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Thyroid Hormone Resistance in Hibernating Ground Squirrels, *Spermophilus richardsoni*

I. Increased Binding of Triiodo-L-thyronine and L-Thyroxine by Serum Proteins

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Thyroid function was examined during the annual cycle of Richardson's ground squirrel, *Spermophilus richardsoni*. A number of facets were measured to facilitate comparison of thyroid function in active, dormant, and aroused animals. This report discusses changes in the serum thyroid hormone and binding as assessed by radioimmunoassay, equilibrium dialysis, and competitive binding assays. During the hibernation phase (both dormant and aroused), total serum T_3 (triiodo-L-thyronine) and T_4 (L-thyroxine) are elevated over active levels, two- to fivefold and four- to sixfold, respectively. However, in dormant squirrels, both free T_3 and free T_4 are reduced compared with both active and aroused phases of the annual cycle, while in aroused squirrels there is an increase in free T_3 but no change in free T_4 compared with active squirrels. The difference between changes in total and free thyroid hormone levels in the three groups is due to changes in serum binding of thyroid hormone. There is a more than twofold increase in the capacity of a saturable T_3-binding site in serum of both dormant and aroused squirrels, and there is an increase in serum binding affinity at the low core temperature of dormant squirrels (6°). Therefore, even though serum total T_3 and T_4 are elevated during dormancy, free T_3 and T_4 levels are reduced to half of the levels in active squirrels as a consequence of increased serum binding capacity and affinity. In aroused animals, however, increased serum binding capacity only partially buffers the increase in total T_3 and T_4, so that free thyroid hormone levels exceed those of active squirrels.

Richardson's[1] ground squirrel, *Spermophilus richardsoni,* is a seasonal hibernator. Its annual cycle is divided into two phases, a summer active phase, during which the animals reproduce and accumulate fat, and a hibernation phase. The latter phase is composed of alternating dormancy and arousal bouts.

Differences in hibernation patterns among species reflect varied adaptations to diverse habitats (reviewed by Hudson and Wang, 1979; Hudson, 1981; Wang, 1982). From many earlier studies, it was concluded that thyroid function is depressed during the dormancy phase in hibernators. Our studies of thyroid function in Richardson's ground squirrel were stimulated by the surprising finding that the circulating levels of the thyroid hormones are sharply increased during the hibernation phase (both dormancy and arousal) compared with the concentrations in active animals at any time of the year (Demeneix and Henderson, 1978a). In hibernating ground squirrels, serum levels of total triiodo-L-thyronine (T_3) and L-thyroxine (T_4) are two to seven times the values in active animals. Fluctuations in hormone concentrations occur within dormancy and arousal bouts, but the minimal levels are at least double those in active animals (Demeneix and Henderson, 1978a). Subsequent work showed the elevated hormone levels in hi-

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bernators were the consequence of greatly reduced clearance rates (Demeneix and Henderson, 1978b) and continued, albeit reduced, hormone synthesis and secretion (Winston and Henderson, 1981). In woodchucks, *Marmota monax*, Young and her colleagues found the circulating levels of T_3 and T_4 were maximal in midhibernation (Young *et al.*, 1979, personal communication).

Thyroid hormones are prime regulators of metabolic rate and influence numerous aspects of carbohydrate, lipid, and protein metabolism. In dormancy bouts, physiological functions are radically depressed and the animals are severely hypometabolic. Wang (1979) reported minimum core temperatures of *S. richardsoni* between 1 and 4° with minimum ambient (burrow) temperature ranging from 0 to −2°. During arousal bouts, following resumption of euthermic core temperatures, ground squirrels are lethargic and their resting rates of oxygen consumption do not exceed those of active animals over an ambient temperature range of 18 to 34° (Huestis *et al.*, unpublished observations). Thus in hibernating ground squirrels, particularly during dormancy periods, the animals are unresponsive to the increased circulating levels of T_3 and T_4 and can be described as being in a thyroid hormone resistant state.

We investigated the mechanism of thyroid hormone resistance in hibernating ground squirrels by asking whether increased total serum T_3 and T_4 levels lead to increased availability of hormone to target sites. Most of the circulating thyroid hormones are bound to serum proteins. According to the free hormone hypothesis of Robbins and Rall (1960), it is the free fraction which enters cells to exert biological effects, the bound fraction serving as an inert reservoir that buffers changes in the free levels. With time, the original postulate has undergone modification and the concentration of free hormone is recognized now to be an important, but not the only, determinant of the amount of hormone available to the tissues which can exert metabolic effects (Ekins, 1985; Ingbar, 1985; Pardridge, 1987).

The purpose of this study was to investigate free hormone levels at three points of the annual cycle, the active phase and during dormancy and arousal bouts. Analyses were extended to investigate the mechanism by which any changes in free hormone levels are realized. The effects of temperature and changes in binding protein concentration were examined.

MATERIALS AND METHODS

Animals. Procedures for sampling and maintaining the animals were similar to those used previously (Demeneix and Henderson, 1978a). Ground squirrels were live-trapped in a 2-ha field in the northwest outskirts of Calgary, Alberta. Animals were either (1) anesthetized immediately upon capture and blood samples collected (field samples) as described below or (2) they were transferred to the laboratory in July and August to establish hibernating colonies. The latter group were in their first year, having been born during the first few weeks of the preceding May. In the laboratory, squirrels were held under 12L:12D at 21° in standard rat cages until early September when they were transferred to a controlled environmental room where they were housed individually in pigeon cages (Model LC131, Wahman Manufacturing Co., Baltimore, MD). They were given rat chow (Wayne Rat Chow, Lab-Blox, Allied Mills, Chicago, IL) and tap water *ad libitum*. Cotton batting was provided as bedding. A waste adsorbent (Pel-e-Cel, Paxton Processing Co., Paxton, IL) was placed in the cage trays and replaced as required.

At the time of transfer of animals to the environmental room, conditions were set at 18°, 12L:12D with a light intensity of 25–100 lux. At 3-day intervals, the temperature was decreased by 3°, the photoperiod by 2 hr, and the light intensity was gradually reduced until a regime of 6°, 2L:22D, and 0.2–1 lux was attained. This regime was maintained for the duration of the observation period. Maintenance and daily observation of the animals were done during the 2-hr light period. Daily records of dormancy and arousal bouts were kept for each animal using the sawdust technique of Pengelley and Fisher (1961) and by examination of the respiration rate.

All animals (active, dormant, and aroused) were anesthetized with ether for 2 min. The thorax was opened and 5–10 ml blood was withdrawn via cardiac puncture using a sterile syringe fitted with a 1" (2.54

cm), 21-ga needle. Blood was transferred to a centrifuge tube, covered, and stored on ice for 1.5–2 hr. Clotted samples were centrifuged at 2000*g* for 15 min. Serum was collected and stored in ½-ml aliquots in polypropylene screw-top vials at −80°. While anesthetized, animals were killed by cervical dislocation. Carcasses were weighed and sex was recorded. All field and laboratory animals were sampled between 10:00 and 13:00 hr.

Active animals were sampled in the field between May and September. Core (abdominal) temperatures varied between 37 and 39°. In the laboratory, animals entered hibernation in late September or October. Hibernation began with short dormancy bouts (2–6 days), followed by longer bouts midway through the winter, 19 days being the longest observed. Shorter bouts occurred again before hibernation terminated in late February. Samples from dormant animals were taken on the second day of the second or a subsequent dormancy bout when core temperatures were 7–8°. Arousal bouts varied from 12–48 hr in length. Samples were taken after completion of a spontaneous arousal when core temperatures ranged from 35 to 38°.

Radioimmunoassays. Serum concentrations of total T_3 (TT_3) and total T_4 (TT_4) were measured by radioimmunoassay (RIA) using the procedures of Brown and Eales (1977) with modifications reported previously (Demeneix and Henderson, 1978a). T_3 and T_4 standards (sodium salts) were purchased from Sigma Chemical Co. (St. Louis, MO) ^{125}Iodine-labeled T_3 and T_4 ($[^{125}I]T_3$ and $[^{125}I]T_4$) were obtained in 100- to 250-μCi lots from New England Nuclear Corp. (Lachine, Quebec). Specific activities of carrier-free T_3 and T_4 were >3300 and >5500 μCi/μg, respectively. All preparations were repurified before use by the method of Green (1972) using disposable Sephadex columns (G-25 fine). The detection limit for both assays was 0.1 to 0.2 ng/ml.

Free thyroid hormones. Free or dialyzable fractions of T_3 ($\%FT_3$) and T_4 ($\%FT_4$) were estimated by equilibrium dialysis, using the Mg precipitation technique of Sterling and Brenner (1966) with minor modification as reported by Demeneix and Henderson (1978b). High specific activity $[^{125}I]T_3$ and $[^{125}I]T_4$ were repurified (Green, 1972) to provide preparations with <1% free ^{125}iodide. All dialyses were carried out for 24 hr. Each serum sample was analyzed in duplicate at both temperatures.

Serum T_3-binding assays. Endogenous hormone was extracted from serum. To a 0.5-ml sample of serum was added 30 μl of tracer $[^{125}I]T_3$, approximately 10,000 cpm (2.8 fmol). The mixture was incubated for 15 min at room temperature (RT). Each sample was diluted to 5.0 ml (1:10 serum dilution) with charcoal–dextran (1 g% charcoal, 1 g% dextran, 0.05 *M* phosphate, 0.1 *M* NaCl, pH 7.4) and incubated for 1 hr at RT with inversion mixing. This procedure extracts endogenous iodothyronines (bound and free) and $[^{125}I]T_3$ (Larsen, 1972). Samples were centrifuged at 1650*g* and the supernatant collected as extracted serum. The extraction efficiency for $[^{125}I]T_3$, determined by comparing counts in the charcoal pellet to total counts before extraction, was 85–98%. T_3 was not detectable by RIA in samples of extracted serum prepared without the addition of $[^{125}I]T_3$.

For binding assays, $[^{125}I]T_3$ and T_3 were added to 200-μl aliquots of extracted serum to give a final volume of 400 μl. In the assay, $[^{125}I]T_3$ was 40 n*M* (20,000 cpm/tube) and T_3 ranged from 0 to 1000 n*M*, dilutions being made with 0.05 *M* phosphate buffer, 0.1 *M* NaCl, pH 7.4. Tubes were incubated at RT (shaking) for 60 min, transferred to ice, and 0.5 ml charcoal–dextran was added to separate bound and free fractions (Sterling *et al.*, 1978). After 15 min, tubes were centrifuged for 10 min at 1650*g*, 0°. Total binding was determined by measuring $[^{125}I]T_3$ in ½-ml aliquots of supernatant and correcting for the specific activity of the label. Nonspecific binding, defined as that which could not be displaced by a 100-fold excess of unlabeled hormone (4 μ*M* T_3), was subtracted from total binding to calculate specific binding. All samples were assayed in triplicate.

In plots of specifically bound versus free or unbound hormone concentration (total hormone minus total binding), it was apparent that 40 n*M* T_3 was sufficient to almost saturate binding sites (Fig. 1). Further increases in T_3 did not significantly increase the amount of hormone specifically bound and thus the values obtained over the range 40 to 1040 n*M* T_3 were averaged to determine the binding capacity (three or four concentrations).

As 40 n*M* $[^{125}I]T_3$ was used together with increasing amounts of unlabeled hormone, the data were used to generate competition displacement curves (Fig. 2). In

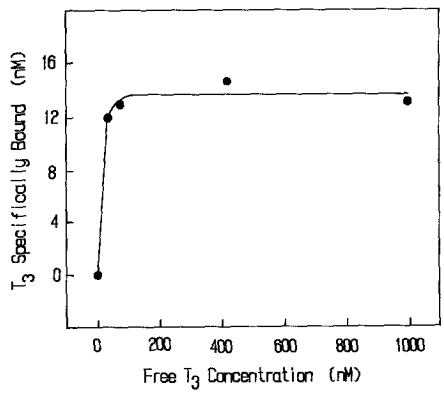

FIG. 1. Effect of increasing T_3 concentrations on the quantity of T_3 specifically bound by serum proteins. The example shown is for serum from a dormant squirrel.

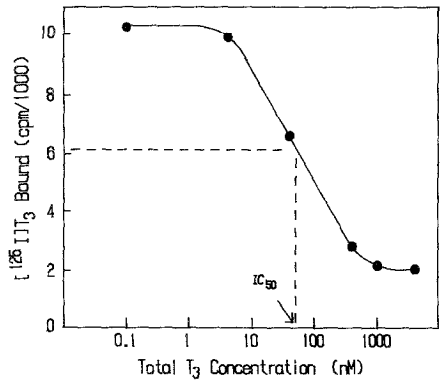

FIG. 2. A typical competition displacement curve showing the effect of increasing T_3 concentration on the displacement of $[^{125}I]T_3$ from saturable binding sites. IC_{50} refers to the concentration of T_3 required to displace 50% of specifically bound $[^{125}I]T_3$. Values were derived from data reported in Fig. 1.

these, the labeled hormone represents the agonist and the unlabeled parent hormone the competitive inhibitor. The concentration of T_3 required to displace 50% of specifically bound $[^{125}I]T_3$, defined as the IC_{50}, was determined graphically and used to calculate the inhibitor dissociation constant (K_i) for T_3 using the equation given by Hollenberg (1985):

$$K_i = \frac{IC_{50}}{(1 + L^*/K^*)}.$$

L^* is the concentration of $[^{125}I]T_3$ and K^* is the equilibrium dissociation constant for $[^{125}I]T_3$. Because T_3 and $[^{125}I]T_3$ are almost identical, K^* was assumed to be equal to K_i.

Blood and serum analyses. Total serum protein was assayed using the Bio-Rad protein assay (Kit II, Richmond, CA) and serum albumin was measured with Sigma Albumin Kit 630-A. Hemoglobin was measured using Sigma Hemoglobin Kit 525-A. ODs for standards and samples were measured with a Pye Unicam SP8-150 spectrophotometer (Canlab, Calgary, Alberta). Hematocrits were determined using conventional procedures.

Data analysis. Data were analyzed for significant differences using ANOVA and the Student–Newman–Keuls test.

RESULTS

Hibernation behavior in laboratory colonies of Richardson's ground squirrels has been described earlier (Demeneix and Henderson, 1978a).

Serum thyroid hormone levels. Tables 1 and 2 report the total and free levels of T_3 and T_4 in serum from active, dormant, and aroused ground squirrels. Both total T_3 and T_4 were elevated in hibernating animals, as has been demonstrated in more detailed studies of monthly samples collected over several annual cycles (Demeneix and Henderson, 1978a; Magnus, 1986). Total serum T_3 in aroused squirrels was one-half that of dormant squirrels, but four times the level in active squirrels. Total serum T_4 was elevated in dormant and aroused squirrels compared with active animals.

TABLE 1
TT_3, FT_3, AND $\%FT_3$ CONCENTRATIONS IN SERUM FROM GROUND SQUIRRELS

Group	TT_3 (ng/ml)	FT_3 37° (pg/ml)	FT_3 6° (pg/ml)	$\%FT_3$ 37°	$\%FT_3$ 6°
Active					
$n = 11$	0.95 ± 0.12^{1}	$2.51 \pm 0.44^{1,a}$	0.57 ± 0.12^{3}	$0.255 \pm 0.014^{1,a}$	0.058 ± 0.001^{3}
Dormant					
$n = 8$	8.83 ± 1.02^{2}	[13.30 ± 2.50]	$1.07 \pm 0.15^{3,a}$	0.148 ± 0.020^{2}	$0.012 \pm 0.001^{4,a}$
Aroused					
$n = 10$	4.25 ± 0.36^{3}	$5.51 \pm 0.52^{2,a}$	0.44 ± 0.04^{3}	$0.138 \pm 0.015^{2,a}$	0.011 ± 0.001^{4}

Note. Values are means ± SEM. TT_3 is total serum T_3, FT_3 is serum free T_3, and $\%FT_3$ is serum percentage free T_3. Means with unlike superscripts for each hormone fraction are significantly different ($P < 0.05$). The bracketed value accounted for the major portion of error in ANOVA and so was eliminated from ANOVA for increased resolution.

[a] Values measured at the temperatures approximating the core temperatures of the animals.

TABLE 2
TT_4, FT_4, AND $\%FT_4$ CONCENTRATIONS IN SERUM FROM GROUND SQUIRRELS

Group	TT_4 (ng/ml)	FT_4 37° (pg/ml)	FT_4 6° (pg/ml)	$\%FT_4$ 37°	$\%FT_4$ 6°
Active					
$n = 11$	25.6 ± 1.2^{1}	$11.9 \pm 0.7^{1,a}$	3.30 ± 0.25^{3}	$0.047 \pm 0.002^{1,a}$	0.013 ± 0.001^{3}
Dormant					
$n = 8$	65.8 ± 13.5^{2}	17.5 ± 3.4^{2}	$4.17 \pm 1.04^{3,a}$	0.028 ± 0.003^{2}	$0.007 \pm 0.002^{4,a}$
Aroused					
$n = 10$	48.4 ± 5.5^{2}	$12.8 \pm 0.1^{1,a}$	2.45 ± 0.40^{3}	$0.027 \pm 0.002^{2,a}$	0.005 ± 0.001^{4}

Note. Values are means ± SEM. TT_4 is total serum T_4, FT_4 is serum free T_4, and $\%FT_4$ is serum percentage free T_4. Means with unlike superscripts for each hormone fraction are significantly different ($P < 0.05$).

[a] Values measured at the temperatures approximating the core temperatures of the animals.

For each animal, the estimate of percentage free was multiplied by the total serum hormone level to yield the concentration of either FT_3 or FT_4. Comparisons of free hormone levels were restricted to values obtained from serum dialyzed at the respective core temperatures of the animals. Both FT_3 and FT_4 were reduced in dormant animals to one-half the levels in active squirrels. In aroused squirrels, FT_3 was doubled over the levels in active animals. FT_4 in aroused squirrels did not differ from active squirrels. There were no apparent differences in free and total hormone levels between male and female squirrels.

When dialyzed at temperatures corresponding to the respective core temperatures of the animals, the $\%FT_3$ and $\%FT_4$ were reduced in hibernating squirrels compared with active animals (Tables 1 and 2). The reduction was greater in dormant than in aroused squirrels. Within all groups (active, dormant, and aroused), the $\%FT_3$ and $\%FT_4$ were considerably lower when dialyzed at 6° than at 37°. When samples were dialyzed at either 6 or 37°, the $\%FT_3$ and $\%FT_4$ were significantly reduced in dormant and aroused animals compared with those of active squirrels. No differences were observed between sera from dormant and aroused animals when the sera were assayed at either temperature.

Serum T_3-binding assays. Binding capacities and K_is calculated for T_3 in squirrel serum are shown in Table 3 together with data for human serum. The binding capacities of saturable T_3-binding sites in serum from squirrels in both dormant and aroused states were more than twice those of active squirrels. The capacity value for human high affinity binding sites was within the range 11–33 μg T_3/100 ml calculated from data reported by Hoffenberg and Ramsden (1983) for thyronine binding globulin.

K_is, measured *in vitro* under the same assay conditions for all samples, were not significantly different among active, dormant,

TABLE 3
CAPACITIES AND K_iS FOR GROUND SQUIRREL AND HUMAN SERUM

Group	n	Capacity (μg T_3/100 ml serum)	K_i (nM)
Active	8	7.5 ± 0.4^{1}	12.0 ± 1.7^{1}
Dormant	9	18.8 ± 1.8^{2}	8.2 ± 1.1^{1}
Aroused	6	17.3 ± 1.4^{2}	7.1 ± 1.3^{1}
Human[a]	7	14.6 ± 1.2	18.0 ± 2.7

Note. Values are means ± SEM. Means with unlike superscripts within each column are significantly different ($P < 0.05$).

[a] Human serum was provided by healthy volunteers.

and aroused groups. The K_is were lower than the K_i estimated for human serum. A K_i for human serum of 18 n*M* (Table 3) overestimates an *in vivo* K_d for TBG binding to T_3 of 2 n*M* (Harpen *et al.*, 1982).

Blood and serum analysis. There were no differences in either blood hemoglobin content or in hematocrit between active and dormant squirrels (Table 4).

Serum protein was significantly increased during hibernation compared with the levels in active squirrels, and, within the hibernation phase, the values were higher during arousal than in dormancy bouts. Serum albumin was also increased during hibernation but there was no significant difference between dormant and aroused squirrels (Table 4).

DISCUSSION

The hormone levels for aroused squirrels in the current report correlate with the early arousal group described by Demeneix and Henderson (1978a). They reported significantly lower TT_3 in early arousal, followed by an increase in TT_3 between early and late arousal to levels seen during dormancy. By contrast, TT_4 is highest in early dormancy, drops by late dormancy and remains at a constant level during arousal (Demeneix and Henderson, 1978a). Therefore, it is assumed that the aroused animals in the current study are in early arousal, soon after reaching euthermic core temperature, when TT_3 levels are about half the levels in dormant animals.

Because of the variable core temperatures of hibernating animals, only equilibrium dialyses of serum dialyzed at the corresponding temperatures provide estimates of conditions *in vivo*. Thus measurements of the free fractions of T_3 and T_4 at 6° in serum from dormant animals should be compared with those measured at 37° in serum from aroused and active animals. Compared with active squirrels, FT_3 is decreased in dormant animals but increased in aroused animals ($P < 0.05$). FT_4 is decreased in dormant animals compared with active and aroused squirrels ($P < 0.05$), with the latter two groups not being significantly different. The reduction in free hormone during dormancy translates to less hormone available to exert biological effects, while the amount of active hormone is increased during arousal bouts. Young *et al.* (1979) demonstrated a marked reduction in FT_3 and FT_4 levels in dormant compared with active woodchucks. Previously, Demeneix and Henderson (1978b) reported FT_4 was increased in dormant *S. richardsoni*. The discrepancy between Demeneix and Henderson and the present results may be due to their use of a $[^{125}I]T_4$ preparation of lower specific activity and/or contamination with $[^{125}I]T_3$.

It is clear that changes in total serum hormone levels are not paralleled by changes in free thyroid hormone levels in ground

TABLE 4
BLOOD AND SERUM PARAMETERS FOR GROUND SQUIRRELS

Group	Hemoglobin (g%)	Hematocrit (%)	Serum protein (mg/ml serum)	Serum albumin (mg/ml serum)
Active	14.0 ± 0.2^1 (7)	51.2 ± 2.0^1 (7)	45.2 ± 1.8^1 (22)	30.8 ± 1.4^1 (11)
Dormant	16.7 ± 0.8^1 (6)	55.9 ± 2.6^1 (6)	60.9 ± 2.7^2 (22)	37.7 ± 2.9^2 (4)
Aroused	—	—	77.3 ± 3.6^3 (9)	39.5 ± 1.2^2 (7)

Note. Values are means ± SEM, with the number of animals in parentheses. Means with unlike superscripts within each column are significantly different ($P < 0.05$).

squirrels. This is explained by significant changes in the $\%FT_3$ and $\%FT_4$ (1) among active, dormant, and aroused squirrels at either temperature or (2) between temperatures for each group ($P < 0.05$). This could be a consequence of changes in either or both the number (capacity) and affinity of available binding sites.

Changes in serum binding are suggested from a comparison of percentage free in active serum with percentage free in hibernator serum (both dormant and aroused) at either 6 or 37°. These changes were further investigated with T_3 competitive displacement assays in which capacities and affinities (K_i) of the serum binders were estimated using the same thermal conditions for samples from all animals. These showed that the concentration of saturable T_3-binding sites was doubled in serum from dormant and aroused squirrels compared with active animals (Table 3; $P < 0.05$). In all groups, the measured capacity exceeded the total concentration of hormone in serum (T_3 plus T_4). The affinities were similar in dormant, active, and aroused animals. It is recognized that these results are suggestive only of conditions *in vivo* as they do not take into account an effect of temperature on the binding properties of serum proteins. In fact, the $\%FT_3$ and $\%FT_4$ measurements on the same serum samples at different temperatures (Tables 1 and 2) suggest that temperature does effect the affinity of the serum binding proteins for thyroid hormone.

In all groups, $\%FT_3$ and $\%FT_4$ were several times greater when serum was dialyzed at 37° than when the same samples were dialyzed at 6° ($P < 0.05$). It is well known that a decrease in temperature promotes binding of thyroid hormones to proteins in human and fish serum *in vitro* (e.g., Davis and Gregerman, 1971; Pederson, 1974; Eales and Shostak, 1986). This suggests that a decrease in temperature further enhances binding (affinity) of thyroid hormones to serum proteins. Results similar to ours were obtained by Young *et al.* (1979) with the woodchuck (*M. monax*), another hibernating species.

Polyacrylamide gel electrophoresis of samples containing tracer amounts of $[^{125}I]T_3$ or $[^{125}I]T_4$ suggested labeled T_3 and T_4 bind to the same serum proteins in ground squirrels (Magnus, 1986). On the bases of their mobilities, these proteins were identified as an α-globulin and albumin. The labeling pattern for electrophoresis suggests that the estimates of the saturable T_3-binding sites reflects the binding capacity of the globulin fraction.

The increases in TT_3, TT_4, and T_3-binding capacity are not a consequence of hemoconcentration as neither hemoglobin concentration nor hematocrit differed between dormant and active squirrels (Table 4). There were increases in both serum protein and albumin in hibernating animals compared with active squirrels, but the relative increases were less than the increase in saturable T_3-binding sites. In 13-lined ground squirrels, *S. tridecemlineatus,* Galster and Morrison (1966) described changes in the concentration of serum protein components over the year. Values for α-globulin and albumin appeared to be increased during hibernation. In the woodchuck, total serum protein was highest before and during the hibernation period which was partly explained by an increase in the albumin fraction (Wenberg and Holland, 1972).

In the only two species of hibernators in which total and free thyroid hormone levels have been studied, the woodchuck (Young *et al.*, 1979) and Richardson's ground squirrel, increased binding of T_3 and T_4 to their serum binding proteins occurs during the hibernation phase. In earlier studies, $[^{125}I]T_3$ binding by serum proteins was found to be doubled during hibernation in 13-lined ground squirrels (Bauman and Anderson, 1970), and the T_4 binding globulin levels were reported to be increased in hibernating woodchucks (Wenberg and

Holland, 1973). Thus, it may be possible that in deep hibernators generally, the concentration of free thyroid hormones is dampened by increased binding to serum proteins during the dormancy phase.

In Richardson's ground squirrels, binding is increased in dormant animals to the extent that free thyroid hormone levels are halved ($P < 0.05$). In aroused animals binding is also increased; however, the FT_3 levels are twice those in active animals while FT_4 levels do not differ. We propose that increased serum binding of thyroid hormone is a factor contributing to thyroid hormone resistance during hibernation in Richardson's ground squirrels.

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