Influence of Lymphocytic Thyroiditis on Iodine Metabolism in the Beagle[1] (34811)

T. E. Fritz, W. P. Norris, and N. D. Kretz
(Introduced by A. M. Brues)
Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439

Lymphocytic thyroiditis (Hashimoto's type) has been recognized and studied in dogs only in the past few years (1–5). Although it has a familial incidence in the dog (3, 5) no overt physiologic changes or clinical signs have been useful in its diagnosis (1, 2, 4, 5). Even in dogs with severe microscopic lesions no nonsurgical method of diagnosis has been useful although a number of laboratory tests have been used to diagnose thyroid disease (6–14). When several of these tests are applied simultaneously in clinical cases in man, Hashimoto's thyroiditis can be distinguished from other types of goiter or tumors of the thyroid (11, 12). Reports of clinical tests on dogs, unfortunately, have been limited; the only favorable results are correlations between PBI and ^{131}I uptake values in dogs with severe thyroiditis (1). Also, these results were obtained when the dogs were first put on a restricted iodide diet.

The high incidence of thyroiditis in beagles (1, 3–5) and the lack of reliable diagnostic procedures makes selection of unaffected animals for experimentation difficult. Previous experience with ^{131}I metabolism in dogs in this Laboratory suggested that the rate at which their thyroid glands lose injected ^{131}I may be significantly altered by thyroiditis or by hyperplasia of thyroid epithelium (15). Similar alterations in ^{131}I metabolism in thyroiditis of man also have been noted (8). This study was conducted to determine if changes in iodine metabolism could be used as a diagnostic procedure for lymphocytic thyroiditis.

Materials and Methods. The Argonne Beagle colony and its maintenance are described elsewhere (16). The diet is estimated to provide a minimum of 500 μg of iodide per dog per day (15). Previously we estimated that approximately 20% of the adult population has lymphocytic thyroiditis (5). Twelve dogs were selected for this experiment on the basis of their pedigrees and the known incidence of thyroiditis in their ancestors, descendents, and siblings. All 12 had a high probability of having thyroiditis (5).

Each dog was injected intravenously with 20 μCi of ^{131}I (as essentially carrier-free $Na^{131}I$ in sterile, isotonic, saline solution).[2] The ^{131}I content of their thyroid glands was followed, using a collimated NaI(Tl) crystal coupled to a 400-channel, γ-ray spectrometer, until ^{131}I could no longer be measured (15). Depending on the ^{131}I uptake of the glands and the subsequent rate of loss of ^{131}I from them, the period of observation ranged from 7 to 30 days.

Thirty days after ^{131}I administration, one thyroid lobe from each dog was surgically removed, weighed, and fixed in Technicon solution.[3] Sections were prepared for microscopic examination by the paraffin method.

Fourteen months later, six of these dogs were similarly injected with 20 μCi of ^{131}I and their thyroid activity was measured as before. Thirty days after this second dose of ^{131}I, the remaining lobe was removed from each of the six reinjected dogs. The tissues were weighed, fixed, and sectioned as were the first lobes.

For each set of measurements, the ^{131}I content of thyroid tissue was plotted against time and fitted visually to a single first-order exponential term, to provide an estimate of

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[2] Abbott Laboratories, North Chicago, Illinois

[3] Technicon Co., Chauncey, New York.

TABLE I. Summary of Thyroidal ^{131}I Metabolism, Histology, and Related Data.

		First ^{131}I tracer test (intact dogs)					Second ^{131}I tracer test (hemithyroidectomized dogs)				
Dog No.	Sex	Age (days)	Left thyroid weight (g)	Thyroid histology[a]	Max. uptake (% of dose)	BT 1/2[b] (days)	Age (days)	Right thyroid weight (g)	Thyroid histology[a]	Max. uptake (% of dose)	BT 1/2[b] (days)
0234	M	2151	.20	3+	—[c]	—					
1176	F	1397	.24	3+	0.39	2.0					
1084	F	1078	.52	3+	1.20	3.3	1505	.49	3+	0.25	2.8
1131	M	1043	.52	3+	0.30	3.5	470	.45	3+	0.40	2.6
0306	F	3000	.37	3+	0.42	3.5					
1129	F	1043	.46	3+	0.70	4.2	1470	2.56	3+	0.80	2.0
0495	F	2572	1.80	3+	0.85	4.4					
Mean			.59 ± .21		.56 ± .15	3.1 ± .5		1.17 ± .70		.48 ± .16	2.47 ± .2
0403	M	2780	.43	0	2.00	7.4					
0215	M	2759	.49	1+	1.90	12.2	3186	.37	1+	1.30	6.8
0193	M	2778	.49	1+	1.70	12.8	3205	.38	1+	1.40	9.5
1231	M	852	.48	0	1.80	13.4	1279	.46	0	1.40	9.8
1719	M	580	.37	0	1.45	14.2					
Mean			.45 ± .02		1.77 ± .09	12.0 ± 1.2		.40 ± .03		1.37 ± .03	8.7 ± .9

[a] 0 = no evidence of disease, essentially normal gland; 1+ = focal lymphocytic infiltration with no epithelial involvement; 2+ = grade 1+ plus epithelial involvement, i.e., acinar atrophy, early disintegration of follicular cells, presence of macrophages, and reduction of colloid; 3+ = grade 2+, plus parafollicular cell hyperplasia, severe destruction of follicles, and loss of colloid (Hürthle cell changes).

[b] Biological half-time of ^{131}I in the thyroid gland.

[c] No measurable uptake; for statistical analysis the value was assumed to be 0.5.

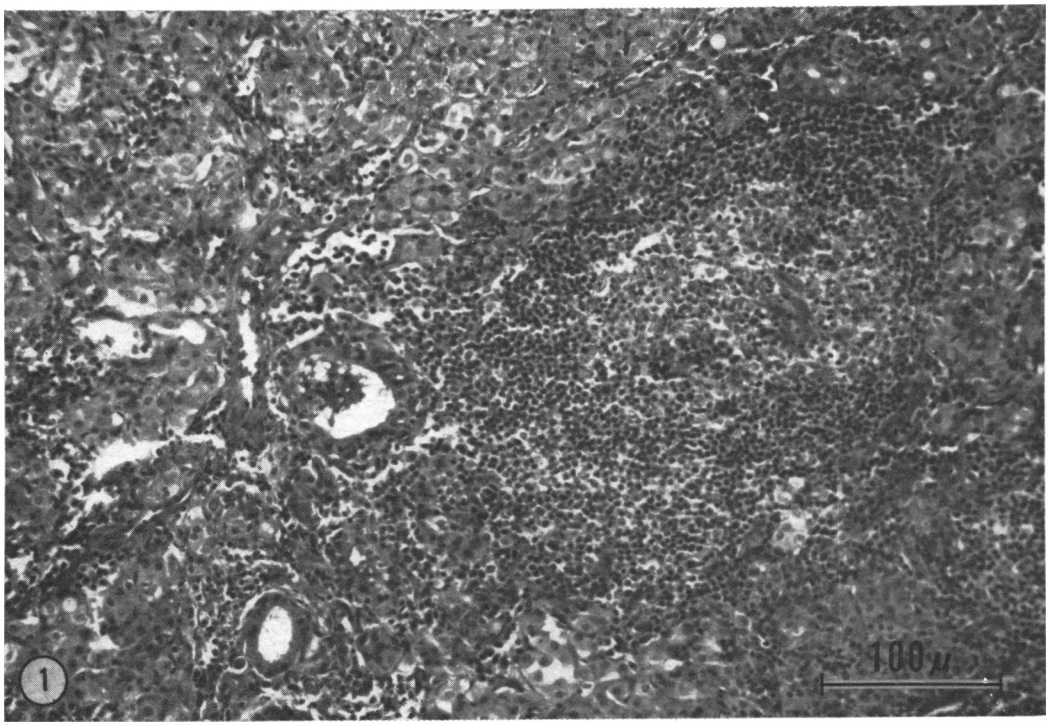

FIG. 1. Thyroid gland from a dog with severe (3+) thyroiditis. There is extensive lymphocytic infiltration, increased numbers of parafollicular cells, and destruction of follicular epithelium.

the rate of loss of ^{131}I from the thyroid which is expressed as the biological half-time (BT ½) in days (Table I).

H and E-, periodic acid–Schiff-, and azan-stained sections of each excised thyroid lobe were examined and scored using the criteria of Beierwaltes and Nishiyama (1) to evaluate and compare the severity of any lesions present (see footnotes, Table I). Sections of each thyroid lobe were also measured microscopically by the method of Uotila and Kannas (17) to obtain a quantitative estimate of the proportions of the principal components of the glands (*i.e.*, percentage of total section area presented as epithelium, colloid, and stroma). The amount in grams of colloid, epithelium, and stroma in the entire gland were computed from the percentage composition and weight of one lobe. To compute weights of the tissue components when the first ^{131}I injection was made, the weight of the left thyroid lobe was doubled because the weight of the right gland was not known.[4]

The BT ½ of ^{131}I in the thyroid, the percentage of colloid, epithelium, and stroma, and the grams of colloid, epithelium, and stroma at the time of each ^{131}I tracer injection for dogs with 3+ thyroiditis were compared with similar data from the dogs found to have normal thyroids. Significant differences were determined by Student's t test.

Results. Histologic examination of the thyroid lobes removed after the first tracer dose of ^{131}I revealed nine dogs with lymphocytic thyroiditis and three with no lesions. Of the nine dogs with thyroiditis, two had such mild (1+) involvement that they were undetected on the initial examination; the other seven had severe (3+) cases. The histologic features of the glands with thyroiditis were

[4] There was no difference in the weights of the left and right thyroid glands of 16 dogs from this colony when they were analyzed for significance by Student's t test [$t_{(31\ DF)} = .67$; $p < .50$].

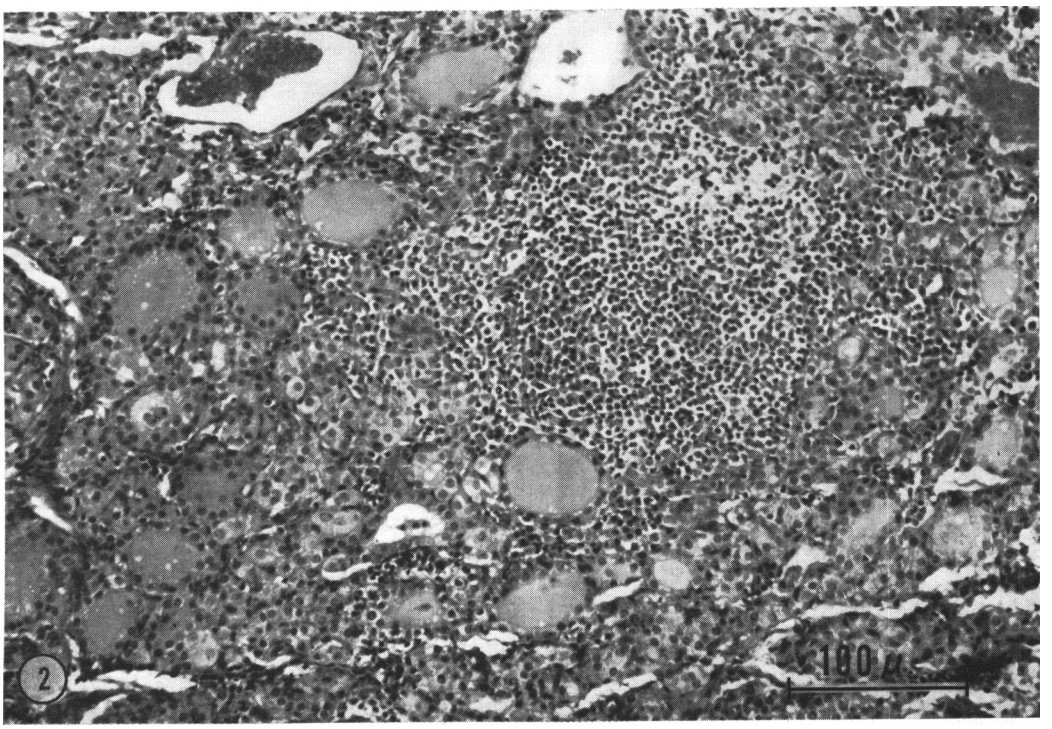

FIG. 2. Severe (3+) thyroiditis in another dog. Compared to Fig. 1, there are more normal-appearing follicles and fewer parafollicular cells.

similar to those described previously in other dogs in this colony with thyroiditis (5). In each 3+ case of thyroiditis there was lymphocyte infiltration, reduction in size of follicles and quantity of colloid, and increased numbers of parafollicular cells (Figs. 1 and 2).

Table I, which summarizes the incidence and severity of lesions in the thyroid gland of the 12 dogs studied, also presents the results of the thyroidal ^{131}I uptake and retention studies; these indicate that the thyroid glands of dogs with 3+ thyroiditis took up only about ⅓ as much of the test dose of ^{131}I as did the glands of normal dogs. After the maximum uptake, thyroid glands with 3+ thyroiditis lost ^{131}I 3–4 times faster than normal glands. There were no significant differences in the ^{131}I metabolism of dogs with normal glands and those with mild (1+) thyroiditis; the rate at which the thyroid glands lost ^{131}I was correlated with the presence or absence of severe (3+) thyroiditis in each dog (Fig. 3). Also, as shown in Fig. 3, the time of maximum uptake also correlated with the presence or absence of thyroiditis; in dogs with severe thyroiditis maximal thyroidal uptake occurred earlier. The BT ½ of ^{131}I in the thyroid gland of dogs with severe (3+) thyroiditis was significantly shorter than in dogs with minimal (1+) or no lesions [mean ± SE for dogs with 3+ thyroiditis = 3.06 ± .52; dogs with normal glands and 1+ thyroiditis = 12.0 ± 1.20; $t_{(11\ DF)} = 7.65$; $p < .005$] when examined by Student's t test.

The BT ½ of ^{131}I in the thyroid gland, 14 months after the first lobe had been removed, was reduced below the earlier values in all six dogs, both in those with severe thyroiditis and those with mild cases or normal glands (Table I). The BT ½ in dogs with severe thyroiditis, however, remained significantly shorter than those with normal or nearly normal glands.

The second lobe removed from dog No. 1129 was five times heavier than the first. The one lobe removed from dog No. 0495

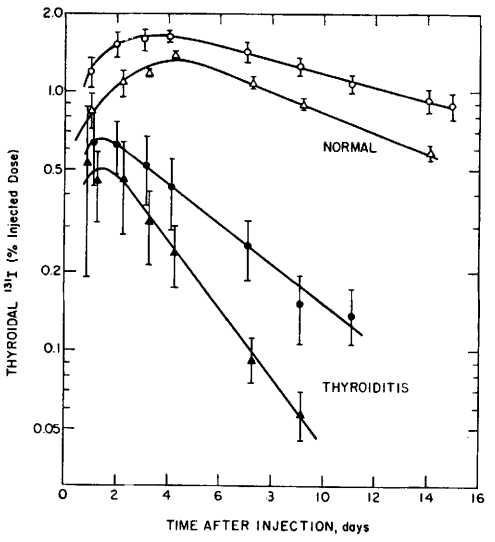

FIG. 3. Comparison of the uptake and subsequent rates of loss of tracer doses of ^{131}I in thyroid glands of four groups of dogs. The mean and its associated standard error are indicated for each measurement. Beyond the point of maximum uptake, the lines were drawn according to least-square fits of the data. ○ = dogs with normal glands or mild (1+) thyroiditis (data from five dogs); △ = dogs with normal glands or mild (1+) thyroiditis 14 months after removal of one lobe (data from three of five ○ dogs above); ● = dogs with severe (3+) thyroiditis (data from six dogs); ▲ = dogs with severe (3+) thyroiditis 14 months after removal of one lobe (data from three of six ● dogs above).

was nearly twice the mean weight of the two thyroids from beagles in this colony.[5] The bulk of the thyroid from each of these dogs consisted of epithelial cells.

Analyses of the proportions of epithelium, and colloid in each thyroid gland are presented in Table II. The percentage of colloid in the thyroid gland in dogs with 3+ thyroiditis was significantly smaller than the other dogs (19.9% ± SE = 6.8% vs 66.8% ± SE = 6.0%; $t_{(11\ DF)} = 4.37$; $p < .005$). Similarly, dogs with 3+ thyroiditis had a significantly smaller amount of colloid, calculated in grams, than did the other dogs (.19 g ± SE = .07 g vs .62 g ± SE = .08 g; $t_{(11\ DF)} = 47.4$; $p < .005$). Although several glands were scored as 0% colloid, each gland had a few follicles. The true percentage was less than 1 but the test will not detect such small quantities. The proportion of colloid to epithelium was altered in the second gland as compared to the first gland removed 14 months earlier; in each dog, the percentage of colloid decreased, while the epithelial component increased. Dogs with unaffected glands and those with 1+ thyroiditis showed increases in the follicular epithelium (Figs. 4 and 5) while the others (those with 3+ thyroiditis) showed an increase in the parafollicular cells at the expense of both the follicular structures and the colloid (Figs. 6 and 7).

Discussion. Severe lymphocytic thyroiditis in the beagle is accompanied by a large reduction to almost complete absence of colloid (1–5). The mean size and weight of the gland is not significantly altered (5), and the loss of colloid is obviously compensated by

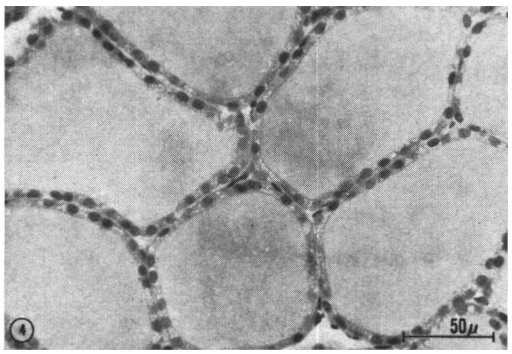

FIG. 4. First thyroid lobe removed from dog No. 1231. It is normal.

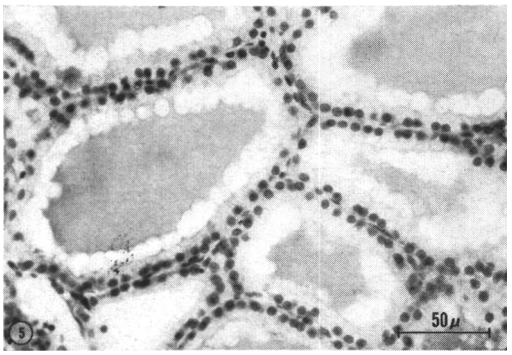

FIG. 5. Second thyroid lobe removed from dog No. 1231 14 months after the first lobe was removed. Compared to Fig. 4 there is hypertrophy and hyperplasia of the follicular epithelium.

[5] Mean ± SE for 17 dogs = 0.98 ± 0.07.

TABLE II. Comparison of Thyroid Structure and Thyroidal ^{131}I Metabolism.

	First ^{131}I tracer test (intact dogs)						Second ^{131}I tracer test (hemithyroidectomized dogs)					
		Thyroid composition						Thyroid composition				
		Epithelium		Colloid				Epithelium		Colloid		
Dog no.	Left thyroid weight (g)	%	g	%	g	BT ½[a] (days)	Right thyroid weight (g)	%	g	%	g	BT ½[a] (days)
0234	.20	83.97	.34	7.41	.03	—[b]						
1176	.24	94.68	.46	0.00	.00	2.0						
1084	.52	62.60	.65	28.50	.30	3.3	.49	72.40	.36	19.26	.10	2.8
1131	.52	78.74	.82	12.00	.12	3.5	.45	96.22	.44	0.00	.00	2.6
0306	.37	34.98	.26	59.50	.44	3.5						
1129	.46	74.02	.68	13.08	.12	4.2	2.56	91.62	2.34	0.00	.00	2.0
0495	1.80	83.45	3.00	6.68	.24	4.4						
Mean	.59±.21	73.21±7.38	.89±.36	18.17±7.65	.18±.06	3.1±.5	1.17±.70	86.75±7.35	1.50±.65	6.45±6.40	.08±.03	2.5±.2
0403	.43	41.22	.36	48.97	.42	7.4						
0215	.49	16.73	.16	76.02	.75	12.2	.37	55.59	.20	39.90	.15	6.8
0193	.49	11.83	.12	83.18	.81	12.8	.38	31.91	.12	61.43	.23	9.5
1231	.48	23.39	.22	72.15	.69	13.4	.45	35.28	.16	57.79	.26	9.8
1719	.37	38.91	.29	57.68	.43	14.2						
Mean	.45±.02	26.42±5.88	.23±.04	67.60±6.24	.62±.08	12.0±1.2	.39±.03	40.93±7.40	.16±.02	53.04±6.65	.21±.34	8.7±.95

[a] Biological half-time of ^{131}I in the thyroid gland.

[b] No measurable uptake; for statistical analysis the value was assumed to be 0.5.

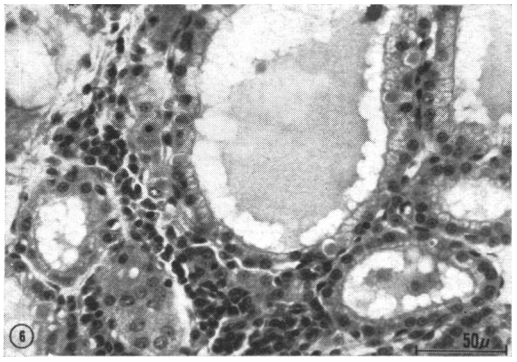

FIG. 6. First thyroid lobe removed from dog No. 1131. There is severe (3+) thyroiditis.

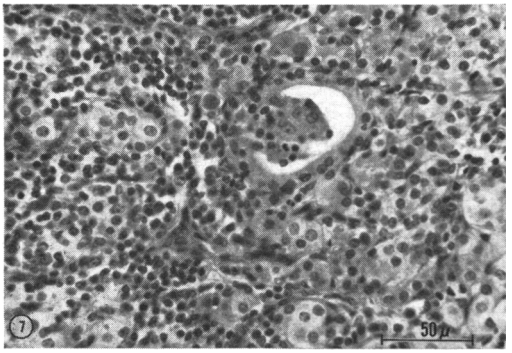

FIG. 7. Second thyroid lobe removed from dog No. 1131 14 months after the first lobe was removed. Compared to Fig. 6 there is loss of follicular structure and increase in numbers of parafollicular cells.

increases in the number of epithelial cells and the occurrence of inflammatory cells. Also, in severe thyroiditis the more numerous epithelial cells have the characteristics of parafollicular cells (1–5); residual follicular cells are morphologically distinct and arranged in colloid-containing follicles.

After the first tracer injection, the BT ½ of ^{131}I in the thyroid gland was diagnostic for severe (3+) thyroiditis with the animals dividing into two clearly distinct groups. One group contained seven dogs with a BT ½ between 0 and 4 days and severe (3+) thyroiditis, the other group contained five dogs with BT ½ between 7 and 14 days and had normal glands or mild (1+) thyroiditis. It first appeared that the BT ½ of ^{131}I might provide a critical quantitative measure of colloid content of the thyroid gland and thus a nondestructive method of estimating the severity of thyroiditis. The BT ½ does provide an index of the severity of lymphocytic thyroiditis as there is no overlap at all in results from dogs with 3+ involvement and those having 1+ thyroiditis and normal glands. Within these two groups, however, there is not a good correlation between grams of colloid and the BT ½ of ^{131}I. Unfortunately, no dogs had 2+ thyroiditis, in which moderate lymphocytic invasion and epithelium changes are characteristic. Data from dogs with 2+ lesions would allow a more conclusive interpretation as to the correlation between the BT ½ of ^{131}I in the thyroid gland and the severity of thyroiditis or the quantity of colloid. We believe that the BT ½ of ^{131}I in the dog is a measurement of the degree of microscopic pathology in the gland, *i.e.*, variations from the normal histologic pattern and, particularly, the loss or reduction in the amount of colloid. This interpretation is supported by results of our previous study (15) in which we found that the BT ½ of ^{131}I becomes extremely short in dogs fed a low-iodide diet for approximately 1 year. In response to this restricted iodide intake there was marked follicular hyperplasia and nearly complete loss of colloid. When the thyroid glands later accommodated to the diet and again stored colloid, the BT ½ of ^{131}I also reverted to a more normal value. In the present study, when a second ^{131}I tracer dose was given to 6 of 12 dogs 14 months after one of their thyroid lobes had been removed, the BT ½ values were parallel to those obtained with the first tracer dose, but in each case the second BT ½ for ^{131}I was shorter than the first. This difference was highly significant in the three dogs with mild (1+) thyroiditis or normal glands [$t_{(5\ DF)} = 4.03$; $p < .01$] but not as significant for the three dogs with severe (3+) thyroiditis [$t_{(5\ DF)} = 3.30$; $p < 0.5$] probably because their ^{131}I retention values were lower and the variance was larger.

The minimally adequate iodine requirement of the dog is poorly defined. The iodine content of commercial dog diets reflects recommendations (18) that may exceed the minimally adequate requirements by as much

as a factor of 10 (15). Excess dietary iodide intake has generally interfered with thyroid function tests, particularly when tracer doses of ^{131}I were used (1, 14). A correlation between lowered serum PBI values, subnormal thyroidal ^{131}I uptake, and severe thyroiditis has been demonstrated in dogs when they were fed a diet containing restricted amounts of iodide (1). We found that maximum uptake of ^{131}I in the thyroid is clearly less in dogs with 3+ thyroiditis than in the dogs with 1+ thyroiditis or normal glands as determined in both the first and second tracer tests [first tracer test: $t_{(5\ DF)} = 6.33$; $p < .005$; second tracer test: $t_{(5\ DF)} = 5.27$; $p < .005$]. Because the commercial diet regularly fed these dogs contains a large amount of iodine (15), all ^{131}I uptake values as a whole are very low. This generally reduced uptake, however, does not obscure the fact that the thyroids of dogs with severe (3+) thyroiditis have significantly lower ^{131}I uptakes.

Another significant aspect of this study is that thyroid glands with severe (3+) thyroiditis responded in a manner essentially similar to structurally normal glands; *i.e.*, removal of one lobe resulted in epithelial hyperplasia and a reduction in the BT ½ of ^{131}I. Glands with severe thyroiditis had increased numbers of parafollicular cells rather than hyperplasia and hypertrophy of the follicular epithelium. We conclude that the decrease in BT ½ of ^{131}I is related to decreased storage of iodine in the gland, and this decreased storage is expressed as a loss of colloid. The lower maximum ^{131}I uptake values in dogs with thyroiditis appears to be related to the large proportion of parafollicular cells characteristic of this disease. These cells do not seem capable of concentrating iodine. The large iodide content in the diet normally fed these dogs did not interfere with the diagnosis of histologically severe lymphocytic thyroiditis on the basis of the uptake, retention, and rate of loss of tracer doses of ^{131}I.

Summary. Tracer studies of thyroidal ^{131}I metabolism were conducted in beagles some of which had lymphocytic thyroiditis. The percentage of each thyroid lobe represented as epithelium, colloid, and stroma, as well as the severity of the lesions, was determined by microscopic examination. The high level of iodide in the commercial diet fed resulted in low thyroidal ^{131}I uptake values in all animals. Dogs with severe thyroiditis had biological half-times (BT ½) of ^{131}I significantly shorter than dogs with mild or no lesions. The maximum thyroidal uptake of ^{131}I was significantly lower and also occurred earlier in dogs with severe thyroiditis. Fourteen months after one thyroid lobe was removed the BT ½ of ^{131}I was shortened compared to values obtained in the intact dog.

There is little colloid in thyroid glands affected with severe lymphocytic thyroiditis; hemithyroidectomy also results in a reduction of the relative amount of colloid with a corresponding increase in the percentage of epithelium in the opposite lobe. Thus, the BT ½ of ^{131}I in the thyroid gland appears to be related to the amount of follicular colloid. Lymphocytic thyroiditis in the dog can be diagnosed by tracer studies with ^{131}I even when the dietary level of iodide is high.

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