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The Intracellular Metabolism Of Iodine In Carcinogenesis

BERNARD A. ESKIN,* CHARLES E. SPARKS, AND BRUCE I. LAMONT

The Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129

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ABSTRACT

Research from this laboratory and others have concluded that significant glandular atypia, and often neoplasia, occurs in the breast tissues of rodents and humans under conditions of iodine deprivation. These cellular changes caused by iodine deficiency are intensified by aging, steroid hormones, and pituitary hormones. There has been controversy concerning the effect of iodine deficiency on stimulation and maintenance of cancer of the breast in rodents when the cancer is induced chemically or by transplantation. However, neither within this induced neoplastic framework nor with the dysplastic changes seen by deficiency alone, have laboratory studies of the *pathway* of intracellular iodine been previously possible.

The new research data addresses the question of whether organification occurs and whether iodine significantly affects the intracellular structures. An hypothesis will be presented that places the inorganic element, iodine, into association with receptor protein complexes that may be responsible for intracellular sex hormone activity. The relationship of this mechanism to carcinogenesis in breast tissue will be considered.

Key Words: Metabolism, intracellular, of iodine in carcinogenesis; iodine, intracellular metabolism in carcinogenesis; carcinogenesis, intracellular metabolism of iodine in.

INTRODUCTION

Iodine and iodinated compounds have been found to be remarkably ubiquitous in the body. Once considered to have activity limited only to thyroid hormone metabolism, it is still difficult to disassociate extra-thyroidal

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functions of this element from thyroid physiology and metabolism. Iodine most often appears as iodide and organified as T_3, T_4, and iodothyronines in the blood plasma and tissues of mammals. Iodine has been found in measurable amounts in the parenchyma of the breasts, salivary glands, gastric mucosa, liver, skin, tumors, and the ovaries and testes (*1*, *2*). Recent studies concern the response of iodinated compounds at nerve endings and brain tissues (*3*, *4*). Iodine has potential significance in receptor physiology (*5*). Organified products of iodine metabolism (i.e., iodothyronines and iodotyrosines) have been studied extensively at the tissue and organ level (*6*). With the exception of the thyroid little work has related iodine metabolism to protein organification in endocrine organs.

The rat breast served as our model for research on the significance of iodine in the etiology of tissue dysplasia and neoplasia. Limited experimentation on iodine metabolism has been described in liver, skin, and gastric mucosa (*2*, *7*, *8*), but most of the work in these tissues has consisted of clinical and histopathological observations. Similar work in the female breasts of rodents and humans indicates glandular atypia occurs under conditions of iodine deprivation (*9*, *10*). The cellular changes caused by iodine deficiency are intensified by aging (*11*, *12*). Additionally, some evidence favors further modifications of breast histology in iodine deficiency by steroid hormones (estrogen and testosterone) (*9*, *13*), and pituitary hormones (gonadotropins and thyrotropins) (*14*, *15*). Considerable controversy has arisen concerning the effect of iodine deficiency on stimulation and maintenance of breast neoplasia in rodents when DMBA is employed as a carcinogen (*16*, *17*). However, neither within this induced neoplastic framework nor with the dysplastic changes seen in iodine deficiency alone has a consideration of the *pathway* of intracellular iodine been studied in the breast tissues.

Many questions remain unanswered:

1. Does iodine directly affect cellular structure of the breast?
2. Is iodine taken up by breast tissue cells?
3. Are organic iodides produced *de novo* in the breast?
4. Are organic iodides transported from the thyroid?
5. What are the pathways for iodine metabolism within the breast cells?
6. Are there changes in breast histologic appearance mediated by organic iodide?

The main objective of this presentation is to offer current knowledge concerning intracellular iodine metabolism in breast tissues. The techniques described have been used in our laboratories and are described in our recent publications (*18*, *19*). The metabolic events are compared with the histopathology obtained from the same experimental animal wherever possible. Pilot results are presented to provide an insight into a mechanism where iodine may directly influence breast growth and development following cellular uptake and protein organification. Direct influence of iodide on breast glandular tissue, possibly mediated by an intracellular iodoprotein,

may help explain why abnormal tissue development occurs with iodide deprivation. A direct effect of iodide on breast tissue may help resolve conflicting evidence relating thyroid to breast function.

HISTOPATHOLOGY

Iodine Restriction

Research initiated here, and confirmed by others, has shown that reduced breast iodine produces glandular changes in the rat. Iodine deficiency by diet alone (*9*) or by blockade with perchlorate treatment (*20*) induces mild atrophy with significant atypia and hyperplasia in 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. The morphologic appearance includes a general reduction in cell size in the atrophic areas. Throughout the atrophic areas there are scattered foci of marked hyperplastic activity where the epithelial cells lose their relatively small uniform appearance concurrent with other abnormalities including increased cellularity, variability of nuclear shape and size, and hyperchromatism. In areas there is atypical lobular hyperplasia, not unlike that seen in the human breast (*10*).

When perchlorate-treated rats are given thyroxine replacement and compared with perchlorate alone, the histology is similar to that of iodine deficient animals. Thus, the breast changes represent direct iodine restriction rather than reduction in serum thyroxine. Hypothyroidism induced by propylthiouracil (PTU) or thyroidectomy provides mild generalized hyperplasia of mammary glands without remarkable atypia (*9*, *10*). The variant response caused by iodine deficiency appears to be histologically different from those changes in hypothyroidism. When concomitant replacement of iodine is given with the several iodine-deficient diets used normal control breast histology results (*14*). However, when thyroxine (T_4) in doses approximately three to four times greater than replacement are given, an enhancement of the already existing abnormal changes occurs (*21*, *22*). Termination of either dietary iodine restrictions or perchlorate therapy results in a variable, but modest, return toward the normal histologic appearance of breast tissue. Additional iodine of two to six times normal following deficiency results in no measurable improvement compared to simple termination of iodide deprivation (*21*).

Recent research from our group employs animals whose ages correspond to stages in mature human adults. Iodine deficiency results in even more evidence of atypical changes in the epithelium as the rat age increases (*11*). In the "menopausal" age group many focal changes occurred. Using perchlorate to create iodine blockade there are significant increases in periductal fibrous overgrowth with entrapment of adjacent lobules and aberrant ductular

proliferation, sclerosing adenosis, and microcyst formation compared to similar studies in younger rats. The changes in the menopausal group strongly resemble fibrocystic disease in the human. There are areas in some breast tissues in the menopausal group showing atypical lobules with hyperchromatism, enlargement of nuclei, altered nuclear–cytoplasmic ratios, increased mitosis, and loss of polarity of the epithelium. Some of the breast lobules exhibited even more dysplastic changes including papillomatosis with poorly polarized epithelium.

The morphologic evidence suggests that breast iodine deficiency by itself may result in hyperplasia, dysplasia, and even neoplasia in the rat breast tissue that are age-related in terms of breast response. Once found, the changes are apparently not completely reversible by dietary iodide replacement.

Dimethylbenzanthracene (DMBA)

There is an earlier onset of breast cancer in prepubertal rats, resulting from the carcinogen DMBA as well as an increase in size and numbers of lesions when DMBA is administered after an iodine deficient state is reached (*16*, *17*). The growth of the cancers in size and numbers is greater in the iodine deficient than in the normal rats. However, the onset of lesions and tumor responses to DMBA appear to differ according to the techniques used and whether the carcinogen is given before or after the animals have been iodine deficient (*16*). Prepubertal rats are given an iodine-deficient diet (Remington or low iodine, both less than 2 ppm iodine salts) for approximately 7 days prior to a pulse dose of DMBA by gavage. Following this, the onset of neoplasias is earlier and the number of tumors seen by 60 days after DMBA is increased (*17*).

The dysplastic and neoplastic changes occur sooner in perchlorate-treated DMBA rats than in the DMBA control. To be certain that an euthyroid status is obtained, perchlorate treatment is supplemented by replacement thyroxine therapy. DMBA is an unique carcinogen for it appears to produce an estrogen-dependent neoplasia. Intracellular iodine uptake may relate to estrogen receptor status according to evidence that is described later. The basis for this relationship may be important, but requires further study.

Summation of Histologic Research

The requirement of intracellular iodine for histologic normalcy of the breast tissues is shown by dietary deficiency or cellular blockade. The lesions caused by iodine inadequacy are primarily atrophy, but focal areas of atypia and peripheral hyperplasia also occur. Aging rats respond morphologically to iodine deprivation, with an increase in the focal atypia and an intensification of the dysplasia when compared with younger animals. When DMBA is used for induction of breast carcinoma in rats that are already iodine deficient after 60 days, there is a significantly earlier onset and a greater number of tumors than in noniodine restricted controls.

INTRACELLULAR METABOLISM

Radionuclide Studies

Radioactive iodide (^{125}I) uptakes are determined in rat breasts under varying conditions of iodine availability, both physiologic and pharmacologic (*14*, *20*). In dietary iodine deficient rats breast uptake increases when ^{125}I is injected (*14*). Blockade produced by perchlorate treatment, however, continues to prevent the radioactive iodide from entering the cells. Therefore, although the blocked gland is depleted of its iodine, the radioactive iodide uptakes do not increase but may decrease (*12*).

In mice, the uptake of ^{125}I iodide by transplanted hormone responsive (HR) mammary tumors is shown to be significantly greater than the uptake of ^{125}I iodide by transplanted hormone independent (HI) mammary tumors (*23*). As anticipated, uptake of ^{125}I by HR mammary tumors is greatly reduced by the simultaneous injection of an excess of nonradioactive iodide. Perchlorate treatment blocks the breast iodide uptake.

Histologic normalcy parallels iodine availability in rat studies (*13*, *20*). Both human and rat studies confirm the findings that dysplasia and neoplasia show enhanced radioactive uptakes in the breast tissues (*24–26*). Again, the need for iodine is indicated.

Autoradiography

The presence of iodine in breast tissue is confirmed by the utilization in our laboratory of autoradiography. Using the emulsion-dipping method of autoradiography with ^{125}I as the radionuclide, the presence of iodine was studied in control and dietary iodine-deficient rat breasts. The results show iodine shadows are localized in patchy areas with some locales remarkably heavier than others. This randomized response permits hypothesizing that iodine deficiency results in focal changes.

The iodine deficient cell takes up more ^{125}I, but the increased iodide uptakes are the result of focal changes that may relate to the focal atypical areas described earlier (*27*). Most inorganic iodide is washed out in the staining procedures so these results are consistent with increased iodide organification in these focal areas, but the results are preliminary and are under further investigation.

DNA/RNA Studies

Nucleic acid ratios may be correlated with intracellular activity. Quantitation of changes of DNA/RNA ratios in dysplasia and neoplasia in mammary glands of rodents occur when the tumors are induced by DMBA (*28*). Generally, increased DNA reflects rapid growing undifferentiated breast tumors and increased RNA occurs with more benign neoplasias (*14*).

Both DNA and RNA increase threefold over normal values in iodine-deficient rat breast tissues. Although hypothyroidism also causes an increase in DNA, iodine deficiency alone produces an increase in RNA and DNA (*5, 14*).

Estrogen Receptor

Estrogen end-organ responses of the breast are important and can be measured both histologically and biochemically. The response of estrogen of intracellular metabolism is dependent on estrogen receptor protein (ERP). Triiodothyronine has been shown to cause increased estrogen receptor levels in rodent breast tissues in cultures (*29*). Considering that estrogen results in the increased atypical responses of the rat breast in iodine deficiency, ERP was studied.

Cytosol preparations from mammary glands of virgin female rats are incubated with ^{3}H-estradiol-17-beta and subjected to sucrose density gradient centrifugation and minute but definite amounts of estrogen receptor (maximum concentration 14 fmol/g wet tissue) are detected. Both 4-5S and 8-9S components are present. The binding of ^{3}H-estradiol to both forms is eliminated when the anti-estrogen CI-628 is included in the incubation. The mammary glands from iodine deficient rats contain estrogen receptor in concentrations that are not significantly different from those in control animals. However, the protein is significantly less aggregated than the controls. In the control animals the 4-5S form is the minor component (mean = 41%); in the treated rat the 4-5S form accounts for a significantly higher proportion of the receptor (mean = 68%) (*5*).

It would appear that estrogen receptor may be modified by alterations in intracellular iodide metabolism. Receptor changes may cause variations in estrogen response which are observed in iodide deficiency.

Protein Iodination and Organification

We hypothesize that if iodine, known to be present in breast tissue, is biologically active, it must organify *within* the cell. Earlier investigations (*30, 31*) using TCA/tissue techniques showed organification especially in lactating rat breasts. When breast tissue is stimulated by estrogen, an increase in organification is shown (*31*). In light of the vagaries of the TCA technique and the need for further evidence of protein iodination, the first step in this research program is confirmation of protein iodination before beginning to unravel the intracellular pathway(s) involved. Studies were performed using homogenized breast tissue following injection or perfusion of ^{125}I. Organified iodide was studied using gel filtration and electrophoretic techniques (*19*).

Injection Experiments: Controls and Lactating Rats. Female age-paired Sprague-Dawley control and lactating rats were employed in this experiment. Lactation provided "active" glandular breast tissues and was the condition previously employed in TCA-organification experiments (*30, 31*). In this

series, the rats were injected with ^{125}I via the tail vein and after an appropriate time, anesthetized and sacrificed, with tissues subsequently being removed and homogenized for analysis. Sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis was carried out on a vertical slab apparatus and further analysis by sodium dodecyl sulfate–molecular exclusion column chromatography was performed similarly to the method of Sparks et al. (*18*). The methods of analysis for organified iodide employed electrophoresis or column chromatography to fractionate the SDS–protein complexes according to their molecular size, and both techniques depend on molecular sieving effects. The electrophoretic technique accomplished the molecular sieve by creating uniform pores through the controlled polymerization of acrylamide. The columns were packed with a molecular exclusion gel (Sepharose-6B-CL-Pharmacia) with a uniform known pore size.

In each set of experiments matched groups of experimental and control animals were analyzed. The tissue analysis was performed on serum, lung, breast, and thyroid glands. The organified iodide was determined by column or gel mobility.

RESULTS

There were significant differences between the control and the lactating groups of rats. Figure 1 and Table 1 summarize some of these differences. In all groups the serum radioactivity migrated entirely as ^{125}I. The radioactivity remaining in the serum 120 min after iodide injection was 13.6 ± 1.6 in the controls compared to 12.4 ± 3 in the lactating animals. The control lung homogenates showed only iodide radioactivity with no radioactivity in column mobility characteristic of proteins. Representative radioactivity

TABLE 1

Molecular Size Distribution of ^{125}I-radioactivity in the Breast and Thyroid[a]

Organ	Time, min	Less than 50,000 daltons	Greater than 50,000 daltons	Iodide
Thyroid	60	15.5 ± 1.5	71.0 ± 1.0	13.0 ± 2.0
Thyroid	120	15.5 ± 2.5	75.5 ± 3.5	9.0 ± 1.0
Thyroid-L	120	15.0 ± 0.7	73.3 ± 1.0	11.8 ± 0.6
Breast	60	0.7 ± 0.5	—0—	99.0 ± 1.0
Breast	120	0.5 ± 0.3	—0—	99.0 ± 0.5
Breast-L	120	19.8 ± 5.7	—0—	80.2 ± 5.7

[a] Molecular size distribution expressed as a percentage ± SEM of the injected ^{125}I radioactivity. Time indicates tissue distribution in minutes after intravenous injection of ^{125}I. -L indicates that the animals were lactating at the time of sacrifice (actively nursing). From Eskin, Sparks, LaMont, and Kolansky, 1978 (*19*).

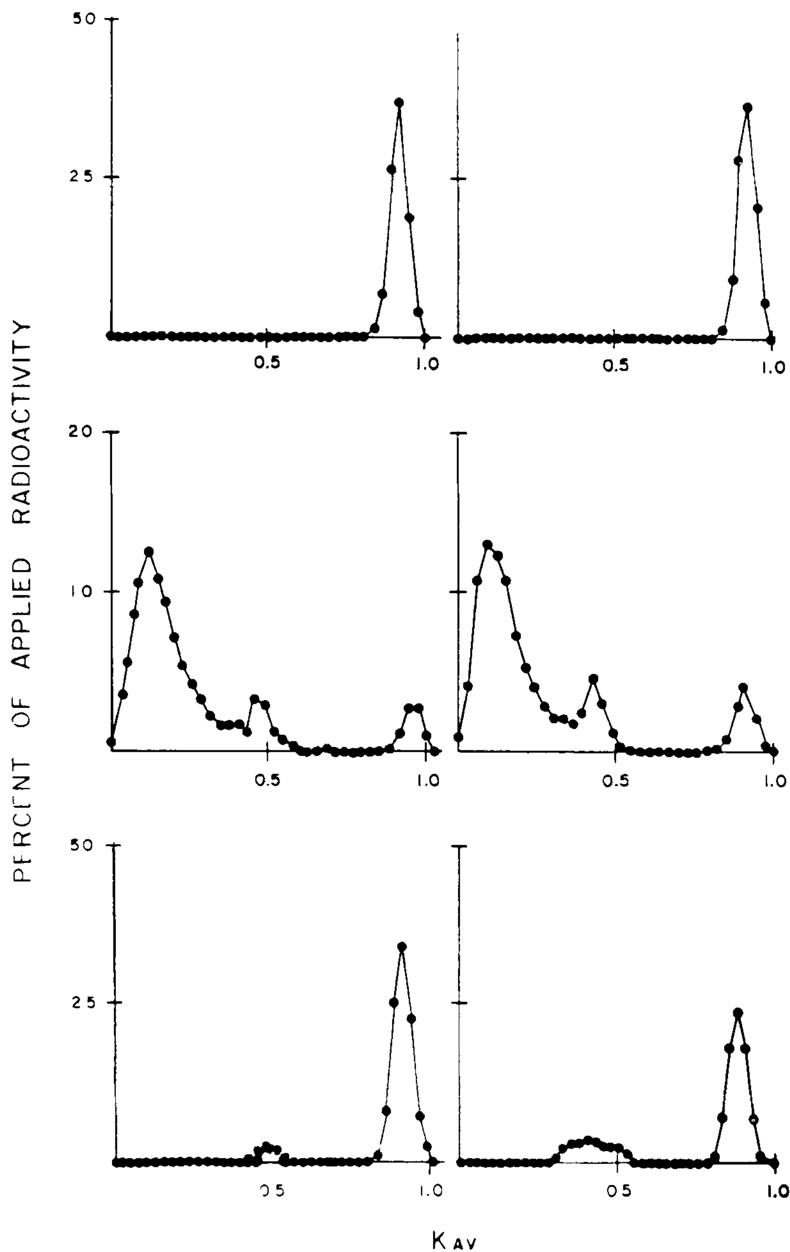

FIG. 1. Radioactivity distribution plotted against column mobility (K_{av}) by SDS–MECC. Left panels represent control rats and right panels represent lactating rats. All animals were studied 120 min after intravenous injection of ^{125}I. Upper panels represent serum; middle panels the thyroid; and lower panels the breasts. Description of results in text. Modified from Sparks and Eskin, 1979 (*32*).

distribution curves are presented in Fig. 1 (*19, 32*). The upper panels demonstrate the radioactivity distribution of rat serum and the single peak of $K_{av} = 0.92$ is iodide. The middle panels demonstrate the radioactivity distribution of thyroid homogenate. The peak between $K_{av} = 0$ and $K_{av} = 0.25$ corresponding to greater than 50,000 daltons in subunit molecular size is most likely thyroglobulin. The subunit molecular size is estimated at 100,000–200,000 daltons, comparing to proteins of known column or gel mobility. The second peak of $K_{av} = 0.4$ corresponds to 10,000–20,000 daltons in molecular size. Again the $K_{av} = 0.92$ peak is iodide. In the lower panels there is a peak in the lactating breasts between $K_{av} = 0.3$ and $K_{av} = 0.55$ that is minimally present in the control animals. The peak became better resolved following delipidation and corresponds to 10,000–20,000 daltons in subunit molecular size. Again the $K_{av} = 0.90$ peak is iodide.

The thyroid tissue showed significant uptake of the injected radioactivity of 2.0, 4.1, and 5.1% in the 60-min control, 120-min control, and the lactating rats, respectively. The breast tissue showed uptake of 0.25 and 0.23% of the injected radioactivity in the 60- and 120-min control groups and the average breast weight was 0.8 g. The lactating group had an uptake of 6.7% of the injected radioactivity after 120 min and the average breast weight was 2.0 g. It is interesting that the total uptake in the lactating rats is 30 times that in the matched controls, which was even greater than the total uptake into the thyroid. No correction was made for blood in the tissue, but 2 ml (2 g) of blood would only have contained 1.5% of the injected radioactivity. Using the ^{51}Cr correction the plasma iodide contamination was at most 0.2% in the breast tissues (*32*).

There was organification of the injected radioactivity in all of the thyroids and the lactating breast. The data is presented in Table 1 and in the middle and lower panels of Fig. 1. In all of the thyroids there was significant organification of the injected iodide and in the three groups there was 1.8, 3.8, and 4.5% organification in the 60-, 120-, and 120-min lactating groups, respectively. Uniformly, there was organification of about 73% of the radioactivity to a 100,000–200,000 dalton protein subunit (thyroglobulin). There was about 15% iodination to a protein of 10,000–20,000 daltons in subunit molecular size. The protein peaks remained following delipidation.

In the breast tissue of lactating rats there was significant iodination to a protein of 10,000–20,000 daltons in subunit molecular size; i.e., the same column mobility as the smaller protein in the thyroid. In contrast there was no iodination to a protein larger than 50,000 daltons. The protein peak became better resolved following delipidation.

In all of these experiments the results were confirmed by SDS–PAGE although the sensitivity of this technique is not as great as SDS–MECC. There was *no* iodoprotein in the serum of any of the animals at any time making iodoprotein transport from the thyroid or other tissues extremely unlikely in the times chosen for study.

Summary of Organification Experiments with Lactation

Iodide organification to protein was studied by analyzing tissue homogenates solubilized by SDS using molecular exclusion column chromatography and polyacrylamide gel electrophoresis in the presence of SDS. Our results showed significant organification of injected radioactivity in thyroids and lactating breasts.

Perfusion Experiments—DMBA and Controls

There was a small quantity of iodoprotein formed in the breasts of the control animals in the injection studies. The low levels of radioactivity in control breasts made the results difficult to interpret but the organification was to a protein smaller than 50,000 daltons. The question of protein iodination in normal and tumor-bearing rat breasts are extremely important to our hypothesis concerning the mediating role of iodoproteins as previous iodide uptake studies were performed on nonlactating animals and DMBA-treated animals (*32*). If absence of iodoprotein were to play a role in the neoplasia development, presence of iodoprotein should be conclusively determined and quantitated.

Iodide organification was demonstrated in both controls and tumor-bearing animals using perfusion techniques employing levels of radioactivity which would allow conclusive results.

TECHNIQUES

Three groups of Sprague-Dawley female rats were studied using an infusion technique with constant infusion of 90–92 μCi of ^{125}I in prefilled 1-mL syringes over 120 min. Each animal was injected ip with Nembutal 30 mg/kg. Cannulation was performed on the femoral artery and vein with preheparinized PE 50 tubing with 3-way stopcocks (Pharmacia) available for sampling. The venous catheter was flushed with heparin to avoid coagulation. The Harvard pump was placed on an infusion schedule of 0.1 mL/min. Infusion was begun after the animal was recovered from anesthesia and immobilized.

The groups consisted of:

1. DMBA-treated rats with one or more 1-cm palpable breast tumors (female rats, 200–250 g, received from Hazelton Research Animals, Inc.).
2. Control animals.
3. Control animals (tissue analysis 120 min after discontinuing perfusion).

The samples were prepared similarly except that the tissue was delipidated following homogenization and the delipidated tissue homogenate was immediately dissolved in SDS solution. The SDS–protein complex was then

evaluated by molecular exclusion column chromatography in the presence of SDS. Recoveries of radioactivity were monitored during extraction and preparatory procedures. The serum contamination was determined by ^{51}Cr techniques and in the control animals was less than 1.5% of the breast tissue radioactivity.

RESULTS

The results in Table 2 indicate the feasibility of using infusion techniques in the study of iodide uptake and organification with the following advantages:

1. Exposure of the tissues to high levels of ^{125}I.
2. Control and monitoring of the tissue exposure to ^{125}I.
3. Study of the system under conditions approximating equilibrium of ^{125}I in the nonvascular space.

It is important to note that even at high levels of radioactivity the ^{125}I dose would not significantly alter the iodide pool size because of the high specific activity of the injected ^{125}I. The results in Table 2 show that ^{125}I is being added to the iodide pool more rapidly than its clearance with resultant increases in

TABLE 2
Serum ^{125}I during Perfusion of ^{125}I into Control and DMBA-Breast Tumor Rats[a]

Time into perfusion	cpm/20 μL			
	Control 1	Control 2	DMBA 1	DMBA 2
15	1095	1057	3179	1565
30	2025	4799	7359	5119
45	5487	9853	12071	9806
60	11496	15755	17540	13158
75	16799	19734	20886	17732
90	21722	23403	23887	23442
105	25691	27679	26210	31583
120	29026	30288	35220	39181
30X	22503	22972		
60X	16995	21437		
90X	15512	21805		
120X	16006	20679		

[a] Results expressed as averages of triplicate cpm/20 μL serum during perfusion of 90–92 μCi of ^{125}I. In two control animals the serum ^{125}I was measured for 120 min after stopping the 120-min perfusion. Modified from Sparks and Eskin, 1979 (*32*).

the iodide pool specific activity. The data also indicate a fairly rapid equilibrium with the nonvascular space (i.e., the delay initially and the delay following 120-min perfusion). There is good duplication of the controls and DMBA and there are only slight differences between the groups.

The results are presented in Table 3, with the following conclusions:

1. The total ^{125}I uptake in the breasts of DMBA-treated animals was significantly increased, which was primarily a reflection of the increase in breast mass since the specific activities of ^{125}I/g breast tissue did not increase.
2. The breast ^{125}I uptake was less than serum. The post-perfusion controls showed that iodide seems to reach equilibrium with the serum. Although an active uptake may exist in the breast tissue possibly this is balanced by diffusion of cellular iodide back to the tissue space. When these results are contrasted to thyroidal uptake, the thyroid continues to concentrate ^{125}I in spite of clearance of the ^{125}I pool in the post-perfusion controls.

The result of ^{125}I diffusion into the eye is of interest for comparison to the breast.

In these experiments organification of ^{125}I in the breasts was significant, as can be seen in Table 4. The ^{125}I radioactivity coincided with the mobility of proteins smaller than 50,000 daltons in subunit molecular size, as was seen previously. The total breast uptakes ranged from 8×10^5 to more than 3×10^6 cpm, which means that 1% organification represented a minimum of 8×10^3 cpm. *All* breast tissue analyzed organified ^{125}I. It is of interest that the results expressed in Table 1 correspond quantitatively to control results in Table 4.

Preliminary evidence suggests variability of iodine uptake in DMBA-breast tissue and there is also apparent variability in ^{125}I organification. The

TABLE 3

Tissue ^{125}I Following Perfusion of ^{125}I in Control and DMBA-Breast Tumor Rats[a]

	Breast	Thyroid	Serum	Eye
DMBA	2946/474	1754/—	—/1767	85/423
DMBA	3778/583	1587/—	—/1950	79/415
Control	730/373	1136/—	—/1541	45/314
Control	1214/636	1694/—	—/2033	68/518
Control[b]	841/339	3030/—	—/850	26/207
Control[b]	753/364	2083/—	—/1050	36/271

[a] The results are expressed as cpm $\times 10^{-3}$ of tissue ^{125}I (counting efficiency 82%) following perfusion of 90–92 μCi of ^{125}I over 120 min. The results are expressed as cpm total on the left and cpm/g tissue or serum on the right uncorrected for vascular iodide. Modified from Sparks and Eskin, 1979 (*32*).

[b] These two control animals were analyzed 120 min after perfusion was stopped and are the same controls presented in Table 2.

TABLE 4
Organification of ^{125}I in Breasts of Control and DMBA-Breast Tumor Rats[a]

	Anterior breast, %	Posterior breast, %	Breast tumors, %	Breast tumors, %
Control 1	1.0	0.4	—	—
Control 2	0.6	0.8	—	—
DMBA 1	1.4	2.6	3.1	2.3
DMBA 2	0.7	1.8	0.4	0.5

[a] Results expressed as the percent of tissue iodide organified to a protein smaller than 50,000 daltons. (No significant organification to protein larger than 50,000 daltons is seen.) Modified from Sparks and Eskin, 1979 (*32*).

^{125}I organification in DMBA animals affected not only the tumor, but also the surrounding "nontumor" tissue. Actually, histologically the surrounding "nontumor" tissue exhibited focal hyperplastic and neoplastic tissue proliferation quite similar in appearance to the ιumor.

DISCUSSION

We hypothesize that uptake and organification of iodide by breast tissue may be important to cellular function possibly by balancing hormonal influences at the cellular level. The breast has the capability to take up and organify iodide to protein in a process that is *not thyroid dependent.* The breast is a complicated secretory organ that responds to endocrine influences in the postpartum period by producing milk. Although iodoprotein formation is greatest during lactation, it is demonstrated at lower levels in the nonlactating gland. The described studies indicate a requirement of the breast for iodine to maintain tissue normalcy. The described iodoprotein may be the active compound and interaction with the estrogen receptor protein is a possible mechanism for iodide effects on breast tissue.

The breast secretes milk in the postpartum period under endocrine influence produced by estrogen, progesterone, and prolactin. It is apparent that iodinated compounds are utilized by the breast in preparation for milk secretion, possibly promoting growth and development by increased protein synthesis (*33*). The hormones involved in iodine/thyroid metabolism are described in Fig. 2. Milk contains iodide, iodinated tyrosyls, and iodothyronines that may be produced locally or derived from the mother's blood (*34*). Iodoprotein appears to be produced locally, but its role in maintaining tissue normalcy in the lactating state has not been studied, nor is it known whether iodoprotein appears in milk. Further studies in this area would be particularly interesting as they relate to the estrogen receptor protein.

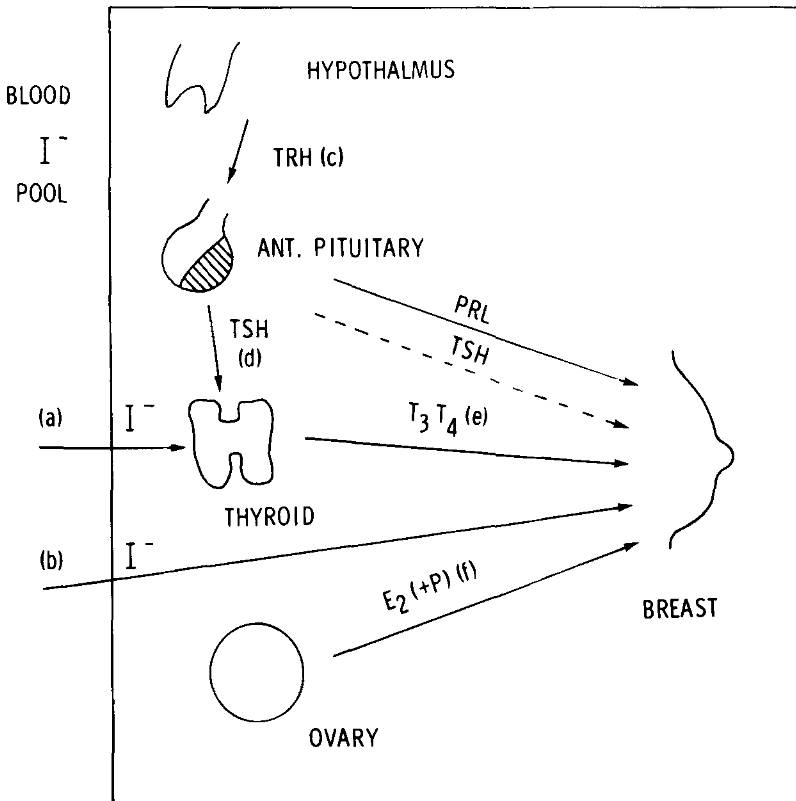

FIG. 2. Endocrine influences on the breast. (a) Iodide enters the thyroid leading to final secretion (e) of T_3 and T_4 hormones and iodotyrosyls. The syntheses are mediated by TRH (c) and TSH (d). In the breast iodide (b) enters from blood. TRH (c) releases both PRL and TSH to the breast secretory action. Ovarian secretion of both estrogen and progesterone (f) stimulate breast tissue. From Eskin (*36*).

In the breast at least two distinct mechanisms of iodine metabolism appear to be functioning intracellularly, as shown in Fig. 3. The first, in conjunction with insulin, cortisone, thyroxine, and prolactin, may involve iodine and/or iodoprotein in milk secretion from the lactating mammary gland (*35*). The second may involve iodide and/or iodoprotein in the nonlactating breast. Thus, iodine may play a role in end-organ response to cyclic changes of sex hormones, and in particular may involve the estrogen receptor. Although thyroid hormone certainly influences breast tissue especially in the process of lactation, iodine may have a direct effect mediated by an iodoprotein. The study of thyroid hormone in breast tumorigenesis has produced conflicting results in the past and consideration of direct iodide effects on breast tissue may involve some of the inconsistencies.

There is a poor understanding of trace element metabolism. Studies of how iodide might directly effect tissue metabolism may provide insight into at least the metabolic role of this important trace element.

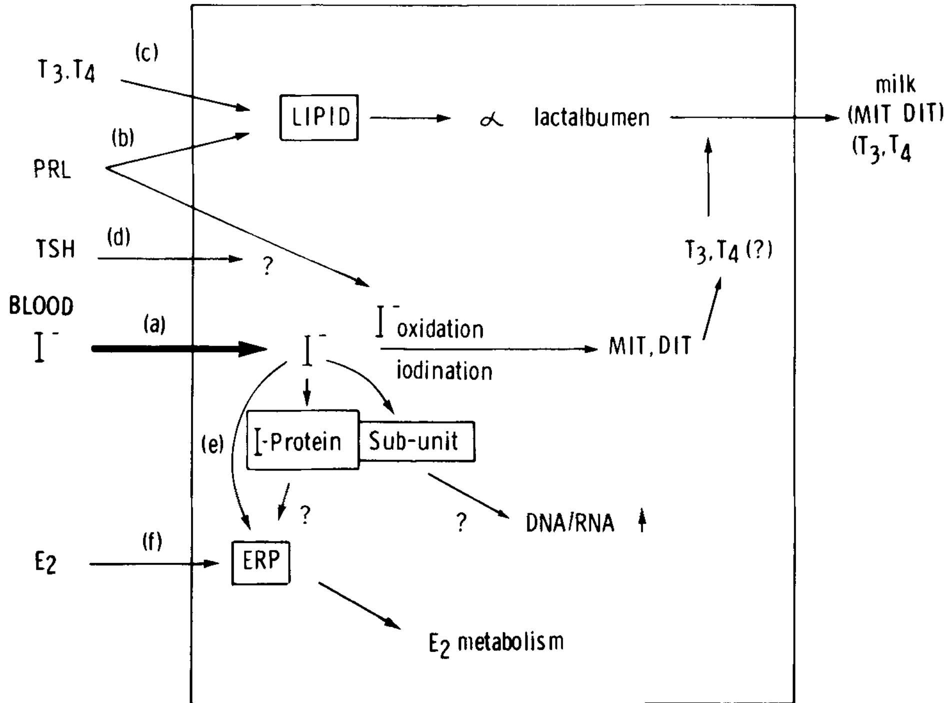

FIG. 3. Iodine intracellular metabolism in the breast (hypothetical). Blood iodide (a) enters parenchymal cell and after oxidation and iodination results in MIT, DIT (T_3T_4?) secretion with milk. T_3, T_4 entering breast (c) reacts with PRL (b) and lipid to form alpha-lactalbumen for secretion as milk. Additionally, iodine iodinates a small protein (e) which may increase DNA/RNA or change estrogen receptor protein response with estrogen (f). From Eskin (*36*).

CONCLUSIONS

1. The active (lactating) breast glandular tissue significantly takes up and organifies iodide.
2. Both inactive and DMBA-treated breast tissues also take up iodide and organify the element, but to a lesser extent.
3. The presence of iodoprotein in the breast indicates coupling of iodide uptake to a breast peroxidase.
4. The iodination is to a small protein subunit which is similar in size to an iodinated protein subunit in the thyroid in low concentration.
5. Iodine appears to be required for breast tissue normalcy and is in some way related to intracellular metabolic functions, possibly by interaction with estrogen receptor protein.

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