Uptake and Metabolism of Iodine Is Crucial for the Development of Thyroiditis in Obese Strain Chickens

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Abstract

To assess the importance of the role of thyroidal iodine in the pathogenesis of thyroiditis in the obese strain (OS) chicken, a model of spontaneous and severe disease, we studied the effect of antithyroid drugs that reduce thyroidal iodine or prevent its metabolism. Reduction of thyroidal iodine was achieved with $KClO_4$, an inhibitor of iodine transport and mononitrotyrosine (MNT), a drug that promotes loss of thyroidal iodine as iodotyrosines. A regimen consisting of $KClO_4$ and MNT administration beginning *in ovo* and continuing after hatching reduced thyroidal infiltration to 2% of control values and decreased thyroglobulin antibody (TgAb) production for as long as 9 wk. Untreated birds had severe disease by 5 wk of age. The suppression of disease was independent of TSH, not mediated by generalized immunosuppression and reversed by excess dietary iodine. Two drugs that inhibit the metabolism of iodine, propylthiouracil (PTU) and aminotriazole, reduced thyroidal infiltration and TgAb levels, although to a lesser extent. When splenocytes from OS chickens with thyroiditis were transferred to Cornell strain (CS) chickens, a related strain that develops late onset mild disease, only the recipients that were iodine supplemented developed thyroiditis. In conclusion, autoimmune thyroiditis in an animal model can be prevented by reducing thyroidal iodine or its metabolism and optimal effects require intervention at the embryonic stage. (*J. Clin. Invest.* 1991. 88:106–111.) Key words: autoimmune disease • antithyroid drugs • thyroid • immune suppression • etiology

Introduction

There is accumulating evidence which suggests that iodine, an environmental element that is an integral component of thyroid hormone and the prohormone thyroglobulin, plays an important role in the induction of autoimmune thyroiditis in certain animal models and in humans. Our studies with the Cornell strain (CS)[1] chicken (1) and other studies with the BB/W rat (2) and the Buffalo rat (3) have shown that dietary iodine supplementation exacerbates thyroiditis in animals genetically susceptible to mild, late-onset disease. Clinical reports have also shown that an increase in iodine intake is associated with the production of thyroid autoantibodies (4, 5).

The results of converse experiments, namely, the attempts to demonstrate a decrease in the incidence and severity of disease by iodine depletion in the obese strain (OS) chicken were minimally successful (1). The OS chicken develops severe autoimmune thyroiditis characterized by complete destruction of the thyroid gland and production of autoantibodies to thyroglobulin (Tg), thyroxine (T_4), and triiodothyronine (T_3) ~ 4 wk after hatching (6, 7). When OS chickens were provided a diet containing $KClO_4$ and T_4, antibodies to thyroglobulin, T_3 and T_4 were reduced but there was no diminution in infiltration (1). It was hypothesized that thyroiditis develops in the absence of thyroidal iodine or that iodine depletion regimens initiated after hatching are ineffective due to the accumulation of iodine by the embryonic thyroid gland.

We undertook the present study to determine if early intervention with agents that inhibit the uptake and organification of iodine would result in more effective prevention of OS thyroiditis. We found that the time at which iodine depletion regimens were initiated had profound effects on their ability to diminish thyroiditis. One regimen initiated *in ovo* and continued until sacrifice was particularly effective and virtually prevented thyroiditis for as long as 9 wk, the longest time period studied.

Methods

Experimental animals and diets. Fertile OS eggs were obtained from hens maintained at Cornell University on a T_4-supplemented diet (10 μg/100 g feed). Normal strain fertile eggs were obtained from a local supplier. Chickens hatched from these eggs were maintained on regular diets or food supplemented with T_4, (10 or 20 μg/100 g) and aminotriazole (0.1 or 0.2%) and water supplemented with mononitrotyrosine (MNT) (10 mM), $KClO_4$ (0.1%), propylthiouracil (PTU) (0.1%), and KI (20 mg/dl). All reagents were purchased from Sigma Chemical Co., St. Louis, MO. In some experiments, 13-d chick embryos were given a single injection of $KClO_4$ (0.05, 0.5, 5.0 mg) into the albumin of the egg. At hatching each bird then received a single subcutaneous dose of 5 mg $KClO_4$ and 11 mg MNT.

Iodine content of thyroid. Chemical analyses of the iodine content of thyroid glands were performed in the laboratory of Dr. Lewis Braverman at the University of Massachusetts Medical Center by a semiautomated colorimetric assay (8).

Measurement of thyroglobulin autoantibody. In early experiments Tg antibody (Tg Ab) was measured by a direct binding radioassay (9). Briefly, 1 μl of each serum specimen was added to assay tubes with 500

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1. *Abbreviations used in this paper:* CS, Cornell strain; MNT, mononitrotyrosine; OS, obese strain; PTU, propylthiouracil; T_4, thyroxine; Tg, thyroglobulin.

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μl PBS, pH 7.4, containing 0.1% BSA and ^{125}I Tg. The tubes were incubated for 1 h at 37°C and overnight at 4°C. Separation was achieved by bringing the mixture to 2% with polyethylene glycol and centrifuging at 2,500*g* for 30 min. Values were expressed as the percentage of the total radioactivity that was specifically precipitated.

In later experiments an ELISA was used. Briefly, microtiter well plates (Dynatech, Chantilly, VA) were coated with high iodine Tg (10 μg/ml) and blocked with 2% BSA. The Tg was obtained from chickens that had been provided drinking water supplemented with KI (20 mg/dl) for 1 mo. Sera (100 μl of a 1:1,000 dilution) were incubated in the wells for 3 h at room temperature. The plates were then washed and phosphatase-labeled goat anti-chicken IgG (Kirkegaard and Perry, Gaithersburg, MD) was added. 1 h later the plates were washed and *n*-nitrophenylphosphate substrate (Kirkegaard and Perry) was added. Absorbance at 407 nm was determined using a microplate reader (Molecular Devices, Menlo Park, CA). Arbitrary units were assigned based on a calibration curve of dilutions of pooled OS sera previously determined to have high Tg antibody titers. A cutoff value of 8–12 units was set to minimize false negative and false positive values. The within assay CV at 1:1,000 serum dilutions was < 8%.

Thyroid histology. Paired thyroid lobes of each bird were fixed and embedded in paraffin. Multiple sections were cut and stained with hematoxylin and eosin. These sections were then examined by one of the authors (A. Dhar) who determined the percent of each cross-section of thyroid gland that was replaced by mononuclear leukocytes. The results for multiple sections, usually 10, were then averaged and this value was the percent infiltration for each chicken. When all of the slides were read the slide numbers were then decoded and grouped according to treatment protocol. The mean percent infiltration was then calculated by averaging the percent infiltration of all birds in that group.

Thyroidal distribution of radioiodine. To determine the optimal dose of proplythiouracil (PTU) and aminotriazole, we determined their effect on thyroidal iodine metabolism by examining the distribution of radioiodine in the thyroids of birds receiving these treatment regimens. Birds were injected i.p. with ^{125}I (20 μCi/bird) and 20 h later their thyroids were weighed, homogenized in 0.05 M Tris-HCl, pH 7.5 containing 0.05 M methimazole and hydrolyzed with pronase (5 mg/ml) (Calbiochem-Behring Corp., San Diego, CA). The hydrolysates were analyzed by ascending paper chromatography using butanol:acetic acid (2 M) (1:1, vol/vol). The bands were detected by autoradiography and counted.

Tests of generalized immunosuppression. Chickens were given an i.p. injection of heat killed *Brucella abortus* (2.7×10^9 bacilli) kindly supplied by the National Veterinary Services Laboratory, Ames, IA) (Table IV, experiment 2). The animals were bled 10 d after the injection and the sera were tested for antibodies to *Brucella abortus* (10). Briefly, serially diluted sera (25 μl) were incubated with 25 μl of 1:100 diluted stock suspension of heat killed and stained *Brucella abortus* at 4°C overnight, and the wells were then read for agglutination.

In other experiments 3- or 4-wk-old birds were injected i.m. with 1 mg of bovine gamma globulin (BGG) in complete Freund's adjuvant (Table IV). Antibodies to BGG were measured by ELISA according to the details described above for Tg antibody except that BGG was used to coat the plates and results were expressed in O.D. units.

Adoptive transfer. Recipient CS chicks homozygous for the major histocompatibility allele B^{13} obtained from Cornell University as fertile eggs were given 1 mg cyclophosphamide i.p. at hatching, 1 mg the next day and two injections of 1 mg each on the third day. At hatching half of these birds were placed on a high iodine regimen consisting of KI (20 mg/dl in the drinking water) and the other half were placed on a low iodine regimen consisting of $KClO_4$ (0.1%) and MNT (10 mM in the drinking water). On the fourth day they were injected i.v. with 5×10^7 splenocytes in 0.2 ml culture medium isolated from 5-wk-old untreated OS donor chickens of the same major histocompatibility type. They were bled on day 10 and killed on day 23 at which time they were tested for TgAb and histology.

Results

Effect of iodine depletion on thyroid autoimmunity. Iodine depletion was produced by treatment with $KClO_4$ and MNT. $KClO_4$ inhibits active iodine transport, whereas MNT, an inhibitor of iodotyrosine deiodinase, prevents recycling of thyroidal iodine and causes iodine depletion by promoting loss of iodine as iodotyrosines (11). A single injection of $KClO_4$ and MNT at hatching followed by dietary $KClO_4$ and MNT reduced thyroidal infiltration of 5-wk-old OS chickens to approximately half the value of the T_4-fed group (Table I). When this regimen was preceded by a single injection of $KClO_4$ *in ovo,* infiltration was dramatically reduced. The most effective reduction of thyroid infiltration was achieved with 5 mg $KClO_4$ *in ovo.* Subsequent experiments using *in ovo* $KClO_4$ injections employed the 5-mg dosage.

Effect of $KClO_4$ in ovo on thyroidal iodine content at hatching. A single injection of 5 mg $KClO_4$ *in ovo* at 13 d of age caused a significant reduction in the thyroidal iodine content at hatching 7 d later from 443±54 to 86±17 μg/g±SEM (Student's *t* test, unpaired, $P < 0.002$). Thyroid weights were unchanged. The time of hatching was not delayed by this dose of $KClO_4$ indicating that the embryos remained euthyroid.

Effect of dietary combinations of $KClO_4$, MNT, T4, and KI on autoimmune thyroiditis. The effect of various combinations of dietary $KClO_4$, MNT, T_4, and KI in chickens treated *in ovo* and at hatching with $KClO_4$ and MNT injections are shown in Table II. Dietary $KClO_4$ without MNT did not have a significant effect on thyroidal infiltration or TgAb production. Dietary $KClO_4$ + MNT significantly reduced thyroiditis when compared with birds receiving $KClO_4$ alone. The addition of thyroxine to the diet suppressed TSH as indicated by lower thyroid gland weights but it did not alter the ability of $KClO_4$ and MNT to prevent infiltration and antibody production. The addition of excess iodine to the $KClO_4$ + MNT + T_4 regimen increased infiltration toward control values thus providing evi-

Table I. Effect of $KClO_4$ Administered In Ovo on Thyroiditis in OS Chickens Examined at 5 wk of Age

Treatment of 13-d embryos	$KClO_4$ + MNT at hatching	Additions to diet		Thyroid infiltration
		Food	Water	
				mean (%)±SEM
None	−	T_4	None	78±9
None	+	T_4	$KClO_4$ + MNT	43±13
$KClO_4$, 0.05 mg	+	T_4	$KClO_4$ + MNT	13±5
$KClO_4$, 0.5 mg	+	T_4	$KClO_4$ + MNT	17±15
$KClO_4$, 5 mg	+	T_4	$KClO_4$ + MNT	1±1

13-d embryos were given a single injection of $KClO_4$. At hatching each chick received a single subcutaneous dose of 5 mg $KClO_4$ to prevent thyroidal uptake of iodine and 11 mg MNT to prevent thyroidal reutilization of iodine. Thyroxine (20 μg/100 g), MNT (10 mM), and $KClO_4$ (0.1%) were added to the diet for the duration of the experiment. There were six to eight birds per group and the data were analyzed by ANOVA + Fisher PLSD at $P < 0.05$ significance level). The infiltration in all experimental groups was significantly less than that of the T_4 control. In addition, infiltration in the 5 mg $KClO_4$ group was significantly less than the group receiving $KClO_4$ + MNT at hatching.

Table II. Effect of Combinations of Antithyroid Drugs, Thyroxine, and Excess Iodine on Thyroiditis in 5-wk-old OS Chickens

Group number	$KClO_4$ + MNT injections	Additions to diet	Thyroid weight	Thyroid infiltration	TgAb (incidence)
			mg	%	
1	No	T_4	ND	78±9	9/13
2	Yes	None	48.2±12.0	88±4	8/8
3	Yes	$KClO_4$	67.2±17.9	70±14	7/10
4	Yes	$KClO_4$, MNT	134±11	2±1	2/7
5	Yes	$KClO_4$, MNT, T_4	31±3	6±2	0/7
6	Yes	$KClO_4$, MNT, T_4, KI	20±2.5	40±8	0/6

OS chickens were treated as above and killed at 5 wk. The values for thyroid weight and infiltration are mean±SEM of 6–13 chickens per group. TgAb was measured by ELISA. The details of treatment with $KClO_4$, MNT, and T_4 are provided in Table I utilizing the 5 mg $KClO_4$ protocol. KI (20 mg/dl) was added to the drinking water of group 6 for the duration of the experiment. Data were analyzed by ANOVA + Fisher PLSD at $P < 0.05$. Summary of infiltration: Groups 1, 2 and 3 do not differ from each other, but each differs from groups 4 and 5. Group 5 differs from group 6. Thyroid weight: Group 4 had the largest thyroids and the thyroids in groups 2 and 3 were larger than those in groups 5 and 6. TgAb incidence data was analyzed by multiple Chi-square analyses and subjected to a Bonferroni correction. Groups 4–6 had lower incidence of antibody than the control group 1 ($P < 0.025$). Groups 2 and 3 did not differ from group 1.

dence that the likely mechanism for the prevention of thyroiditis by $KClO_4$ + MNT was the reduction of thyroidal iodine rather than indirect effects at other sites.

The suppression of OS thyroiditis by iodine depletion over time. The details of treatment with $KClO_4$, MNT, and T_4 are provided in Table I utilizing the 5 mg $KClO_4$ protocol. Infiltration and TgAb incidence data were analyzed by multiple Chi-square analyses and subjected to a Bonferroni correction. Because chicks in this experiment had either severe disease or no disease, infiltration data were expressed as the incidence of chicks with thyroids exhibiting > 50% infiltration. The mean value of the infiltration negative birds (< 50%) at 9 wk was 7.9%.

Iodine depletion obtained with injections of $KClO_4$ + MNT and dietary additions of $KClO_4$ + MNT + T_4 produced a dramatic suppression of disease for up to 9 wk, the longest time period studied when compared with control birds given T_4 alone. Most of the control birds had severe disease by 5 wk of age. Their incidence of infiltration was 7/10 compared with the iodine depleted birds that had 0/9 to 2/9 from 5 to 9 wk of age. TgAb (incidence > 25 units) showed a similar reduction. Control birds at 5 wk of age had antibodies in 8/10 birds, whereas iodine-depleted birds had antibodies in 0/9 or 1/9 from 5 to 9 wk of age. All experimental groups differed from the control group for infiltration incidence ($P < 0.01$) and TgAb incidence ($P < 0.0005$).

Effect of propylthiouracil (PTU) and aminotriazole on thyroid function in normal strain birds. To determine the optimal dosage of PTU and aminotriazole, preliminary experiments to determine their effects on iodine metabolism in normal birds were performed. Data were analyzed by ANOVA + Fisher PLSD at $P < 0.05$. Both PTU (0.1%) and aminotriazole (0.2%) caused similar changes in ^{125}I metabolism such as significantly increased iodide and decreased DIT and T_3 + T_4 indicative of inhibition of iodination and coupling. Aminotriazole at 0.1% did not alter these parameters. Thyroid gland weight was significantly increased by all treatments including aminotriazole at 0.1%, a concentration which did not block iodination. This may be indicative of an additional hypothalamic-pituitary site of aminotriazole action.

Effect of various antithyroid drugs on thyroiditis in 6-wk-old OS chickens. PTU and aminotriazole given in the diet from hatching significantly reduced thyroid infiltration (Table III, experiment 1). When $KClO_4$ and MNT injections preceded the administration of dietary antithyroid drugs (experiment 2) a reduction in thyroidal infiltration and a decrease in the incidence of TgAb was again observed.

Effect of various antithyroid drugs on the immune response to foreign antigens in 6-wk-old OS chickens. To determine if antithyroid drugs decreased thyroiditis by causing generalized immunosuppression, we tested the effect of these drugs on the immune response to BGG, a T cell–dependent antigen, and to *Brucella abortus,* a T cell–independent antigen. Because hypothyroidism could have effected the immune responses to exogenous antigens, both control and experimental groups received thyroxine. PTU and aminotriazole given in the diet from hatching (Table IV, experiment 1) did not cause a difference in the antibody response to BGG. When PTU, aminotriazole, and $KClO_4$ plus MNT were given in the diets of birds receiving $KClO_4$ and MNT injections (experiment 2) the response to *Brucella abortus* was uneffected. There was also no decrease in antibody response to BGG in birds receiving $KClO_4$, MNT, PTU, or ATA. However, for unknown reasons, there was a significant increase in the anti-BGG response in both the PTU and $KClO_4$ plus MNT groups. It is possible that these drugs, by preventing the severe inflammatory autoimmune response, allowed the immune system to respond more vigorously to a foreign antigen.

Table III. Effect of Various Antithyroid Drug Regimens on Thyroiditis in OS Chickens

In ovo and at hatching	Additions to diet	Infiltration incidence (infiltration > 50%)	TgAb incidence
Experiment 1			
None	T_4	9/10	ND
None	T_4, PTU (0.1%)	4/11	ND
None	T_4, ATA (0.2%)	6/11	ND
Experiment 2			
$KClO_4$/MNT injection	T_4	7/10	9/10
$KClO_4$/MNT injection	T_4, $KClO_4$, MNT	1/9	2/8
$KClO_4$/MNT injection	T_4, PTU (0.1%)	1/7	3/6
$KClO_4$/MNT injection	T_4, ATA (0.2%)	2/8	3/8

OS chickens (8–11 per group) were placed on various antithyroid regimens at hatching plus T_4 (20 μg/100 g food) (experiment 1). In experiment 2 chickens were given $KClO_4$ + MNT injections before the initiation of the dietary regimens. The data were analyzed by multiple Chi-square analyses and subjected to a Bonferroni correction. In experiment 1 PTU and aminotriazole both reduced thyroid infiltration ($P < 0.0002$. In experiment 2, all dietary additions significantly reduced infiltration from the T_4-fed control ($P < 0.015$) and TgAb incidence ($P < 0.001$).

Table IV. Effect of Antithyroid Drugs on the Immune Response to Foreign Antigens in OS Chickens

In ovo and at hatching	Additions to diet	Ab to BGG O.D. mean±SEM	*B. abortus* $\log_2$ titer
Experiment 1			
None	T_4	0.91±0.17	ND
None	T_4, PTU (0.1%)	1.00±0.31	ND
None	T_4, ATA (0.2%)	0.82±0.33	ND
Experiment 2			
$KClO_4$/MNT injection	T_4	1.176±0.116	6.0±0.5
$KClO_4$/MNT injection	T_4, $KClO_4$, MNT	2.067±0.159	7.0±0.3
$KClO_4$/MNT injection	T_4, PTU (0.1%)	1.847±0.154	5.0±0.6
$KClO_4$/MNT injection	T_4, ATA (0.2%)	1.298±0.174	6.8±0.8

OS chickens were treated with various drug regimens as described in Table IV. The birds of experiment 1 were injected with bovine gamma globulin (1 mg in complete Freund adjuvant) at 4 wk and tested at 6 wk for antibody to BGG by ELISA. Birds of experiment 2 received a single injection of BGG (1 mg in CFA) at 3 wk and *Brucella abortus* (2.7×10^9 bacilli) 4 d later. At 5 wk their sera were tested for antibodies. Data were analyzed by ANOVA + Fisher PLSD. There were no differences in antibody titers to BGG in experiment 1, but in experiment 2 birds provided with dietary $KClO_4$ + MNT or PTU (plus T_4) had more BGG Ab than control birds fed T_4 alone ($P < 0.05$). There were no differences between any groups in their response to *B. abortus*.

The effect of dietary iodine on recipient target organ susceptibility to adoptive transfer of thyroiditis. CS chickens develop minimal thyroiditis by 10 wk of age on a high-iodine diet (1) and would be expected to have even less disease upon treatment with cyclophosphamide. However, transfer of OS splenocytes to KI-treated CS recipients caused significant thyroidal infiltration (Table V). Transfer to iodine-depleted CS recipients, on the other hand, resulted in minimal infiltration. These treatment regimens had no effect on TgAb production.

Discussion

The present study provides the first experimental demonstration of the protective effect of iodine depletion in the development of autoimmune thyroiditis in an animal model. Our previous attempt to demonstrate this phenomenon in the OS chicken by $KClO_4$-induced iodine depletion from the time of hatching was minimally successful (1). The iodine depletion regimen consisting of $KClO_4$ and MNT treatments beginning *in ovo* and continuing after hatching caused a fivefold reduction in thyroidal iodine at hatching, reduced infiltration to 2% of untreated controls values and decreased TgAb production for as long as 9 wk. It was important to begin iodine depletion *in ovo* because treatment with these drugs at hatching resulted in only partial improvement. It was also necessary to maintain severe iodine deficiency by continuing $KClO_4$ and MNT administration in the diet after hatching. Even when preceded by the *in ovo* treatment, $KClO_4$ without MNT was ineffective in the present series of experiments. This may be an indication that the dose of $KClO_4$ was not high enough to completely block the thyroidal uptake of dietary iodine. When the diet was supplemented with KI in an attempt to override the iodine deficiency induced by $KClO_4$ and MNT, infiltration increased toward untreated control values demonstrating that the mode of action of $KClO_4$ and MNT is the reduction of intrathyroidal iodine.

Table V. Effect of Dietary Iodine on Recipient Target Organ Susceptibility to Adoptive Transfer of Thyroiditis

Diet of recipient	Thyroid infiltration	TgAb incidence
	%	
$KClO_4$ + MNT	1.0±0.4	4/12
KI	36±8	4/12

24 recipient CS chicks given cyclophosphamide at hatching were placed on a high iodine regimen consisting of KI (20 mg/dl in the drinking water) or a low iodine regimen consisting of $KClO_4$ (0.1%) and MNT (10 mM in the drinking water). They were injected on the fourth day with splenocytes isolated from 5-wk-old untreated OS donor chickens. They were bled on day 10 and killed on day 23 at which time they were tested for TgAb and histology. Infiltration values are mean±SEM. Infiltration in the KI group is significantly greater than the $KClO_4$ + MNT group (*t* test, $P < 0.0001$).

The amount of iodine taken up by the thyroids of embryonic OS chicks in our previous study was relatively high (1). At that time the normally hypothyroid OS breeding hens were fed protamone, an iodinated casein that contains enough T_4 to render the hens euthyroid for breeding purposes. However, this diet caused a 4.5-fold increase in the iodine content of their eggs (12). Initially we reasoned that the birds were receiving a significantly increased iodine load *in ovo* that had a detrimental effect on their disease. However, when the OS flock at Cornell University was subsequently placed on a T_4-supplemented diet containing no protamone they continued to have progressive disease. The present study shows that the iodine in a normal diet taken up by the thyroid during embryonic development is critical in the development of the disease.

Our results indicate that TSH does not play a major role in thyroiditis of OS chickens because significant amelioration was achieved in birds given $KClO_4$ that had increased TSH shown by a fourfold increase in thyroid weight compared to T_4-fed animals. In addition, suppression of TSH by exogenous T_4 did not decrease thyroiditis. Weetman et al. (3, 13) also observed that treatment with T_4 did not influence experimental autoimmune thyroiditis in rats. Some studies, however, have reported a slight and/or transient effect of T_4 in reducing the disease of OS chickens (14, 15). However, neither of those studies addressed the question of whether T_4 acts by reducing TSH, intrathyroidal iodine, or both. The studies of Reinhardt et al. are of particular interest (16). They observed a reduction of thyroidal infiltration in BB/W rats with T_4 treatment for 2–3 mo. The protective effect of T_4 was lost when the rats were additionally treated with iodine, illustrating the key role of iodine in this disease.

The effect of PTU on Hashimoto's thyroiditis has been controversial. McGregor et al. (17) reported a decrease in thyroid autoantibodies in patients treated with carbimazole, however, this observation has not been confirmed. In fact, Jansson et al. (18) and Romaldi et al. (19) have shown that even high doses of antithyroid drugs fail to cause a diminution in autoantibody production. Rennie et al. (20) found lower autoantibody production in thyroglobulin-induced autoimmune thyroiditis in

the rat, however, Davies could not confirm these findings in a similar model (21). In Graves' disease where PTU-induced immunosuppression is well documented, the mechanism of action remains controversial (22). The results presented here show a clear ameliorative effect of PTU on thyroiditis of OS chickens.

To determine whether the effects of $KClO_4$, MNT, PTU, and aminotriazole were due to direct action on the thyroid or to generalized suppression of the immune system, we examined their effects on the responses to unrelated antigens. None of the antithyroid drugs decreased antibody responses to BGG, a T cell–dependent antigen or to *B. abortus,* a T cell–independent antigen. These results argue against a generalized effect on the immune system although they do not rule out the possibility of a local effect of the drugs on the immune cells within the thyroid. On the other hand, both PTU and aminotriazole inhibit iodine organification and may act by decreasing the iodine content of critical thyroidal components such as thyroglobulin as discussed below.

While this study clearly demonstrates that the uptake and metabolism of iodine is critical in the development of autoimmune thyroiditis, the mechanism underlying iodine action remains unclear. There is some evidence for a direct relationship between the iodine content of Tg and its immunogenicity. We have shown that highly iodinated thyroglobulin elicits a greater antibody response in normal chickens than poorly iodinated thyroglobulin (9). Champion et al. have demonstrated that the response to thryoglobulin by murine T cell hybridomas is directly related to its iodine content (23). They also showed that poorly iodinated thyroglobulin was ineffective in causing adjuvant-induced thyroiditis while normally iodinated thyroglobulin was able to induce the disease. However, in the spontaneous disease of OS chickens there is no evidence that highly iodinated Tg is a major etiologic factor. As far as the spontaneously occurring antibodies to Tg, we have recently shown that OS autoantibodies react well with thyroglobulin of high and low iodine content (9). Furthermore, the thyroglobulin from OS chickens contains less iodine than that of normal strains maintained on similar diets and suppressed with T_4 (24).

There is evidence that the iodination of Tg may not be the only mechanism by which iodine affects thyroiditis. Another possible mechanism of iodine action may involve free radical alteration of the thyroid gland. Iodine may interact with reactive oxygen intermediates such as H_2O_2 or O_2^- generated in the thyroid to produce highly reactive oxidized forms of iodine. We have shown that structurally unrelated dietary antioxidants reduce the onset and severity of thyroiditis in OS chickens (10). This suggests that oxidative alteration of the thyroid may play a role early in the development of the disease. Although increased free radical production in OS thyroids containing normal amounts of iodine has yet to be demonstrated, thionamide drugs have been shown to decrease free radical formation (25–27). However, while this may explain the action of PTU, the effect of aminotriazole, a catalase inhibitor, cannot be explained by this mechanism.

Finally, it is possible that iodine depletion by $KClO_4$ and MNT as well as inhibition of iodine metabolism by aminotriazole and PTU may decrease the formation of a putative iodine metabolite, unrelated to thyroglobulin, but important in the immune response. Using SDS/PAGE we failed to find an iodinated protein unique to OS thyroid (24), however, the possibility of a nonprotein iodine-dependent modulator remains.

The results of the adoptive transfer experiment reported here offers experimental evidence for the importance of acquired iodine-induced target organ alteration in the development of OS thyroiditis. Splenocytes sensitized to and specific for thyroid antigens transferred disease only in iodine sufficient recipients. This emphasizes the importance of iodine-induced thyroidal alteration before the autoimmune process begins. Such alteration could result in the expression of altered antigens or an increase in the expression of normal antigens. The precise mechanism remains to be elucidated.

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