IODINE STATUS AND THYROID ACTIVITY OF WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS BOREALIS)

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This is to certify that the

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IODINE STATUS AND THYROID ACTIVITY OF WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS BOREALIS)

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Bruce Ellsworth Watkins

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Duane E. Ullrey
Major professor

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IODINE STATUS AND THYROID ACTIVITY OF WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS BOREALIS*)

By

Bruce Ellsworth Watkins

AN ABSTRACT OF

A DISSERTATION

Submitted to
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ABSTRACT

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The effects of dietary iodine (I) level, moderate feed restriction and starvation on thyroid activity in captive white-tailed deer (Odocoileus virginianus borealis) were studied in 3 experiments. In addition, thyroid activity in 3 wild deer and I concentration of several important deer foods in Michigan were examined. Providing 6 does per group with either +0, +0.2 or +0.7 ppm supplemental I did not significantly affect growth, serum total thyroxine (T4) or free thyroxine (FT4), reproductive performance, serum T4 and triiodothyronine (T3) levels of offspring, milk I concentration, or weight and I concentration of the thyroid gland. Serum T3 differed between groups during 2 of the 14 sampling periods, but a treatment effect did not appear to be involved. These data indicate 0.26 ppm I in a dry diet consumed ad libitum is sufficient for normal reproduction and lactation in white-tailed does. During the 2 year study serum T4 and FT4 followed a consistent circannual pattern with high levels occurring during early winter and spring, and low levels occurring during late winter, summer and fall. Changes in serum T4 and FT4 may have been related

to cyclic changes in body weight, feed consumption, and ambient temperature. Serum T3 did not follow a consistent seasonal pattern. Serum T3 and T4 were significantly higher in fawns than in adults, and in nonlactating versus lactating does. In another experiment, approximately 50% feed restriction (FR) for 4 months did not affect serum T3, T4 and FT4 or thyroid I concentration of 13 weaned fawns as compared to 6 weaned fawns fed ad libitum. Weight gain and thyroid weights were reduced in the FR deer, however. Supplementing the diet (0.28 ppm I) of FR fawns with +0.7 ppm I had no effect on thyroid parameters. Starving 9 to 10 month old fawns for 16 to 20 days resulted in dramatic declines in serum T3, T4 and, to a lesser extent, FT4; serum reverse T3 (rT3) did not change appreciably. Fractional turnover rate, distribution volume and metabolic clearance rate of ^{131}I-T4 did not differ between fed (N=3) and starved (N=2) fawns. T4 secretion rate, however, was greatly reduced. Thyroid ^{131}I and ^{127}I concentrations tended to be lowest and highest, respectively, in the starved versus the fed fawns. Wild adult does collected during February and March from northern lower Michigan weighed less, had much less I in their thyroids, had larger thyroids per $BW_{kg}^{0.75}$, and showed considerably lower serum T4, FT4 and T3 levels than adult captive does fed a nutritious diet. The combined effects of malnutrition and an incipient I deficiency were believed to be the etiology of the thyroid profile observed in the wild deer.

Twigs of browse species were typically low in I

(13-338 ppb); leaves (30-481 ppb), terrestrial herbaceous species (17-836 ppb) and aquatic species (85-3,100 ppb) tended to be higher. Most plants were highest in I during winter and spring and lowest during summer. A method for the determination of I in plants based on the I catalyzed reduction of Ce IV is described.

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Iodine Status and Thyroid Activity of White-tailed Deer in Michigan.

INTRODUCTION

Thyroid hormones, thyroxine (T4) and triiodothyronine (T3), are known to affect a variety of physiological processes including lipid, carbohydrate and nitrogen metabolism, calorigenesis, growth and development, nervous system function and reproductive performance (Myant 1964, Bernal and Refetoff 1977, Underwood 1977). Thyroid activity in white-tailed deer has been studied to better understand the ways in which deer respond to different nutritional and environmental conditions (Hoffman and Robinson 1966, Seal et al. 1972, Byrne et al. 1974, Bahnak 1978, Bubenik and Bubenik 1978, Seal et al. 1978b). It is well established that white-tailed deer undergo cyclic changes in food consumption, body weight and body composition (Holter et al. 1977, Moen 1978). The relationship between thyroid activity and deer physiology, including changes in metabolic rate, nutritional status, catabolic and anabolic states, age, and reproductive phenology is still not fully clear.

Among the endocrine glands the thyroid is unique in that its function is dependent upon a specific

trace element — iodine (I). Insufficient hormone production resulting from I deficiency can reduce survivability and impair reproductive performance. The Great Lakes region is known to be a naturally I deficient area (Eldridge 1924) with a history of endemic goiter in humans (Olin 1924, McClendon 1939) and domestic animals (Unknown 1923, Bell 1931, McCollum 1957) before I supplementation began in the 1920's. To what degree I may be limiting in wild animals is not known. This study examined thyroid activity in captive deer fed different levels of I and in a small sample of wild deer.

METHODS

Part 1.— Eighteen female fawns approximately 6 to 7 months of age were allotted into 3 groups. Beginning December 8, 1977 all animals were given a complete pelleted diet (Table 1) which differed between groups only in the level of supplemental I: +0, +0.2 and +0.7 mg I per kg diet, as pentacalcium orthoperiodate (Morton Salt Co., Chicago IL). These supplemental levels were chosen as they represented recommended I requirements for growth and for reproduction, respectively, in domestic ruminants. I concentrations of different batches of the diets, as analyzed (Section V), are shown in Table 2. In order to reduce the basal I level, the diet was reformulated in May 1978 to exclude cane molasses and a commercial mold inhibitor. The high I concentration of the unsupplemented diet from July-November 1979 was believed to have resulted from an error in feed formulation.

All deer were kept outdoors in pens with dirt floors and shelters at the Michigan Department of Natural Resources'

Table 1. Composition of the basal diet used for iodine studies with white-tailed deer.

Ingredient	Percent
Corn cob product[a]	35
Soybean meal (44% crude protein)	24.5
Shelled corn	18 (23)[b]
Wheat middlings	10
Alfalfa meal (17% crude protein)	5
Cane molasses[c]	5
Soybean oil	1
Limestone (38% calcium)	0.5
Trace mineral salt[d]	0.5
Vitamin A, D, E and selenium premix[e]	0.32
Mold check[c]	0.2
Calcium propionate	(0.2)[b]

[a]Bracts and pith (soft parenchyma without vascular bundles).

[b]Values in parentheses are for the reformulated diet used after May 1978.

[c]Excluded from the reformulated diet

[d]Contained 94.6% noniodized NaCl, 1% ZnO, 0.6% MnO, 2.5% $FeSO_4.7H_2O$, 0.2% $CuSO_4.5H_2O$, 0.044% $CoCO_3$, and 1% corn oil.

[e]Supplied 3300 IU vitamin A, 220 IU vitamin D, 88 IU vitamin E, and 0.2 mg Se (as sodium selenite) per kg of diet.

Table 2. Iodine concentration of different batches of the diet as analyzed (ppm, dry basis).

Initial feeding date	Supplemental iodine		
	+ 0	+ 0.2 ppm	+ 0.7 ppm
December 1977[a]	0.43	0.65	0.82
May 1978	0.29	0.45	0.74
November 1978	0.26	0.49	0.83
July 1979	0.61	0.49	0.93
November 1979	0.28	0.40	0.88

[a]Diet before reformulation (see Table 1).

Wildlife Research Station at Houghton Lake, MI. Two or 3 animals were kept in each pen except during fawning season when each doe was penned individually. Food and water were offered ad libitum; food consumption per pen was recorded daily. Animals were maintained on their respective diets for over 2 years. In the fall of 1978 each doe was mated and their offspring were also included in the study until weaning on September 9, 1979. After weaning the fawns were placed on a feed restriction experiment reported in Section II. In January 1980 all deer were killed by injection with succinyl choline (Sucostrin^{R}, E.R. Squibb and Sons Inc., Princeton, NJ). Thyroids were immediately removed, trimmed of fat, weighed and frozen.

Blood samples and weights were taken at approximately 6 to 8 week intervals throughout the study. Between 9:00 AM and 1:00 PM animals were weighed, run into holding crates, drugged with a combination of ketamine (Vetalar^{R}, Parke, Davis and Co., Detroit, MI) and xylazine (Rompun^{R}, Haver-Lockhart, Shawnee, KS) and bled by jugular puncture. Blood samples were allowed to clot overnight at 5 C before centrifugation (1,000 g) and collection of serum. Serum samples were frozen and stored at -20 C until analyzed.

Hormone analyses were performed after almost all samples had been collected and were randomized across animals, treatments and times. Total T4 (T4) and free T4 (FT4) were determined using a fixed-antibody radioimmunoassay (RIA) (Mean T4 recovery = 95.9 $\pm$ 7.7%, inter-assay CV = 1.5%, intra-assay CV = 1.5%; Gammacoat^{R}, Clinical Assays, Cambridge,

MA). Due to the large number of samples FT4 analysis was performed only for selected times. Total T3 (T3) was determined by the method of Chopra et al. (1972) (Mean T3 recovery = 91.2 ± 5.9%, inter-assay CV = 12.7%, intra-assay CV = 5.6%). Reverse T3 (rT3) analysis was performed by RIA utilizing polyethylene glycol to precipitate the bound fraction (Serono Lab., Braintree, MA).

Milk samples were collected from lactating does following injection with oxytocin. Milk I was analyzed by a procedure similar to that reported in Section V. Dry thyroid weights were determined after freeze-drying for 48 hours. Thyroid I was determined by neutron activation analysis (TRIGA^{R} System, General Atomic, San Diego, CA). Thyroids and KI standards were irradiated in heat-sealed polyethylene vials for 10 minutes at a neutron flux of 10^{12} neutrons per cm^2 per second. Resulting ^{128}I activity was counted in standards and in thyroids using a Ge(Li) detector (Series 80 Multichannel Analyzer, Canberra, Meriden, CN).

Part II.— During March 1979, 3 wild adult does which had been feeding on northern white cedar (Thuja occidentalis) cuttings were trapped in the Houghton Lake area. The collection area was representative of lowland winter range typically used by deer in northern, lower Michigan. Ages, as determined by dentition (Ryel et al. 1961), were 1.7, 1.7 and 8+ years. Blood samples were taken after immobilization with ketamine-xylazine and thyroids were removed after sacrifice as described previously. In addition, the thyroid was taken from a 4-year-old wild doe which had been road killed

in the same area in March 1980.

Statistical Analysis.— Hormone data from the captive does were divided into 2 parts (December 1977 to November 1978, juveniles; November 1978 to January 1980, adults) and analyzed as split-plots in time (Gill 1978). It was considered desirable to divide the data in this fashion due to the different physiological condition of the animals after breeding. When analysis of variance indicated significant time effects but no treatment (i.e., diet) effects or time x treatment interaction, data were pooled across treatments and Tukey's test was used for comparison of time means with significance considered as $P<0.05$ (Steel and Torrie 1960). When treatment effects or interaction were significant, each treatment was analyzed separately for time effects, and one-way analysis of variance (AOV) was used to determine treatment effects within times (Gill 1978).

Weights, thyroid gland and milk I data were analyzed by one-way AOV. Reproductive performance and fawn survivability were analyzed by chi-square analysis (Steel and Torrie 1960). Wild versus captive deer data, lactating versus nonlactating deer data and juvenile versus adult data were analyzed using an unpaired t-test (Steel and Torris 1960). Data for juvenile deer (see Section II) were pooled across treatments and sex for comparison with adult does if AOV indicated these effects were not significant. A paired t-test was used for comparing right and left thyroid lobes (Steel and Torrie 1960). The relationship between T4 and FT4 was determined using simple linear correlation (Steel

and Torrie 1960).

RESULTS

Effect of Iodine Supplementation

Addition of I to the basal diet did not affect T4 or FT4 serum concentrations throughout the study (Table 3, Fig. 1). Serum T3 showed treatment x time interaction ($P<0.01$) in juveniles and effect of iodine level ($P<0.05$) in adults during February 1979 and January 1980 (Tables 4 and 5). Body weights did not differ between treatments within any time. Reproductive performance of does and characteristics of their offspring are shown in Table 6. With the exception of the number of fawns weaned per pregnant doe, which was lowest ($P<0.05$) in the +0.7 ppm I group, there was no difference between groups in any parameter. Mean milk I levels as well as average total I content and concentration of the thyroid glands increased directly with the level of dietary I (Table 7). Differences, however, were not significant due to the large variability within groups and small sample size.

Mean daily feed consumption per deer was very similar for each group except during the summer of 1979. Lower intake at this time by the group offered +0.7 ppm I probably resulted due to decreased lactational demands after high fawn mortality.

Seasonal Effects

Both juvenile and adults displayed typical cyclic changes in food consumption and body weight. Food consumption was highest in early fall and decreased to low levels

Table 3. Degrees of freedom (Df) and F values for split-plot analysis of T_4, FT_4 and T_3 serum concentrations in juvenile and adult does.

	T_4 juveniles			T_4 adults			FT_4 juveniles			FT_4 adults			T_3 juveniles			T_3 adults		
	Df	F	P[a]	Df	F	P	Df	F	P	Df	F	P	Df	F	P	DF	F	P
Diets	2	0.01	NS	2	0.16	NS	2	0.11	NS	2	1.7	NS	2	0.66	NS	2	11.31	<0.01
Deer/Diets ($error_a$)	15			14			15			14			15			14		
Time	7	8.87	<0.001	6	10.82	<0.001	3	8.93	<0.001	3	9.16	<0.001	7	11.15	<0.001	6	8.00	<0.001
Diets x Time	14	1.15	NS	12	0.78	NS	6	1.53	NS	6	0.30	NS	14	2.28	<0.01	12	1.36	NS
$Error_b$	105			84			45			42			105			84		

[a]Probability level. NS = nonsignificant (P>0.05).

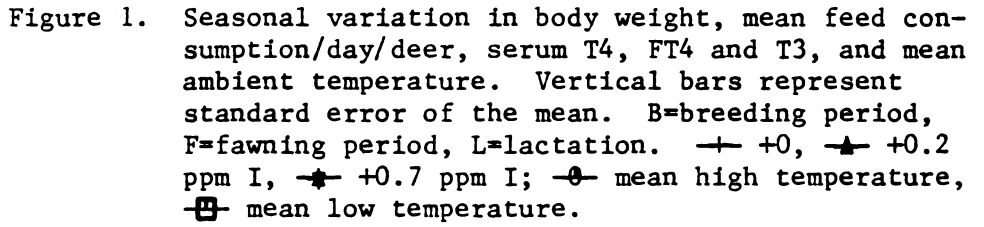


Figure 1. Seasonal variation in body weight, mean feed consumption/day/deer, serum T4, FT4 and T3, and mean ambient temperature. Vertical bars represent standard error of the mean. B=breeding period, F=fawning period, L=lactation. —+— +0, —▲— +0.2 ppm I, —●— +0.7 ppm I; —θ— mean high temperature, —⊟— mean low temperature.

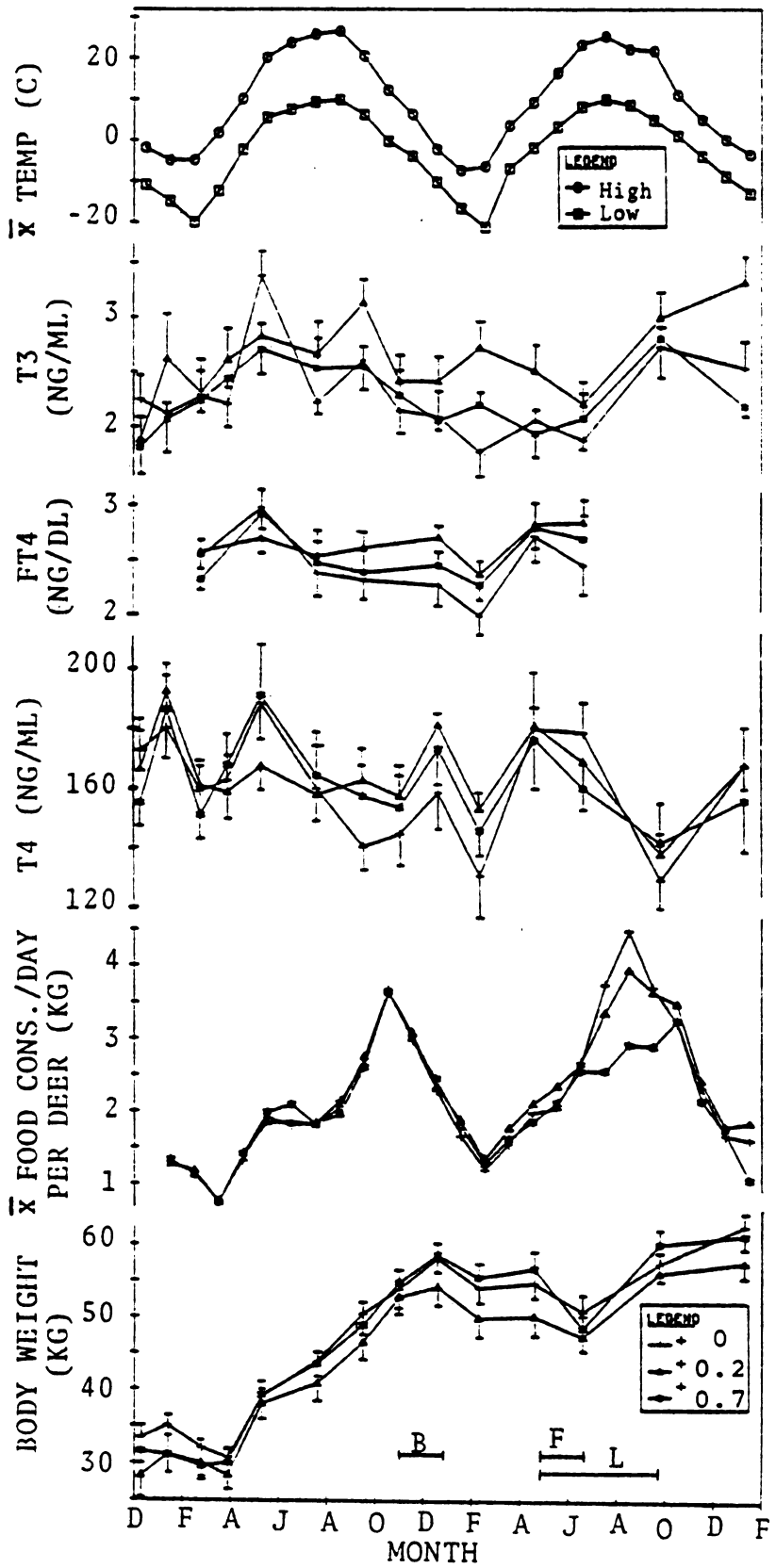

x̄ TEMP (C)
20
0
-20
LEGEND
High
Low
T3 (NG/ML)
3
2
FT4 (NG/DL)
3
2
T4 (NG/ML)
200
160
120
x̄ FOOD CONS./DAY PER DEER (KG)
4
3
2
1
BODY WEIGHT (KG)
60
50
40
30
LEGEND
0
0.2
0.7
B
F
L
D F A J A O D F A J A O D F
MONTH

Table 4. Effect of supplemental iodine on reproductive performance and milk iodine of does and serum T_4 and T_3 of their offspring (June 21, 1979).

Item	Supplemental iodine +0 ppm	+0.2 ppm	+0.7 ppm	P[a]
Does bred	5	6	6	
Barren does	0	1	0	NS
Fawns born/pregnant doe	1.8	1.8	2.0	NS
Fawns weaned/pregnant doe	1.4	1.4	0.5	<0.05
Birth weight (kg)				
Male fawns	2.60 ± 0.23(3)[b]	2.68 ± 0.46(2)	2.70 ± 0.12(5)	NS (all)
Female fawns	2.77 ± 0.30(6)	2.83 ± 0.26(5)	2.32 ± 0.23(7)	NS (all)
Milk iodine (ppm)	0.16 ± 0.03(3)	0.30 ± 0.11(4)	0.48 ± 0.22(3)	NS
T_4 (ng/ml)				
Male fawns	195.0 ± 14 (3)	144.8 ± 29 (2)	184.8 ± 25 (2)	NS (all)
Female fawns	190.1 ± 18 (4)	195.4 ± 35 (5)	247.1 (1)	NS (all)
T_3 (ng/ml)				
Male fawns	2.83 ± 0.01(3)	1.80 ± 0.56(2)	2.85 ± 0.14(2)	NS (all)
Female fawns	3.27 ± 0.16(4)	2.79 ± 0.22(5)	2.42 (1)	NS (all)

[a] Probability level. NS = nonsignificant (P>0.05); NS (all) = no significant effect (P>0.05) of diet, sex or interaction.

[b] Mean ± standard error (N).

Table 5. Effect of supplemental iodine on thyroid characteristics of adult does (January 9, 1980).

Item	Supplemental iodine			P[a]
	+ 0	+ 0.2 ppm	+ 0.7 ppm	
N	5	6	6	-
Body weight (kg)	62.8 ± 1.8[b]	57.8 ± 2.4	61.5 ± 1.8	NS[a]
Thyroid weight (g,dry)	1.59 ± 0.30	1.50 ± 0.13	1.66 ± 0.15	NS
$\frac{\text{Thyroid weight (g,dry)}}{BW_{kg}^{0.75}}$	0.071 ± 0.013	0.072 ± 0.007	0.076 ± 0.007	NS
Thyroid iodine (mg)	15.76 ± 3.78	17.95 ± 2.81	19.75 ± 1.90	NS
$\frac{\text{Thyroid iodine (mg)}}{BW_{kg}^{0.75}}$	0.70 ± 0.16	0.86 ± 0.12	0.90 ± 0.09	NS
Thyroid iodine (ppm,dry)	9,452 ± 897	11,742 ± 1,016	11,869 ± 373	NS

[a]Probability level. NS = nonsignificant (P>0.05)

[b]Mean ± standard error.

during late winter. Body weights followed a similar pattern, although peak weights were reached during late fall. Weight loss also occured in does during fawning.

Time of year affected ($P<0.001$) serum T4, FT4 and T3 concentrations in both juvenile and adult does. T4 levels in juveniles were higher ($P<0.05$) during January and May than at other times of the year. Similarly, FT4 levels were also highest ($P<0.05$) during May. In adults T4 concentrations in December and April were higher ($P<0.05$) than in November, February, and September. FT4 levels in adults were higher ($P<0.05$) in April and June than in February. Throughout the study T4 and FT4 levels were highly correlated ($r=0.74$, $n=138$, $P<0.001$). Changes over time in T3 concentrations did not follow a clear, consistent pattern in either juveniles or adults. T3 levels in juveniles were highest across all groups during May and September and in adults were highest during September and January.

Effect of Age and Lactation

Serum T3 and T4 levels and thyroid characteristics of juvenile versus adult deer are shown in Tables 8 and 9 respectively. Both young (<3 months) and older fawns (6-8 months) had significantly greater serum T3 and T4 concentrations than adult does (2 and 2.5 years, respectively) sampled at the same time. Absolute, but not relative, weights, total I and I concentration of thyroids collected in January 1980 were greater ($P<0.01$) in adults than in fawns.

Table 6. Effect of time of year on serum T_4, FT_4 and T_3 in juvenile does.

Item	Date							
	12/8/77	1/11/78	2/21/78	3/29/78	5/8/78	7/17/78	9/12/78	11/1/78
T_4 (ng/ml)[a]	164.4 ± 6.4^{e}	186.4 ± 6^{d}	153.3 ± 4.9^{e}	162.5 ± 5.5^{e}	182.2 ± 7.4^{d}	160.7 ± 6.9^{e}	154.3 ± 6.4^{e}	152.4 ± 5^{e}
FT_4 (ng/dl)[a]	---[b]	---	2.47± 0.06^{e}	---	2.87± 0.09^{d}	2.47± 0.11^{e}	2.46± 0.10^{e}	---
T_3 (ng/ml)[c]								
+ 0 ppm	2.34± 0.24^{e}	2.09± 0.07^{e}	2.26± 0.23^{e}	2.07± 0.19^{e}	3.38± 0.25^{d}	2.23± 0.09^{e}	2.62± 0.08^{e}	2.16± 0.20^{e}
+ 0.2 ppm	1.88± 0.20^{f}	2.62± 0.44^{de}	2.31± 0.26^{ef}	2.61± 0.29^{de}	2.83± 0.13^{de}	2.67± 0.25^{de}	3.14± 0.22^{d}	2.43± 0.16^{ef}
+ 0.7 ppm	1.81± 0.17^{e}	2.13± 0.27^{de}	2.24± 0.15^{de}	2.44± 0.11^{de}	2.71± 0.24^{d}	2.54± 0.28^{d}	2.56± 0.19^{d}	2.30± 0.18^{de}

[a]Mean ± standard error across all groups (N=18).

[b]Values not determined for these dates.

[c]Mean ± standard error for each group (N=6).

[def]Row means with different superscripts differ significantly ($P<0.05$).

Table 7. Effect of time of year on serum T_4, FT_4 and T_3 in adult does.

Item	Date						
	11/1/78	12/19/78	2/8/79	4/12/79	6/21/79	9/20/78	1/9/80
T_4 (ng/ml)[a]	152.4 ± 5[ef]	171.8 ± 5.8[d]	144.0 ± 5.2[f]	179.1 ± 7.8[d]	168.8 ± 5.2[de]	137.0 ± 5.8[f]	164.0 ± 7.8[de]
FT_4 (ng/dl)[a]	---[b]	2.51± 0.08[de]	2.23± 0.08[e]	2.81± 0.11[d]	2.70± 0.13[d]	---	---
T_3 (ng/ml)[c]							
+ 0 ppm (5)	2.16± 0.20[def]	2.11± 0.23[def]	1.79± 0.17[fh]	2.08± 0.13[def]	1.90± 0.08[ef]	2.76± 0.28[d]	2.57 ± 0.21[deh]
+ 0.2 ppm(6)	2.43± 0.16[ef]	2.43± 0.25[ef]	2.74± 0.23[dfg]	2.53± 0.22[ef]	2.23± 0.18[f]	3.02± 0.23[dc]	3.35 ± 0.18[dg]
+ 0.7 ppm(6)	2.30± 0.18[de]	2.07± 0.13[e]	2.22± 0.15[degh]	1.96± 0.16[e]	2.10± 0.21[de]	2.84± 0.07[d]	2.22 ± 0.14[deh]

[a]Mean ± standard error across all groups (N=17)

[b]Values not determined for these dates.

[c](N) and mean ± standard error for each group.

[def]Row means with different superscripts differ significantly ($P<0.05$).

[gh]Column means with different superscripts differ significantly ($P<0.05$).

Nonlactating does had greater ($P<0.05$) T3 levels than lactating does in September 1979 (Table 10). T4 levels at this time would also have been significantly greater ($P<0.05$) in the nonlactating does if one animal which had a very low T4 value (3.9 standard deviations from $\bar{x}$) was excluded. Otherwise T3 and T4 levels did not differ significantly although nonlactating does consistently had higher mean hormone levels.

Right Versus Left Thyroid Lobe

The left lobe of the thyroid was found to be larger ($P<0.001$) and contain more I ($P<0.001$) than the right lobe. I concentration of the two lobes, however, did not differ significantly.

Captive Versus Wild Does

Wild does weighed less ($P<0.01$), had much less I in their thyroids ($P<0.01$) and showed considerably lower ($P<0.001$) T4, FT4 and T3 levels than adult captive does (Table 11). Thyroid weight per metabolic body size and the ratio of serum rT3 to T4 were greater ($P<0.01$) in the wild deer. Weight (g, dry), I content (mg) and I concentration (ppm, dry) of the thyroid from the wild, road-killed doe obtained in March 1980 were 1.68, 4.5 and 2,701, respectively.

DISCUSSION

Iodine

Studies with monogastric and ruminant mammals have shown the basic clinical manifestations of I deficiency are very similar between species (Struder and Greer 1968, Karmarkar et al. 1974, Belshaw et al. 1975, Riesco et al. 1976,

Table 8. Effect of age on serum T_4 and T_3 of deer.

Item	June 21, 1979			January 9, 1980		
	Adult[a]	Juvenile[b]	P[c]	Adult[d]	Juvenile[e]	P
N	17	17		17	19	
T_4 (ng/ml)	168.8 ± 5.2[f]	195.4 ± 11.4	<0.05	164.0 ± 7.8	187.4 ± 5.9	<0.05
T_3 (ng/ml)	2.09 ± 0.1	2.78 ± 0.15	<0.001	2.72 ± 0.15	3.18 ± 0.72	<0.05

[a]Data from 2 year old does.

[b]Data from male and female fawns <3 months old.

[c]Probability level.

[d]Data from 2½ year old does.

[e]Data from 6 to 8 month old male and female fawns.

[f]Mean ± standard error.

Table 9. Effect of age on thyroid characteristic of deer (January 9, 1980).

Item	Adult[a]	Juvenile[b]	P[c]
Thyroid weight (g,dry)	1.58 ± 0.11(17)[d]	0.81 ± 0.05 (4)	<0.01
$\frac{\text{Thyroid weight (g,dry)}}{BW_{kg}^{0.75}}$	0.073 ± 0.005 (17)	0.060 ± 0.007 (4)	NS
Thyroid iodine (mg)	17.94 ± 1.58 (17)	7.68 ± 1.16 (4)	<0.01
$\frac{\text{Thyroid iodine (mg)}}{BW_{kg}^{0.75}}$	0.83 ± 0.07 (17)	0.57 ± 0.08 (4)	NS
Thyroid iodine (ppm,dry)	11,113 ± 490 (17)	9,104 ± 298 (18)	<0.01

[a]Data from 2 year old does.

[b]Data from 6 to 8 month old female fawns only (N=4) or male and female fawns (N=18).

[c]Probability level.

[d]Mean ± standard error (N).

Table 10. Serum T_4, FT_4 and T_3 in lactating (L) versus nonlactating (NL) does.

	June 21, 1979		Sept 20, 1979[a]		Jan 9, 1980[b]	
Item	L	NL	L	NL	L	NL
N	11	6	11	6	11	6
T_4 (ng/ml)	166.0 ± 7.3[c]	173.8 ± 6.2	130.1 ± 6.1	149.7 ± 10.7	162.7 ± 10.0	166.4 ± 13.4
FT_4 (ng/dl)	2.63 ± 0.18	2.84 ± 0.14				
T_3 (ng/ml)	2.01 ± 0.11	2.22 ± 0.19	2.70± 0.11[d]	3.21± 0.20	2.71± 0.18	2.74± 0.31

[a]Date when fawns were weaned.

[b]Date subsequent to lactation.

[c]Mean ± standard error.

[d]Lactating and nonlactating does differ significantly ($P<0.05$).

Table 11. Thyroid characteristics and serum thyroid hormone levels in wild versus captive adult does.

Item	Wild[a]	Captive[b]	P[c]
N	3	17	
Body weight (kg)	42.6 ± 6.1[d]	57.0 ± 6.1	<0.01
Thyroid weight (g,dry)	1.99 ± 0.35	1.58 ± 0.35	NS
$\frac{\text{Thyroid weight (g,dry)}}{BW_{kg}^{0.75}}$	0.118 ± 0.010	0.073 ± 0.005	<0.01
Thyroid iodine (mg)	5.98 ± 3.39	17.94 ± 1.58	<0.01
$\frac{\text{Thyroid iodine (mg)}}{BW_{kg}^{0.75}}$	0.33 ± 0.16	0.83 ± 0.07	<0.05
Thyroid iodine (ppm,dry)	2,664 ± 1,124	11,113 ± 490	<0.001
T_4 (ng/ml)	53.9 ± 23	144.0 ± 22	<0.001
T_3 (ng/ml)	0.77 ± 0.25	2.28 ± 0.14	<0.001
FT_4 (ng/dl)	0.67 ± 0.25	2.23 ± 0.08	<0.001
rT_3 (ng/ml)	0.35 ± 0.08	0.31 ± 0.03	NS
$T_3/T_4 \times 10^3$	15.9 ± 1.9	15.9 ± 0.8	NS
$FT_4/T_4 \times 10^5$	13.5 ± 1.4	15.8 ± 0.5	NS
$rT_3/T_4 \times 10^3$	12.2 ± 7.4	2.1 ± 0.2	<0.01

[a]Wild deer collected near Houghton Lake, MI between February 15 and March 12, 1979

[b]Captive deer hormone data from February 8, 1979; thyroid gland data from January 9, 1980.

[c]Probability level. NS = nonsignificant (P>0.05).

[d]Mean ± standard error.

Ermans 1978, Naeije et al. 1978, Potter et al. 1980). When insufficient I begins limiting thyroid hormone synthesis, reduced negative feedback on the anterior pituitary causes increased release of thyrotropin (TSH). TSH, in turn, stimulates iodine uptake, hormone synthesis and hormone release by the thyroid (Field 1978, Taurog 1978). When increased TSH stimulation is prolonged, the thyroid may undergo hypertrophy and hyperplasia (goiter) in an attempt to meet demands for maintaining an euthyroid state. In the thyroid, inadequate dietary I results in decreased I concentration, reduced T4 synthesis and increased monoiodotyrosine/diiodotyrosine and T3/T4 ratios (Underwood 1977, Taurog 1978). In the blood, T4 levels may decrease markedly, whereas T3 generally remains normal or near normal and may even be elevated (Fukuda et al. 1975, Ermans 1978, Naeije et al. 1978). Since T3 is several times more metabolically active in mammals than T4 and contains 25% less I, this is believed to be an important adaptive mechanism. Moderately reduced T3 levels have been reported but only in cases where I deficiency was severe and T4 levels were extremely low (Riesco et al. 1977, Potter et al. 1980). It is not clear to what extent peripheral monodeiodination of T4 to T3 versus increased relative synthesis in the thyroid contributes to maintaining T3 levels in I deficiency (Ermans 1978). Other adaptations to I deficiency include more efficient recycling of I by decreasing fecal and renal excretion and reducing secretion in milk (Miller et al. 1975,

Miller 1975).

Generally the most apparent consequence of I deficiency in domestic ruminants is impaired reproductive performance or birth of weak, goitrous offspring (Hemken 1970, Underwood 1977, Hidiroglou 1979). Infertility, irregular or suppressed estrus, abortion, stillbirth and retained placenta have been reported in sheep and cattle in response to I deficiency (Wilson, 1975, Hidiroglou 1979). Dams of goitrous offspring may or may not show overt pathophysiological signs of inadequate I (Aschbacker 1968, Piel 1979, Andrewartha et al. 1980).

In the present study, I supplementation had no apparent effect on thyroid function. Serum T4 and FT4 levels, reproductive success and thyroid data did not provide evidence of inadequate I intake by the +0 group. Significant differences in T3 during February 1979 and January 1980 are not readily explainable. As discussed previously, however, T3 is generally not a sensitive indicator of I nutriture. T3 levels in the +0.2 ppm I group tended to be high throughout the study and the detected differences may be of genetic origin. The high fawn mortality in the +0.7 ppm I group was unexpected. All fawns, except one which developed severe scours, died shortly after birth apparently from cold exposure as a result of unseasonably cold weather which happened to occur when most of the deer in this group were fawning. Necropsy revealed no visible abnormalities in the thyroid glands. It is doubtful that I toxicity was involved. Over 20 times the required level of I has been fed to domestic

ruminants without ill-effect (Newton et al. 1974, Fish and Swanson 1977). In addition, Ullrey et al. (1971) have reported excellent reproductive success and low fawn mortality in white-tailed deer fed a diet supplemented with 0.5 ppm I, as pentacalcium orthoperiodate.

Results obtained in the present study do not agree with those of Byrne et al. (1974, unpublished data) who reported lower T4 in deer fed 0.5 ppm I versus those fed 1 ppm I. The present study, however, had the benefit of improved methodology for measuring T4 and I.

I requirements of domestic animals are highest during gestation and lactation due to increased demands caused by the fetus and secretion of I in milk. The National Research Council has recommended 0.8 ppm I for pregnant and lactating ewes and 0.1 ppm for other sheep (NRC 1975); 0.5 ppm for pregnant and lactating dairy cows and 0.25 ppm for other dairy cattle (NRC 1978); and 0.1 ppm for beef cattle (NRC 1976). The British Agricultural Research Council (1965) has suggested 0.8 ppm I for pregnant and lactating ruminants and 0.12 ppm for nonpregnant ruminants. In all cases the requirements are higher if goitrogenic substances are present in the feed.

During gestation and early lactation the basal diet in the present study was analyzed to contain 0.26 ppm I. Because this level was adequate during the period of greatest demand, 0.26 ppm in a dry diet consumed ad libitum is probably sufficient for all phases of the life cycle in

white-tailed deer assuming goitrogenic compounds are not present.

It is unfortunate that the basal diet was not low enough in I to produce a deficiency. Analysis of individual components in the diet indicated that greater than 0.1 ppm I was being added to the basal diet during mixing and pelleting, presumably due to residual I contamination in the system at the large, commercial mill which produced the feed.

Seasonal Effects

Seasonal changes in thyroid activity have been reported in sheep (Henneman et al. 1955, Sutherland and Irvine 1974, Wallace 1979, Andrewartha et al. 1980), goats (Flamboe et al. 1959), and cattle (Post 1965a, Christopherson et al. 1979) as well as in a large number of other species. An absence of seasonal variation also has been reported for many species and conflicting reports are prevalent in the literature. In order to interpret circannual thyroid patterns it is necessary to consider a plethora of factors which can influence thyroid function. Feed intake, diet composition, temperature, body composition, age, sex and reproductive stage, breed, exercise, altitude, stress, time of day, cyclic physiological alterations (i.e., changes in anabolic-catabolic status, torpor, molting), disease, and photoperiod have all been found to influence or be associated with changes in thyroid activity in different species.

In white-tailed deer and other cervids investigations of seasonal changes in thyroid activity have not elucidated

a consistent pattern. Grafflin (1942) reported no seasonal changes in thyroid gland structure in a small sample of wild, white-tailed deer collected in Massachusetts. Hoffman and Robinson (1966), however, provided histological evidence that thyroid activity was greatest during November and December and again in May and June in free-roaming deer from Maryland. Activity was determined to be lowest during January and February. In northern Michigan, Seal et al. (1972) found no difference between T4 concentrations in December, March and April in captive, pregnant does fed a nutritious diet. Does offered only white-cedar browse, however, showed a significant decrease in T4 levels in March with a slight rebound in April. Bahnak (1978) also investigated thyroid activity in penned adult does in northern Michigan. T4 levels in animals on either a high or low plane of nutrition were found to be highest in late spring, decreased to low levels during late summer and fall, increased again during early winter and decreased in late winter. Although T3 followed a much less consistent seasonal pattern, levels tended to be highest during late spring and in the high diet does during late summer and early fall. In Ontario, Bubenik and Bubenik (1978) also found T4 levels in juvenile and mature bucks and barren adult does to peak, albeit not significant statistically, in the spring. Byrne et al. (1974) and Barth et al. (1980) reported T4 levels in captive, male and female white-tailed and roe deer (Capreolus capreolus), respectively, to be highest during winter and lowest during late summer. Yousef and Luick (1971) reported

no effect of season on thyroxine secretion rate in reindeer (Rangifer tarandus) in Alaska. Ringberg et al. (1978), however, found a significant reduction in serum T4 in winter versus summer in both adult and juvenile reindeer in Norway.

The present data clearly demonstrate a seasonal trend in serum T4 with high levels occuring in early winter and again in spring and low levels occuring during summer, fall and late winter. This pattern is virtually identical to that reported by Bahnak (1978). FT4, which is considered to be the metabolically active T4 fraction available to the tissues (Ingbar et al. 1965, Nicoloff 1978), followed a similar pattern indicating a true shift in thyrometabolic status. Serum T3, however, followed a much less consistent seasonal trend. Bahnak (1978) noted similar variability in T3 levels in adult does.

It is of interest to speculate on the relationship between changes observed in feed consumption, ambient temperature and body weight, and changes observed in serum T4 and FT4 levels (Fig. 1). It must be considered that the ultimate peripheral expression of thyroid hormone is dynamic and depends on the physiological status of the individual. It also must be considered that blood hormone concentrations are not necessarily indicative of true thyrometabolic status.

Early Winter.— Increased T4 during early winter may have resulted due to cold exposure and in turn mediated an increase in metabolic rate to compensate for increased body heat loss. It is well established that acute cold exposure can cause at

least a transient increase in thyroid activity and, presumably, subsequent hypermetabolism in most mammals (Blincoe and Brody 1955, Yousef et al. 1967, Katovich et al. 1974, Westra and Christopherson 1976, Kennedy et al. 1977, El-Nouty et al. 1978, Galton 1978). At this time, body weights and presumably fat stores were at a maximum and copious energy reserves would have been available for fueling increased metabolic activity. Food consumption had recently begun to decline, hence heat increment due to digestive fermentations also would have been decreasing.

In contrast to this hypothesis, however, Silver et al. (1971) and Holter et al. (1975) have proposed that energy expenditure in deer is relatively independent of low ambient temperature (>-20 C) during fall and winter. Similarly, Galton (1978) reviewed studies concerning the relationship between cold exposure and thyroid activity in mammals and concluded that, in most cases, the thyroid probably does not cause an increase in metabolic activity in response to seasonal reductions in temperature.

It is also possible that increased thyroid hormone levels may have been involved with adipose mobilization, irrespective of temperature. The lipolytic effect of thyroid hormone, particularly in conjunction with catecholamines, is well documented (Debons and Schwartz 1961, Krishna et al. (1968, Bernal and Refetoff 1977, Thenan and Carr 1980). This being the case, it follows that some factor other than temperature must act to trigger the apparent change in thyroid activity. Increased T4 levels were observed in

adults at the winter solstice and in juveniles shortly thereafter, thus circumstantially implicating short photoperiod. In another study (Watkins et al. unpublished data) on the effect of abruptly decreasing photoperiod from 16 to 8 hours, however, no increase was observed in serum T4 levels in doe fawns. Accordingly, Brown et al. (1978) reported pinealectomy in male deer did not disrupt typical cyclic patterns in weight change and food consumption. Conversely, photoperiod has been determined to influence thyroid secretion rate in sheep (Hoersch et al. 1961) and antlergenesis in sika deer (Cervus nippon) (Goss 1976).

Another possible triggering mechanism may be body composition. White-tailed deer will voluntarily reduce food consumption and begin catabolizing fat reserves in the winter. It may be that once the fat to lean ratio in deer reaches a certain point, intrinsic mechanisms are triggered which prevent further fat deposition and initiate fat mobilization. Body weight has been proposed to be a regulator of the onset of first estrus in black-tailed deer (Odocoileus hemionus columbianus) (Mueller and Sadleir 1979).

Additionally, changes in other hormones may be involved with the increased T4 levels observed in early winter. Bubenik et al. (1975a) noted that plasma T4 was significantly elevated in male, white-tailed deer treated with cyproterone acetate, an antiandrogenic compound. Testosterone concentrations in adult bucks have been found to decrease markedly during December and January from high levels in November (Mirarchi et al. 1978). Collateral

studies on T4 and testosterone levels in other species have shown an inverse relationship between the two hormones (Maurel et al. 1977). Androgen therapy is generally associated with decreased circulating T4 levels, presumably due to a decrease in T4 binding proteins and an increase in T4 turnover (Federman et al. 1958, Engbring and Engstrom 1959). Plasma progesterone levels in white-tailed does have been found to increase rapidly during late November and December in conjunction with estrus (Plotka et al. 1977, Harder and Moorhead 1980). An increase in protein-bound I has been reported in the goat in association with estrus (Sharma and Sharma 1976). Estrogen therapy is generally associated with an increase in T4 levels due to enhanced hepatic synthesis of T4 binding globulin and decreased T4 turnover (Dowling et al. 1960, Gregerman and Davis 1978).

Late Winter.— The decrease observed in serum T4 and FT4 levels during late winter may have been associated with the marked reduction observed in feed consumption and continued depletion of body stores. As winter progresses energy conservation becomes increasingly critical in white-tailed deer. In order to reduce energy expenditure, a variety of behavioral and physiological mechanisms appear to be employed (Holter et al. 1975, Moen 1976). A decrease in thyroid activity could aid in conserving energy since food consumption, tissue protein turnover and fat catabolism are generally diminished in the hypothyroid state (Loeb 1978). Overall, lowered T4 levels may have the effect of depressing calorigenesis. This scenario is consistent with indirect

calorimetry data provided by New Hampshire Workers (Silver et al. 1969, Silver et al. 1971, Holter et al. 1975) which indicate deer have a reduced metabolic rate in the winter. Similar data have been reported for other wild ungulates in temperate regions (Chappel and Hudson 1978). It should be noted, however, that T4 levels during late winter did not differ significantly from T4 levels measured during late summer and fall.

On the assumption that deer become hypometabolic during winter, it is of interest to consider thyroid function in mammals which hibernate or undergo torpor. Unfortunately, as pointed out by Hudson and Wang (1979), investigations on the role of the thyroid in hibernation have produced an abundance of contradictory information. Although there tends to be a general consensus that metabolic depression during torpor is associated with reduced thyroid activity (Wenberg and Holland 1973, Hudson and Deavers 1976, Azizi et al. 1979), there is also evidence that suggests thyroid activity may not decrease in some hibernating species (Hudson and Deavers 1976, Demeneix and Henderson 1978a,b, Hudson 1980). As shown by Young et al. (1979), assessment of thyroid status in hibernating mammals must take into account body temperature and free hormone levels in order to avoid misinterpretation. It has been hypothesized that the decrease observed in thyroid activity in some hibernating species may serve to lower the temperature limit of membrane phase transition and thus allow body temperature to fall without detrimental changes in membrane fluidity (Hulbert 1978, Augee et al. 1979).

Body temperature in white-tailed deer has been found to be lowest during winter, although the difference from other times of the year was less than 2 C (Holter et al. 1975).

In sheep and cattle, thyroid hypoactivity has been found to result in decreased feed intake and gastrointestinal motility and increased feed retention time (Miller et al. 1974, Westra and Christopherson 1976, Kennedy et al. 1977). It is intriguing to consider that decreased thyroid activity in deer during winter may be an evolutionary adaptation to limited food availability. Although administration of exogenous thyroid hormone has been found to decrease dry matter digestibility (Kennedy et al. 1977), there is no evidence that depression of normal thyroid activity can enhance digestibility. There is, similarly, no evidence that digestibility is improved in deer during winter (Holter et al. 1977).

The foregoing discussion has assumed that decreased thyroid activity during late winter may act to regulate metabolic change. It is also possible, however, that lower T4 levels occurred in response to metabolic alterations and not exclusively vice versa. Feed restriction, for example, generally results in decreased thyroid activity (see Section II). Additionally, prolonged exercise has been found to increase thyroid activity (Refsum and Stromme 1979). During fall and early winter, deer are highly active (Ozoga and Verme 1970). During late winter activity falls off markedly and, in response to cold stress, deer spend much more time lying down (Holter et al. 1975, Moen 1976).

If it is assumed that increased thyroid activity in

early winter occurred due to cold exposure, it follows that the decrease observed in late winter may have been due primarily to cold acclimation. Thyroid activity in several species has been found to return to pre-exposure levels, subsequent to an initial increase, during chronic cold exposure (Bauman et al. 1968, Sterling and Lazarus 1977). It has been proposed that the thyroid is necessary for developing a cold acclimated state but is not necessary for its maintenance (Galton 1978).

Spring.— In the spring, feed consumption, body weights and ambient temperature began to increase. The concomitant increases observed in T4 and FT4 may have been involved in regulating metabolic alterations as the animals switched from a catabolic to an anabolic state. To what extent T4 levels may have responded to, versus regulated increased feed intake and tissue accretion is not known. Feed intake can be elevated by T4 administration (Blair and Forbes 1974, Miller et al. 1974); conversely, increased feed intake following feed deprivation can result in increased T4 levels. A small amount of thyroid hormone administered to hypothyroid individuals has been observed to increase lipogenesis (Llobera et al. 1979) and promote nitrogen retention (Scow 1951, Bernal and Refetoff 1977). Accordingly, energy and nitrogen balances determined in fawns by Holter et al. (1977) indicate deer begin net fat deposition and increase nitrogen retention in the spring. Similar phenomena occur in species arousing from torpor in the spring. It has also been reported that growth hormone increases from low winter levels to a peak in the spring in deer (Bubenik et al. 1975b,

Bahnak 1978). Growth hormone and thyroid hormone are known to be associated in a number of actions. In addition, somatostatin has been found to inhibit TSH secretion (Sterling and Lazarus 1977).

Holter et al. (1975) have reported metabolic rate in deer to be highly sensitive to low temperatures in the spring. It could be proposed that the increase observed in T4 during spring may have resulted primarily from cold exposure, even though temperatures were higher than during winter, due to cyclic physiological changes in the deer which occurred independent of the thyroid.

High T4 and FT4 levels in the spring coincided with the latter part of gestation in adult does. In pregnant humans it has been shown that increased hepatic T4 binding globulin synthesis in response to high estrogen levels can result in elevated serum T4 (Charles et al. 1979, Feely 1979, (Yamamoto et al. 1979). Because increased T4 and FT4 levels were also observed in juvenile does in the spring, it does not appear that pregnancy was a primary factor influencing serum T4 concentration. Pregnant and nonpregnant sheep have similarly shown seasonal changes in plasma T4 which did not appear related to pregnancy (Sutherland and Irvine 1974).

Summer and Fall.— During late summer and autumn, feed consumption and ambient temperatures were at their highest. Fat deposition also probably reached a maximum at this time (Holter et al. 1977). Low T4 levels may have played a permissive role in lipid accretion. Also, high temperature has been found to suppress thyroid activity (Yousef et al. 1967,

Galton 1978).

Triiodothyronine.— The previous discussion excluded T3 levels because no consistent seasonal pattern was detected. It is well established that T3 is the more metabolically active thyroid hormone in mammals; therefore, the absence of an interpretable trend in T3 was disappointing. It may be that serum T3 levels in deer fluctuate up and down, within a certain range, thus making single-point hormone measurements difficult to evaluate. Serum T3 concentrations in humans given exogenous T3 have been found to fluctuate as much as fivefold in a day (Wenzel and Meinhold 1974). The greater intracellular distribution and smaller circulating reservoir of T3, and the lesser binding affinity of serum proteins for T3 as compared to T4 could make serum T3 levels particularly susceptible to fluctuation (Nicoloff 1978). It is of interest to note that short-term variations in T4 levels have been noted by Bubenik and Bubenik (1978) in deer. This finding is somewhat suprising in light ot the buffering capacity of the circulating T4 pool (Nicoloff 1978). It may also be that T3 levels in deer reflect short-term regulation whereas T4 and FT4 levels are more indicative of long-term thyroid activity. In mammals the turnover rate of T3 is much more rapid than that of T4 and its action is expressed more quickly (Chopra 1978, Nicoloff 1978). Recent influences of temperature, feed consumption, and other variables may affect T3 but not T4 levels. Obviously more research is needed to determine the biological significance of circulating T3 levels in deer. It may be necessary to

measure not only total T3 but also free T3 concentrations.

Age and Lactation

Increasing age in most animals is generally associated with decreased thyroid activity after the neonatal period (Flamboe and Reineke 1959, Falconer and Robertson 1961, Abdullah and Falconer 1977, Kahl et al. 1977, Azizi 1979, Leatherland and Ronald 1979, Ooka 1979). Bubenik and Bubenik (1978) reported juvenile male deer between 1.5 and 3 years of age to have significantly higher T4 values than adult bucks. Byrne et al. (1974), however, found T4 levels in weaned fawns to be lower than in adult deer even though T4 levels of nursing fawns were higher than lactating dams.

Data obtained in the present study support an association between age and thyroid hormone levels in deer with T3 and T4 levels being higher in suckling and weaned fawns than in adult does. Although these data are in concert with Bubenik and Bubenik (1978), we do not support these authors' proposed use of T4 as an indicator of age and physiological maturation in deer. As discussed previously, there are too many factors (i.e., diet, temperature) which can influence thyroid hormone levels that would make any such interpretation difficult. Seal et al. (1978a), for example, were unable to detect any significant difference in T4 between free-roaming fawns and adult white-tailed deer.

The finding of generally higher T3 and T4 levels in nonlactating versus lactating does is consistent with

observations reported by Flamboe and Reineke (1959) in dairy goats, Lorscheider et al. (1969) in rats and dairy cattle, Katovich et al. (1974) in horses, and Hart et al. (1979) in dairy cattle. Other researchers, however, have provided evidence suggesting increased thyroid activity during lactation (Henneman et al. 1955, Grosvenor and Turner 1958). To what degree the thyroid is involved in the regulation of lactation is not clear (Hart et al. 1979). It has been known for many years that exogenous T4 or thyroprotein can stimulate milk production at least temporarily (Blaxter et al. 1949, Schmidt et al. 1971). Tucker (1974) suggested that the lactating animal is in a functional hypothyroid state as a result of reduced T4 availability.

Wild Versus Captive Deer

Even though only a small sample of wild deer was available, it is evident that thyroid activity was markedly altered in the wild versus the captive deer. The extremely low levels of T4, FT4 and T3 in the wild deer indicate these animals were in a hypothyroid and presumably a hypometabolic state. The high ratio of rT3 to T4 would also suggest a metabolic decrement. A similar thyroid hormone profile has been produced experimentally in acutely starved deer (Section III), the only difference being a high FT4/T4 ratio in the starved animals. This, however, probably resulted from a rapid shutdown of hepatic T4 binding protein synthesis, due to protein deprivation, which caused proportionately less protein binding of available T4 before equilibrium could again be reached. The non-elevated FT4/T4 ratio in the

wild deer would suggest a chronic condition where equilibration had already occurred.

Evidence that the wild deer were suffering from malnutrition is further supported by their lower body weights and from winter mortality data. An estimated 83,000 deer died during the winter of 1978-1979 in the northern half of Michigan's Lower Peninsula (Borgoyne and Moss 1979). In addition, low productivity of does (Friedrich 1979) and small antler beam diameters of bucks (Vogt 1979) have been reported in the part of Michigan where the wild deer were collected.

Other investigators have also found lower T4 levels during winter in free-roaming deer than in well nourished captive deer (Seal et al. 1972, Byrne et al. 1974). Accordingly, Bahnak (1978) observed T3 and T4 levels to be depressed during winter in penned deer fed northern white cedar browse, an important, yet nutritionally inadequate (Ullrey et al. 1970), winter food in northern Michigan. Both hormones were further reduced when the deer were starved for one week. Seal et al. (1978b) reported a relationship between low T4 levels in deer and poor habitat quality in Minnesota.

To what degree feed deprivation versus feed quality may have contributed to the decrement in thyroid activity is not clear. As pointed out by Verme (1971), severe malnutrition and not starvation is usually the cause of nutrition related mortality in deer. In many cases, however, consumption of a poor quality diet is usually confounded

with reduced feed intake. The effects of feed restriction and diet composition on thyroid activity are discussed elsewhere in greater detail (Section II).

Although the wild deer were smaller in size, their thyroid glands tended to be larger. Conversely, feed restriction in fawns has been found to cause a decrease in the size of the thyroid even when expressed on the basis of metabolic body size (Section II). Acute starvation of fawns has also been found to cause an apparent decrease in thyroid weight (Section III). The greatly diminished concentration of I in the thyroids of the wild deer is also contrary to what has been observed in feed-restricted and starved deer. Moderate feed restriction has been found to have little effect on thyroid I concentration of fawns (Section II), whereas I concentration of the thyroid in starved fawns supplemented with I has been observed to be higher than that in fed animals (Section III). I uptake by the thyroid of acutely starved rats has been found to be increased (Catz et al. 1953) and level of nutrition has not been found to affect the I concentration in thyroids of pigs (Sidor et al. 1973).

Low I content and increased size of the thyroid is indicative of inadequate I (Underwood 1977). It would appear, therefore, that the etiology of the thyroid profile obtained from the wild deer may be best explained by the combined effects of poor nutrition and low I. It is interesting that a similar thyroid profile of low plasma T3 as well as T4 and reduced I concentration of the thyroid has been produced experimentally in sheep fed a low I diet (Potter et al. 1980).

I concentrations of selected plants in the area where the deer were collected seldom exceeded 0.2 ppm during winter (Section IV). At other times of the year, due to the availability of herbaceous and aquatic plants, which tend to be higher in I than woody species, wild deer could probably consume an amount of iodine comparable to or greater than that fed to the +0 captive deer. In addition, reduced food intake and the inability to consume soil would also make winter the most likely time of I insufficiency in northern Michigan deer. Gist and Whicker (1971) reported the highest mean ^{131}I uptake by the mule deer (Odocoileus hemionus) thyroid occurred during winter when food, and hence I, intakes were minimal. The concentration of I in soil can be considerably greater than that of corresponding vegetation (Aston and Brazier 1979, Whitehead 1979). Soil ingestion, which has been documented in mule deer (Arthur and Alldredge 1979), has been found to be associated with a reduced incidence of goiter in lambs (Healy et al. 1972). To what extent naturally occurring goitrogenic compounds, such as thiocyanate, may limit utilization of I by deer is not known.

According to Underwood (1977) the normal healthy thyroid of most mammals contains 2000 to 5000 ppm I (dry). Average thyroid I concentration observed in captive deer in the present study exceeded 10,000 ppm. Considering the relatively low I content of all the diets, the deer thyroid apparently has a remarkable ability to concentrate I. Thyroid glands of sheep have also been reported to contain

as much as 10,000 ppm I, but this occured in animals ingesting seaweed high in I (Wilson 1975). The ability of the deer thyroid to concentrate I may be an adaptation to seasonally fluctuating I intake (Section IV).

I deficiency in wild deer could be a particularly insidious limiting factor. Reproductive failure and neonatal mortality resulting from a lack of sufficient I would be difficult to detect in the field. Goiter has been reported in 2 wild, pregnant does and their fetuses by Hoffman and Robinson (1966). Dams of I-deficient offspring may not themselves always show overt signs of I deficiency, however (Andrewartha et al. 1980). The influence of diet, temperature and other variables which can affect thyroid function, further complicate the task of evaluating I status of free-ranging animals. Because I nutriture is probably most critical during late pregnancy (Wilson 1975, Knights et al. 1979), I deficiency could be a particular problem during a prolonged winter when deer are forced to consume woody browse for an extended period. Even though protein-calorie nutriture might be adequate, the low I content of the woody plants could be detrimental to the fetus. In addition, restricted feed intake, which occurs in deer during the winter, has been reported to exacerbate the effect of low dietary I in sheep (Knights et al. 1979). Establishing the degree to which I may be limiting to white-tailed deer in northern Michigan will require further research.

SECTION II

Effects of Feed Restriction and Supplemental Iodine on Thyroid Activity in White-tailed Deer Fawns.

INTRODUCTION

Because deer in the wild often do not have the opportunity to consume unlimited food and because feed restriction in sheep has been reported to exacerbate the effect of low dietary I (Knights et al. 1979), a study was undertaken to investigate the combined effects of feed restriction and I supplementation in deer. In addition, the effects of feed restriction on thyroid function were of interest in light of the possible use of circulating thyroid hormone levels to assess general nutritional status and habitat condition of deer (Seal et al. 1972, Bahnak 1978, Seal et al. 1978a).

METHODS

Six fawns, from does which had been given the basal diet containing no supplemental I, and 7 fawns, from does receiving either 0.2 or 0.7 ppm supplemental I, were offered the basal and +0.7 ppm I diets, respectively, at approximately 50% the intake level of a group of 5 control fawns given +0.2 ppm I in a diet fed ad libitum. I concentrations, as analyzed, and composition of the diet have been described previously (Section I). Dams of control fawns had been offered a similar diet (Ullrey et al. 1971) containing +0.5 ppm I. Fawns were maintained on their respective diets from weaning in September 1979 until sacrifice in January

1980. All deer were housed outdoors in pens with shelters and dirt floors at Houghton Lake, Michigan. Feed restricted (FR) fawns were penned individually, whereas, all fawns fed ad libitum (AL) were penned together. It is probable that actual feed consumption by FR deer exceeded 50% that of AL animals owing to some feed wastage by the fawns penned together. Sample collection and analytical methods have been described previously (Section I).

Data were analyzed as a 3 x 2 factorial, with unequal replication, for diet and sex main effects (Gill 1978). Contrasts between feed intakes and between the basal and +0.7 ppm I diets were tested using Bonferroni t-statistics (Gill 1978).

RESULTS AND DISCUSSION

Results are summarized in Table 12. I-supplemented and unsupplemented FR fawns did not differ in any parameter except final body weight. This, however, was an artifact resulting from the larger initial size of the fawns in the +0.7 ppm group as these deer actually had the smallest weight gains during the trial.

In does fed ad libitum, 0.26 ppm I has been found to be adequate for normal reproduction and lactation (Section I). In the present study a similar level (0.28 ppm) was fed during the last 2 months of the experiment to the +0 group on restricted intake. Absence of any signs of I insufficiency in these animals would indicate that this level of I was safely above the requirement for growth even when food was limited.

Table 12. Effect of feed intake and iodine supplementation on thyroid characteristics and serum T_4, FT_4 and T_3 of fawns.

Item	Feed restricted (FR) +0 Male	Feed restricted (FR) +0 Female	Feed restricted (FR) +0.7 ppm Male	Feed restricted (FR) +0.7 ppm Female	Ad libitum (AL) +0.2 ppm Male	Ad libitum (AL) +0.2 ppm Female	SEM[a]	Signif. effect[b]	+0 versus +0.7[c] ppm	FR versus AL[d]
N	2	4	3	4	1	4				
Body weight (kg)	29.7	27.8	32.7	29.3	37.2	32.1	0.8	T**, S*	*	**
Weight gain (kg)	5.0	6.1	2.9	4.8	15.4	9.2	1.3	T*	NS	**
Thyroid weight (g,dry)	0.51	0.49	0.45	0.45	0.53	0.81	0.05	T**	NS	**
$\frac{\text{Thyroid weight (g,dry)}}{BW_{kg}^{0.75}}$	0.040	0.041	0.033	0.036	0.035	0.060	0.012	T**, S*	NS	**
Thyroid iodine (mg)	4.91	4.64	4.02	3.78	4.68	7.68	0.60	T**	NS	**
$\frac{\text{Thyroid iodine (mg)}}{BW_{kg}^{0.75}}$	0.387	0.383	0.295	0.304	0.311	0.569	0.047	T**	NS	*
Thyroid iodine (ppm,dry)	9950	9280	8940	8370	8910	9410	566	NS	NS	NS
T_4 (ng/ml)	201.4	200.1	169.4	183.8	205.6	180.7	11.4	NS	NS	NS
FT_4 (ng/dl)	2.42	2.21	1.90	2.35	2.23	2.03	0.12	NS	NS	NS
T_3 (ng/ml)	2.76	3.30	3.10	3.59	3.60	2.73	0.30	NS	NS	NS

[a]Standard error of the mean.

[b]T = treatment, S = sex, * (P<0.05), ** (P<0.01), NS = nonsignificant (P>0.05).

[c]Significance of +0 versus +0.7 ppm contrast.

[d]Significance of feed restricted versus ad libitum contrast.

As expected, FR fawns displayed decreased weight gains and lower final body weights than fawns fed ad libitum. Although weight and I content of the thyroid glands also were significantly lower in FR fawns, serum thyroxine (T4), free thyroxine (FT4) and triiodothyronine (T3) concentrations did not differ with the level of feed intake. This was somewhat unexpected as feed restriction is generally associated with reduced thyroid activity. Dramatic reductions in serum T3 and T4 levels have been observed in starved fawns (Section III), and other investigators have reported decreased serum T4 (Seal et al. 1972, Bahnak 1978) and T3 levels (Bahnak 1978) in feed-restricted deer. Furthermore, wild deer during late winter, a time of nutritional stress, have been found to have markedly lower serum T4, FT4 and T3 values than captive deer fed a nutritious diet (Section I). Conversely, however, Byrne et al. (1974) reported T4 concentrations in feed-restricted deer to generally be higher than levels in deer fed ad libitum. In other species, food restriction, including protein-calorie malnutrition (PCM) and starvation, has typically been found to result in an overall decrease in thyroid activity via a number of different mechanisms. Serum thyrotropin (TSH) may be reduced (Croxson et al. 1977, Azizi 1978) or unchanged (Ingenbleek and Malvaux 1980), and/or the thyroid may become less responsive to TSH stimulation (Graham et al. 1973); the secretion, deiodination and disposal of T4 is generally diminished (Grossie and Turner 1962, Post 1965, Nathanielsz 1970, Ingbar and Galton 1975, Abdullah

and Falconer 1977); circulating T3 and, in some cases, T4 levels are decreased (Abdullah and Falconer 1977, Carlson et al. 1977, Blum et al. 1979, Hastings and Zeman 1979, Ingenbleek and Malvaux 1980); peripheral conversion of T4 to T3 is reduced and shifted toward less thyrometabolically active pathways, i.e., reverse T3 (Vagenakis et al. 1975, Burman et al. 1979, Harris et al. 1979); and there may be fewer nuclear T3 receptor sites (Schussler and Orlando 1978). Overall, the alterations which occur in thyroid status as a result of feed restriction are believed to be adaptive responses to conserve limited energy and protein, and to maintain homeostatic integrity. The extent to which these alterations are manifested appears to depend not only on the duration and severity of the food restriction but also on the diet, environment, age, species and body composition of the individual, as well as other factors. For example, consumption of small amounts of carbohydrate has been reported to prevent or reverse the decline observed in T3 levels during food deprivation in obese humans, whereas, consumption of protein had no effect (Spaulding et al. 1976, Azizi 1978, Burman et al. 1979). Although plasma T4 has been found to be markedly depressed in chronic PCM children (Ingenbleek and Malvaux 1980), T4 levels in chronic PCM adults have been found to be unaffected (Chopra and Smith 1975). In acutely starved, obese subjects, T4 levels have even been observed to increase during fasting (Azizi 1978). FT4 levels in PCM may increase, decrease or remain the same depending on changes which occur in the

thyroxine-binding proteins (Ingenbleek et al. 1976, Ingenbleek and Malvaux 1980). Plasma T4 has been observed to increase as a result of feed restriction in beef cattle exposed to low ambient temperature but remains unchanged in feed-restricted animals maintained under moderate temperature (Christopherson et al. 1979).

The present data indicate that depending on the time of year and physiological condition, thyroid hormone levels may not always reflect moderate differences in nutritional status of deer. Based on fat measurements, Verme and Ozoga (1980a) suggested that autumn lipid deposition in deer is an obligatory event whereby fawns lay down fat in anticipation of winter, even when food is limited and growth is depressed. These authors also observed feed-restricted fawns to feed more efficiently and be less active during winter than well-fed deer, thus conserving energy. When fawns in the present study were killed in early January it was noted that FR animals appeared to have copious depot fat. By this time AL fawns had markedly reduced their feed intake and it is likely that all deer were shifting to a net catabolic energy state (Holter et al. 1977) and becoming increasingly reliant on body stores for fueling metabolic activity. It is possible, therefore, that FR and AL fawns during early winter were essentially in the same physiological condition even though body size and presumably lipid reserves of AL deer were superior. Had the experiment continued until early spring FR fawns may have shown lower T3 and T4 levels than AL fawns because,

as winter progressed, FR fawns would have been more likely to reach a critical point of energy depletion than AL deer. At this theoretical threshold, thyroid activity would have decreased to an abnormally low level in order to conserve dwindling energy reserves.

Alternatively, it is also possible that T4 secretion and peripheral metabolism, and hence thyroid status, actually differed between the two groups even though circulating thyroid hormone levels did not. Moderate feed restriction in goats has been observed to decrease T4 secretion rate even though plasma T4 levels remained essentially unchanged (Abdullah and Falconer 1977). In addition, it is possible that some other factor, such as cold exposure, influence of other hormones or metabolites, photoperiod, or body composition, could have temporarily overriden any effect that feed restriction might have had on thyroid activity. An increase in serum T4 levels during early winter has been noted previously in deer fed ad libitum (Section I).

The different effects that starvation, feed restriction, protein malnutrition and caloric insufficiency may have on thyroid function warrant brief discussion. Seal et al. (1978b) reported serum T3, but not T4, levels to be significantly reduced in fawns in December when a low energy versus a moderate energy diet was offered ad libitum. Body weight differences between groups in this study were very similar to body weight differences observed between FR and AL fawns in the present study. Bahnak (1978) found both T3 and T4 levels to generally be lower during winter in deer given

a low protein-low energy diet, ad libitum, as compared to deer fed a high protein-moderate energy diet. It would appear that feed restriction and consumption of a hypocaloric diet ad libitum do not produce tantamount effects on thyroid activity in deer. A difference in fecal mass may be involved as food deprivation has been found to result in decreased excretion of thyroxine conjugates via the bile as a consequence of reduced fecal output (Ingbar and Galton 1975, Abdullah and Falconer 1977). There may also be factors affected in the gut which modulate thyroid activity. For example, Westgren et al. (1977) reported that oral but not intravenous glucose was capable of increasing T3 levels in fasted humans. In addition, T3 and T4 are known to affect gastrointestinal motility and feed retention time (Johansson 1966, Levin 1969, Miller et al. 1974, Westra and Christopherson 1976, Kennedy et al. 1977), and heating the rumen in cattle has been found to depress thyroid activity (Yousef et al. 1968).

Seal et al. (1978b) also investigated the effects of feeding low dietary protein to fawns. Serum T3 was not affected; serum T4 was reduced although not significantly. Reports on thyroid function during protein insufficiency in other species have been conflicting. In rats, protein malnutrition has been found to result in an increase in plasma T3 and T4 concomitant with an increase in thermogenesis (Tulp et al. 1979). This has been suggested as a means of dissipating excess calories in order to enhance protein consumption. In the same study, T3, T4 and TSH

levels in pair-fed controls (approximately 50% feed-restricted) did not differ significantly from levels in full-fed rats even though mean weight gain decreased 40%. Other studies have indicated thyroid hypofunction as a result of protein restriction (Singh et al. 1971, Atinmo et al. 1978). There is evidence that protein deficiency in neonatal and young animals may cause long term impairment in the function of the thyroid gland (Atinmo et al. 1978). Starvation and protein depletion have also been found to cause involution of the thyroid gland (Cowan and Margossian 1966).

It is of interest to note the smaller size of the thyroids taken from FR deer. A similar finding has been reported by Verme and Ozoga (1980a) in feed-restricted fawns. Conversely, however, these same authors (1980b) reported that thyroid weights of fawns fed diets low in protein and/or in energy, ad libitum, were not significantly affected even though body weights were significantly reduced. This would further suggest that poor diet quality and inadequate food intake may have different effects on the thyroid in deer.

Growth of the thyroid gland in deer is apparently not independent of overall body growth when weight gain is limited by feed restriction. In view of the fact that thyroid weights in FR fawns were significantly smaller even when expressed on the basis of metabolic size, it appears that thyroid growth is more sensitive to feed inadequacy than overall body growth.

Thyroid iodine content followed a pattern similar to that of thyroid weight. Iodine concentrations, however, remained similar regardless of feed intake. This would suggest a functional stasis of the gland irrespective of its smaller size. Similarly, level of nutrition has not been found to affect the I concentration in thyroids of pigs (Sidor et al. 1973). Sex was not found to affect any thyroid parameter except thyroid weight per $BW_{kg}^{0.75}$. This resulted due to the significantly larger size of the male fawns. Based on data provided by Hoffman and Robinson (1966) and Verme and Ozoga (1980a), and results obtained in the present study, it would appear that thyroid weight in male deer does not differ significantly from that of similar aged females during late fall and winter, even though body size of male deer is typically larger. Thyroxine levels of mature bucks and mature barren does have been reported to be virtually identical (Bubenick and Bubenick 1978). This would further suggest that the thyroid can be functionally homologous even when it is smaller relative to body size.

At our present level of knowledge thyroid profiles can give only coarse resolution of the nutritional and physiological status of deer. Obviously more controlled research on the effects of diet, as well as a number of other factors (see Section I), is necessary before thyroid profiles of wild, free-roaming deer can be accurately interpreted. As additional information and more advanced methodology becomes available, thyroid profiles, in conjunction with other hematological and physical data, may prove to

be highly discriminatory of specific nutritional and physiological etiologies in deer.

SECTION III

Thyroid Function and Thyroxine Turnover in Fed and Starved White-tailed Deer Fawns.

INTRODUCTION

Thyroid function in white-tailed deer has gained increasing attention due to the role of thyroid hormone in mediating physiological adjustments to varying environmental and nutritional conditions (Seal et al. 1972, Byrne et al. 1974, Bahnak 1978, Seal et al. 1978a). Investigations thus far have dealt only with hormone concentrations in the blood or histological changes in the gland. Prior to this study no information was available on peripheral thyroid hormone kinetics in white-tailed deer.

Starvation (here used synonomously with severe malnutrition) is a common occurance in northern deer herds during severe winters (Verme and Ozoga 1971, Severinghaus 1972). During the winter of 1978, for example, estimated deer mortality in the northern half of lower Michigan exceeded 83,000 animals (Borgoyne and Moss 1979). Major losses occur in late winter and early spring after deer have depleted body fat reserves. Fawns are more susceptible to winter stress and nutrient deprivation than adults as they are less competitive for limited feed supplies and have smaller energy reserves.

Since the thyroid is intimately involved with metabolic

regulation it plays a critical role in adjustment to nutritional stress. Altered thyroid function in response to feed deprivation or severe malnutrition has been reported in a number of species (Post 1965b, Ingbar and Galton 1975, Vagenakis et al. 1975, Abdullah and Falconer 1977, Ingenbleek and Malvaux 1980). Knowledge of how deer adapt physiologically to starvation will provide a more scientific basis for their management.

It is also of interest to study thyroxine (T4) turnover in deer from a comparative standpoint. Northern white-tailed deer have been found to have considerably higher circulating serum T4 levels (150-250 ng/ml) than domestic ruminants (30-120 ng/ml). In addition, white-tailed deer are indigenous to areas where, before iodine (I) supplementation, goiter and reproductive problems due to I deficiency were prevalent in domestic animals (McCollum 1957).

MATERIALS AND METHODS

Six fawns approximately 9 months old were allotted into two groups each consisting of 1 female and 2 males. On April 2, 1980 (on-test date) the fawns were moved indoors, weighed and penned by group in adjacent rooms maintained at ambient temperature under simulated natural lighting conditions. Throughout the study one group (fed) was given a complete pelleted diet offered ad libitum (Ullrey et al. 1971), containing 0.5 ppm supplemental I as pentacalcium orthoperiodate. The other group (starved) was provided only with wheat straw for bedding. Straw was provided not only for comfort but also to simulate poor quality roughage which is

commonly consumed by malnourished deer in the wild. All animals were given free access to water which had been supplemented with 1 mg I, as KI, per liter.

After 16 days (April 18, 1980), approximately 150 uCi (5.55 megabecquerels) of ^{131}I-T4 in 50% propylene glycol (New England Nuclear, Boston, MA; purity>95%, specific activity 315 uCi/ug) was injected into the right jugular vein of each fawn (injections were actually subcutaneous in two animals). At the same time, to provide a reference dose, an equal volume of tracer solution was injected into a 1 liter volumetric flask and brought up to volume with 1 M KOH. Blood samples were taken on April 2, immediately prior to ^{131}I-T4 injection, and at 1, 2, 4, 6, 12, 24, 36, 48, 74, and 96 hours post-injection via the left jugular vein. For bleeding, the animals were manually restrained upright using a padded squeeze-chute especially designed for handling deer (Schmitt and Cooley unpublished). Each animal was euthanized 96 hours post-injection using T-61^{R} (National Lab., Somerville, NJ) and weighed. Thyroid glands were immediately removed, trimmed of fat and weighed. Radioactivity in each lobe was counted in a well-type gamma counter and expressed as a percentage of the injected dose. Thyroids were then frozen at -20 C until freeze-dried and analyzed for total I content by neutron-activation analysis (Section I).

All hormone analyses were performed on serum extracted after blood had been allowed to clot overnight at 5 C and had been centrifuged at 1,000 g. Serum samples were frozen at -20 C until analyzed. T4, free T4 (FT4), triiodothyronine

(T3) and reverse T3 (rT3) were determined by radioimmunoassay as detailed in Section I. ^{131}I-T4 kinetics were determined by counting radioactivity in trichloroacetic acid precipitated serum proteins (Katovich et al. 1974) in a well-type gamma counter. Radioactivity in each sample was expressed as a percentage of the injected dose and related to the time after injection using a computerized curve-fitting program (Sedman and Wagner 1976). A bi-exponential model was derived for each deer which described distribution (αphase) and elimination (β phase) of the injected ^{131}I-T4. T4 distribution space (TDS) was calculated as the Y-intercept of the β phase (time zero) divided into 100. The fractional turnover rate (k) was calculated from the slope of the β phase, and the T4 half-life (t $\frac{1}{2}$) was determined by dividing 0.693 by k. T4 secretion (degradation) rate (TSR) and metabolic clearance rate (MCR) were calculated as follows:

$$\text{TSR (ug T4/day)} = \text{TDS(l)} \times \text{T4(ug/l)} \times \text{k/day}$$

$$\text{MCR (ml/day)} = \text{TDS(ml)} \times \text{k/day.}$$

Two TSR estimates were calculated using T4 concentrations measured in serum taken just prior to ^{131}I-T4 injection and at 96 hours post-injection.

On samples collected between 24 and 96 hours post-injection, separation of radioactive serum constituents was performed by thin-layer chromatography following ethanol extraction. Butanol-ethanol-ammonia solvent was used as detailed by Eales (1972). Location of I, T3 and T4 on the chromatograms was verified using ^{125}I labeled standards.

Statistical analysis was performed using an unpaired

t-test (Steel and Torrie 1960).

RESULTS

On April 17 the smallest of the starved fawns died of emaciation. Body weights, weight change and thyroid weights of the remaining fawns are shown in Table 13. As expected, all fed animals gained weight whereas both starved fawns displayed net catabolism. During the latter part of the experiment the starved fawns were reluctant to move about and spent much of their time laying down. Thyroid weights, both absolute and relative to metabolic body size, did not differ significantly between groups. Serum thyronine concentrations and changes in hormone levels over time are shown in Tables 14 and 15, respectively. T4 and T3 dropped precipitously in starved fawns. FT4 levels also declined significantly, albeit to a lesser extent, in response to feed deprivation. Reverse T3 levels did not differ significantly between groups.

Data collected after ^{131}I-T4 injection (April 18) were analyzed both including and excluding deer #3. Unlike the other fawns which had been hand-reared and were accustomed to human activity, fawn #3 had been exposed to a minimum of human contact. This deer was far more skittish and less tractable than the others and when confronted by a person it would begin hyperventilating. On April 20 it fractured a hind leg at the phalango-metatarsal joint while being run into the squeeze-chute. From thereon it was necessary to catch and restrain the fawn by hand for bleeding. The 60% drop in T4 levels during the period of repeated bleedings

Table 13. Sex, weights, weight change and thyroid weights of starved and fed fawns.

Fawn	Sex	Weight (kg) April 2[a]	Weight (kg) April 22[b]	Weight change (kg)	Thyroid weight (g) Fresh basis	Thyroid weight (g) Dry basis	Dry thyroid weight (g) $BW_{kg}^{0.75}$
Fed							
1	M	29.0	35.4	+6.35	3.44	1.11	0.077
2	M	34.9	40.8	+5.89	4.02	1.00	0.062
3	F	35.4	38.8	+3.40	2.37	0.65	0.042
$\bar{x}\pm SD$[c]		33.1 ± 3.5	38.3 ± 2.8*	+5.21 ± 1.6**	3.28 ± 0.84	0.92 ± 0.24	0.060 ± 0.018
$\bar{x}\pm SD$[d]		---	---	---	3.73 ± 0.41	1.06 ± 0.08	0.070 ± 0.011
Starved							
4	M	32.7	29.9	-2.72	3.17	0.80	0.063
5	F	34.9	30.6	-4.31	3.24	0.86	0.066
$\bar{x}\pm SD$		33.8 ± 1.6	20.3 ± 0.5	-3.52 ± 1.1	3.21 ± 0.05	0.83 ± 0.04	0.065 ± 0.002

[a]On-test date.

[b]Termination date.

[c]Mean ± standard deviation; *differs significantly ($P<0.05$) from value for starved fawns;

**($P<0.01$).

[d]Fawn number 3 omitted.

Table 14. Serum T_4, FT_4, T_3 and rT_3 in fed and starved fawns.

	T_4 (ng/ml)			FT_4 (ng/dl)		T_3 (ng/ml)		rT_3 (ng/ml)	
Fawn	April 2[a]	April 18[b]	April 22[c]	April 2	April 18	April 2	April 18	April 2	April 18
Fed									
1	213.6	167.6	162.0	2.23	2.12	1.93	2.28	0.79	0.33
2	238.5	231.2	234.6	2.56	2.28	1.52	2.12	1.26	0.53
3	241.3	187.9	77.7	2.50	2.01	3.32	2.24	0.39	0.23
$\bar{x}\pm SD$[d]	231.1 ± 15	195.6 ± 33*	158.1 ± 79	2.43 ± 0.2	2.14 ± 0.1	2.26 ± 0.9	2.12 ± 0.1**	0.81 ± 0.4	0.36 ± 0.2
$\bar{x}\pm SD$[e]	---	---	198.3 ± 51*	---	---	---	---	---	---
Starved									
4	278.4	98.0	42.4	3.05	1.80	2.90	0.56	0.70	0.74
5	204.7	38.6	30.5	2.12	0.94	3.53	0.41	0.39	0.26
$\bar{x}\pm SD$	241.6 ± 52	68.3 ± 42	36.5 ± 8	2.59 ± 0.7	1.37 ± 0.6	3.22 ± 0.5	0.49 ± 0.1	0.55 ± 0.2	0.53 ± 0.3

[a] On-test date.

[b] ^{131}I-T_4 injection date.

[c] Termination date.

[d] Mean ± standard deviation; * differs significantly ($P<0.05$) from value for starved fawns; ** ($P<0.01$).

[e] Fawn number 3 omitted.

Table 15. Serum T_4, FT_4, T_3 and rT_3 levels in fed and starved fawns expressed as a percentage of prior levels (i.e., 100 denotes no change from previous hormone concentration).

	T_4				
Fawn	April 2 to April 18	April 18 to April 22	FT_4[a]	T_3[a]	rT_3[a]
Fed					
1	78.5	96.7	95.1	118.1	41.8
2	96.9	101.5	89.1	139.5	42.1
3	77.9	41.4	80.4	67.5	59.0
$\bar{x} \pm SD$[b]	84.4 ± 11*	79.9 ± 33	88.2 ± 7*	108.4 ± 37*	47.6 ± 10
$\bar{x} \pm SD$[c]	-	99.1 ± 3	-	-	-
Starved					
4	35.2	43.3	59.0	19.3	105.7
5	18.9	79.8	44.3	11.6	66.7
$\bar{x} \pm SD$	27.1 ± 12	61.6 ± 26	51.7 ± 10	15.5 ± 5	86.2 ± 28

[a]April 2 to April 18.

[b]Mean ± standard deviation; *differs significantly ($P<0.05$) from value for starved fawns.

[c]Fawn number 3 omitted.

(April 18 to April 22) indicates thyroid activity was greatly altered as a result of handling stress.

Serum FT4, T3 and rT3 levels relative to T4 levels and change in these ratios over time are shown in Tables 16 and 17, respectively. FT4/T4 and rT3/T4 ratios increased in starved fawns whereas T3/T4 tended to decrease.

Compartmental analysis and ^{131}I-T4 kinetics are given in Table 18. Injections in fawns #2 and #4 were inadvertantly subcutaneous with first-order absorption occurring in the circulation before the β-phase was reached. K, t $\frac{1}{2}$, and TDV did not differ significantly between fed and starved fawns (Table 19). In fawn #3, k exceeded twice that in the other fawns.

TSR in the starved deer was significantly less than in the fed animals (Table 20). Lower TSR resulted due to lower circulating hormone levels. TSR in fawn #3 was much higher than in the other fed deer owing to the high k. Using the T4 concentration of serum collected on April 22, TSR of fawn #3 approached that of the other fed fawns due to the drop in serum T4 which occured during the handling.

Chromatographic separation data are not presented because they were not considered sufficiently quantitative. Qualitatively, radioactivity on the chromatograms was located primarily at the T4 position with a lesser peak occuring at the I position. A small peak from an unidentified compound was also consistently detected between the origin and the T4 peak. No radioactivity associated with the T3 position was detected.

Table 16. Thyroid hormone ratios in fed and starved fawns.

	$FT_4/T_4 \times 10^5$		$T_3/T_4 \times 10^3$		$rT_3/T_4 \times 10^3$	
Fawn	April 2[a]	April 18[b]	April 2	April 18	April 2	April 18
Fed						
1	10.4	12.6	9.0	13.6	3.7	2.0
2	10.7	9.9	6.4	9.2	5.3	2.3
3	10.4	10.7	13.8	11.9	1.6	1.2
$\bar{x} \pm SD$[c]	10.5 ± 0.2	11.1 ± 1.4*	9.7 ± 3.8	11.6 ± 2.2	3.5 ± 1.9	1.8 ± 0.6**
Starved						
4	11.0	18.4	10.4	5.7	2.5	7.6
5	10.4	24.4	17.2	10.6	1.9	6.7
$\bar{x} \pm SD$	10.7 ± 0.4	21.4 ± 4.2	13.8 ± 4.8	8.2 ± 3.5	2.2 ± 0.4	7.2 ± 0.6

[a]On-test date.

[b]^{131}I-T_4 injection date.

[c]Mean ± standard deviation; * differs significantly ($P<0.05$) from value for starved fawns; ** ($P<0.01$).

Table 17. Serum thyroid hormone ratios in fed and starved fawns expressed as a percentage of prior levels (i.e.,100 denotes no change in thyroid hormone ratio).

Fawn	FT_4/T_4	T_3/T_4	rT_3/T_4
Fed			
1	121.2	151.1	54.1
2	92.5	143.8	43.4
3	102.9	86.2	75.0
$\bar{x} \pm$ SD[a]	105.5 ± 15*	127.0 ± 36	57.5 ± 16**
Starved			
4	167.3	54.8	304.0
5	234.6	61.6	352.6
$\bar{x} \pm$SD	201.0 ± 48	58.2 ± 5	328.3 ± 34

[a]Mean ± standard deviation; * differ significantly ($P<0.05$) from value for starved fawns; ** ($P<0.01$).

Table 18. Compartmental analysis of $^{131}I\text{-}T_4$ distribution and elimination in fed and starved fawns.

Fawn	Model type	Model	R^2
Fed			
1	2 compartment open, IV injection	% dose = $0.028e^{-0.01018t} + 0.01743e^{-0.2703t}$	0.996
2	1 compartment open, 1st order absorp.	% dose = $0.0306e^{-0.00996t} - 0.0189e^{-0.0686t}$	0.956
3	2 compartment open, IV injection	% dose = $0.01964e^{-0.0239t} + 0.01819e^{-0.0818t}$	0.993
Starved			
4	1 compartment open, 1st order absorp.	% dose = $0.029e^{-0.00864t} - 0.0245^{-0.134t}$	0.986
5	2 compartment open, IV injection	% dose = $0.0224e^{-0.0103t} + 0.0085^{-0.0695t}$	0.997

[a]% dose equals the percentage of the injected $^{131}I\text{-}T_4$ dose in 1 ml of serum at t hours post injection.

Table 19. Fractional turnover rate (k), half-life ($t_{½}$) and T_4 distribution volume (TDV) of ^{131}I-T4 in fed and starved fawns.

Fawn	k/day	$t_{½}$ (hr)	TDV (l)	TDV (l/kg)
Fed				
1	0.244	68.1	3.57	0.101
2	0.239	69.6	3.27	0.080
3	0.574	29.0	5.09	0.131
$\bar{x}\pm SD$[a]	0.352 ± 0.19	55.6 ± 23	3.98 ± 0.98	0.104 ± 0.03
$\bar{x}\pm SD$[b]	0.242 ± 0.00	68.9 ± 1	3.42 ± 0.21	0.091 ± 0.02
Starved				
4	0.207	80.2	3.45	0.115
5	0.247	67.4	4.46	0.146
$\bar{x}\pm SD$	0.227 ± 0.03	73.8 ± 9	3.96 ± 0.71	0.131 ± 0.02

[a]Mean ± standard deviation.

[b]Fawn number 3 omitted.

Table 20. Thyroxine secretion rate (TSR) and metabolic clearance rate (MCR) in fed and starved fawns.

Fawn	TSR (ug/day)		TSR / $BW_{kg}^{0.75}$ (ug/day)		MCR (ml/day)
	April 18	April 22	April 18	April 22	
Fed					
1	146.0	141.1	10.06	9.72	871.1
2	180.7	183.3	11.19	11.34	781.5
3	545.7	227.0	35.12	14.61	2921.7
$\bar{x} \pm SD$[a]	290.8 ± 221	183.8 ± 43*	18.8 ± 14.2	11.9 ± 2.5*	1524.8 ± 1211
$\bar{x} \pm SD$[b]	163.4 ± 25*	162.2 ± 30*	10.6 ± 0.8*	10.5 ± 1.2*	826.3 ± 63
Starved					
4	70.0	30.3	5.47	2.37	714.2
5	42.5	33.6	3.26	2.58	1101.6
$\bar{x} \pm SD$	56.3 ± 19	32.0 ± 2	4.4 ± 1.6	2.5 ± 0.2	907.9 ± 274

[a] Mean ± standard deviation; * differs significantly ($P<0.05$) from value for starved fawns.

[b] Fawn number 3 omitted.

Radioactivity and I content of the thyroid glands are shown in Table 21. No significant difference between groups was detected in any parameter although percent dose in the thyroids of fawns #1 and #2 tended to be higher, and I concentration of their thyroids tended to be lower than that of the starved fawns.

DISCUSSION

The dramatic changes observed in thyroid hormone levels in the starved fawns are in accordance with hormone alteration observed in feed-restricted goats (Abdulla and Falconer 1977), calorie-restricted cattle (Blum et al. 1979) and protein-calorie malnourished children (Ingenbleek and Malvaux 1980). Although the low T4 levels would suggest greatly diminished thyroid secretion, decreased T4 turnover was not evident in the starved fawns. Increased fractional turnover rate of T4 in acutely protein-calorie-malnourished children has been attributed to a decreased concentration of circulating T4 binding globulin (Ingenbleek and Malvaux 1980). The high ratio of FT4 to T4 observed in the starved fawns supports their view. It would appear that the acute deprivation of protein may have sharply inhibited hepatic T4 binding protein synthesis and resulted in greater serum FT4 than would have occurred had equilibrium between decreased available binding proteins and circulating T4 been reached. The further decline in T4 levels between April 18 and April 22 indicates that falling serum T4 still had not leveled off even after 16 days of starvation. Whether this was due to a persistent decline in secretion and/or a further decline

Table 21. ^{131}I and ^{127}I content of the thyroids of fed and starved fawns (April 22).

Fawn	Percent dose[a] / Thyroid weight (g,dry)	Total iodine (mg)	Total iodine (mg) / $BW^{0.75}$ kg	Iodine (ppm,dry)
Fed				
1	0.62	8.88	0.61	8,000
2	0.87	6.43	0.40	6,430
3	0.10	6.04	0.39	9.294
$\bar{x}\pm SD$[b]	0.53 ± 0.39	7.12 ± 1.54	0.47 ± 0.13	7,908 ± 1,434
$\bar{x}\pm SD$[c]	0.75 ± 0.18	7.66 ± 1.73	0.51 ± 0.15	7,215 ± 1,110
Starved				
4	0.40	8.46	0.66	10,570
5	0.16	11.04	0.85	12,840
$\bar{x}\pm SD$	0.28 ± 0.17	9.75 ± 1.83	0.76 ± 0.13	11,705 ± 1,605

[a]Percent of injected ^{131}I-T4 dose.

[b]Mean ± standard deviation.

[c]Fawn number 3 omitted.

in binding proteins is not known. In studies with goats and rats, feed restriction has been found to result in decreased fractional turnover of T4 (Yousef and Johnson 1968, Abdulla and Falconer 1977). In these cases, T4 binding proteins and circulating T4 levels may have been proportionately reduced and at, or near, equilibrium. In contrast to these studies, TDV in the fawns also did not appear to be reduced as a result of starvation.

To what extent fecal excretion of T4 may have influenced hormone disposal is not known. Feed deprivation has been found to result in decreased excretion of T4 conjugates in the bile due to reduced fecal mass (Ingbar and Galton 1975, Abdulla and Falconer 1977). It was evident from numerous pellet groups that the starved fawns were ingesting some straw; this may have contributed somewhat to the excretion of T4 and thereby enhanced turnover rate.

The greater decrease observed in serum T3 as opposed to T4 in the starved fawns may have been due to reduced peripheral monodeiodination of T4 (Nathanielsz 1970, Ingbar and Galton 1975, Suda et al. 1977). In mammals, T3 has been found to be several times more metabolically active than T4 (Chopra 1978). Maintenance of rT3 levels in the starved fawns, even though T4 was markedly depressed, may have been the result of a shift in peripheral T4 deiodination away from the T3 pathway (Vagenakis et al. 1975). Reverse T3 is considered to be a relatively inactive T4 metabolite (Chopra 1978).

Starvation-induced hypothyroidism probably played a

critical role in energy and protein conservation and enhanced survivability in fawns #4 and #5. The hypothyroid state is generally associated with reduced metabolic rate and decreased lipid and protein catabolism (Loeb 1978). Recent studies in humans and rats have provided evidence that reduced T3 production in response to starvation can have a significant protein-sparing effect (Vignati et al. 1978, Lancer et al. 1979). Along with reduced T4 secretion, lower serum T3 and T4 levels and decreased peripheral conversion of T4 to T3, a reduction in the number of nuclear T3 receptors (Schusslar and Orlando 1978) appears to be yet another part of an elaborate multileveled adaptive response to starvation in animals.

During early spring, when the present study was conducted, lipid reserves of the fawns would have been at a minimum as a result of reduced feed consumption during winter (Holter et al. 1977). Deer are particularly susceptible to starvation at this time. Bahnak (1978) noted a pronounced drop in T3 and T4 levels in poorly nourished white-tailed does as a result of a one week fast in the spring. Wild deer during late winter have been found to have low T3 and T4 levels similar to those observed in the starved fawns (Section I).

Chromatographic data provided evidence that T4 was the primary radioactive constituent in the blood of the fawns. Absence of detectable radioactive T3 following ^{131}I-T4 injection has also been reported by Katovich et al. (1974) in horses. Hays et al. (1980) suggested that little ^{131}I-T3

can be detected after ^{131}I-T4 injection due to rapid turnover and large distribution volume of T3. The small peak of radioactivity which occured between the origin and T4 peak on the chromatograms may have represented a T4 derivative such as tetraiodothyroacetic acid (Pittman et al., 1980).

With the exception of fawn #3, thyroid weights tended to be less in the starved fawns. ^{131}I concentration of the thyroids also was less in the starved fawns, possibly reflecting a decrease in peripheral T4 deiodination. Total I concentration in the thyroids of the starved deer tended to be higher, however. Apparently, with the provision of supplemental I in the drinking water, the deer thyroid does not lose its ability to trap I during starvation. I uptake by the rat thyroid has been found to be increased during fasting (Catz et al. 1953, Donati et al. 1963).

Based on assumptions used by the NRC (1978) to calculate I requirements of dairy cattle from TSR estimates, I requirements of the fed fawns (#1 and #2) would be approximately 0.2 mg per kg of feed (assumes: average body weight = 37 kg, average daily feed intake = 1.5 kg, average TSR = 4.4 ug T4/kg body weight/day). A previous study (Section I) indicated that 0.26 ppm I in a diet consumed ad libitum was sufficient to support normal reproduction and lactation in white-tailed does.

It was apparent that thyroid function in fawn #3 was greatly altered, presumably due to the stress of handling during repeated bleedings. Stress has been shown to inhibit

thyroid hormone release in rabbits, rats and guinea pigs apparently via suppression of thyrotropin (Gregerman and Davis 1978). In contrast, an increase in protein-bound I in response to stress has been reported in sheep and cattle (Falconer and Hetzel 1964, Post 1965b). The fall in serum T4 along with the extremely high k and MCR in fawn #3 are difficult to explain. It may be that the fawn was drawing off the circulating T4 reservoir and was in a transient hyperthyroid state, even though hormone release by the thyroid gland may have been reduced. These data are of particular interest because they indicate that metabolic status of deer might be significantly altered by prolonged harassment.

Comparison of various T4 parameters from fawns #1 and #2 with those from other species is shown in Table 21a. Although the fawns had considerably higher circulating T4 levels, TSR per metabolic body size was comparable to that of the other species owing to the long $t_{\frac{1}{2}}$ in the deer. It is interesting to speculate that deer, through adaptation, developed a high serum T4 concentration and long $t_{\frac{1}{2}}$ in order to provide a larger extrathyroidal reservoir of T4. This reservoir would help buffer perturbations in T4 secretion and disposal resulting from environmental and nutritional extremes cyclically encountered by deer. Maximizing the size of the T4 reservoir would also increase I storage capabilities of deer. This may be particularly important when I intakes are low during winter (Section I).

Table 21a. Interspecific comparison of thyroxine (T4), T4 distribution volume (TDV), T4 half-life ($t_{½}$) and T4 secretion rate (TSR).

Species	Age[a]	T4 (ng/ml)	TDV (l/kg)	$t_{½}$ (hr)	TSR ($ug/BW_{kg}^{0.75}$)	Ref.
Fawn	10 mo	199.4[b]	0.09	68.9	10.6	Present study
Goat	7 mo	82.8	0.23	26.0	26.7	Abdullah and Falconer 1977
Goat	6 wk-3 yr	65.3[b]	0.10	28.8	8.3	Anderson and Harness 1975
Cow	9-12 mo	88.7[c]	0.06	42.8	6.8	Anderson et al. 1973
Horse	4-6 yr	22.4	0.09	47.5	3.3	Katovich et al. 1974
Pig	6.5 mo	27.4	0.12	25.2	6.7	Marple et al. 1980
Reindeer	<1 yr	73.4[c]	--	72.2	-	Yousef and Luick 1971
Llama (Lama glama)	>1 yr	106.0	0.10	81.5	7.2	El-Nouty et al. 1978
Burro (Equus asinus)	8-10 mo	47.5	0.14	46.9	7.4	El-Nouty et al. 1978

[a]Age in weeks (wk), months (mo) and years (yr).

[b]Serum T4 concentration. All other values determined in plasma.

[c]Calculated from protein-bound iodine. T4 = PBI x 1.53.

SECTION IV

Iodine Concentration in Plants Used by White-tailed Deer in Michigan.

INTRODUCTION

Because (1) supplemental I is easily and routinely provided in the diets of humans and domestic animals, (2) plants do not require I, and (3) analytical techniques for determining I have traditionally been as much art as science, very little is known about the I content of native plants in the Great Lakes region. The historical prevalence of goiter in humans (Olin 1924, McClendon 1939) and domestic animals (McCollum 1957) in Michigan, which has been ameliorated with supplemental I (Trowbridge et al. 1975), indicates a paucity of I in native soils and plants. Ground and surface waters throughout the state also have been found to be very low in I (Eldridge 1924).

Virtually no information is available concerning the I status of deer, or for that matter any other wild animal, in low I regions. Whether I requirements of deer are similar to those of domestic ruminants, but due to different dietary habits deer can obtain adequate I whereas domestic animals cannot, or whether deer have developed physiological mechanisms for existing on extremely low I intakes is not known. It is also possible that

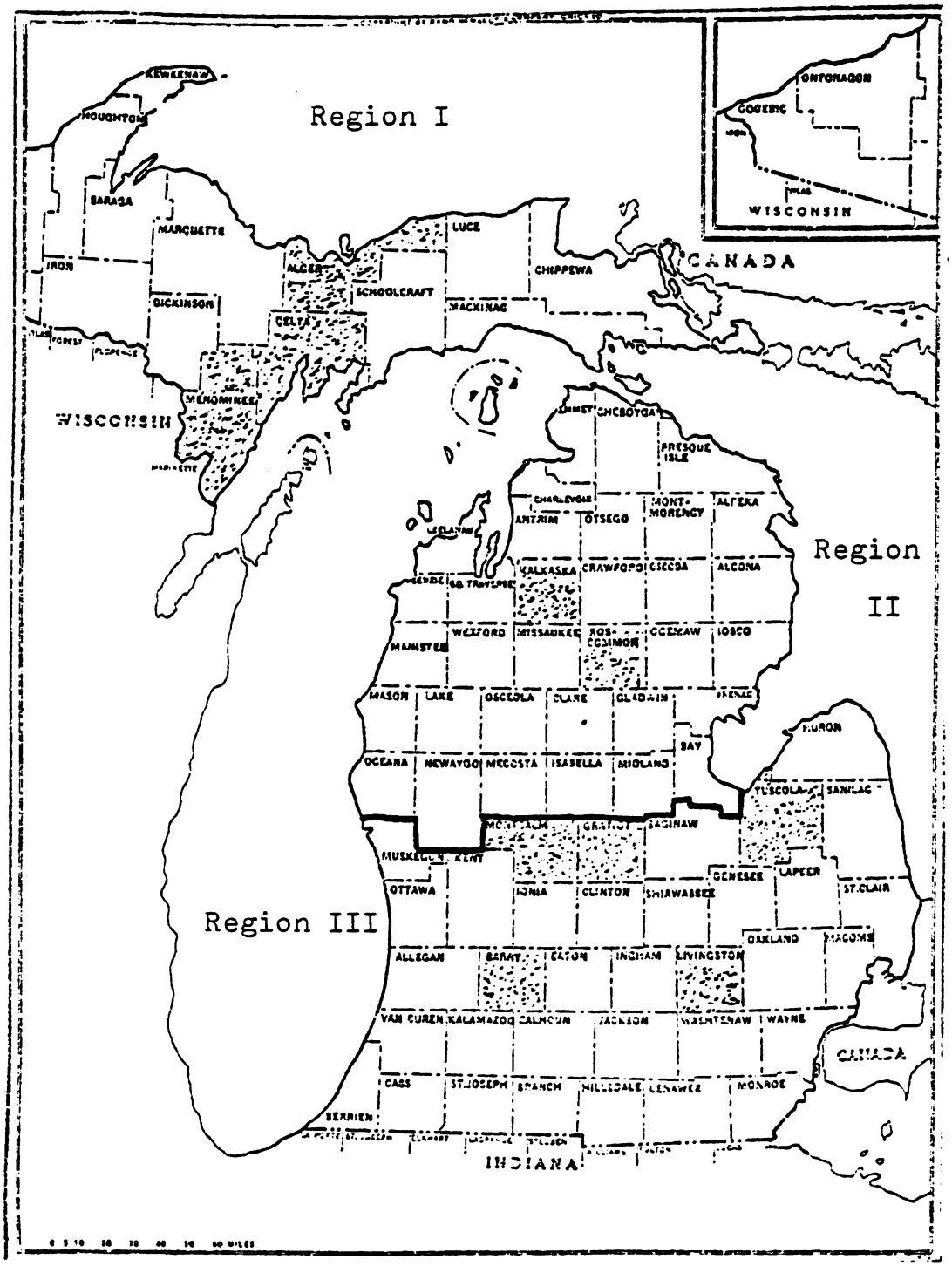
deer do, periodically, suffer from I deficiency. This study determined I concentration in plants commonly used by deer in Michigan and evaluated the results with respect to I feeding-trials conducted with captive deer (Section I). Seasonal and regional differences in I concentration of plants were also examined.

METHODS

Counties where the samples were collected are shown in Figure 2. The state was divided into three regions consistent with the classification used by the Michigan Department of Natural Resources. Region I was the Upper Peninsula; Regions II and III were the northern and southern halves of the Lower Peninsula, respectively. Samples were collected during the first week of February, August and November 1979. In May 1979, samples were collected during the first, second and third weeks in Regions I, II, and III, respectively, in order to account for latitudinal differences. Leaf-fall had occured in all regions when the November samples were gathered; leaf buds were present on all woody samples collected in May.

Within each region, 5 areas, specified by township and section, were selected for collection of each species. In most cases, sampling areas were 3 or more miles from one another. Samples were taken consecutively from the same area. Each sample (usually > 200g wet weight) was a composite of at least 5 individual plants from a given area. All samples were gathered within 5 feet of the ground in areas known to be used by deer. Twig ends of

Figure 2. Michigan counties where plant samples were collected for iodine analysis.


Region I
Region II
Region III
WISCONSIN
CANADA
INDIANA
ONTONAGON
GOGEBIC
HOUGHTON
KEWEENAW
BARAGA
MARQUETTE
IRON
DICKINSON
MENOMINEE
ALGER
SCHOOLCRAFT
LUCE
CHIPPEWA
MACKINAC
DELTA
CHEBOYGAN
PRESQUE ISLE
ANTRIM
OTSEGO
MONTMORENCY
ALPENA
LEELANAU
GRAND TRAVERSE
KALKASKA
CRAWFORD
OSCODA
ALCONA
WEXFORD
MISSAUKEE
ROSCOMMON
OGEMAW
IOSCO
MANISTEE
MASON
LAKE
OSCEOLA
CLARE
GLADWIN
ARENAC
BAY
HURON
OCEANA
NEWAYGO
MECOSTA
ISABELLA
MIDLAND
TUSCOLA
SANILAC
SAGINAW
MUSKEGON
KENT
GENESEE
LAPEER
OTTAWA
IONIA
CLINTON
SHIAWASSEE
ST.CLAIR
OAKLAND
MACOMB
ALLEGAN
BARRY
EATON
INGHAM
LIVINGSTON
VAN BUREN
KALAMAZOO
CALHOUN
JACKSON
WASHTENAW
WAYNE
CASS
ST.JOSEPH
BRANCH
HILLSDALE
LENAWEE
MONROE
BERRIEN

woody species were taken to a diameter of 3.5 mm. Deciduous leaves, including petioles, were analyzed separately from twigs. All above-ground parts of herbaceous species, unless otherwise specified, were collected. Samples were placed in plastic bags with a minimum of handling, and stored at -20 C until they could be freeze-dried and ground in a Wiley mill.

Iodine analysis was performed using the method described in Section V.

Two-way analysis of variance was used to examine seasonal effects within regions (Steel and Torrie 1960). Regional differences were examined using a one-way analysis of variance or an unpaired t-test; a paired t-test was used for comparing leaves and twigs (Steel and Torrie 1960). Mean comparisons were made using Tukey's procedure (Steel and Torrie 1960).

Scientific names of the plants are provided in Appendix Table A1.

RESULTS

I concentration of largetoothed aspen twigs tended to be highest during February and May, lowest during August and intermediate in November (Table 22). Leaves were generally higher in I than August twigs. Region III samples tended to be higher in I than those from Regions I and II. With the exception of Region III, red-osier dogwood followed a seasonal pattern similar to that of aspen (Table 23). Region III samples were very consistent across seasons. Leaves tended to be higher than August

Table 22. Effect of season and region on the iodine concentration of largetoothed aspen (ppb, dry basis).

	TWIGS										LEAVES	
	Feb			May			Aug		Nov		Aug	
Region	N	$\bar{x} \pm$ SE	Range	N	$\bar{x} \pm$ SE	Range	N	$\bar{x} \pm$ SE	Range	N $\bar{x} \pm$ SE	Range	N $\bar{x} \pm$ SE	Range
I	-	--	-	4	77 ± 7^{a}	63-94	5	40 ± 7^{b}	25-64	5 53 ± 9^{bc}	20-68	5 48 ± 4^{c}	38-63
II	5	82 ± 5^{a}	70-101	5	70 ± 9^{ab}	35-90	5	49 ± 9^{b}	31-85	5 58 ± 3^{abc}	51-66	5 120 ± 47^{cde}	65-309
III	4	111 ± 11	84-131	5	91 ± 8	63-116	5	76 ± 16	31-131	5 86 ± 7^{d}	60-101	5 165 ± 27^{de}	101-233

[ab]Row means having different superscripts differ significantly ($P<0.05$).

[cd]Column means having different superscripts differ significantly ($P<0.05$).

[e]Leaves differ significantly ($P<0.05$) from August twigs.

Table 23. Effect of season and region on the iodine concentration of red-osier dogwood (ppb, dry basis).

	Twigs												Leaves		
	Feb			May			Aug			Nov			Aug		
Region	N	$\bar{x} \pm$ SE	Range	N	$\bar{x} \pm$ SE	Range	N	$\bar{x} \pm$ SE	Range	N	$\bar{x} \pm$ SE	Range	N	$\bar{x} \pm$ SE	Range
I	-	--	--	5	154 ± 50	72-338	5	36 ± 6[c]	21-57	5	35 ± 10[c]	17-67	5	53 ± 8[e]	30-75
II	5	82 ± 7[a]	62-103	5	76 ± 5[ab]	63-92	5	36 ± 4[bc]	29-48	5	58 ± 7[bcd]	37-73	5	156 ± 56[e]	51-353
III	4	99 ± 19	62-152	5	100 ± 3	89-106	5	101 ± 20[d]	59-105	5	100 ± 20[d]	64-162	5	196 ± 73	76-481

[ab]Row means having different superscripts differ significantly ($P<0.05$).

[cd]Column means having different superscripts differ significantly ($P<0.05$).

[e]Leaves differ significantly ($P<0.05$) from August twigs.

twigs. During August and November red-osier dogwood twigs collected in Region III tended to have more I than twigs from Regions I and II. I concentration of white clover also was generally lowest during August (Table 24). No regional effect was evident. I content of strawberry, collected in Region II, was similarly lowest in August (Table 25). Region III samples, however, tended to be highest in August. Strawberry plants collected in Region II during May and November were higher in I than those collected in Region III. Mountain maple, red maple, blueberry and alfalfa all tended to be highest in I in February and May and lowest in August (Table 26). Wintergreen and thornapple tended to be highest in February and lowest in November. With the exception of blueberry, I was generally higher in leaves than August twigs.

The highest I concentrations were found in aquatic species (Table 27). The lowest concentration was found in thornapple fruit and corn grain.

DISCUSSION

Seasonal Differences

I concentration of most species was highest during winter and spring, decreased during summer and increased again in autumn. Similar seasonal patterns in I concentrations have been reported for Welch pasture grasses (Alderman and Jones 1967), for orchardgrass (Dactylis glomerala) in West Virginia (Horn et al. 1974), and for ryegrass (Lolium perenne) pasture in the Netherlands (Hartmans 1974). Seasonal variation may be due to

Table 24. Effect of season and region on the iodine concentration of white clover (ppb, dry basis).

	May			August			November		
Region	N	$\bar{x} \pm$ SE	Range	N	$\bar{x} \pm$ SE	Range	N	$\bar{x} \pm$ SE	Range
II	5	132 ± 23^{ab}	70-190	5	88 ± 19^{b}	50-110	5	240 ± 58^{a}	110-381
III	4	156 ± 62	67-336	4	101 ± 24	65-168	4	169 ± 51	99-321

[ab]Row means with different superscripts differ significantly ($P<0.05$).

Table 25. Effect of season and region on the iodine concentration of strawberry (ppb, dry basis).

	May			August			November		
Region	N	$\bar{x} \pm$ SE	Range	N	$\bar{x} \pm$ SE	Range	N	$\bar{x} \pm$ SE	Range
II	5	241 ± 25^{ac}	182-325	5	121 ± 26^{b}	75-215	5	232 ± 24^{ac}	158-295
III	5	81 ± 15^{d}	47-132	5	141 ± 37	79-276	5	128 ± 13^{d}	112-180

[ab]Row means with different superscripts differ significantly ($P<0.05$).

[cd]Column means with different superscripts differ significantly ($P<0.05$).

Table 26. Effect of season on the iodine concentration of selected Michigan deer foods (ppb, dry basis).

	Twigs												Leaves		
	Feb			May			Aug			Nov			Aug		
Species	N	$\bar{x} \pm$ SE	Range	N	$\bar{x} \pm$ SE	Range	N	$\bar{x} \pm$ SE	Range	N	$\bar{x} \pm$ SE	Range	N	$\bar{x} \pm$ SE	Range
Region I															
Mountain maple	-	--	--	5	68 ± 4	58-80	5	37 ± 10	13-63	5	69 ± 13	57-121	5	71 ± 13[c]	50-120
Red maple	-	--	--	4	70 ± 10	42-88[a]	5	38 ± 6[b]	18-51	-	--	--	5	71 ± 7[c]	49-87
Region II															
Blueberry	5	178 ± 24[a]	84-213	5	137 ± 7[ab]	117-161	5	93 ± 11[b]	62-120	5	136 ± 34[ab]	56-247	5	62 ± 9	43-93
Wintergreen[d]	5	128 ± 30	80-247	5	98 ± 15	74-154	5	103 ± 27	67-207	5	91 ± 6	72-104	-	--	--
Region III															
Alfalfa[d]	4	418 ± 148[a]	158-836	5	116 ± 45[ab]	49-292	5	84 ± 26[b]	43-197	4	107 ± 20[b]	56-153	-	--	--
Thornapple	4	207 ± 44	121-313	5	201 ± 55	98-411	5	148 ± 20	78-200	5	134 ± 14	95-167	5	181 ± 39	101-311

[ab]Row means with different superscripts differ significantly ($P<0.05$).

[c]Leaves differ significantly ($P<0.05$) from August twigs.

[d]Includes all parts above the ground.

Table 27. Iodine concentration of seasonally important deer foods (ppb, dry basis).

Species	Month	Iodine conc.
Region I		
Bush - honeysuckle	Aug	31
Sugar maple	Feb	220
Northern white cedar	Feb	96
Region II		
Quaking aspen leaves	Aug	80
Smartweed	Aug	2,280
Coontail	Aug	1,660
Spirogyra	Aug	3,100
Yellow water lily	Aug	270
Pondweed	Aug	1,520
Fine Sedge	Aug	85
Red clover	Aug	17
Northern white cedar	Feb	140
Staghorn sumac	Feb	190
Bracken fern (fiddle heads)	May	26
White oak acorns[a]	Nov	52
Red oak acorns[a]	Nov	32
Hills oak acorns[a]	Nov	35
Region III		
Jewelweed	Aug	175
Thornapple fruit	Aug	8
Corn grain	Feb	9
Rye	May	69
Soybeans and hulls	Nov	28

[a]Includes meat and shell but excludes cups.

a dilution effect caused by summer growth. This is exemplified by the decremental effect increasing yield with nitrogen fertilization has on I concentration of grasses (Alderman and Jones 1967, Hartmans 1974). Low I concentration of August twigs may have been due primarily to the increase in biomass resulting from foliation.

Regional Differences

Iodine concentrations of red-osier dogwood and large-toothed aspen samples were typically highest in Region III. Eldridge (1924) found I concentration of ground water supplies to generally be highest in that part of Michigan which corresponds with Region III. No consistent regional differences were found in white clover or strawberry. Wide variability in the concentration of other elements in these species (Watkins et al. unpublished) suggests local site factors may have been important determinants of elemental composition.

I is not required by higher plants, therefore its uptake is secondary to that of vital nutrients. A variety of factors can influence the I content of plants. A relationship between soil I levels and plant I levels has been clearly demonstrated by amending soils with I (Hartmans 1974). I content of the soil is determined by I content of parent rock, rainfall, contribution of marine aerosols, degree of vegetative recycling, industrial contamination and edaphic properties, as well as other factors (Shacklette and Cuthbert 1967, Aston and Brazier 1979, Whitehead 1979). Retention of I by soil against the

effects of leaching and, possibly, volatilization has been found to be related to the occurrence of aluminum and iron oxides, organic matter and soil pH (Whitehead 1978, Aston and Brazier 1979, Whitehead 1979). Soil I concentration and I content of corresponding vegetation often show little relationship (Newton and Toth 1951, Whitehead 1979). A number of factors have been found to influence the availability of I in soil, including the chemical form of the I, pH and organic matter (Whitehead 1975). In addition to uptake through the soil, plants can apparently accumulate I directly from the atmosphere (Shacklette and Cuthbert 1967, Whitehead 1979).

Interspecific Differences

There is little published information on the I content of woody plants. In the present study, twigs were found to generally be low in I (13-338 ppb); leaves were somewhat higher (30-481 ppb). Newton and Toth (1951) found leaves from trees native to New Jersey, which is not considered a low I area, contained 100-1,220 ppb. Gist and Whicker (1971) reported the I content of 6 important browse species used by mule deer in Colorado ranged between 700 and 1,100 ppb. Shacklette and Cuthbert (1967) reported coniferous trees and deciduous trees, of unspecified origin, contained 2,900-6,900 and 1,100-6,200 ppb, respectively. It is believed, however, that the analytical method used by these last authors grossly overestimated true I concentration.

Terrestrial herbaceous species (17-836 ppb) tended to be higher in I than woody plants, although there was considerable variation. I concentrations were comparable to those reported by Hartmans (1974) for white clover (170 ppb) and assorted pasture grasses (60-140 ppb) in the Netherlands, Whitehead (1979) for ryegrass (<300 ppb) in England, Alderman and Jones (1967) for assorted pasture grasses (80-480 ppb) in Wales, Horn et al. (1974) for orchardgrass (50-650 ppb) in West Virginia, and Hemken et al. (1971) for corn silage (340-700 ppb) and hay (620-1,020 ppb) in Illinois. Values reported by Shacklette and Cuthbert for plants in Wisconsin are much higher (2,200-10,000 ppb). Although no grasses were analyzed in the present study, dicotyledons have generally been found to be higher in I than grasses (Hartmans 1974).

Aquatic species had the highest I concentrations of all the plants tested (85-3,100 ppb). Very little published information is available on the I content of fresh-water plants. McClendon (1939) reported fresh-water algae from Switzerland, a goitrous area, contained 340-8,350 ppb I. Shacklette and Cuthbert (1967) reported 3,000 to 6,200 ppb I in green algae - values which did not differ appreciably from those of land plants also reported.

Consistent with previous reports (McClendon 1939), fruits, nuts and grains were found to be very low in I (8-52 ppb).

Deer Nutrition Perspectives

Overall, the deer foods analyzed were neither clearly deficient in I nor were they clearly adequate relative to what is known about the I requirements of deer and what can be surmised from domestic ruminant data. As reported previously (Section I) 0.26 ppm in a diet offered ad libitum has been found to be adequate for normal reproduction and lactation in captive white-tailed does. It is possible that free-ranging deer in Michigan could consume a comparable amount of I between spring and fall. At this time leaves and herbaceous plants such as white clover and strawberry, which generally contain more I than twigs, are available. In addition, aquatic plants may be important sources of I for deer. Deer have been observed to eat each of the aquatic species analyzed (Fassett 1966).

It is somewhat paradoxical that, although I concentration of most of the plants was greatest during winter, this is probably the time when I intakes of deer in northern Michigan are lowest. Only browse species, which were low in I, would be available to deer during winter. Also, I consumption would be decreased as a result of reduced feed intake. Northern white cedar, a very important winter food in parts of northern Michigan, contained 96-140 ppb I. Other browse species during winter typically contained less than 200 ppb.

SECTION V

A Method for the Determination of Microquantities of I in Plant Samples

INTRODUCTION

Due to their sensitivity in the parts per billion range, neutron-activation analysis and spectrophotometric quantification of the iodide-catalyzed reduction of Ce IV by As III have been the most widely used methods for determining the I content of plants (Binnerts and Das 1974). Although both methods have been used for years to determine I, there are still no standard, fully accepted procedures for either method. Extreme variability in results obtained between laboratories performing I analyses, as well as poor precision within laboratories, are persistent problems (Heckman 1979).

In order to measure the I content of plants used by white-tailed deer in Michigan, a Ce-As method was developed utilizing a two-stage alkaline ashing procedure.

METHOD

Reagents

J. T. Baker Analyzed reagent grade acids (J.T. Baker Chemical Co., Phillipsburg, NJ) and deionized, distilled water (DDH_2O), exclusively, were used for reagent preparation. All glassware was acid-washed, soaked in 2 M KOH and rinsed thoroughly with DDH_2O. Arsenious acid (As-R)

and ceric ammonium sulfate (Ce-R) reagents were adapted from Wilson and van Zyl (1967).

As-R.— Dissolve 2.475 g As_2O_3 in 25 ml 1 M KOH with the aid of low heat. Combine with approximately 600 ml DDH_2O in a liter volumetric flask followed by 54 ml concentrated HCl. Gradually add 170 ml concentrated H_2SO_4. Allow to cool to room temperature and adjust final volume to 1 l.

Stock Ce-R.— Gradually add 45 ml concentrated H_2SO_4 to approximately 600 ml DDH_2O in a liter volumetric flask. Dissolve 9.5 g $(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$ (G. Frederick Smith Chem. Co., Columbus, OH), cool to room temperature and adjust the final volume to 1 l.

Working Ce-R.— Dilute Ce-R (stock) such that the absorbance of the 0 standard is between 0.9 and 1.0 (approximately 1.5:10 DDH_2O).

Stock iodide solution (100 ug/ml).— In a liter volumetric flask, dissolve 130.8 mg KI (dry basis) in 10 ml 0.1 M NaOH and bring to volume with DDH_2O. Store at 5 C in the dark; replace bi-monthly.

Working iodide solution (5 ug/ml).— Dilute 5 ml stock I solution to 100 ml. Store at 5 C in the dark; replace bi-weekly.

Stock 4 M KOH solution.— Dissolve 264 g of 85% KOH in 1 l DDH_2O. All other KOH solutions are made from this stock.

Procedure

1. Weigh 0.5 g of dry sample into a 40 ml, heavy-duty centrifuge tube (Corning8400) and saturate with 5 ml 0.5 M KOH.
2. Spike a duplicate sample with 0.25 ug I (50 ul working I solution) for calculation of recovery.
3. Place the tubes in a Zn-Cr coated rack and rinse the sides of each tube with a small amount of DDH_2O. Cover loosely with aluminum foil and dry at 95-100 C.
4. After drying, cap each tube with a porcelain crucible cover (Coors 24003) and place the racks upright in a cold muffle furnace. Gradually bring the temperature to 250 C (approximately 45 minutes) and ash at 250 C for 2 hours, 350 C for 2 hours and 500 C for 4 hours. Do not vent the furnace with air.
5. After ashing, 1 ml of 1 M KOH + 1% KNO_3 is added to each tube. Samples are again dried and capped, and ashed for 5 hours at 550 C in a preheated muffle furnace. Again the furnace is not vented.
6. Allow to cool and add 5 ml DDH_2O, washing down the sides of the tubes. Thoroughly break-up and mix the ash with a quartz rod. Centrifuge at 1300 g for 15 minutes.
7. Without entraining carbon particles, transfer 1 ml of supernatant to a 13 x 100 mm Pyrex test tube and add 4 ml DDH_2O. To each test tube, gradually add 1 ml As-R down the side of the tube to prevent excessive evolution of CO_2.
8. Cover the samples with plastic film and store

overnight at 5 C.

9. Standards equivalent to 0, 0.125, 0.25 and 0.5 ppm I are used. To prepare, dilute 0, 25, 50 and 100 ul of working I solution to 5 ml with DDH_2O. Mix and transfer 1 ml to 13 x 100 mm Pyrex tubes containing 0.5 ml 1.4 M KOH. Dilute to 5 ml. Add 1 ml As-R, cover and store overnight at 5 C as described previously.

10. After refrigeration, place the samples and standards in a water-bath and allow to equilibrate at 37 C. Add 1 ml working Ce-R to 6 tubes at time 0. Cover each tube with Parafilm (American Can Co., Greenwich, CT) and invert 3 times. Immediately return the tubes to the water bath. Repeat this procedure every 2 minutes such that Ce-R will be added to 30 tubes after 10 minutes.

11. At exactly 10 and 20 minutes following Ce-R addition, absorbance readings are taken at 360 nm using a spectrophotometer equipped with a vacuum operated, flow-thru cuvette. Absorbance is taken as the highest reading which immediately precedes a steady decline.

12. Calculate Δ log A_{360} between 10 and 20 minute readings. Linear regression is used to determine the standard curve. Samples are adjusted for weight (g), recovery, and for the amount of I determined in reagent blanks, which have been carried through the entire procedure, as follows:

$$\text{Sample ppm} = [(Y/g)/\text{Recov.}] - \text{Blank ppm},$$

where Y is the value predicted from the standard curve.

RESULTS AND DISCUSSION

Precision and recovery of the method as determined on a sample of dehydrated alfalfa meal are shown in Table 28. The method showed satisfactory reproducibility and virtually total recovery of added I, as KI and thyroxine.

A number of factors were found to influence the method. With plant samples, no problem with cross-contamination was evident. Contamination of reagents also was not a problem. Blanks consistently contained less than 10 ppb I. I loss during the preparatory phase and uncontrolled factors influencing Ce IV reduction were the primary problems which required circumvention.

Factors influencing I loss.— As detailed by Foss et al. (1960) and Binnerts and Das (1974), it was necessary to raise the temperature of the muffle furnace gradually during the first ashing in order to reduce I volatilization. Contrary to Lauber (1975), and in agreement with Binnerts and Das (1974) and Bellanger et al. (1979), admitting air into the furnace, especially during the rapid decomposition phase, was found to cause significant I loss and is not recommended. Capping the tubes as well as restricting air entry into the furnace, was found to result in a slight further improvement in recovery, possibly due to a greater reduction in air flow into the tubes.

KOH helps retain I in samples during drying and ashing, possibly by preventing formation of volatile HI (Binnerts and Das 1974). As more KOH was added to the samples, there was less oxidation of carbon. When 5 ml of 0.1 M KOH was

Table 28. Determination of iodine in dehydrated alfalfa meal: precision and recovery.

Item	Value
Sets[a]	4
Replications/set	5
Mean ± standard error (ppm)	0.083 ± 0.001
Range	0.070 - 0.091
Coefficient of variation (%)	6.87
Mean recovery ± standard error (%)[b]	
+ 0.125 ug as KI	99.6 ± 2.2
+ 0.25 ug as KI	99.6 ± 0.6
+ 0.125 ug as thyroxine	102.3 ± 1.7
+ 0.25 ug as thyroxine	96.1 ± 1.7

[a]The sets were run over a 4 week period.

[b]New tubes were used to determine recoveries.

added before the first ashing, there was very little residual C; with 0.5 M KOH there was considerable C and recoveries were generally 8-12% higher.

Recoveries of added I were 5-10% less when old versus new centrifuge tubes were used for ashing. KOH etches glass tubes at high temperatures. Lower recoveries with the old tubes may be due to adsorption of I to the glass. Foss et al. (1960) found badly-etched tubes retained up to 10% of added ^{131}I. After adjusting for recovery, old and new tubes were found to yield very similar results. Old tubes were used, therefore, until they cracked (6-10 runs), but were kept segregated depending on the degree of etching so appropriate recoveries could be calculated. In order to reduce the likelihood of I adsorption to glassware throughout the method, Pyrex glassware and quartz stirring rods were used in all cases.

Factors influencing Ce IV reduction.— One of the foremost limitations of the Ce-As method is the sensitivity of the reaction to factors other than I. A number of these interferences have been reviewed (Rodriguez and Pardue 1969, Binnerts and Das 1974). Interfering elements such as Os, Ru, Hg, and Ag are generally not a problem because they do not occur to a significant extent in plant samples. Cl ions are provided in excess, as HCl, to cancel out possible interference by sample Cl. K ions depress the rate of Ce IV reduction, therefore it was undesirable to add excess KOH to the samples for ashing. It is necessary that the same amount of KOH be added to the standards as is added

to the samples. Under the conditions specified for the method, 0.5 ml of 1.4 M KOH, equivalent to that in a 1 ml sample aliquot, resulted in the steapest possible curve that was linear to 0.5 ppm. When less KOH was used the reaction would no longer be first-order after 20 minutes for the 0.5 ppm standard. If dilutions are made it is necessary to adjust KOH accordingly.

Several researchers have favored obtaining a C-free ash (Binnerts 1954, Foss et al. 1960, Riesco et al. 1976). Foss et al. (1960) reported C could accelerate Ce IV reduction. In the present method, C in the ash was found to be desirable not only because recoveries were higher, but also because there were fewer interference problems. After centrifugation, an aliquot of supernatant can be extracted which is essentially free of C and has an A_{360} similar to that of a DDH_2O blank. Authors recommending a C-free ash describe a white-ash endpoint. Green, blue, pink and brown-ash endpoints, as well as white, were obtained when a C-free ash was used in the present study. The most common interference encountered when using a C-free ash was Mn. Plants such as blueberry, wintergreen and strawberry were found to be high in this element, and I determinations were elevated considerably due to the accelerating effect Mn has on Ce IV reduction. When 5 ml 0.5 M KOH was used for the first ashing, resulting in residual C, the Mn problem disappeared.

KNO_3 is added to help insure release of organically-bound I. Residual KNO_3 can cause rapid Ce IV reduction.

It is desirable to add a small amount (0.1-0.2 g) of starch to the reagent blanks during the second ashing to break down residual KNO_3. I determinations performed on duplicate samples ashed with and without KNO_3 were very similar, indicating KNO_3 may not be necessary. Omission of KNO_3 has been suggested by Jones et al. (1979).

As demonstrated by the effect of KNO_3 and Mn it is desirable to use a rate measurement, i.e., Δ log A_{360}, as opposed to a single time measurement, to avoid error resulting from the occurrence of non-specific reducing agents.

Overnight refrigeration prevented bubbles from forming when the samples were aspirated into the spectrophotometer. Although very little change in I activity was noted as a result of 24 hour storage, it is considered desirable to store the standards along with the samples.

Examples of typical I recoveries determined for various species during routine analysis are shown in Table 29. Using a combination of old and new tubes, recoveries were generally between 85 and 95%.

Table 29. Recovery of 0.25 ug iodine from various plant samples.

Sample	N	Mean ± standard error (%)
Red-osier dogwood twigs	17	90.4 ± 1.4
Blueberry twigs	8	93.1 ± 1.9
Blueberry leaves	2	91.4 ± 1.0
Largetoothed aspen twigs	14	86.8 ± 2.7
Largetoothed aspen leaves	5	76.3 ± 3.0
Corn grain	3	94.7 ± 0.4
Soybean meal	2	94.5 ± 0.6
Strawberry plant	7	93.8 ± 1.3
Thornapple twigs	6	88.8 ± 3.1
Mountain maple twigs	6	82.5 ± 1.5
White clover	12	93.9 ± 1.9
Northern white cedar	2	94.5 ± 1.9

CONCLUSIONS

1. Assuming goitrogenic compounds are not present, 0.26 ppm I (dry basis) in a diet offered adlibitum is considered adequate for all phases of the life cycle in white-tailed deer.

2. Serum T4 and FT4 appear to follow a consistent circannual pattern in juvenile and adult does. High levels occur in early winter and again in spring, and low levels occur during summer, fall and late winter. Changes in serum T4 and FT4 may be related to cyclic changes in feed consumption, body weight and composition, and ambient temperature. A lack of consistent seasonal variation in serum T3 may be due to short-term fluctuations in the circulation.

3. Suckling and weaned fawns appear to have higher serum T3 and T4 levels than adults.

4. Nonlactating does appear to have higher serum T3 and T4 levels than lactating does.

5. Very low serum T3, T4 and FT4 levels, large thyroid size and markedly reduced thyroid I concentration in wild deer versus well-nourished captive deer, probably resulted from the combined effects of malnutrition and incipient I deficiency.

6. Even during moderate feed restriction, 0.28 ppm I in the diet appears to be adequate for growing fawns.

7. Moderate feed restriction may not affect the I concentration of the thyroid or serum T3, T4 and FT4 levels in deer, even though weight gain and thyroid weight are significantly reduced. Feed restriction and poor diet quality may affect the thyroid in different ways.

8. Starvation in fawns causes a dramatic drop in serum T3, T4 and, to a lesser extent, FT4; serum rT3 does not change appreciably. Fractional turnover rate, distribution volume and metabolic clearance rate of T4 may not differ between fed and starved fawns even though T4 secretion rate in starved fawns is greatly reduced. Changes in thyroid activity probably play a critical role in energy and protein conservation during starvation.

9. Thyroid hormone profiles similar to those observed in wild deer during late winter can be produced experimentally by starvation. In contrast to observations on wild deer, however, starvation appears to reduce the size of the thyroid gland and causes no decrease in thyroid I concentration.

10. Prolonged stress may have profound effects on thyroid activity in deer.

11. Most plants analyzed appeared to be highest in I during winter and spring, and lowest during summer, possibly due to a dilution effect resulting from summer growth.

12. Overall, the deer foods examined were neither clearly deficient nor clearly adequate in I. It is believed

that deer could probably consume sufficient I when herbaceous and aquatic plants and leaves are available. I intakes are probably lowest during winter due to the low I concentration in twigs and due to reduced feed consumption.

13. In the analytical procedure developed for I, recoveries of added I were found to be higher when the furnace temperature was raised gradually during the first ashing, when air-flow into the furnace was restricted, when the incineration tubes were capped, when 0.5 M versus 0.1 M KOH was used for the first ashing, and when new versus old tubes were used. Interfering factors were found to be minimized by using enough KOH to retain carbon in the final ash.

APPENDIX

APPENDIX

Table A1. Common and scientific plant names.

Common Name	Scientific Name
Alfalfa	(*Medicago sativa*)
Aspen, largetooth	(*Populus grandidentata*)
Aspen, quaking	(*Populus tremuloides*)
Blueberry	(*Vaccinium* sp.)
Bracken fern	(*Pteridium aquilinum*)
Bush-honeysuckle	(*Diervilla lonicera*)
Cedar, northern white	(*Thuja occidentalis*)
Clover, red	(*Trifolium pratense*)
Clover, white	(*Melilotus alba*)
Coontail	(*Ceratophyllum demersum*)
Corn	(*Zea mays*)
Dogwood, red-osier	(*Cornus stolonifera*)
Jewelwood	(*Impatiens* sp.)
Maple, mountain	(*Acer spicatum*)
Maple, red	(*Acer rubrum*)
Maple, sugar	(*Acer saccharum*)
Oak, hills	(*Quercus ellipsoidalis*)
Oak, red	(*Quercus rubra*)
Oak, white	(*Quercus alba*)
Pondweed	(*Potamogeton* sp.)
Rye	(*Secale cereale*)
Sedge, fine	(*Salix* sp.)
Smartweed	(*Polygonum* sp.)
Soybean	(*Glycine max*)
Spirogyra	(*Spirogyra* sp.)
Strawberry	(*Fragaria virginiana*)
Sumac, staghorn	(*Rhus typhina*)
Thornapple	(*Crataegus* sp.)
Waterlily, yellow	(*Nuphar* sp.)
Wintergreen	(*Gaultheria procumbens*)

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