation in cellular and nuclear size and shape, with increased staining intensity of the nuclei, but striking abnormalities were limited in most animals (Fig. 16). The degree of glandular development throughout the remainder of the breast generally conformed to the pattern described in thyroxine- and estrogen-treated animals in the absence of perchlorate.
Radioactive iodine uptake in rat thyroid, blood serum, and the thyroid-serum ratio following treatment with hormones alone and with perchlorate

<table>
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<th>Group</th>
<th>Treatment</th>
<th>Thyroid uptake (Counts/min/mg)</th>
<th>Blood serum uptake (counts/min/mg)</th>
<th>T/S x 100</th>
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<td></td>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
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<td>Normal</td>
<td>485.593</td>
<td>91.073</td>
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<td>Normal + 1-thyroxine</td>
<td>435.715</td>
<td>49.086</td>
<td>9,256.493</td>
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<td>1c</td>
<td>Normal + estradiol</td>
<td>471.415</td>
<td>122.842</td>
<td>9,080.357</td>
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<td>1d</td>
<td>Normal + 1-thyroxine + estradiol</td>
<td>464.672</td>
<td>112.351</td>
<td>7,189.123</td>
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<td>Perchlorate</td>
<td>25.151</td>
<td>2.640</td>
<td>7,000.000</td>
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<td>68.253</td>
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<td>102.079</td>
<td>51.294</td>
<td>7,792.718</td>
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* T/S, thyroid-serum ratio.
N. J.), yet they still showed a 55% iodine blockade and atypical histological development. This enhanced blockade of iodine over the control level could be the result of the influx of exogenous L-thyroxine sodium salt, which depresses the thyrotropin level (12) and, correspondingly, the ability of the thyroid and extrathyroidal tissue to synthesize thyroxine (12). Thyroxine influences on normal breast development have been alluded to by others (16, 17). No specific evidence, however, shows thyroxine to be a requirement. Thyroxine nuclear receptors have been shown in tissue culture studies to have an effect on increasing RNA polymerase activities, nucleoside transport, and glucose utilization (18). These are crucial to protein metabolism within the cytosome (5).

The effect of estradiol, seen in this experiment, on the perchlorate breast is ambiguous. We had previously found that dietary iodine deficiency enhanced the responsiveness of the breast tissue to sex steroid stimulation (7). In this study it was seen that the state induced by chemical blockade of iodine greatly reduced the estrogenic effect on the breasts. There was significantly less lobular proliferation in the perchlorate animals receiving estradiol than there was in normal controls. In light of our recent findings suggesting the necessity of iodine in the synthesis of estrogen receptor protein (H. I. Jacobson and B. A. Eskin, unpublished data), a decrease in the amount of iodine available in the breast because of the perchlorate blockade would diminish the amount of iodine available for use by the estrogen receptor proteins, without which the estradiol would be less able to exert the anticipated proliferative effects on the tissues.

A 2nd possible explanation for this apparent disparity is that the mechanism by which the breast tissue would be affected by an iodine deficiency could be different in these 2 cases. In the 1st case, the animals were made iodine deficient by dietary restriction, and thus hypothyroinemic (21). In the 2nd case, the iodine was available but could not be utilized because it was chemically blocked by the perchlorate. The substitution of the perchlorate ion could affect the receptivity of the breast tissue to estrogen (20).

The combined effect of estradiol and thyroxine on the blockaded breast in uptake studies showed intermediate reduction of the effects of these 2 substances alone. It would seem that the enhancing effects of estradiol overcame the reducing effects of thyroxine administration. Again, the replacement of the thyroid hormone to normal serum levels did not reverse the abnormal hyperplasia obtained, so that the need of the breast tissues for iodine was reinforced (6, 7).

The result of this research shows that iodine plays a vital role in the maintenance and development of normal breast tissues. The reduction of available iodine to the breast has produced distinctive atypia. Research continues in order to determine the site of iodine metabolism, the extent of atypia produced, and whether an additional factor is required for progression of the tissues to cancer.

REFERENCES

Figs. 1 and 2. Normal rat (Group la).

Fig. 1. Low-power view of untreated rat breast showing scattered small mammary lobules consisting of prominent ducts and occasional relatively quiescent alveolar clusters in an abundant adipose stroma. Note the moderate amount of periductular and periacinar connective tissue. H & E, x 70.

Fig. 2. High-power view of the acinar structures lined by nonsecretory, small cuboidal cells and myoepithelial cells. H & E, x 425.

Figs. 3 and 4. Normal rats, thyroxine effect (Group 1b).

Fig. 3. Breast lobules of thyroxine-treated animals showing focally increased alveolar development. H & E, x 70.

Fig. 4. High-power photomicrograph of the better-developed lobules showing considerable proliferative and secretory activity of ductal and acinar epithelium. H & E, x 425.

Figs. 5 and 6. Normal rats, estrogen effect (Group 1c).

Fig. 5. Low-power magnification illustrating extensive lobular development with consequent replacement of the intervening interlobular adipose tissue in estrogen-stimulated rats. Note the lobular acinar hyperplasia exhibiting both proliferative and secretory activity. H & E, x 70.

Fig. 6. High-power magnification of an active lobule showing a high degree of normal epithelial proliferation and secretory changes. Glandular tissue assumes a pregnancy-like effect. H & E, x 425.

Figs. 7 and 8. Normal rats, thyroxine plus estrogen effect (Group 1d).

Fig. 7. Low-power photomicrograph showing lobular changes resembling those seen in Fig. 5, but with increased secretion spilling into dilated ducts and acini. Note so-called "scrambling" of nuclei, seen also in Fig. 6, probably resulting from different phases of secretory activity. H & E, x 425.

Fig. 8. High magnification of lobular epithelial activity with secretory material in dilated ducts and acini. Note so-called "scrambling" of nuclei, seen also in Fig. 6, probably resulting from different phases of secretory activity. H & E, x 425.

Figs. 9 and 10. Perchlorate rats (Group 2a).

Fig. 9. Low-power view of s.c. fat pad with scattered main ducts and collateral and terminal branches. Main ducts are invested by circular bundles of fibrous connective tissue. Note prominent lateral and end budding with occasional acinar cluster formation. H & E, x 70.

Fig. 10. High-power view showing a single lobule with minimal but distinctive epithelial atypia involving terminal ductular and acinar epithelium. Epithelial lining cells are slightly hyperplastic and somewhat pleomorphic, with intensely staining nuclei. Marginal orientation of cells is fairly well preserved. H & E, x 425.

Figs. 11 and 12. Perchlorate rats, thyroxine effect (Group 2b).

Fig. 11. A low-power photomicrograph showing minimal stimulatory effect of thyroxine on mammary gland development. Irregular foci of ductal and lobular hyperplasia alternate with areas of minimal atrophy. Note prominent connective tissue surrounding ducts. Picture corresponds to Fig. 3. H & E, x 170.

Fig. 12. A high-power photomicrograph illustrating slight but distinctive atypia of acinar epithelium comparable to Fig. 10. Note abundant secretion in the gland lumina. H & E, x 425.

Figs. 13 and 14. Perchlorate rats, estrogen effect (Group 2c).

Fig. 13. A low-power photomicrograph showing prominent lobular alveolar and ductal growth and development of breast tissues with combined perchlorate and estrogen administration. H & E, x 70.

Fig. 14. A high-power photomicrograph of a hypertrophied lobule showing minimal epithelial atypia. Note increased cellularity and hyperchromaticity and slight variation in nuclear size and shape. Overall pattern is slightly more florid than with estrogen alone in the absence of perchlorate. H & E, x 425.

Figs. 15 and 16. Perchlorate rats, thyroxine plus estrogen effect (Group 2d).

Fig. 15. A low-power view of mammary lobular development reflecting full manifestations of estrogen and thyroxine stimulation. Compare with photomicrograph in Fig. 7. Note 2 isolated, abnormal-appearing lobules at bottom of photomicrograph. Elsewhere, lobules are more normal appearing and present a "honeycomb" effect. H & E, x 170.

Fig. 16. A high-power view of an abnormal-appearing lobule from Fig. 15 showing "atypical lobular hyperplasia." Glandular lumina are partly obliterated by enlarged, somewhat pleomorphic cells. H & E, x 425.
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