PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **188,** 219–228 (1988)

Characterization of Canine Triiodothyronine (T_3) Autoantibodies and Their Effect on Total T_3 in Canine Serum (42731)

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Abstract. A study of 3,5,3'-L-triiodothyronine autoantibody (T_3 AA) in 18 dogs revealed an average apparent affinity constant for T_3 of $2.24 \pm 1.78 \times 10^{10}$ M^{-1}, an average T_3 binding capacity of 639.3 ± 666.5 ng/dl and a low thyroxine (T_4) cross-reactivity (<1%) in all samples tested. A valid radioimmunoassay (RIA) procedure which involved heat treatment of samples for 1 hr at 70°C and assay on Sephadex minicolumns was developed for measuring T_3 in the presence of T_3 AA. Total T_3 was elevated ($\bar{x} = 374.8 \pm 158.4$ ng/dl) in samples in which T_4 was in the normal canine range, but T_3 was lower ($\bar{x} = 96.1 \pm 63.3$ ng/dl) in samples with T_4 values in the hypothyroid range. For each sample the concentration of T_3 not bound by T_3 AA was calculated from the total T_3 concentration, the affinity constant, and the binding capacity. In dogs with normal total T_4 concentrations the average calculated T_3 not bound by T_3 AA was 147.2 ± 144.4 ng/dl while in dogs with low total T_4 the value was 15.7 ± 26.3 ng/dl (normal canine range is 45–150 ng/dl). Canine samples containing T_3 AA were compared to serum from three rabbits actively immunized against T_3 to provide anti-T_3 for commercial RIA. The rabbit T_3-antisera had an average T_3 affinity constant similar to those of the canine samples (1.57×10^{10} M^{-1}), but had average titer, T_3 binding capacity, and total T_3 values more than 10-fold higher. Our findings indicate that, in dogs with serum containing T_3 AA and normal total T_4 concentrations, a compensatory mechanism appears to exist to maintain non-T_3 AA bound T_3 within the range of normal total T_3. This compensatory mechanism does not operate in those dogs with insufficient thyroid activity to maintain normal total T_4 values. © 1988 Society for Experimental Biology and Medicine.

Autoimmune phenomena appear to be involved in many aspects of thyroid disease. Hyperthyroidism in Graves disease, due to thyroid stimulating autoantibodies specific to the TSH receptor, and hypothyrodism due to autoimmune destructive thyroiditis with associated antithyroid autoantibodies are examples (1). Autoimmune phenomena have also been reported in the dog where autoantibodies to thyroglobulin were found in 59% of hypothyroid dogs and autoantibodies to thyroid microsomes in 29% (2).

Spontaneous thyroid hormone autoantibodies have been reported in human patients, both clinically euthyroid (3–5) and hypothyroid (5, 6). These individuals are singled out since thyroid hormone autoantibodies interfere with RIA procedures for thyroid hormone measurement resulting in dramatically elevated apparent values using solid phase RIA methods (3) or lowered apparent values using charcoal separation methods (5). Interference in double antibody procedures depends on whether the labeled hormone bound to the autoantibody is precipitated in the RIA procedure (4). Elevated total T_3 levels have been reported in human patients with T_3 autoantibody (T_3 AA) (3, 5) and in rabbits (7) and rats (5) immunized against T_3 or T_4. In an attempt to determine whether total T_3 was elevated in canine samples containing T_3 AA, an RIA method was developed which accurately measured T_3 in these samples. Various authors, who have reported measuring T_3 in the presence of T_3 AA, either extracted serum or plasma samples in ethanol (3, 5), assayed samples in the presence of cold T_4 to saturate the autoantibody (8), or used an RIA involving Sephadex minicolumns (2, 9).

Abnormal canine T_3 binding factors were shown in a previous report to exhibit a high apparent affinity constant for T_3, a relatively high heat stability compared with that of human thyroxine binding globulin (TBG), a lack of inhibition of binding to T_3 by 8-anilino-1-napthalene sulfonic acid (ANS), and precipitability by goat anti-canine IgG (10). The purpose of the present study was to provide additional evidence that these T_3 bind-

0037-9727/88 $1.50
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ing factors are autoantibodies, to characterize T_3 AA in more detail, and to develop a procedure to accurately measure total T_3 in the presence of T_3 AA.

Materials and Methods. *Samples.* Canine serum samples (controls and samples containing T_3 AA) were obtained as samples submitted by veterinarians to the Endocrine Diagnostic Service of Auburn University, College of Veterinary Medicine for thyroid hormone analysis. Total T_3 and total T_4 measurements were requested for evaluation of the thyroid status of pet dogs. Samples yielding extremely high apparent total T_3 values, or inappropriately high total T_3 relative to total T_4, were screened for the presence of T_3 AA by determining the capacity of the serum to bind [^{125}I]T_3. Samples used in this study were taken from dogs which had not received thyroid medication.

Basic binding assay. Triiodothyronine binding was determined under a variety of conditions by variations on the basic binding assay. Samples (usually 100 μl) were incubated with 0.5 ml phosphate-buffered saline (PBS), pH 7.4, containing 0.1% gelatin (gel PBS), 100 μl [^{125}I]T_3 (Clinical Assays; Cambridge, MS) containing 0.025 μCi and 100 μl unlabeled thyroid hormone solution (where appropriate) at 37°C for 1 hr, then at 5°C for 2 hr. Bound and free fractions were separated by adding 0.5 ml of 1.25% dextran-coated charcoal in PBS (DCC), incubating at 5°C for 10 min, then centrifuging at 2000*g* for 10 min. The supernatant (bound fraction) was decanted and radioactivity determined using a 4/200 Automatic Gamma Counter (Micromedic Systems; Horsham, PA). Under these conditions undiluted sera from dogs without T_3 AA bound less than 10% of [^{125}I]T_3.

Second antibody precipitability. Canine sera were diluted to bind approximately 50% of [^{125}I]T_3 when 25 μl of diluted serum was assayed in the basic binding assay (total T_3 binding). In addition 25 μl of a series of 1:2 dilutions of three different "second antibodies" [goat anti-canine IgG (Cooper Biomedical; Malvern, PA), goat anti-canine IgM, and anti-canine IgA (Pel-Freeze Biologicals: Rogers, AR)] was added and the tubes incubated for 48 hr at 5°C. After the addition of 2 ml of saline containing polyethylene glycol the tubes were centrifuged at 2000*g* for 20 min. The supernatants were decanted and the pellets, representing precipitable T_3 binding, were counted.

Electrophoresis. Duplicate serum samples suspected of containing T_3 autoantibodies were applied to 2.5 × 15 cm Sepharose III cellulose polyacetate strips (Gelman Instrument Co; Ann Arbor, MI) and electrophoresed for 1 hr at 250 V in Tris barbital high resolution buffer, pH 8.6 (Helena Laboratories, Beaumont, TX), in a Deluxe electrophoresis chamber with regulated power supply (Gelman Instrument Co). One lane was stained in amino black and scanned with a Quick Scan Jr. densitometer (Helena Laboratories). The remaining lane was cut into 32-mm fractions and each fraction eluted in 0.7 ml gel PBS by shaking for 30 min. Each eluted fraction was assayed for T_3 binding using the basic binding assay.

Routine T_4 and T_3 RIA. Total T_4 was measured in canine serum samples with a double antibody Canine T_4 RIA kit procedure (Diagnostic Products Corp; Los Angeles, CA) validated for use in the dog (11). Apparent total T_3 was measured using the Diagnostic Products solid phase Canine T_3 method validated for canine samples (unpublished data).

T_3 affinity constant and T_4 cross-reactivity determination. Apparent T_3 affinity constants were determined as described in a previous report (10). Sera diluted to bind 50% of [^{125}I]T_3 in the basic binding assay were incubated in the presence of [^{125}I]T_3 and unlabeled T_3 at seven concentrations from 0 to 100 ng/dl. Scatchard analysis (12) of the resulting data was performed to determine the affinity constant and binding capacity in each sample.

T_4 cross-reactivity was determined using the ED_{50} in similar experiments except that [^{125}I]T_3 binding was measured in the presence of five concentrations of nonradioactive T_4 (0 to 1250 ng/ml) and 5 m*M* ANS to prevent binding of T_4 to TBG.

Autoantibody titer determination. Serial 1:2 dilutions of serum were carried out in gel PBS and the percentage binding was determined in the basic binding assay. Titer, the dilution factor that results in exactly 50% binding, was extrapolated from the data.

Ethanol extraction. Ethanol extraction of canine samples was performed at various ratios of ethanol/serum in an attempt to remove or inactivate T_3 AA and allow accurate RIA for total T_3. The effect of ethanol extraction on T_3 binding activity, and apparent total T_3 using the routine solid phase RIA, was determined in samples containing T_3 AA and in sera from normal dogs.

Radioimmunoassay of T_3 in the presence of T_3 autoantibody. RIA of total T_3 in the presence of T_3 AA was based on a modification of the method of Alexander and Jennings (9). Serum samples and T_3 standards prepared in charcoal stripped canine serum were diluted 3:5 in 100 μg/ml thermolysin protease (Sigma Chemical Co.; St. Louis, MO) in distilled water and heated at 70°C for 1 hr. Heat treated samples and standards were diluted 2:3 with [^{125}I]T_3 (0.025 μCi/100 μl) solution in 80 m*M* NaOH, and 0.3 ml of this solution was added in duplicate to small columns containing 1.5 ml of Sephadex G25–50 (Pharmacia, Uppsala, Sweden) equilibrated in 80 m*M* NaOH. After samples had penetrated the gel, the columns were washed with 2.0 ml of 75 m*M* sodium barbital buffer (pH 8.6) to remove proteins (including T_3 AA). T_3 antiserum (Wien Laboratories, Inc; Succasuma, NJ), diluted to result in 50% binding in the 0 standard columns, was added (0.5 ml) and allowed to incubate for 1 hr at room temperature. The antibody bound fraction was then eluted with 1.5 ml barbital buffer, and the radioactivity measured using a gamma counter. Total T_3 was calculated using a log–logit transformation. The columns were regenerated by rinsing with 4 ml of charcoal stripped canine sera diluted 1:20 in distilled water.

Results. A major portion of the abnormal T_3 binding activity in serum from five dogs was precipitable by goat anti-canine IgG, but was not precipitable with goat anti-canine IgA or goat anti-canine IgM (Table I). Additionally, the T_3 binding activity in these dogs was found to migrate with the β- and γ-globulin fractions on electrophoresis (Fig 1). In 2 of the dogs a fast migrating peak of T_3 binding activity was observed, but we were unable to determine the nature of this material.

A study of serum from 18 dogs with T_3 AA was performed to investigate the nature of the T_3 binding and its possible effect on thyroid physiology. Results of this study are presented in Table II. T_4 levels were clearly in the hypothyroid range in 11 out 18 of the dogs. The normal range for canine serum samples is 45–150 ng/dl for the routine total T_3 RIA and 1.5–4.0 μg/dl for total T_4 RIA. T_4 binding in the presence of 5 m*M* ANS (data not shown) was above normal in only one of the samples and there was no indication of interference in the T_4 RIA in any sample. Apparent total T_3 values as measured with the routine solid phase RIA were spuriously elevated and ranged from values which were only slightly above normal to values greater than 5000 ng/dl. T_3 AA titer ranged from 17.6 to 1,223.0 ($\bar{x}$ = 357.0). The T_3 apparent affinity constant averaged 2.24 $\times 10^{10}$ M^{-1} indicating a high affinity for T_3. Cross-reactivity with T_4 was <1% in all 18 samples examined.

TABLE I. SECOND ANTIBODY PRECIPITABLE [^{125}I] T_3 AS PERCENTAGE OF TOTAL [^{125}I] T_3 BOUND IN CANINE SERUM CONTAINING T_3 AA

Dog	Anti-canine IgG	Anti-canine IgA	Anti-canine IgM
1	69.6	0.4	0
2	92.3	0.3	0
3	116.0	0	0
4	65.8	0.1	0
5	84.8	0	0.5

Efforts to accurately measure total T_3 in canine samples containing T_3 AA by extracting in ethanol were not successful. Ethanol extraction, while efficiently extracting [^{125}I]T_3, resulted in less efficient and highly variable results when comparing assayed values of total T_3 in canine samples without T_3 AA before and after extraction. Hehrmann *et al.* (5) using extraction in 1 vol 95% ethanol measured total T_3 in serum from rats immunized to T_3. In canine samples containing T_3 AA extracted in an identical manner, the binding of [^{125}I]T_3 was reduced to 40% of the initial value. This level of binding would cause falsely elevated values using the routine total T_3 RIA.

Although heat treatment of T_3 AA at 60°C was totally ineffective in altering binding activity, treatment of samples from five dogs

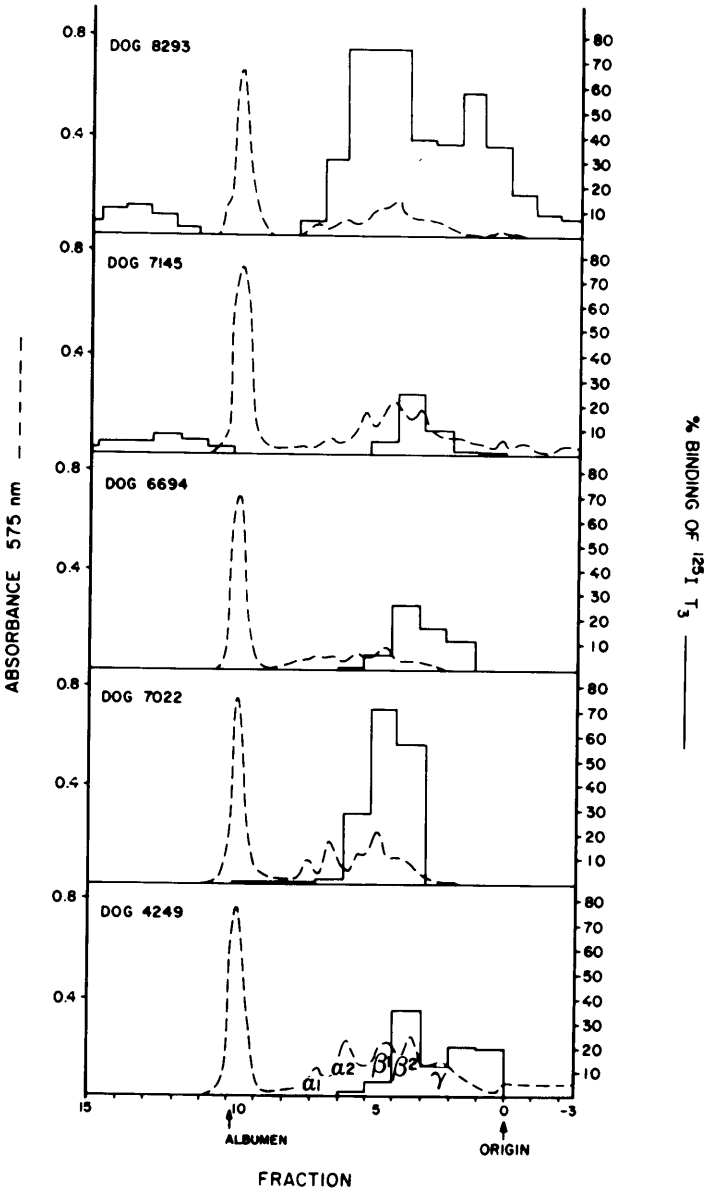

FIG. 1. Electrophoresis pattern of serum from five dogs with T_3 AA.

(diluted to bind 50% of [^{125}I]T_3 in the basic binding assay) at 70°C for 1 hr essentially eliminated T_3 AA binding (Fig. 2). Heat inactivation of samples followed by RIA for total T_3 using the routine solid phase RIA proved impractical since the heat treatment itself in canine samples not containing T_3 AA interfered with the assay resulting in elevated values (Table III).

The Sephadex total T_3 RIA method (9), designed to remove interfering proteins after release of T_3 at alkaline pH, in combination with treatment of samples diluted in thermolysin at 70°C for 1 hr, was validated for measurement of total T_3 in canine samples containing T_3 AA. Sample dilution was minimized due to limitations of assay sensitivity, and temperature of treatment was mini-

TABLE II. THYROID HORMONE CONCENTRATIONS AND THYROID HORMONE BINDING CHARACTERISTICS OF SERUM FROM DOGS WITH T_3 AA

Dog	Total T_4 (μg/dl)	Apparent T_3[a] (ng/dl)	Total T_3[b] (ng/dl)	T_3 AA titer	T_3 Affinity constant ($\times 10^{10}\ M^{-1}$)	Binding capacity (ng/dl)	Non-T_3 AA bound (T_3 ng/dl)
Normal T_4							
1	1.6	1039.6	353.0	808.4	5.79	284.7	71.2
2	1.6	272.5	185.2	148.1	0.63	334.3	11.6
3	1.9	205.1	566.8	74.3	3.98	82.8	424.3
4	1.9	415.3	568.7	256.0	1.88	388.5	192.5
5	1.8	231.9	449.4	65.3	0.99	321.7	142.3
6	2.2	584.6	211.3	49.2	0.31	1560.0	3.4
7	2.0	509.4	289.1	61.9	4.12	104.5	185.2
$x \pm$ SD	1.86 ± 0.215	465.5 ± 290.9	374.8 ± 158.4	209.0 ± 274.2	2.53 ± 2.11	396.3 ± 507.7	147.2 ± 144.4
Low T_4							
A	0.6	>600[c]	52.4	1223.0	1.87	2376.9	0.072
B	0.3	6158.0	104.2	>1000[c]	1.75	1352.7	0.326
C	0.3	891.0	54.0	412.4	0.55	1530.4	0.451
D	0.3	2646.5	85.2	948.8	6.18	714.8	0.250
E	0.3	1666.5	176.3	431.1	1.09	1096.7	1.17
F	0.7	1368.4	229.6	475.1	3.39	163.3	70.7
G	0.5	152.0	97.2	17.6	1.54	47.3	53.5
H	0.8	427.3	46.7	82.3	2.61	379.6	0.36
I	0.2	199.0	—	148.4	1.65	209.4	—
J	0.3	617.3	93.3	191.3	0.20	504.8	28.6
K	0.2	226.1	22.2	32.0	1.87	55.3	2.03
$x \pm$ SD	0.41 ± 0.21	1359.3 ± 1763.0	96.1 ± 63.3	451.1 ± 425.0	2.06 ± 1.62	766.5 ± 744.8	15.7 ± 26.3

[a] Routine solid phase RIA.
[b] Modified Sephadex RIA.
[c] For statistical purposes, these values were taken as exact.

mized to prevent coagulation of samples. Thermolysin (100 μg/ml) did not enhance inactivation of T_3 AA during the heat treatment, but did prevent coagulation of samples. The modified Sephadex total T_3 method was less sensitive to the 70°C heat treatment and to the presence of thermolysin than the solid phase RIA (Table III).

Elevated T_3 binding, which could cause interference in the modified Sephadex method, would appear as increased [^{125}I]T_3 in the initial 2 ml rinse prior to incubation with antibody and in the 1.5 ml rinse after incubation. The results shown in Table IV indicate that the 70°C heat treatment eliminates both indications of elevated nonspecific binding when canine samples containing T_3 AA are assayed for total T_3.

Triiodothyronine added to canine serum containing T_3 AA was recovered with greater efficiency in 70°C heat treated samples by the Sephadex method than with the standard solid phase RIA (Table V). Serum containing T_3 AA was diluted in charcoal stripped canine serum and the samples were analyzed by the modified Sephadex method. The standard and sample inhibition curves are depicted in Fig. 3. Recoveries of T_3 at dilutions of 1:2, 1:3, and 1:4 were 88.5%, 129.9%, and 98.7%, respectively.

In order to substantiate that the modified Sephadex RIA method provided accurate results, samples from normal and hypothyroid dogs (not containing T_3 AA) and from hyperthyroid cats (a source of endogenously elevated T_3) were assayed using both the solid phase and Sephadex methods (Fig. 4). There was good correlation between the assay methods in samples not containing T_3 AA.

Analysis of sera from 18 dogs with T_3 AA indicated no significant difference ($P > 0.1$) in apparent total T_3 (solid phase RIA), autoantibody titer, T_4 cross-reactivity, and binding capacity between dogs with normal total T_4 values and those with low total T_4 (Table II). The total T_3 determined by the

TABLE III. EFFECT OF 70°C HEAT TREATMENT IN THE PRESENCE AND ABSENCE OF THERMOLYSIN ON ASSAYED TOTAL T_3 VALUES IN A CANINE SERUM POOL NOT CONTAINING T_3 AUTOANTIBODY

			Modified Sephadex RIA		Solid phase RIA	
	TL	70°C treatment	Corrected total T_3 ng/dl	% recovery	Corrected total T_3 ng/dl	% recovery
Control I	−	−	202.8	95.3	122.0	67.7
Control II	−	+	226.4	106.3	143.0	79.4
Control III	+	−	231.2	108.5	107.8	59.8
Treatment	+	+	188.2	88.4	267.6	148.5
Undiluted pool	−	−	212.9	—	180.2	—

modified Sephadex method and the calculated total T_3 not bound by T_3 AA were significantly higher in the dogs with normal T_4 levels ($P < 0.001$ and $P < 0.05$, respectively).

In addition, samples from three rabbits actively immunized to T_3 (Wein Laboratories; Succasunna, NJ) were assayed by the Sephadex method. Rabbit samples diluted 1:1000 were analyzed for T_3 binding and total T_3 (Table VI). T_3 antibody in the rabbit antisera was not inactivated by heating at 70°C for 1 hr, but was inactivated by a 10-min treatment at 100°C, therefore rabbit samples were given the latter treatment prior to RIA. Although the modified Sephadex method has not been validated for use in anti-T_3 rabbit sera, values obtained for total T_3 (using heat treatment at 100°C) were in agreement with those of Nieschlag *et al.* (7). In the rabbit samples, total T_3 levels were profoundly elevated (63,013 ± 102,703 ng/dl). The T_3 AA titer was much higher (40,363 ± 29,136) than in spontaneous canine T_3 AA samples (357.0 ± 384.1) while the average T_3 affinity constant ($1.57 \pm .42 \times 10^{10}$ M^{-1}) was lower than in the canine samples which contained T_3 AA.

Since a large volume of serum was available from a dog with T_3 AA of high T_3 affin-

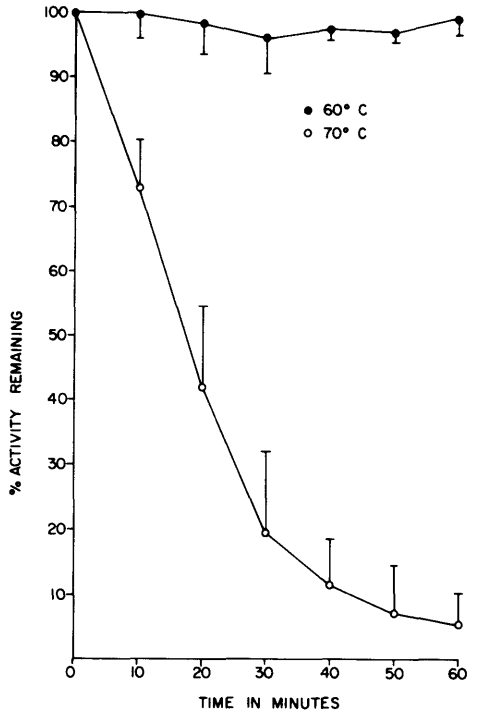

FIG. 2. Effect of heat treatment on inactivation of T_3 AA in serum from five dogs.

TABLE IV. EFFECT OF 70°C HEAT TREATMENT IN THE PRESENCE OF THERMOLYSIN ON NONSPECIFIC BINDING IN THE MODIFIED SEPHADEX RIA USING CANINE SAMPLES WITH AND WITHOUT T_3 AA

		cpm as % of total count	
Treatment	Sample	2.0 ml rinse prior to antibody addition	1.5 ml rinse after antibody addition
Control	0 standard	4.0	0.2
	Canine serum[a]	5.1	0.4
	T_3 AA sample	21.4	1.5
70°C	0 standard	4.3	0.2
	Canine serum[a]	4.4	0.9
	T_3 AA sample	4.8	0.6

[a] Values are mean of four canine samples not containing T_3 AA.

TABLE V. TOTAL T_3 ASSAYED VALUES AND PERCENTAGE RECOVERY OF ADDED T_3 IN A CANINE SERUM SAMPLE CONTAINING T_3 AA HEAT TREATED AT 70°C FOR 1 hr IN THE PRESENCE OF THERMOLYSIN

	Modified sephadex RIA		Solid phase RIA		
T_3 added (ng/dl)	T_3 observed (ng/dl)	% recovery	T_3 observed (ng/dl)	% recovery	T_3 observed (ng/dl) control[a]
0	34.0	—	31.7	31.7	1022.6
25	51.8	87.8	38.8	68.4	1483.3
50	90.3	107.5	42.8	52.4	1363.8
100	113.2	84.5	48.2	36.6	1466.7
200	179.3	76.6	58.2	28.1	1527.2
400	361.9	83.4	134.7	28.7	1474.0
800	724.4	86.9	238.5	28.7	1304.9

[a] Not heat treated.

ity (dog D, Table II), a high sensitivity T_3 RIA using canine serum containing T_3 AA as the source of antibody was designed. This RIA utilizing "spontaneous anti-T_3 antibody" and based on the basic binding assay had a sensitivity of 0.08 ng/dl (based on the apparent T_3 concentration at 2 standard deviations below the 0 bound count), and an ED_{50} of 1.71 ng/dl. This T_3 RIA was more than 10 times as sensitive as most conventional T_3 RIA procedures.

In dogs with T_3 AA there was a clear correlation between the total T_4 value and clinical signs reported. Dogs with total T_4 values in the normal range exhibited few symptoms typical of hypothyroidism (13), while dogs with low total T_4 values all exhibited one or more of these symptoms (Table VII). Due to limited access to the dogs we were able to confirm hypothyroidism by TSH response test (14) in only two of the dogs (dogs D and E, Table II).

Discussion. Abnormal T_3 binding factors in canine serum samples submitted for routine clinical thyroid hormone analysis have

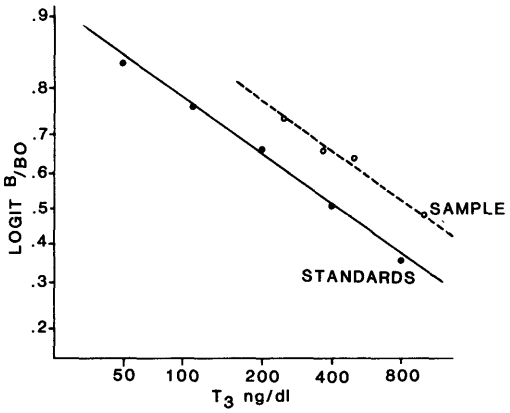

FIG. 3. Inhibition curves for standards and dilutions of a sample containing T_3 AA using the modified Sephadex total T_3 RIA.

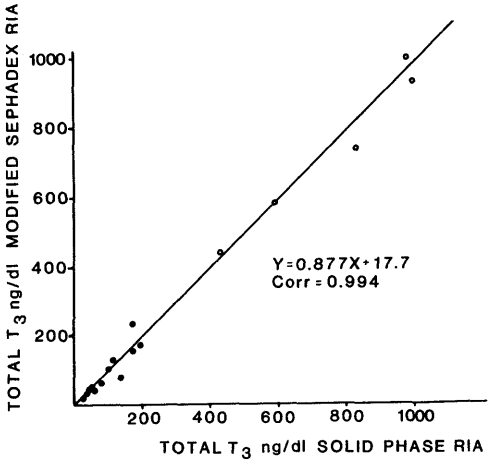

FIG. 4. Total T_3 concentration in samples assayed by a solid phase T_3 RIA and the modified Sephadex RIA. (Canine samples, not containing T_3 AA—closed circles; feline samples—open circles).

TABLE VI. CHARACTERISTICS OF T_3 ANTISERUM FROM THREE RABBITS IMMUNIZED TO T_3

Rabbit	Titer	T3 binding capacity (ng/dl)	T3 affinity constant ($\times 10^{10}$ M^{-1})	Total T_3 (ng/dl)
1	73,720	627,500	1.20	181,600
2	27,480	64,000	2.03	46,600
3	19,890	141,800	1.48	27,900
$x \pm$ SD	40,363	277,767	1.57	85,333
	±29,136	305,366	±0.42	±83,886

been identified as T_3 autoantibody (T_3 AA). Autoantibody in these samples was precipitable by goat anti-canine immunoglobulin G (IgG) and co-migrated with canine IgG during electrophoresis. T_3 AA appears to be present in approximately 0.2% of canine serum samples submitted for thyroid hormone analysis (10). Interestingly, T_3 AA occurred in the same frequency in a human clinical endocrinology laboratory (5). The occurrence of T_3 AA, unlike autoantibodies to thyroglobulin or thyroid microsomes, was rare even in hypothyroid dogs.

The current study was performed on samples from 18 dogs with T_3 AA to characterize these antibodies and measure total T_3 in their presence. The average affinity constant of 2.24×10^{10} M^{-1} was in the same range as T_3 antisera produced in rabbits for use in T_3 RIA. The T_4 cross-reactivity was less than 1% in all 18 dogs, and only one sample had T_4 binding greater than normal values.

The low T_4 cross-reactivity brings into question the nature of the antigens that stimulated the production of T_3 AA. The antibodies may have been initially directed at some portion of the T_3 receptor. Then by an anti-idiotype mechanism as described by Bottazzo (1), antibodies specific to T_3 could result. Indeed, this possibility is supported by a recent study (15) in which purified rat liver nuclear thyroid hormone receptor (THR) was used to raise antibody to THR in mice.

Another possible antigen might be T_3-enriched thyroglobulin. Data in dogs suggested that thyroid hormone secretion involved the uptake and hydrolysis of thyroglobulin molecules containing a higher ratio of T_3/T_4 than the average population of thyroglobulin molecules in the colloid (16). However, no specific studies have been performed to address this question.

The fact that T_3 AA was found both in dogs with low total T_4 values and clinical signs of hypothyroidism and in dogs with normal total T_4 values and few symptoms of hypothyroidism may indicate that the latter group represents dogs in the early stages of thyroid gland degeneration. We have not followed the progress in individual dogs with T_3 AA to confirm such speculation.

Euthyroid human patients with T_3 AA generally have elevated total T_3 levels based on ethanol extracted serum, and have T_3 AA with low T_4 cross-reactivity (3–5). Patients with autoantibodies that predominatly bind T_4 tend to be hypothyroid (6, 8, 17, 18). A case of "peripheral resistance," however, was reported in a human patient in which a hypothyroid state developed apparently due to the presence of an autoantibody that bound T_3 predominantly. The thyroid gland was normal in terms of [^{131}I] uptake and biopsy

TABLE VII. CLINICAL SIGNS OF DOGS WITH T_3 AA

	Hypothyroid symptoms*		
	Alopecia	Obesity	Lethargy
Normal T_4 group			
1	–	+	–
2	–	–	–
3	–	–	–
4	–	–	–
5	–	–	–
6	–	–	–
7	–	–	–
Low T_4 group			
A	+	+	+
B	–	+	–
C	+	–	–
D	+	–	–
E	+	+	–
F	+	–	–
G	+	+	–
H	+	–	–
I	+	+	+
J	–	+	–
K	+	–	–

* These clinical signs were reported by veterinarians submitting samples to the Endocrine Diagnostic Laboratory.

findings. A rapid increase in autoantibody titer without concurrent elevation in thyroid hormones was thought to have led to hypothyroidism (19).

Results of total T_3 measurement by the modified Sephadex method indicated that T_3 levels in dogs with T_3 AA were elevated in the samples where total T_4 was in the normal range. Total T_3 values were not elevated above the normal range in samples with low total T_4 (Table II). Total T_3 in samples from three rabbits actively immunized against T_3 was more than 100 × higher than in the canine samples. However, considering the higher titer and binding capacity in the rabbit samples, the situation in the canine samples with normal T_4 levels may be analogous, in that free T_3 concentrations in both situations may be in the normal range.

The concentration of T_3 not bound by T_3 AA can theoretically be calculated from the mass action equation when the total T_3, T_3 binding capacity, and T_3 affinity constant are known. That such a calculation is physiologically meaningful was suggested by the fact that in human patients, the free T_3 calculated from the total T_3 and T_3 uptake is highly correlated with free T_3 as measured by equilibrium dialysis (20).

In the dogs with normal T_4 levels, the estimated concentration of T_3 not bound to T_3 AA is in the normal canine range for total T_3. This suggests that a compensatory increase in total T_3 had overcome the binding by T_3 AA resulting in a normal effective total T_3.

It is interesting that total T_3 levels are elevated to a greater extent than total T_4 in animals immunized to T_3 or T_4. Rabbits immunized to T_3 had a 94.0-fold increase in total T_3 and 1.3-fold increase in total T_4; T_4 immunization resulted in a 11.0-fold increase in total T_3 and a 2.1-fold increase in T_4 (7). Similar results were obtained in rats (5). These results indicate a similar thyroid compensatory process may occur in dogs with T_3 AA that have thyroid gland activity capable of maintaining normal total T_4 levels. This suggests that a mechanism was present to maintain a constant free T_3 blood level. In the samples from dogs with T_3 AA in which T_4 concentrations are below normal, estimated levels of available T_3 (T_3 not bound to T_3 AA) are so low that the presence of T_3 AA may have a significant deleterious effect on thyroid status and may cause an exaggeration of clinical hypothyroid symptoms, as well as provide apparently elevated T_3 values in routine total T_3 assays.

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Received September 17, 1987. P.S.E.B.M. 1988, Vol. 188.
Accepted March 2, 1988.