CHAPTER 20

IODINE AND MAMMARY CANCER

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I. INTRODUCTION

While iodine has been found in lower prechordates, no identifiable physiologic influence of the element is recognized until the level of amphibians. Organified iodine is required for the metamorphosis of the tadpole to the adult frog. Iodinated compounds are essential to growth and development of most vertebrates, and iodine levels temper these metabolic changes.

In order to properly function and form the characteristic iodinated secretions of thyroxine (T_4) and triodothyronine (T_3), iodine must be adequately present in the circulating blood. Iodine deficiency is expressed by the development of a "goiter", which is hypertrophy or hyperplasia of the thyroid parenchyma. The formation of a "goiter" is an effort of the body to conserve the available iodine stores and secrete the T_4 and T_3 needed to maintain a euthyroid state. Kidney excretion of iodine is minimized and recycling of the precious element, in various forms, continues until iodine is made available again to the system. Activity of the thyroid gland is controlled by pituitary thyroid-stimulating hormone (TSH). Release of T_4 from the pituitary is, in turn, dependent on the levels of free T_4 and T_3. The pituitary/thyroid axis is unique in that there is greater intrinsic pituitary control than in other endocrine systems. TSH secretion is inversely proportional to metabolic effects of T_4 and T_3. The hypothalamic effluent, thyroid-releasing hormone (TRH), exerts only a modulatory influence on the pituitary and is not the final regulator of feedback.

In the higher vertebrates, it was thought that the synthesis of iodinated compounds was limited to the thyroid gland. Since the thyroid gland can trap the greatest load of iodine into its cell transport system, it normally serves as the active reservoir for iodine. Currently, the biochemical and physiological action of the iodinated compounds have been studied at end organs as well.(*1*) In addition, iodine has now been found, in measurable amounts, in the salivary glands, gastric mucosa, liver, certain tumors, and in the ovaries and testes.(*2*)

In several laboratories, including our own, iodine has been shown to be present in breast tissues. Using tissue slices,(*3*) autoradiography,(*4*) and radioactive iodine uptakes,(*5,6*) iodine has been measured and found to be present, in moderate amounts, but not in the abundance seen in the thyroid gland. The biochemical transport and metabolism of iodine by the breasts resemble, at a quantitatively lower level, those seen in the thyroid gland.

Laboratory experiments show that an inadequate supply of iodine, due to an iodine-deficient diet or perchlorate blockade, results in focal atypia and dysplasia of the breast in rodents.(*6,7,8*) It thus appears that maintenance of the optimum structure and function of the breast requires the presence of continuous and specific amounts of iodine. This *iodine effect* has been shown to depend on a secretion of TSH from the pituitary and responds negatively to relative and absolute reduction in TSH.(*5*)

From this basic information, it became apparent that iodine serves not only as an incidental element, responsible for growth and development, but may in itself, be involved in the intracellular metabolism of certain tissues, and hence, in the normalcy of these structures. The research from our laboratory concerns the activities of iodine in mammary glands and, specifically, the carcinogenicity and cocarcinogenicity of the conditions caused by modifications in iodine availability. The work to be reviewed and presented will be divided into (1) the experimental histology; (2) metabolic activity; and (3) clinical responses in altered iodine conditions.

II. EXPERIMENTAL HISTOLOGY

A. Carcinogenicity

Basic research performed in our laboratory, and since confirmed by others, has shown that iodine deficiency from dietary restriction produces specific changes in pubertal rat breasts.(*7,8*) The results have consisted of dysplastic changes, either atrophic or hyperplastic, and sometimes atypical. In the presence of estrogen treatment, the responding histology approaches that of a neoplastic state. Concomitantly with this work, the possibility that the dysplastic changes are secondary to hypothyroidism caused by iodine deprivation

was tested by inducing hypothyroidism in the presence of iodine.(7) The histologic changes seen in the mammary glands of the hypothyroid rats appeared to be distinctly different from those that were seen in the breasts of the iodine-deficient animals which were euthyroid, as indicated by serum thyroid function measurements. The results of this work suggested the probability that iodine itself directly influences breast histology.

Further confirmation of these conclusions are presented by the utilization of perchlorate to prevent iodination of the affected tissues in rats.(6) Breast changes were related to the percentage of the effectiveness of the blockade, increased atypia occurring with reduced iodine availability. These histologic changes were greatest in those breasts rendered euthyroid by thyroxine replacement, thus clearly indicating the necessity of iodine itself for maintenance of normal breast development.

Recent research from our group employed animals whose ages corresponded to mature human adults. With increasing age of the rats, in iodine-deficient breasts, even more evidence of atypical changes in the epithelium occurred.(9) This is seen in many foci in the "menopausal" age group. With the use of perchlorate and, hence, the iodine blockade, statistically significant increases in periductal fibrous overgrowth in trapping adjacent lobules with aberrant ductular proliferation, sclerosing adenosis, and microcyst formation takes place. All are strongly reminiscent of the analogous fibrocystic disease in the human.

But further, there are areas in this group of atypical lobules showing hyperchromatism, enlargement of nuclei, altered nuclear-cytoplasmic ratios, increased mitosis, and loss of polarity of the epithelium. Some of the lobules exhibited papillomatosis with poorly polarized epithelium. In one breast from this menopausal group, there is a focus of a lobule to a histologically malignant pattern, completely apart from the adenosis.

In all these studies, termination of either dietary iodine restriction or perchlorate results in variable modest return toward the normal tissue structure. Use of thyroxine (T_4) results in enhancement of the already existing abnormal changes, particularly if the amount given is greater than maintenance requirements.(10) Parallel replacement of iodine with the several iodine-deficient diets used results in normal control breast histology.(5) Additional iodine levels (2-6 x normal) show no measurable change.

Steroidal estrogen therapy as estradiol-17β(E_2) causes hyperplasia of the mammary gland ducts in normal estrus rats; however, E_2 enhances the glandular dysplasias seen in iodine deficiency, and to a lesser extent, in iodine blockade therapy.(11) The consideration of estrogen-iodine interaction in breast tissue will be dis-

cussed later.

Thus, breast iodine inadequacy by itself causes disturbing changes in the rat tissues that, once formed, are not reversed completely by replacement methods.

B. Cocarcinogenicity

Basic research findings indicate that there is an earlier onset of breast cancer in prepubertal rats, due to a carcinogen (DMBA), as well as an increase in size and numbers of lesions when the carcinogen is administered after an iodine-deficient or hypothyroid state is reached.(*12,13,14*) The growth of the cancers in size and numbers is greater in the iodine-deficient than in the hypothyroid rats.(*12*) The onset of lesions and tumor responses to DMBA appear to differ(*12,14*) according to techniques used and whether the carcinogen is given before or after the animals have become hypothyroid or iodine deficient. All rats used were pubertal before medications were given.

These results are seen also in long-term perchlorate therapy. Again, the dysplastic and neoplastic changes occur more rapidly in perchlorate-treated DMBA rats than in the control groups. Perchlorate treatment is supplemented by replacement thyroxine therapy, in order to maintain a euthyroid condition. The blockade remains at the breast tissue level; hence, the changes seen would represent only cellular effects of the breast, not affected by any perpheral reduction in available thyroxine.

C. Autoradiography

The histologic presence of breast iodine was confirmed by the utilization, in our laboratory, of autoradiography employing ^{125}I. Findings indicate that in iodine deficiency there is a decrease in the iodine in the breast tissues and intracellular iodine, diminished further in the chronic deficiency state, leading to the dysplastic changes described. The same response occurs when perchlorate is given where iodine, again, does not enter the breast tissue itself.(*4*)

Particularly exciting in these studies was the fact that not all glandular cells showed the iodine-effect simultaneously. The response appeared to be randomized, which may permit some speculation on the cause of the *focal* atypia that was described in iodine-deficiency carcinogenesis.

The histologic evidence of both carcinogenesis and cocarcinogenesis that has been presented relates to a requirement for intracellular iodine. How the iodine is biochemically employed within the cells has received some recent attention.

III. METABOLIC ACTION

A. Radionuclide Studies

Radioactive iodine uptakes were determined in rat breasts under varying physiologic and pharmacologic conditions of iodine availability.(*17*) This utilized the fact that when radioactive iodine is given, uptake is greatest where iodine is deficient; the exception would be in total blockade. In rat breast tissues with dysplasia, the uptake was increased, a finding that was employed in our clinical diagnostic research project(*18*) as described later.

Total breast ^{125}I uptake results are the sum of both vascular and cellular radioactive content. The breast parenchyma ^{125}I uptake can be calculated by subtracting the ^{125}I count in blood present in the breast from the total ^{125}I breast count. The amount of blood in the breast tissue has been determined by ^{51}Cr serum dilution studies in our laboratory. Preliminary ^{51}Cr studies have confirmed the feasibility of measuring this absolute breast parenchyma uptake.

The results of these most recent calculations have permitted us to compare the amount of iodine contained in the breast tissues with the related histologic findings. From these data we hope to construct a quantitative level required for breast normalcy in our rat model.

B. Intracellular Studies

Several biochemical studies have been performed to understand how iodine acts within the breast cells. By using the experimental rat model and modifying iodine therapies, our laboratory has shown variations in DNA/RNA, estrogen receptor protein (ERP), and cytosol radionuclide uptakes. Changes in these moieties with added thyroid and estrogen were also studied.

When total body iodine levels are lowered, breast DNA/RNA ratios increased threefold over normal values, and the DNA/RNA ratios shift contrary to those of the hypothyroid state ($p<.001$).(*5,19*)

The aggregation properties of estrogen receptor protein (ERP) is altered in iodine deficiency.(*19*) Sucrose density gradient profiles were employed and showed the protein significantly less aggregated in iodine deficiency and the 4-58 form remarkably increased in the deficient group as compared with the controls. The ERP of breast tissue is used as a diagnostic test for assessing the dependence of tumor tissue on the presence of estrogen for growth and development.(*20*) A consideration of our findings permits speculation on estrogen interaction with the iodine present. The increase in receptor may enhance the tumorigenesis of breast tissues when estrogen is present.

In mice, the uptake of ^{125}I iodine by transplanted hormone responsive (HR) mammary tumors has been shown to be significantly greater than the uptake of ^{125}I iodide by transplanted hormone independent (HI) mammary tumors.(21) As anticipated, uptake of ^{125}I by HR mammary tumors was greatly reduced by the simultaneous injection of an excess of nonradioactive iodide. In addition, perchlorate treatment blocked the uptake in the breasts.

This interaction between iodine and estrogen has been described in castrated female rats made iodine deficient.(11) In these animals, breast ^{125}I uptake was greater than in intact rats, and when given estrogen, the breast dysplasia resulting was significantly greater than in the ovariectomized controls. When both iodine and estrogen were given to these animals, the breast tissues approached normalcy. It would appear, then, that estrogen activity in the breast requires the presence of an adequate level of iodine.

When rat breast cells are fractionated by ultracentrifugation, the content of iodine in the soluble portion can be determined by ^{125}I uptakes.(19) The soluble blood fraction is calculated by the ^{51}Cr method previously described. Under these circumstances, the uptakes of both iodine deficiency and perchlorate blockade are significantly lower than the uptake found in the control animals and in certain altered thyroid states. These findings show that cytosol iodine has been reduced by dietary and blockade methods, while the levels obtained by hypothyroidism, hyperthyroidism, and additional iodine are not significant. These intracellular results offer evidence that iodine acts at the breast tissue level and that biochemical homeostasis is disturbed when iodine is reduced.

IV. CLINICAL

A. Epidemiology

While a correlation between thyroid/iodine and breast cancer has been considered and presumably investigated since 1890, no definitive statements about a relationship between iodine and mammary gland physiology were published until 1956.(3) Consideration of the relationship of iodine metabolism to breast neoplasia was first described in 1967(7) and reviewed in 1970.(5)

Published statistics by others and by us indicate high rates of breast cancer in regions of known endemic goiter and lower rates where iodine is adequate.(22) The incidence of breast cancer is high in Mexico and Thailand, both of which are regions of endemic goiter, while the incidence of breast carcinoma is low in Japan and Iceland where goiter is not endemic.(23) Higher incidence rates of breast cancer have been published in localized pockets of endemic goiter in Poland, Switzerland, Australia, and the Soviet Union.(24,25)

In the United States, close correspondence exists between regions of high mortality rates due to breast cancer (American Cancer Society statistics) and areas of endemic goiter described by literature from several sources including the World Health Organization.(*26,27*)

A recent report from a radiology screening program in a United States metropolitan area with known iodine deficiency(*26*) provoked much controversy concerning the safety of thyroid medication for women by concluding that thyroid therapy increases the incidence of breast cancer, particularly in nulliparous women.(*28*) The authors stated that the increased incidence of breast cancer in their patients was either a function of hypothyroidism itself or of the thyroid supplements. The breast cancer morbidity described in the article was much higher than would be found in nongoitrous regions. Studies in rats have shown that *l*-thyroxine, in the presence of iodine deficiency, causes an increase in breast dysplasia. These clinical data, however, lacked firm diagnostic evidence of the thyroid/iodine abnormality for which the patients were treated.

Variations in iodine-deficiency levels differ within given populations. The evolution of the human organism permits the adaptation of thyroid function and structure to respond to a lower-than-normal supply of iodine. Goiter formation serves only as a symptom of the deficiency and therapeutics that decrease the thyroid growth do not necessarily restore the body metabolic iodine balance.(*29*) The need for further epidemiologic studies in regions of endemic goiter is evident.

B. Endocrinology

There has been a good deal of recent clinical interest in the thyroid/iodine axis as it relates to breast cancer. It is suggested that breast cancer patients, as a group, have a level of thyroid function that is lower than that found in women in hospitals with conditions unrelated to the breast, and that this lowered function is of primary thyroid, and not of pituitary or hypothalamic origin as shown by TSH values.(*30*) In addition, it has been shown that women with breast cancer may exhibit higher resting levels of TSH and a greater TSH response to TRH than women admitted with disorders unrelated to the breast.(*31*)

A hypothesis for dietary iodine and risk of breast cancer has been presented in which it is suggested that relatively low dietary iodine intake might produce an evanescent intermittent primary hypothyroidism that would lead to changes in the hypothalamic/pituitary pool.(*32*) This, in turn, results in increased gonadotrophic stimulation, which in turn, may produce a hyperestrogenic state; a condition theorized as basic to increased risk of breast carcinoma. From these factors, it has been concluded that women in areas of the

world where iodine intake is relatively low should be encouraged to increase their iodine intake.

C. Radionuclide Studies

Using a data base that we have obtained in our animal research, we have begun a clinical research program. Initially, we have evaluated patients in our breast cancer screening unit to determine whether iodine uptakes and scans might be useful in the diagnosis of breast cancer. The research program was designed to obtain the history, breast examination, xeromammography, radioiodine uptake of breasts and thyroid, and scan of the breasts.(*18*)

With data collated from the experimental design, we conclude that it is possible to determine the breast uptake of an administered tracer dose of radioactive iodine. After experimenting with several techniques, reproducible results are being obtained. Using our latest method in 181 cases, radioactive iodine uptakes were significantly greater in malignant or atypical breasts by tissue diagnosis than in normal breasts. Statistical analysis also showed:

1. thyroid uptakes and breast uptakes appeared to be dependent and to vary directly with one another.

2. iodine and estrogen interacted by the fact that radioactive iodine uptake is higher in postmenopausal breasts than it is in premenopausal breasts ($p<0.05$).

3. gestational status and breast feeding do not seem to change the uptake of the breasts by this method.

In other aspects of this clinical research, we have shown that ^{125}I or ^{99m}Tc radionuclide imaging shows reduced tracer in areas of the scans where clinically malignant or equivocal breast disease is diagnosed by breast examination, mammography, and/or tissue biopsy. Increased uptake of iodine in breast cancer tissue (biopsy or mastectomy) has been described.(*33*)

D. Estrogen and Estrogen Receptors

It has been suggested that the presence of estrogen receptor in a breast tumor in women would indicate whether the tumor was hormone dependent, and therefore might be made to regress following appropriate ablative therapy of estrogen-secreting glands.(*20*) On the other hand, a large number of patients may thus be spared unrewarding major endocrine ablative surgery if estrogen-receptor assays are performed and the tumor is hormone resistant. In mice, the uptake of iodine is much greater in hormone-responsive than in

hormone-independent breast cancer.(*21*) Therefore, the effectiveness of estrogen on growth and metastases of a given breast tumor may be determined by iodine evaluation by uptakes or other methodology in the parenchyma of the involved tissues.

Earlier work in rats showed that breast tissue radioiodine uptake increases in ovariectomized rats. The presence of an increased uptake of iodine in menopausal women may indicate that menopausal women have become more estrogen responsive and that possibly, hormone resistance is lost.

The reciprocal actions between estrogen and iodine in the breast are not clear. Whether estrogen metabolism requires iodine or whether iodine deficiency in itself prevents normal growth and development of the breast tissues remains to be determined. We are continuing our present research on the effect of estrogen on the uptakes of women at high risk for breast cancer.

E. Therapeutic Use of Iodine

The use of iodine in therapeutics to prevent breast cancer has been historically described since the late 19th century.(*34*) However, as indicated by basic research, replacement of iodides to iodine-deficient animals after breast abnormalities have occurred, seems to result in a relatively slow and incomplete return to normal.(*5*) The utilization of thyroid medication, in lieu of iodine replacement in these cases, seems ineffectual and perhaps harmful. Large amounts of iodine do not seem to have any valid treatment basis from the research that has been done.(*35*)

At present there are several medical groups who are studying, by double-blind technique, treatment of fibrocystic disease and other dysplasias and carcinoma of the breast with both iodides and organic iodine forms. The treatment of iodine deficiency *per se* throughout the world is a huge, and presumably, almost impossible task. Publications by the World Health Organization and the Pan American Health Organization have shown that the employment of various iodine therapeutic regimens has been successful in reducing fetal and neonatal abnormalities due endemic goiter.(*26,27*) However, as stated in all of these volumes, the problem of eradicating endemic goiter seems insoluble at present.(*27*)

V. SUMMARY

From laboratory studies presented, iodine appears to be a requisite for the normalcy of breast tissue in higher vertebrates. When lacking, the parenchyma in rodents and humans show atypia, dysplasia, and even neoplasia. Iodine-deficient breast tissues are also more susceptible to carcinogen action and promote lesions earlier and in greater profusion. Metabolically, iodine-deficient

breasts show changes in RNA/DNA ratios, estrogen receptor proteins, and cytosol iodine levels.

Clinically, radionuclide studies have shown that breast atypia and malignancy have increased radioactive iodine uptakes. Imaging of the breasts in high-risk women has localized breast tumors. The potential use of breast iodine determination to determine estrogen dependence of breast cancer has been considered and the role of iodide therapy discussed.

In conclusion, iodine appears to be a compulsory element for the breast tissue growth and development. It presents great potential for its use in research directed toward the prevention, diagnosis, and treatment of breast cancer.

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