CONFIDENTIAL

THE EFFECTS OF IODINE AND IODIDE ON IODINE DEFICIENT RAT BREAST AND THYROID TISSUES

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July 1989

EXPERIMENT XII:10

Background

Both breast cancer and hypothyroidism are disorders that are prevalent in regions of low dietary consumption of iodine such as Great Britain and the United States (1). The incidence of breast cancer in Japan is one-tenth that of Great Britain (2). The average dietary consumption in Japan is 5 grams per day while that of Great Britain is only 0.1 milligrams per day. As a result of these and other findings, many investigations have been conducted to determine the relationships between hypothyroidism and breast diseases and iodine deficiency alone and breast diseases.

It was discovered in the 1950's that the ability to iodinate and the mechanisms by which iodination occurs are present in mammary tissues (3). Originally, this process in the mammary glands was assumed to be similar to the iodine trapping and organification process of the thyroid gland(4). Iodine generally circulates in the bloodstream as iodide salts. Iodide trapping occurs when the thyroid accumulates these iodides against a concentration gradient, oxidizes the iodides to iodine, and then organifies them to iodoproteins. The initial organification process produces moniodotyrosines and diiodotyrosines. These hormone precursors are then coupled together to synthesize triiodothyronines and thyroxine.

The entire process of iodine metabolism in the thyroid is under the control of thyroid stimulating hormone (TSH), which is secreted by the adenohypophysis. An iodine deficiency results in an inadequate amount of thyroid hormone which causes continued secretion of TSH which then causes the thyroid gland to enlarge and a goiter to develop (5, 14).

The major enzyme involved in iodine metabolism in the thyroid is peroxidase (3). When peroxidase is denatured or inhibited with

goitrigens such as perchlorate or thiocyanate, neither iodination nor organification can occur. Consequently, no organified products are produced (9). The discovery of a mammary peroxidase is correlated with the ability to iodinate (7). In the breast, these procedures are more apparent during metabolically active conditions such as pregnancy and lactation (13). This suggests that in the breast oxidation and organification are mediated by hormones, specifically, estrogen (8). Resting human breasts also have the ability to iodinate compounds but these tissues have serious limitations in the ability to convert iodide to iodine (8). It is believed that in the resting condition, the ability to iodinate is related to hormone influences associated with the menstrual cycle (8). Therefore, in both active and resting breast conditions, estrogen plays a significant role in iodine metabolism.

Mammary tumor peroxidase activity is probably an estrogen induced characteristic of a hormone dependent mammary tumor (9). Breast cancer may be enhanced in the presence of estrogen and high levels of estrogen receptors (10). Changes in breast dysplasia are enhanced by sex steroid therapy (11).

Hypothyroidism alone does not induce breast tissue atypia but iodine deficiency does (12). Eskin has found that when laboratory rats showing mammary gland atypia due to perchlorate blockade are given thyroxine replacement, the atypia was not alleviated (4). Therefore, by the work of Eskin and others, it was discovered that it is clearly the iodine blockade of the breast and not hypothyroidism that causes mammary gland dysplasia (4). From the same studies, Eskin also discovered that in order for the perchlorate influence to be effective, there is a need for estrogen and that estrogen requires iodine for

normal breast function.

Because the thyroid is totally dependent on iodides for hormone synthesis (2), and iodide metabolism is more efficient in the thyroid than the breast (6), the availability of iodides to the breast for oxidation and organification is limited.

If iodine in its diatomic form is given, it diffuses into the breast cells and the initial oxidative step of the metabolic pathway is eliminated (6). Because the thyroid does not trap iodine into the cells as well as the breast and the breast does not trap iodides as efficiently as the thyroid, diatomic iodine becomes more available to the breast (13) and competition for iodine is reduced significantly. Assuming that the thyroid has a greater affinity for iodides than diatomic iodine and the breast, vice versa, a further investigation was made to prove that iodine therapy is efficacious in improving the histological pathology of mammary gland dysplasia.

Introduction

Prior work has shown that iodine deficiency has induced breast tissue atypia and dysplasia, as well as hypothyroidism in the thyroid. The breast traps iodine and organifies it although not as efficiently as the thyroid (1). The thyroid traps iodides in the blood by diffusion and oxidizes it with peroxidase (1), which is the major enzyme involved in organification and oxidation. It has been found that perchlorate blocks the activities of peroxidase, which would lead to an increase in breast and thyroid abnormalities. Eskin has found that iodine is more significant in improving mammary tissue abnormalities than iodides because the initial step of the metabolic process is eliminated, and the iodine can be organified without impairment by peroxidase blockade (6).

Our investigation was designed to determine iodine association with the breast and thyroid gland, and their affinities for the chemical forms. We used experimental animals to study the changes in both mammary and thyroid tissues after both iodine and iodide therapies. A conclusion was drawn from comparison of physical, histological and biochemical evidence.

Materials and Methods

Ten Sprague-Dawley albino virgin female rats weighing initially 220-250 grams were divided into three groups for the two week study.

All rats were put on an iodine-deficient diet and deionized distilled drinking water for two weeks. They were also given perchlorate treated water for the first four days of the experiment and estrogen injections every other day.

Group I, the control group, consisted of two rats. They were given the iodine-deficient diet for 2 weeks, the perchlorate treated water for the first 4 days, the estrogen injections every other day, and then no further treatment.

Group II consisted of four rats. They received the iodine deficient diet for 2 weeks, the perchlorate treated water for the first 4 days and estrogen injections every other day. In addition, group II was also given nascent iodine for the second week of the experiment.

Group III consisted of four rats. This group was given the iodine deficient diet for 2 weeks, perchlorate treated water for the first 4 days and estrogen injections every other day. In addition, group III was treated with potassium iodide for the second week of the experiment.

Perchlorate treatment consisted of 400 mg/100 ml $NaClO$ in deionized, distilled water for the first 4 days.

Estrogen treatment consisted of intramuscular injections of 2.5 ug of estradiol suspended in 0.1 ml of sesame oil administered every other day for the entire two weeks. In order to insure estrus cycling in the animals, vaginal smears were taken every other day and the day of necropsy.

Iodine therapy consisted of 80 ug of nascent iodine per 100 ml of

doubly distilled water. Each rat consumes about 25 ml per day, therefore, the dosage for each rat was 20 ug of iodine per day or 80 ug per kilogram of body weight.

Iodide therapy consisted of 120 ug of potassium iodide per 100 ml of doubly distilled water. The dosage per rat per day was approximately 30 ug per day or 120 ug per kilogram of body weight. The difference between iodine and iodide therapies were calculated using the molar differences of the iodine formulations. The final iodine availability of both compounds were similar.

The rats were weighed initially and then daily to confirm proper growth patterns.

At the end of the two week study, the rats were killed with carbon dioxide. The mammary glands and the thyroid glands were dissected free and weighed. They were then fixed in a 10% formalin solution for histological studies. Venous blood was removed from the inferior vena cava. Immediately following removal, the blood was centrifuged for ten minutes and the serum was collected and frozen for chemical studies.

The microscopic slides of the thyroid and mammary tissues were stained with hemotoxylin and eosin and then were later observed with the aid of a pathologist.

The dosage of iodine, 80 ug per kilogram of body weight, was derived from clinical data (Dr. William Ghent) as representing improvement of fibrocystic disease in women.

Results

Weights Figure 1 displays the average weight gain per group. The results of our selectively randomized groups show that the rat weights are comparable to normal rats of the same age. There was no significant change in average total weights throughout the experiment. The average weight gain was greater in the controls by 17.5 grams (Figure 1). Iodide treated rats lost 28 grams initially, which may have caused acute hyperthyroidism. The controls and iodine treated rats had a gradually increasing weight gain supporting normal thyroid function, weight gain from iodine therapy, and no significant changes after perchlorate treatment.

The weights of the dissected tissues are displayed in table 1. These weights are given per kilogram animal weight. The results show that group 3 had the most normal weight while that of groups 1 and 2 were higher than normal. The statistical comparison of thyroid weights proved to be significant only in the comparison between groups 1 and 3. The difference in breast tissue weights between group 3 and each other was statistically significant. Group 3 had the highest breast weight, while groups 1 and 2 had lower weights with group 2 being closest to normal.

Vaginal Smears Figures 2-4 demonstrate the stages of the estrus cycle in the rats during the two week period, as a response to estrogen administration every other day. All rats achieved estrus at least two days before necropsy as verified by the vaginal smears taken on alternating days of estrogen administration.

Histological Evaluation of Breast Tissue

The evaluation of breast tissue histology was graded on a scale of 1 to 4, with 1 being a normal, inactive, non-lactating breast, and 4 being the most atypical, overactive breast. These evaluations of the breast tissue were made with respect to lobular hyperplasia, cyst formation, evidence of secretion, perilobular fibrosis and periductal fibrosis. These gradings are listed in tables 2 and 3.

Group 1, the control, demonstrated the greatest amount of periductal and perilobular fibroses as compared to the other two groups. This group appeared to have minimal lobular hyperplasia and secretions. There was also definite cyst formation in this group. It is evident that the animals in this group have fibrocystic disease of the breast.

Group 2, the iodine treated group demonstrated no evidence of significant histological change as compared to groups 1 and 3. There was some focal inflammation and dilation of ducts in one of the animals. This, however did not prove to be fibrocystic disease. Overall, the animals in this group appeared the most normal in terms of breast activity.

Group 3, the iodide treated group, demonstrated the most significant cases of fibrocystic disease. These animals had the greatest amount of secretions and lobular hyperplasia as well as cyst formation as compared to the other two groups. There was also evidence of adenomas and macrophages were present. Significant perilobular and periductal fibrosis appeared in this group, but this was to a lesser degree than that of group 1.

Histological Evaluation of the Thyroid Tissue

The evaluation of the thyroid tissue histology was made with respect to follicle size, presence of colloid, scalloping of colloid and the epithelial condition regarding the presence of papillary areas, metaplasia, hyperplasia, and adenomas. These evaluations are illustrated in Table 5.

The animals in group 1, the control group, had medium to large sized follicles with abundant colloid and significant colloid scalloping. There was also focal metaplasia present in this group. This indicates that the animals in this group have relatively inactive thyroid glands.

The animals in group 2, the iodine treated group, had more large follicles than those in group 1. While there was abundant colloid for the most part, it was difficut to determine colloid scalloping. This was due to the sloughing appearance if the epithelium which was characteristic of this group. This indicates that the animals in this group exhibit reduced thyroid activity.

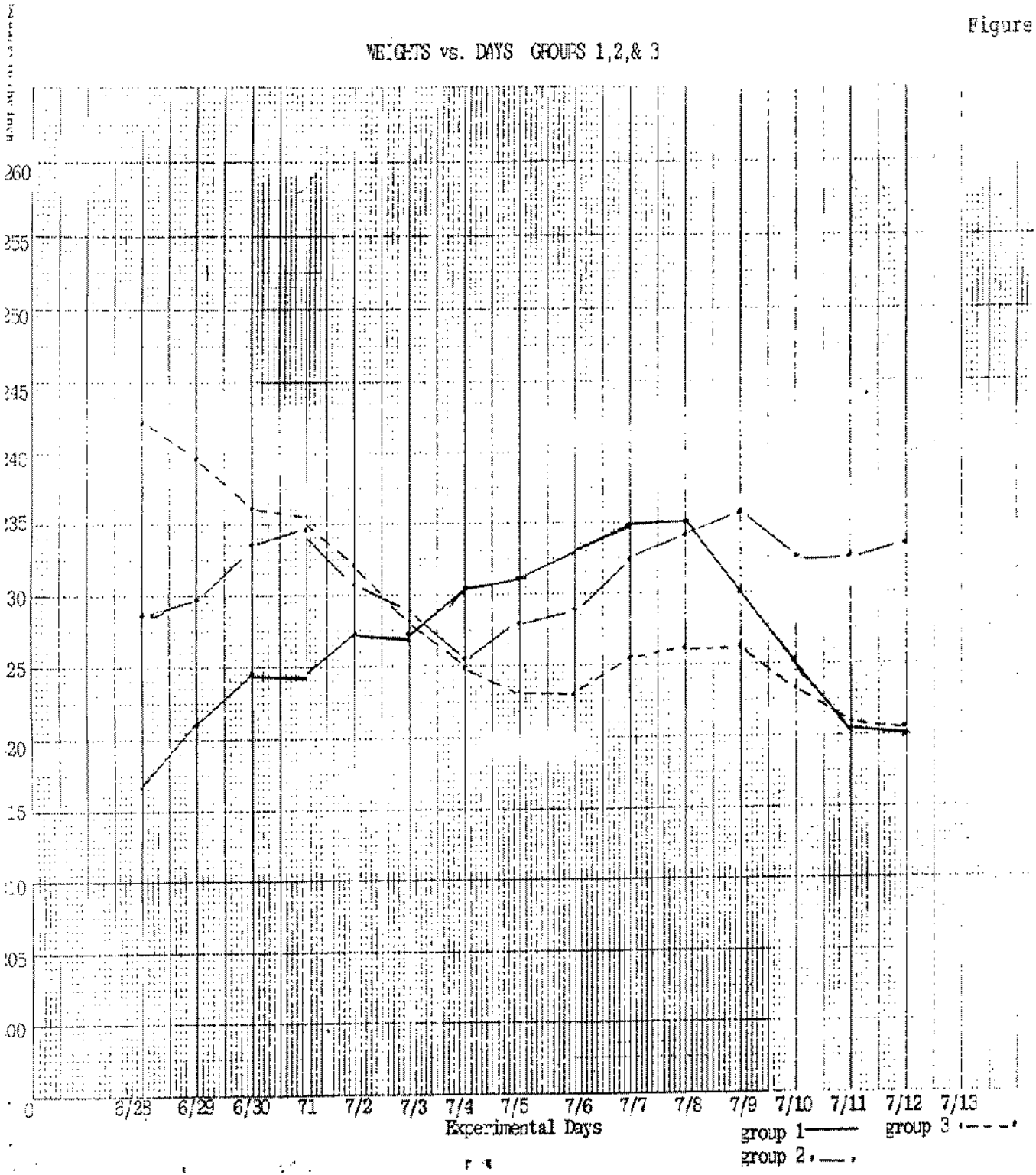
The animals in group 3, the iodide treated group, had the most normal appearance out of all the groups with few exceptions. The follicle size of this group was large for the most part with colloid and colloid scalloping present. Two out of the four animals had adenomas, but had otherwise normally functioning, active thyroid glands.

Table 1: Dissected Thyroid and Breast Weights

Group #	Rat #	Thyroid Weight (mg/kg rat)	Breast Weight (mg/kg rat)
1	1	105.50	3467.89
1	2	98.65	3479.82
		x = 102.1	x = 3473.86
		SD = 4.844	SD = 8.436
		SEM = 3.425	SEM = 5.97
2	1	102.46	4311.48
2	2	89.29	3513.39
2	3	92.11	4298.25
2	4	106.84	5576.92
		x = 97.68	x = 4425.01
		SD = 8.330	SD = 853.80
		SEM = 4.165	SEM = 426.9
3	1	105.50	6834.86
3	2	91.74	4954.13
3	3	81.08	6531.53
3	4	79.65	6150.44
		x = 89.49	x = 6117.74
		SD = 11.96	SD = 824.73
		SEM = 5.979	SEM = 412.37

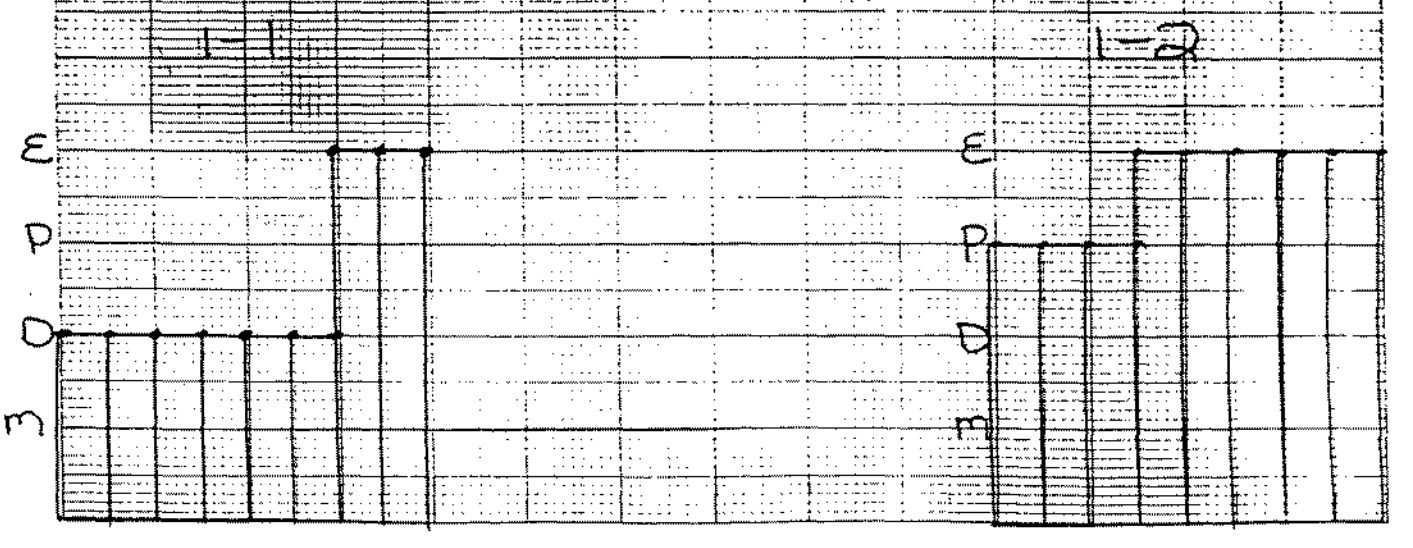
x - Arithmetic mean of weights for the respective group
SD - Standard deviation of the weights for the respective group
SEM - Standard error of the arithmetic mean

Figure 1


WEIGHTS vs. DAYS GROUPS 1,2,& 3
260
255
250
245
240
235
30
25
20
15
10
05
00
0
6/28
6/29
6/30
71
7/2
7/3
7/4
7/5
7/6
7/7
7/8
7/9
7/10
7/11
7/12
7/13
Experimental Days
group 1
group 2
group 3

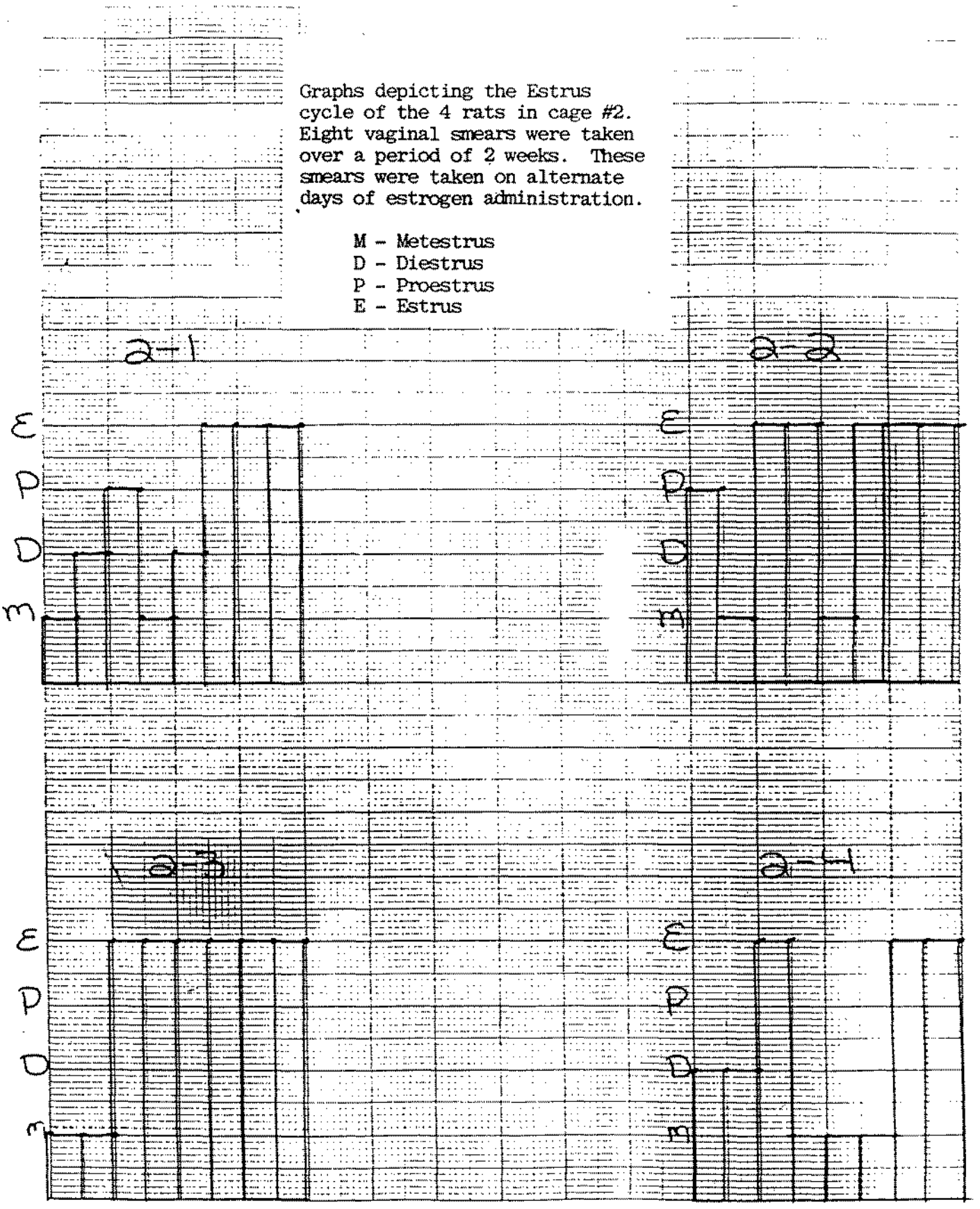
Graphs depicting the Estrus cycle of the 2 rats in cage #1. Eight vaginal smears were taken over a period of 2 weeks. The smears were taken on alternate days of estrogen administration.

M - Metestrus
D - Diestrus
P - Proestrus
E - Estrus



Graphs depicting the Estrus cycle of the 4 rats in cage #2. Eight vaginal smears were taken over a period of 2 weeks. These smears were taken on alternate days of estrogen administration.

M - Metestrus
D - Diestrus
P - Proestrus
E - Estrus



Graphs depicting the Estrus cycle of the 4 rats in cage #3. Seven vaginal smears were taken over a period of 2 weeks. These smears were taken on alternate days of estrogen administration.

- M - Metestrus
- D - Diestrus
- P - Proestrus
- E - Estrus

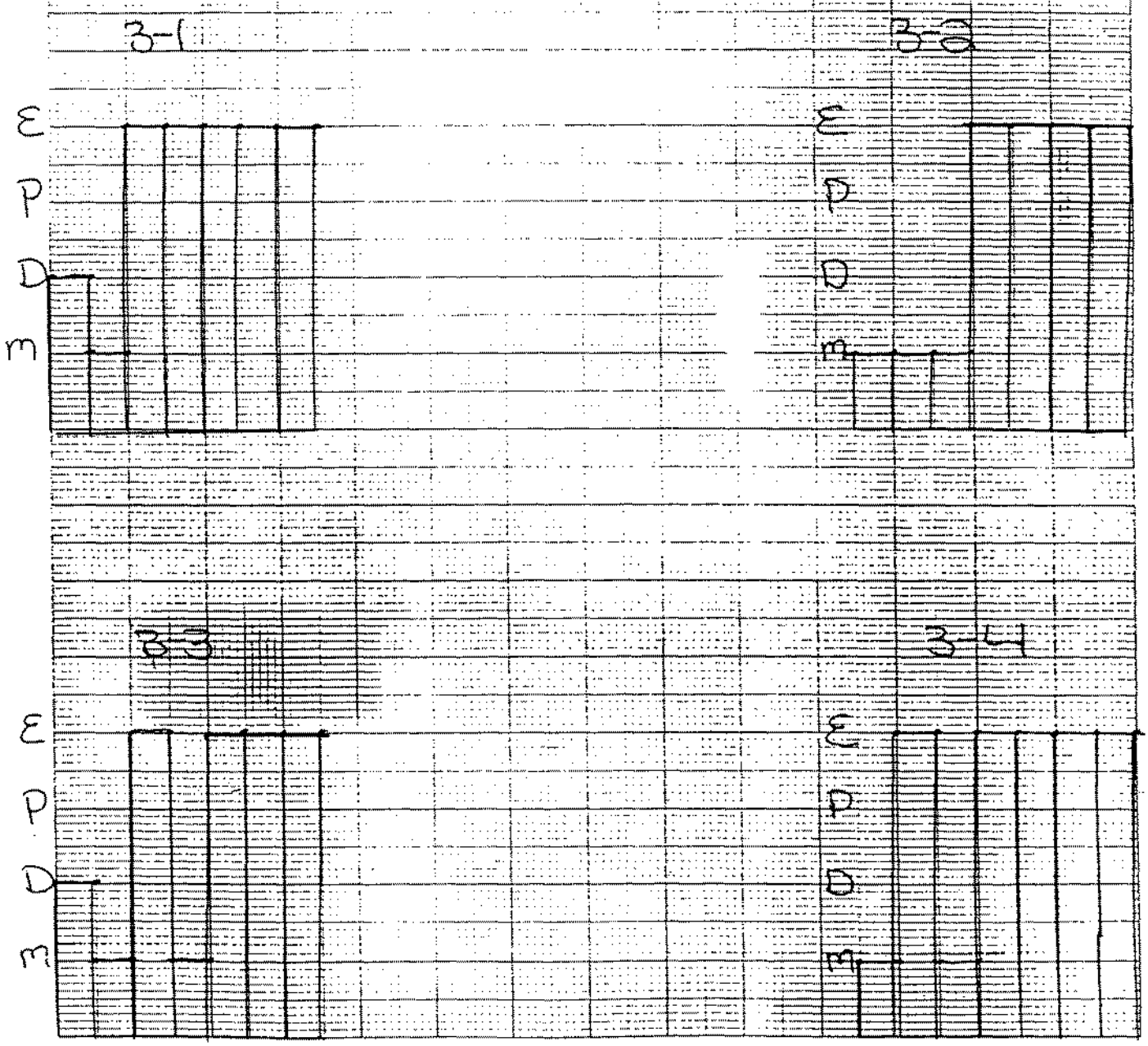


Table 2: Histologic Grading of Mammary Tissue

Group #	Rat #	Lobular Hyperplasia	Secretion	Cyst Formation	Perilobular Fibrosis	Periductal Fibrosis
1	1	1	2	3	3	3
1	2	1	1	1	3	3
2	1	1	1	2	2	1
2	2	1	1	1	1	1
2	3	1	1	1	1	1
2	4	1	2	1	1	1
3	1	4	4	2	2	3
3	2	1	1	2	2	2
3	3	2	4	1	1	2
3	4	1	3	2	2	2

Low to High (1-4)

Table 3: Comparative Histologic Grading of Mammary Tissue

Group #	Lobular Hyperplasia	Secretion	Cyst Formation	Perilobular Fibrosis	Periductal Fibrosis
1	1	1.5	2.5	3	3
2	1	1.25	1	1	1
3	2	3	2.25	1.75	2.25

Table 1: Histological reading of the thyroid

Group #	Rat #	Presence of Colloid	Colloid Scalloping	Epithelial Condition	Follicle Size
1	1	abundant	present	focal metaplasia	medium
1	2	minimal	present	none	medium
2	1	minimal	**	papillary areas, focal metaplasia, hyperplasia	medium
2	2	minimal	none	focal metaplasia	medium
2	3	abundant	**	focal metaplasia	large
2	4	abundant	present	none	large
3	1	minimal	present	none	large
3	2	abundant	present	adenoma	large
3	3	abundant	present	adenoma	large
3	4	minimal	present	none	medium

** Could not be determined due to sloughing appearance of epithelium

Discussion

In this study, the animals were made iodine deficient by two methods: an iodine deficient diet and a perchlorate blockade of peroxidase, the enzyme involved in oxidation and organification of iodine. As we and others have observed, iodine must be processed by a certain mechanism or it is otherwise useless. The question remained as to which tissue breast or thyroid, would respond best to which chemical form of therapeutic iodine, diatomic or ionic as a treatment for the histological pathology caused by the iodine deficiency.

Although iodine deficiency causes breast atypia, it has been discovered by others that the condition and the treatments are enhanced by estrogen (8). The estrogen dose in this study was pharmacophysiological, building up to a constant estrus within a few days. Figures 2-4 show that estrus was not immediately achieved which proves that the estrogen was not immediately achieved which proves that the estrogen was not given in a saturated quantity. Reasons for the rats being in other stages of estrus include: irritation of the vaginal wall causing diestrus, and an improper estrogen quantity in relation to weight and physiology which may have caused the rat to be in metestrus or proestrus by the time the vaginal smear was taken.

As we observed by the mammary tissue and thyroid tissue weights and the histological results from both tissues, iodine is clearly the more effective of the two therapies for fibrocystic disease of the breast in rats. It was apparent that iodine in its diatomic form relieved all of the pathological disorders. Iodide treatment, however, appeared to worsen the condition in all aspects recorded except in periductal and perilobular fibrosis. In these areas, the histology was

worse in the controls indicating that iodide relieves only limited periductal and perilobular fibrosis. In the thyroid gland, we observed the reverse. The iodide treated rats, with exception of adenomas that are unexplainable at this time, had the most normal thyroid glands as seen by the weights and histology. The control group and the treated group (group 2) had similar thyroid weights and histological appearances which indicates that iodine had little or no therapeutic effect on the thyroid glands of rats.

The fact that the treatments evoked such different responses in both types of iodine dependent tissues suggests that the mechanisms for iodination differ in the mammary glands and the thyroid glands of rats, an perhaps, humans.

This difference would seem to correlate with the amount of peroxidase in each tissue. The mammary gland has a lesser amount of peroxidase as compared to the thyroid. Therefore, iodide trapping and oxidation is much more efficient in the thyroid because it contains adequate peroxidase. The breast, on the other hand, with its limited amount of peroxidase can initiate only a minimal amount of iodide for oxidation.

For women with fibrocystic disease, iodine would seem to be the treatment of choice. Not only does it relieve breast tissue atypia, but also it has no damaging effect on the thyroid gland, as opposed to excess iodides which may cause hyperthyroidism.

For future experiments, we suggest a longer period of time for iodine deficiency to increase the amount of breast atypia and determine whether or not iodine would be as effective under more chronic atypical conditions.

Conclusions

1). Iodine deficiency is correlated with mammary tissue atypia, which resembles fibrocystic disease in humans.

2). Estrogen enhances the active condition of the breast and any therapies acting upon the breast tissue in rats.

3). Iodine reverses the histology of fibrocystic disease in rat breasts.

4). Iodine is more therapeutic in the rat breast than iodide.

5). Iodide has a minimal effect on the rat breast but is effective in the thyroid.

6). Potassium iodide improves the iodine deficient thyroid condition, but proves to be an inadequate therapy for the fibrocystic disease and other breast atypia that occurs.

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