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Plasma Thyroxine-Binding Proteins and Thyroid Hormone Levels in Primate Species; Is Callithricidae Thyroid Hormone Resistant?

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Abstract

Thyroxine(T_4)-binding to serum proteins in primates; catarrhini, prosimiae, and platyrrhini were studied by polyacrylamide gel electrophoresis T_4 binding analysis. From the electrophoretic analysis, it was shown that thyroxine-binding proteins similar to human thyroxine-binding globulin (TBG) and thyroxine-binding prealbumin (TBPA) were present in catarrhini and prosimiae species, but not in platyrrhini (callithricidae and cebidae). T_4-binding analysis also revealed that catarrhini and prosimiae have a high affinity T_4-binding protein similar to human TBG. The association constant (Ka) for T_4 of the plasma proteins in these species was approximately $2.0\times10^{10}M^{-1}$. On the other hand, it was unable to demonstrate a high affinity binding site for T_4 in the plasma of platyrrhini species. Both the total and free thyroid hormone concentrations in catarrhini and prosimiae were similar to those in human. Total T_4 in cebidae, one of the platyrrhini species, was extremely low. Among 8 animals examined, T_4 in 6 was undetectable by radioimmunoassay and the mean T_4 of the other two was 2.8 μg/dl. However, free thyroid hormone concentrations were similar to those in human. In callithricidae, another platyrrhini species, T_4 in plasma was 6.90 ± 2.11, which is comparable to the level in normal human subjects. However, in this species, high-affinity T_4-binding protein was lacking and free thyroid hormone concentrations were extremely high (most were higher than the assay limit). Although the thyroid function of callithricidae remains to be studied, it will be interesting if callithricidae is resistant to thyroid hormone action.

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In human plasma, more than 99% of total thyroxine (T_4) is present in a protein bound form (Refetoff, 1979). The major T_4-binding proteins are thyroxine-binding globulin (TBG), thyroxine-binding prealbumin (TBPA), and albumin (Refetoff, 1979). In primates other than human, catarrhini have been shown to possess T_4-binding proteins similar to human TBG and TBPA

(Tanabe *et al.*, 1974, Tanabe, 1981). Although it was reported that TBPA is not found in platyrrhini and prosimiae (Tanabe *et al.*, 1974, Tanabe, 1981), the presence or absence of a TBG-like high affinity T_4 binding protein in these species has not been confirmed.

The present study was therefore undertaken to characterize the plasma T_4-binding protein of primates including catarrhini, platyrrhini and prosimiae by gel electrophoresis and T_4 binding analysis. It was found that a high affinity T_4-binding protein similar to human TBG is present in catarrhini and prosimiae but not in platyrrhini; and plasma free thyroid hormone concentrations in callithricidae, one of the platyrrhini species, were extremely high.

The significance of this observation will be discussed in conjunction with thyroid hormone receptor.

Materials and Methods

1. Animals and sample preparation

Monkeys which belong to catarrhini, platyrrhini and prosimiae species were used in the present study and are listed in Table 1. They were maintained at the Primate Research Institute, Kyoto University, Inuyama, Japan.

Blood samples were obtained from the saphenous vein under ketamine anesthesia. After separation of plasma, they were stored at −20°C.

2. Polyacrylamide gel electrophoresis (PAGE)

Polyacrylamide gel electrophoresis was carried out under non-denaturing conditions with a vertical slab gel apparatus (Protean, Japan Bio-Rad Laboratories, Tokyo) as previously described by Murata *et al.* (1985a). T_4-binding was detected by adding ^{125}I-T_4 (specific activity: 1,300 μCi/μg from New England Nuclear, Boston, MA, U.S.A.) to the plasma samples and autoradiography after the electrophoresis. To reveal the protein bands, the gels were stained with Coomassie blue R after the autoradiography.

3. Isoelectric focusing (IEF)

IEF was carried out on prefocused horizontal polyacrylamide gels over a pH range of 4.0 to 6.5 at 4°C with a flat bed electrophoresis apparatus (FBE 2000 produced by Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.). The details of gel preparation and electrophoretic conditions were described previously (Murata *et al.*, 1985b).

Table 1. Monkeys used in the study

Suborder, family and species of the monkeys	Number of animals
Catarrhini	
Cercopithecidae	
Cercopithecus aethiops (Savannah monkey)	2
Erythrocebus patas (Patas monkey)	2
Macaca radiata (Bonnet monkey)	2
Macaca mulatta (Rhesus monkey)	5
Macaca cyclopis (Formosan monkey)	1
Macaca assamensis (Assamese monkey)	2
Macaca nemestrina (Pig-tailed monkey)	2
Macaca speciosa (Stump-tailed monkey)	2
Macaca fuscata (Japanese monkey)	5
Papio hamadryas (Hamadryas baboon)	2
Pongidae	
Hylobates lar (White-handed gibbon)	1
Hylobates agilis (Agile gibbon)	1
Pongo pygmaeus (Orang-utan)	1
Pongo troglodytes (Chimpanzee)	3
Hominidae	
Homo Sapiens (man)	5
Platyrrhini	
Callithricidae	
Callithrix Jacchus (Marmoset)	2
Saguinus oedipus (Cotton headed tamarin)	3
Cebidae	
Aotes trivirgatus (Night monkey)	2
Cebus apella (Tufted capuchin monkey)	2
Cebus capuchinus (White throated capuchin)	4
Prosimiae	
Lemuridae	
Lemur catta (Ring-tailed lemur)	2
Lorisdae	
Galago crassicaudatus (Thick tailed galago)	1

After application of five microliter plasma samples containing 10,000 cpm of ^{125}I-T_4 onto the gel, IEF was carried out for 3 hours at a constant voltage of 1000 V. After the electrophoresis, the gel was dried in a vacuum at 60 °C for 2 hours and exposed to Kodak X-Omat AR film for a few days. The pH gradient in the gel was determined with a micro-pH meter (Bio-Rad, Richmond, CA, U.S.A.).

4. *Determination of T_4 binding to serum protcins*

The method used was previously described in detail (Murata *et al.*, 1985b). In brief, one ml of diluted plasma samples (1,600 fold) in 0.075 M barbital buffer pH 8.6 containing 3,000 cpm of ^{125}I-T_4 was added to 50 μl of the same buffer containing 0.01% BSA and various amounts of unlabeled T_4. After incubation for 15 min at room temperature, dried resin was added to the tubes (Murata *et al.*, 1985b). The tubes were shaken vigorously for 2 min and 2 ml of ice-cold distilled water was then added. Radioactivity in 1 ml aliquot of the supernatant was counted.

The association constant (Ka) and maximal binding capacity were calculated according to Scatchard (1949).

5. *Determination of total and free thyroid hormone concentrations in plasma by radioimmunoassay*

Total T_4 and T_3 concentrations were determined by radioimmunoassay with kits from Mallinckrodt Diagnostica (GmbH, Germany). Free T_4 and T_3 levels were analyzed with kits from Amersham International PLC (Buckinghamshire, England).

6. *Determination of free T_4 by equilibrium dialysis*

The assay was performed as described by Yoshida *et al.* (1980). Plasma samples were diluted 200 times with phosphate buffered saline (PBS) and dialyzed against PBS in the presence of a tracer amount of ^{125}I-T_4.

Results

1. *Polyacrylamide gel electrophoresis*

As shown in Fig. 1, ^{125}I-T_4 binding was observed

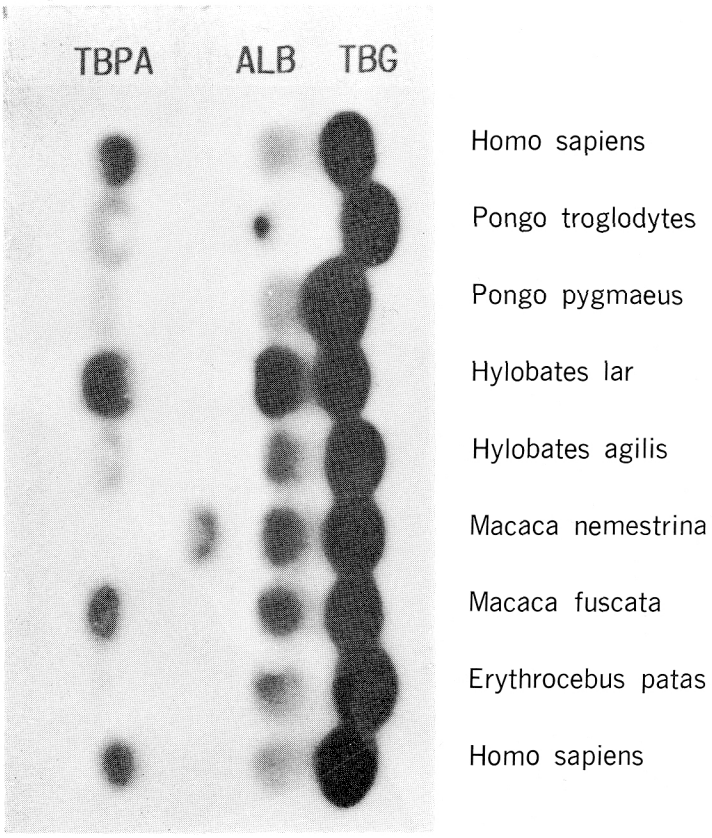

Fig. 1. Polyacrylamide gel electrophoresis of T_4-binding proteins in the plasma of catarrhini species.
^{125}I-T_4 (10,000 cpm) was mixed with 5 μl of plasma samples and subjected to PAGE. Radioautograph revealed the presence of T_4-binding proteins in all catarrhini species similar to those in homo sapiens.

in the TBG zone in all catarrhini species. However, no T_4-binding protein similar to human TBG was observed in platyrrhini species but was observed in prosimiae (Fig. 2). The presence of TBPA was also detected in all catarrhini and prosimiae species except galago crassicaudatus.

2. *Isoelectric Focusing*

TBG in human plasma exhibited microheterogeneity as reported by Marshall *et al.* (1973) and Gartner *et al.* (1981). It was focused at pH 4.2 to 4.5 (Fig. 3, 4). As revealed by ^{125}I-T_4-binding of TBG deficient human serum or normal serum added with an excess amount of unlabeled T_4, albumin was focused at pH 4.6 to 5.3.

In catarrhini, T_4-binding proteins exhibited microheterogeneity at pH 4.0–4.5 similar to human TBG (Fig. 3). As shown in Fig. 4, T_4-binding proteins in Lemur catta were distributed in almost an identical pH range to that observed in human TBG. T_4-binding proteins in galago crassicaudatus migrated in a more acidic pH range (4.0–4.2) than human TBG.

In platyrrhini, the major radioactivity migrated in a more alkaline pH range (5.8–6.0) in the absence of cold T_4. The addition of cold T_4 displaced this binding to the albumin zone, indicating the presence of T_4-binding protein with limited capacity in these species. It should be noted that no microheterogeneous T_4-binding protein band was observed in the 4.0–4.5 pH range in these species.

3. *Determination of T_4 binding*

Scatchard analysis of T_4 binding in the plasma samples of human, catarrhini and prosimiae revealed a high affinity binding site. The affinity of T_4 binding for human

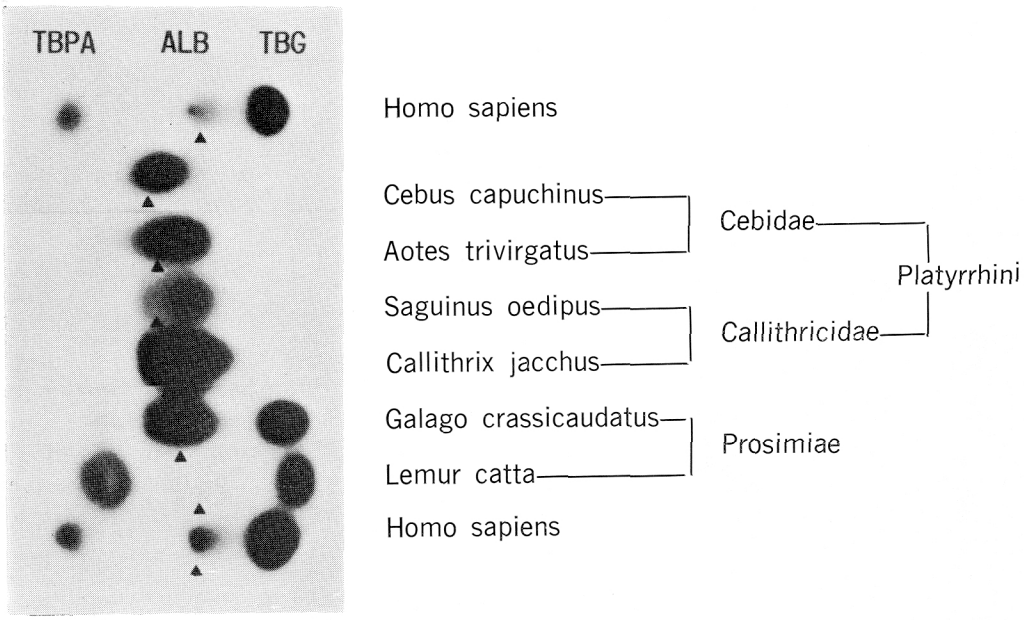

Fig. 2. Polyacrylamide gel electrophoresis of T_4-binding proteins in the plasma of platyrrhini and prosimiae. No T_4-binding protein band similar to human TBG was observed in platyrrhini species, while it was present in prosimiae. Arrows indicate the position of albumin observed by staining the gel.

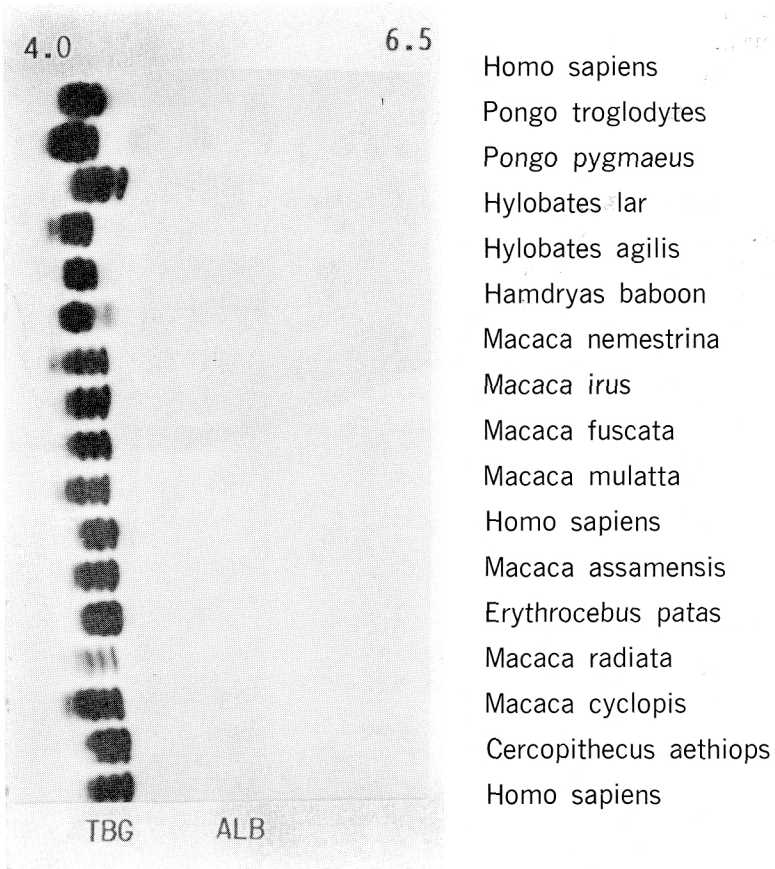

Fig. 3. Isoelectric focusing of T_4-binding proteins in the plasma of catarrhini species. Electrophoretic mobility of human TBG and albumin was indicated in the bottom of the figure. Human TBG was focused at pH 4.2 to 4.5 and albumin at 4.6 to 5.3. T_4-binding proteins in catarrhini species exhibited microheterogeneity similar to human TBG.

Table 2. Scatchard analysis of T_4-binding proteins in plasma of primate species.

Suborder	Species	Number of animals	Total T_4 (μg/dl)	Ka ($\times 10^{10} M^{-1}$)
Prosimiae	Lemuridae	3	3.88±0.87	1.75±0.15
Platyrrhini	Cebidae	5	0.73±0.33	*
	Callithricidae	3	6.90±2.11	*
Catarrini	Homo sapiens	5	7.01±1.55	2.00±0.19

The resin method utilized does not detect T_4-binding to human albumin due to relatively high affinity of the resin for T_4.

* High affinity T_4-binding was not detected in platyrrhini species.

TBG as well as plasma proteins in prosimiae was calculated to be approximately $2.0\times 10^{10}\ M^{-1}$ (Table 2). In contrast, it was not possible to detect such a high affinity binding site in the plasma of platyrrhini species.

4. *Thyroid hormone concentrations in plasma* (Fig. 5)

Both total and free thyroid hormone concentrations in catarrhini were similar to those in man (data not shown).

In prosimiae, total T_4 concentrations were 4.53 ± 1.68 μg/dl. Total T_4 concentrations in cebidae, one of the platyrrhini species, were not detectable in 6 among 8 animals. The mean T_4 level in the rest was 2.8 μg/dl. In callithricidae, another platyrrhini species, total T_4 concentrations were 6.90 ± 2.11 μg/dl. Free T_4 concentrations determined by radioimmunoassay in cebidae were similar to those in man, while in callithricidae they were extremely high. Free T_4 concentrations in 4 out of 5 callithricidae were more than 10.7 ng/dl and 5.0 ng/dl in the last one. Free T_3 concentrations were more than 22.0 pg/ml in three animals and 18.0 and 17.0 pg/dl in the rest.

Free T_4 and T_3 determinations by radioimmunoassay utilize derivatives of T_4 and T_3 which bind antibody to T_4 and T_3 but do not bind to human TBPA and TBG. Since the binding of these derivatives to

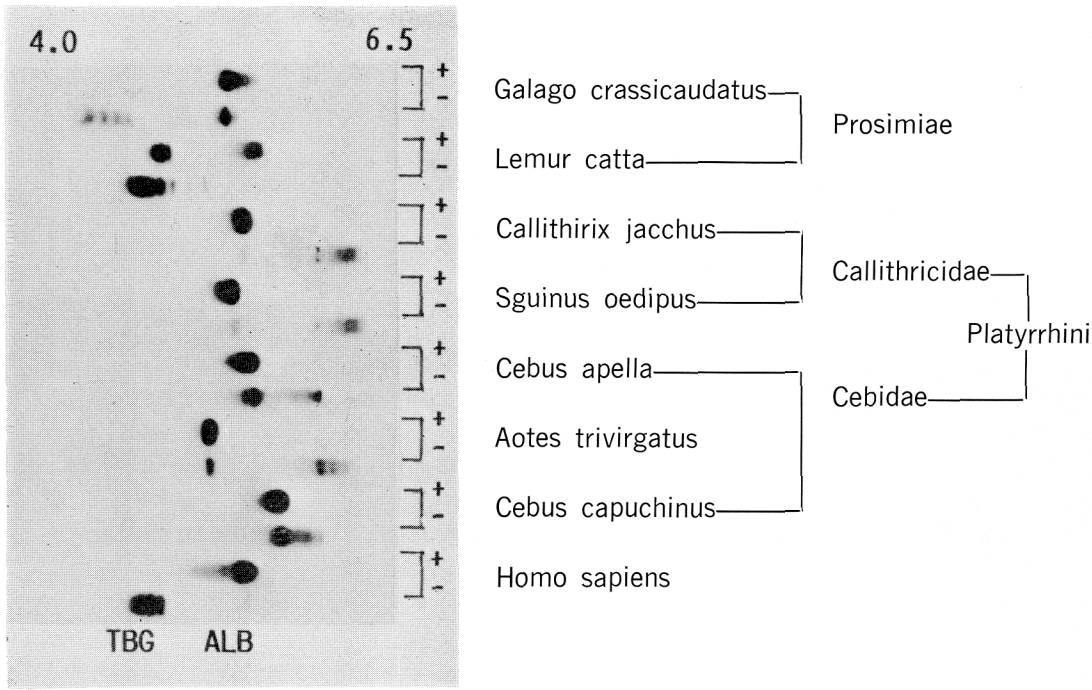

Fig. 4. Isoelectric focusing of T_4-binding proteins in the plasma of prosimiae and platyrrhini. Plasma samples were electrophoresed with ^{125}I-T_4 in the presence (+) or absence (−) of cold T_4. T_4 added was equivalent to 150 μg/dl of plasma. No T_4-binding proteins in the human TBG zone were demonstrated in platyrrhini species (both cebidae and callithricidae). In these species, weak ^{125}I-T_4- binding around pH 6.0 was demonstrated and the binding was displaced by adding cold T_4..

albumin could interfere with the assay system (Amino *et al.*, 1983, Stockigt *et al.*, 1981), we examined the binding of these derivatives to serum proteins from human, catarrhini and platyrrhini by PAGE. It was revealed that slight binding to albumin was observed in all species but there was no significant difference among the species.

The free T_4 fraction in plasma samples of callithricidae was also determined by equilibrium dialysis. The percentage of free T_4 in plasma samples obtained from 3 callithricidae was 0.065 ± 0.012 while that from 5 euthyroid human subjects was 0.020 ± 0.003. Thus, it was indicated that free T_4 concentrations in callithricidae were approximately 3 times higher than those in man.

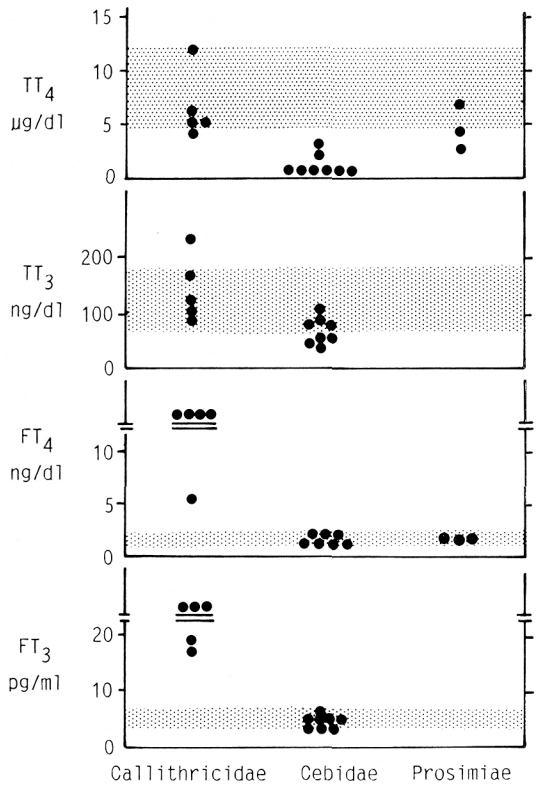

Fig. 5. Thyroid hormone concentrations in the plasma samples of platyrrhini (callithricidae and cebidae) and prosimiae.
The abbreviations are as follows. TT_4: total T_4; TT_3: total T_3; FT_4: free T_4; and FT_3; free T_3. The shaded area represents the normal range in human samples.

Discussion

Our present study revealed that catarrhini and prosimiae have T_4-binding proteins similar to human TBG and TBPA. A T_4-binding study demonstrated the presence of high affinity binding site in the plasma. IEF revealed microheterogeneous T_4-binding proteins migrating in a pH zone similar to human TBG. Although Tanabe *et al.* (1981) reported that TBPA is present only in catarrhini species but not in prosimiae, our data from PAGE indicated the presence of TBPA in prosimiae. This finding could be brought about by the different electrophoretic system employed in the present study.

In platyrrhini (callithricidae as well as cebidae), T_4-binding proteins similar to human TBG or TBPA were not observed in different electrophoretic systems, PAGE and IEF. Also by analyzing T_4 binding it was not possible to demonstrate a high affinity T_4-binding site in the plasma of platyrrhini (callithricidae as well as cebidae). In accordance with this observation, total plasma T_4 in cebidae was very low. The concentrations were comparable with T_4 concentrations in TBG deficiency in humans. And free T_4 concentrations in this species were similar to those in euthyroid humans. Thus, this species lacks high affinity T_4-binding proteins. On the other hand, total T_4 concentrations in callithricidae were much higher than those in cebidae. Moreover, free thyroid hormone concentrations determined by radioimmunoassay were extremely high. We measured free thyroid hormone concentrations in this species by a dialysis method. Free T_4 levels in callithricidae

determined by the method were invariably higher than those in man (about 3 times). Although it was not possible to determine what causes the differences between the free T_4 concentrations determined by radioimmunoassay and by the dialysis method, both methods indicated high free thyroid hormone concentrations in callithricidae.

To assess the thyroid function in callithricidae, it is necessary to determine serum TSH concentrations as well as metabolic indices. It was not possible to measure TSH with a currently available kit for human and rat TSH, probably due to the lack of cross reactivity. Also, metabolic indices were not determined in these animals. Thus, we cannot determine whether callithricidae species are hyperthyroid or thyroid hormone resistant. Since callithricidae have been shown to be resistant to glucocorticoid hormone action (Chrousos *et al.*, 1982), it is interesting to speculate that callithricidae may be resistant to thyroid hormone action. Recently, the thyroid hormone receptor gene has been shown to be identical with the C-erb-A gene (Sap *et al.*, 1986, Weinberger *et al.*, 1986) and the glucocorticoid receptor gene is similar to the C-erb-A gene (Hollenberg *et al.*, 1985, Weinberger *et al.*, 1985). Thus, C-erb-A related genes in callithricidae may have evolved quite differently from those of other primate species.

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