0013-7227/90/1272-0857$02.00/0
Endocrinology

Vol. 127, No. 2
Printed in U.S.A.

Familial Goiter in Bongo Antelope (*Tragelaphus eurycerus*)

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ABSTRACT. Congenital defects in thyroglobulin (Tg) synthesis in animals have proven to be useful models for the study of Tg synthesis and regulation. Defects in Tg synthesis have been well described in Afrikander cattle, Australian Merino sheep, and goats in The Netherlands. This report describes a study of goiter in a nondomesticated bovine species, bongo antelope (*Tragelaphus eurycerus*), an African bovid. Three animals housed at the National Zoological Park, Washington, D.C. were studied; two had visible goiters, and a third bongo had microscopic evidence of goiter. Tg extracted from thyroid glands or thyroid colloid from these animals had a high mol wt component that was greater than 220K daltons and differed in apparent mol wt from 19S Tg from domestic cattle. Thyroid extracts also had thyroid albumin; albumin was more than half the total protein in colloid extract. The animals with goiter were euthyroid according to their circulating levels of thyroid hormones. (*Endocrinology* **127:** 857–864, 1990)

NEARLY 100 yr ago, hereditary goiter in humans was first described by Osler (1) and Pendred (2) independently in the United States and Great Britain. Fifty years later, three different groups in South Africa, Australia, and Holland described congenital goiter in three different animals: the Afrikander cow (3), the Merino sheep (4, 5), and the Dutch goat (6). Biochemical studies on the goiters developing in these animals became the basic model for some forms of the disease affecting humans. While this particular goiter condition in humans is rare (reviewed in Refs. 7 and 8), the work has contributed to our understanding of thyroid hormone synthesis and thyroid function in general.

All three animal models had no normal 19S thyroglobulin (Tg), but all had varying amounts of Tg-like proteins (9–14). Goiter is associated with marked hypothyroidism in the Dutch goat and Merino sheep (6, 14), but not in Afrikander cattle (15), whose goiter compensates and provides sufficient synthesis of hormones. The example of the Afrikander cow serves as a model of what is necessary to enable an animal to synthesize a sufficient amount of thyroid hormone despite the absence of 19S Tg.

Our study was begun in 1988 on a bongo with goiter housed at the National Zoological Park. The following report describes an abnormal Tg, the presence of iodoalbumin in the thyroid, and the thyroid hormone content in the serum of a number of bongos. The findings in this exotic species are unique and separate from those in the three domestic species.

Materials and Methods

Animals studied

Thyroid glands were obtained at necropsy from two bongos. One (bongo A) had adenocarcinoma of the cervix, and the other (bongo B) had severe degenerative osteoarthritis. Bongo A had an enlarged thyroid; the thyroid of bongo B was not enlarged, but had microscopic areas with enlarged follicles and colloid cysts. A third living bongo (bongo C) also has an enlarged thyroid; this bongo has a history of reproductive problems, including prolonged gestations and a high incidence of neonatal mortality. When the thyroids of two of the offspring of bongo C were examined at necropsy, they were not enlarged, but there were a few microscopic colloid cysts. Colloid from bongo C, studied in this report, was aspirated from a large thyroid cyst during surgery for repair of a prolapsed cervix. In addition, there were two bongos, progenitors of bongos A and C, that had massive colloid goiters at necropsy. The thyroid glands

Received February 2, 1990.
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from all four animals, bongos A and C and the two progenitors, were enlarged bilaterally (up to 10 × 20 cm) with multiple colloid cysts. Microscopically, the thyroid tissue was characterized by follicular cyst formation, with areas of follicular hyperplasia and varying degrees of fibrosis. The animals studied are shown in the pedigree chart in Fig. 1. The consanguinity, age, and sex of the bongos studied are also indicated in Fig. 1.

Isolation of Tg from bongos A, B, and C

Tg was prepared from the thyroids of bongos A and B by a modification of previously described procedures (16, 17). Thyroid tissue was partially thawed, minced with a razor, suspended in 0.1 M KCl (2 ml/g tissue), and stirred overnight at 0–4 C. After centrifuging at 30,000 × *g* for 15 min, ammonium sulfate was added to a saturation of 30%. After 1 h, the mixture was spun at 4,300 × *g* for 15 min, and the supernatant was brought to 50% saturated ammonium sulfate and left overnight. The pellet collected by centrifugation (4,300 × *g* for 15 min) was suspended in 0.05 M ammonium bicarbonate buffer, pH 8.0, at a protein concentration of 38 mg/ml and stored at 0–4 C; it is referred to in this report as thyroid extract. Tg from bongo C was prepared by adding KCl (final concentration, 0.1 M) to 25 ml colloid. Material precipitated with ammonium sulfate, as described above, was stored at 0–4 C at a protein concentration of 72 mg/ml. All procedures were performed at 0–4 C in the presence of phenylmethylsulfonylfluoride (20 μg/ml) and aprotinin (2 U/ml; Sigma Chemical Co., St. Louis, MO). The 19S bovine Tg used as a standard is the same preparation as that described in Ref. 18. Protein was measured by the micro-BCA method, obtained from Pierce Chemical Co. (Rockford, IL).

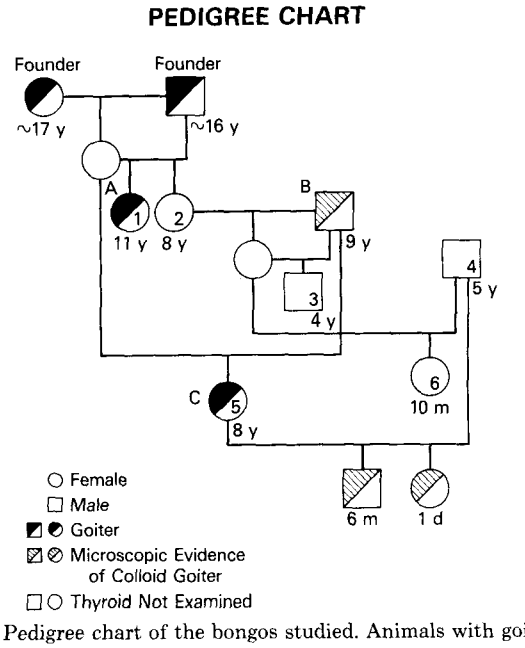

FIG. 1. Pedigree chart of the bongos studied. Animals with goiter are indicated by *solid symbols*; animals whose thyroids were grossly normal but had microscopic evidence of colloid goiter are indicated by *hatched symbols*. A, B, and C correspond to animals whose Tg is studied in this report. The *numbers within the symbols* indicate animals whose serum thyroid hormones were measured. The age of the animal when studied is indicated by the *number under the symbol*.

Sepharose CL6B gel filtration

Thyroid and colloid extracts (34–36 mg protein in 1 ml) were passed over a column (1.6 × 82 cm) of Sepharose CL6B (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ) and eluted with 0.05 M ammonium bicarbonate, pH 8.0, at a flow rate of 0.6 ml/min. The effluent was collected in 1.5-ml fractions, and the protein in the effluent was monitored by measuring the 280-nm absorbance. UV absorption spectra were recorded on peak fractions using a Hewlett-Packard computerized spectrophotometer (model 8452A, Palo Alto, CA). Fractions in each 280-OD peak were pooled, lyophilized, and stored at 0–4 C.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Samples (5–15 μg protein) in Laemmli buffer (19) were separated by PAGE using a minigel electrophoresis apparatus and precast gels (NOVEX, Encinitus, CA). The percentage of SDS used is indicated in the figure legends. Proteins were detected by staining with 0.1% Coomassie brilliant blue R-250 in 50% methanol, 10% acetic acid, and destained with 20% methanol and 8% acetic acid.

Immunoblots

After electrophoresis, proteins were electroblotted by a previously described method (20), onto polyvinylidene difluoride (PVDF) membranes (Immobilon, Millipore, Bedford, MA) using NOVEX apparatus and CAPS buffer (10 mM 3-[cyclohexyl amino]1-propanesulfonic acid and 10% methanol, pH 11). Proteins were detected by staining with 0.1% amido black in 45% methanol and 10% acetic acid and destaining with 10% acetic acid.

Immunodetection

A Streptavidin HyBRL Screen Kit (Bethesda Research Laboratories, Gaithersberg, MD) was used for immunodetection according to the protocol provided by the supplier, except that nonfat dry milk replaced BSA; 4CN was used for detection. Antibovine Tg was the same preparation as that described in Ref. 18 and was used at a dilution of 1:2000; rabbit anti-BSA was purchased from Calbiochem (La Jolla, CA) and used at a dilution of 1:1000.

Determination of thyroid hormone content

Pronase digestion was carried out using previously described procedures with slight modification (21, 22). In brief, 50–200 μg protein were incubated at 37 C for 20 h with Pronase (Calbiochem) at a enzyme/substrate ratio of 1:100 in a total volume of 200 μl. The reaction mixture was passed over an acid-cation exchange column (Dowex-50W; 2% cross-linked; 100–200 mesh; Sigma). The column was made in a Pasteur pipette by adding 200 mg resin in 30% ethanol and washing with 10 ml of the same. The sample was applied and washed

again with 10 ml of the same solvent. Thyroid hormones were eluted with 10 ml NH_4-ethanol-water (50:45:55). The samples from the column were dried and dissolved in 1 ml 1 N NH_4OH. Recovery of hormone from the Dowex column was measured by adding 1 μCi [^{125}I]T_3 or [^{125}I]T_4 (New England Nuclear, Boston, MA) to duplicate samples before being passed over the column. After washing the column as described above, more than 85% of the added radioactivity was eluted with NH_4-ethanol-water.

The T_3/T_4 content was measured by RIA using Magic Total T_4 and T_3 Kits from Ciba-Corning Immunodiagnostics (Medfield, MA). T_3/T_4-free human serum was added to the samples from the Dowex column to make them comparable to serum samples in the RIA. Addition of comparable amounts of NH_4OH or serum to the standards did not change the standard curve. rT_3 was measured by a RIA kit from Serono (Braintree, MA).

Determination of iodine content

Thyroid extract from bongo B (1.27 mg) and 2.17 mg from colloid extract from bongo C were suspended in 1 ml 0.1 M Tris-HCl and 5 mM MMI, pH 8.5. The total iodine content was determined in triplicate using a semiautomatic Zak chloric acid procedure, as described by Benotti and Benotti (23).

Materials

Chemicals for electrophoresis and staining were obtained from Bethesda Research Laboratories. Mol wt standards were purchased from Bethesda Research Laboratories and Boehringer-Mannheim (Indianapolis, IN). Chemical reagents were the highest commercial grade available.

Results

Tg

Thyroid glands from bongo A and bongo B were extracted with salt, and the Tg was precipitated with ammonium sulfate by the procedure described in *Materials and Methods*. The protein precipitated with 50% saturated ammonium sulfate was passed over a column of Sepharose CL6B, and the elution pattern is shown in Fig. 2. The pattern of thyroid proteins from bongos A and B are similar; the first protein to elute from the column appears as a hypersharp peak, with its maximum elution at fraction 37; this protein peak eluted in the void volume of the column. The second major protein to elute from the column is in the same position as an authentic sample of 19S bovine Tg and is labeled IA and IB in Fig. 2.

Colloid from bongo C was also extracted with salt, and ammonium sulfate was added to 50% saturation, as detailed in *Materials and Methods*. Precipitated protein was passed over a column of Sepharose CL6B, and the elution pattern is shown in Fig. 2. Only a small fraction of the total amount of protein recovered from the column

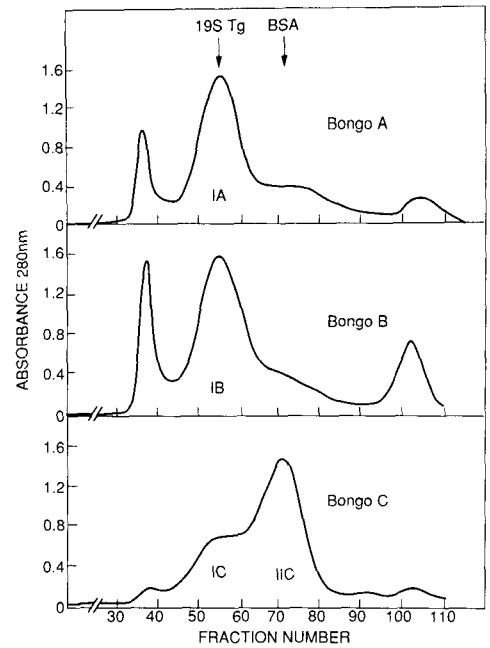

FIG. 2. Sepharose CL6B gel filtration of thyroid proteins from bongos A, B, and C. One milliliter of thyroid protein extract from bongo A (34 mg protein) or bongo B (34 mg protein), or colloid extract from bongo C (36 mg) was separated on a column of Sepharose CL6B, as described in *Materials and Methods*. The absorbance at 280 nm measured in each 1.5-ml fraction is shown. The elution position of authentic bovine 19S Tg is fraction 55, and that for BSA is fraction 72.

eluted in the position where authentic 19S Tg appears (peak IC). The largest quantity of protein to elute from the column appears in the same fraction as authentic BSA and is labeled IIC in Fig. 2.

More than 95% of proteins extracted from bongo thyroid precipitated with 50% saturated ammonium sulfate. This was not the case with colloid extract from bongo C, where an equal amount of protein remained soluble under these conditions; when this protein was passed over a column of Sepharose CL6B, it eluted as a single symmetrical peak in the same fraction as authentic BSA and was immunologically related to BSA (not shown).

Pooled fractions that eluted in the position of Tg were separated by 4% SDS-PAGE under nonreducing conditions, and the proteins were stained with Coomassie blue. The same amount of protein was applied to each lane, but the amount of protein that remained in the stacking gel varied for each animal (Fig. 3). The major protein components in peaks IA, IB, and IC were not the same, and they differed from the major protein component in bovine 19S Tg as well. Peak IA was most like bovine Tg, as it had one major band with an apparent mol wt of 250K. The apparent mol wt of bovine Tg was 245K, and the mol wt was consistently, although only slightly, less than the M_r of the protein in peak IA. In peak IC, the most intense band had an apparent mol wt of 250, but unlike peak IA, there were several prominent protein

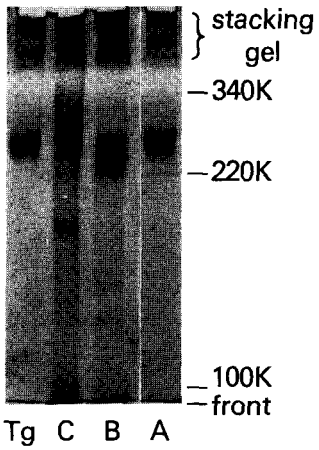

FIG. 3. SDS-PAGE of peaks IA, IB, and IC identified in Fig. 2. Proteins were separated by SDS-PAGE using 4% gel and stained with Coomassie brilliant blue R250, as described in *Materials and Methods*. The samples were prepared under nonreducing conditions, *i.e.* without mercaptoethanol and without boiling. Mol wt standards were prestained markers from Bethesda Research Laboratories. Lane 1 contains 8 μg standard bovine 19S Tg, lane 2 contains 14 μg peak IC (combined fractions 50–58 in Fig. 2), lane 3 contains 15 μg peak IA (fractions 49–63), and lane 3 contains 14 μg peak IB (fractions 49–63).

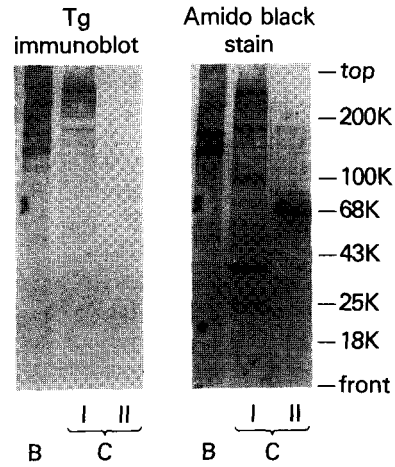

FIG. 4. Immunostaining of Tg in peaks IB, IC, and IIC identified in Fig. 2. After separation on 4–20% gradient SDS-PAGE, the proteins were electroeluted to PVDF membranes as described in *Materials and Methods*. Proteins were detected by immunostaining using antiserum raised against authentic bovine 19S Tg and by staining with amido black. Lane 1 contains an aliquot of peak IB (pooled fractions 49–63 shown in Fig. 2), lane 2, peak IC (fractions 50–58), and lane 3, peak IIC (fractions 63–77). Approximately 10 μg protein were applied to each lane. The migrations of mol wt standards are indicated in the margins.

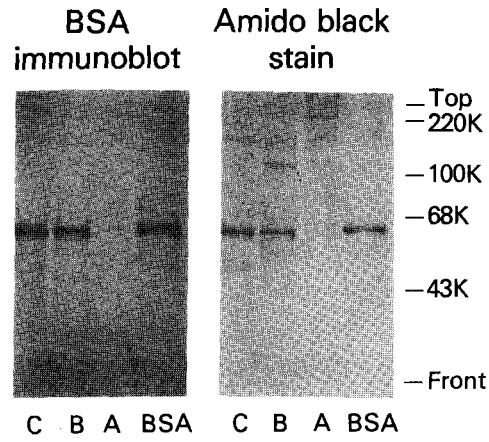

FIG. 5. Immunostaining of BSA in fractions 72 identified in Fig. 2. Proteins were separated by SDS-PAGE using a 8% gel and electroeluted to PVDF as described in *Materials and Methods*. Proteins were detected with anti-BSA immunoglobulins or with amido black staining. Lanes 1, 2, and 3 contain 5 μg fraction 72 from bongos A, B, and C. The migration positions of mol wt standards are indicated; there were 5 μg BSA in the lane indicated.

bands with lower mol wt. The protein in peak IB appeared as a broad band with an apparent mol wt that extended from 220–250K.

To identify proteins in the peaks immunologically related to Tg, peak IB and peaks IC and IIC were separated under reducing conditions by 4–20% gradient SDS-PAGE and transblotted to PVDF membranes, as described in *Materials and Methods*. In preliminary studies bongo Tg interacted with our preparation of antiserum prepared against 19S bovine Tg. All proteins in peak IB that stained with amido black were immunoreactive with anti-Tg antiserum (Fig. 4). Although not shown, all of the proteins in peak IA were also immunoreactive with anti-Tg antiserum, but this was not the case for those in peak IC. Peak IC was very heterogeneous; only the high mol wt protein bands, and none of the lower mol wt protein bands were immunoreactive with anti-Tg (Fig. 4). In peak IIC, there were no Tg-immunoreactive proteins detected. Peak IIC was less heterogeneous than peak IC, with one major protein band with an approximate mol wt of 68K. This protein is immunologically related to BSA (see below).

Thyroid albumin

When the colloid extract from bongo C was passed over a column of Sepharose CL6B, most of the protein eluted in the position of BSA. To determine if this protein was related to BSA, the peak fraction (fraction 72; Fig. 2) was separated under reducing conditions by 8% SDS-PAGE and transferred to PVDF membranes, as described in *Materials and Methods*. The same fraction (no. 72) from the thyroid extracts of bongos A and B was also examined. Proteins detected with antiserum to BSA and with amido black staining are shown in Fig. 5. In both bongos B and C, the major protein in this fraction behaves like authentic BSA. The mobility of the protein band was the same as BSA and was close to the 68K standard. The protein was immunoreactive with antiserum to BSA. Fraction 72 from bongo A, although it had mostly high mol wt proteins, also had a minor band

with the same apparent mol wt as BSA and was immunoreactive with anti-BSA (Fig. 5).

Quantitation of albumin and Tg in thyroid extracts

The fractions from Sepharose CL6B shown in Fig. 2 that elute in the position of BSA were pooled. For bongo C these were fractions 63–77, for bongo A they were fractions 68–77, and for bongo B they were also fractions 68–77. The amount of immunoreactive BSA in these pooled fractions for bongo C was 35% of the total protein. When the amount of BSA-like protein that was soluble in 50% saturated ammonium sulfate was included, 52% of the total protein was albumin. The amount of immunoreactive BSA in the thyroid extracts from bongos A and B was less than 2%.

The amount of immunoreactive Tg in the thyroid and colloid extracts was also estimated. For bongo C, there was only 0.9% immunoreactive Tg; in thyroid extracts from bongos A and B, 55–60% of the total protein was immunoreactive Tg.

Thyroid hormone content in Tg and albumin

The major protein peaks from Sepharose CL6B that elute in the position of Tg and BSA were treated overnight with Pronase to release thyroid hormones, and the thyroid hormone content was determined by RIA, as detailed in *Materials & Methods* As shown in Table 1, the T_3 and T_4 contents in peak IA and the T_4 content in peak IB were similar to those in authentic bovine 19S Tg. The T_3 content in peaks IB and IC were depressed (65% and 53% of the T_3 content found in 19S bovine Tg). The T_4 content from peak IC was markedly reduced; it was only 13% of the bovine Tg content. Peak IIC from goiter colloid also had low levels of T_3 and T_4 despite there being no immunoreactive Tg associated with this peak.

TABLE 1. Thyroid hormone content of Tg peaks from bongos A, B, and C and albumin peak from bongo C

	Thyroid hormone content[a]	
	T_3 (ng/mg protein)	T_4 (ng/mg protein)
19S Tg (bovine)	74.6	2354
Peak IA, Fig. 2 (pooled fractions 49–63)	67.2	2070
Peak IB, Fig. 2 (pooled fractions 49–63)	48.6	2424
Peak IC, Fig. 2 (pooled fractions 50–58)	39.8	307
Peak IIC, Fig. 2 (pooled fractions 63–77)	4.0	24

[a] Pooled fractions from Sepharose CL6B that elute in the position of Tg and BSA were treated with Pronase, and the thyroid hormone content was determined by RIA, as described in *Materials and Methods*. The 19S bovine Tg was the same preparation as that described in Ref. 18.

Iodine in thyroid extracts

To determine if iodine deficiency contributed to the goiters in these animals, the stable iodine content of the thyroid and colloid extracts was measured as described in *Materials and Methods*. The ^{127}I content in the colloid extract from bongo C was 0.15 μg/mg protein, and it was 0.56 μg ^{127}I/mg protein in the thyroid extract from bongo B.

Absorption spectra of Tg from bongos A, B, and C

Monoiodo- and diiodotyrosyl residues in the Tg molecule are responsible for its unique absorption spectrum (24). Whereas unsubstituted tyrosine has its absorption maximum at 276 nm, the presence of a single iodine atom in a position *ortho* to the phenolic hydroxyl group shifts the asorption maximum; the presence of two iodine atoms on either side of the phenolic hydroxyl group in diiodotyrosine causes an even greater shift in the position of the absorption maximum. Thyroid hormones that form when iodotyrosines are coupled lead to a further shift in the absorption maximum. Thus, the absorption spectrum of Tg (24) is not the spectrum of most globular proteins, where the absorption maximum at 280 nm is due exclusively to the presence of tyrosine and tryptophan residues, with a slight inflection at 290 nm due to tryptophan. The spectra in Fig. 6 were obtained with Tg extracted from the thyroids of bongos A and B and show the features just described, *i.e.* there is an increased absorbance between 310–320 nm and in the 250–260 nm region.

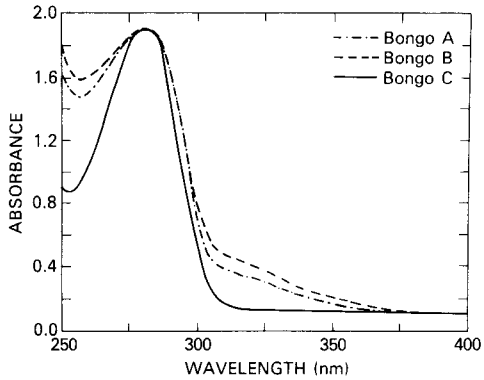

FIG. 6. Absorption spectrum of peak fractions shown in Fig. 2. Thyroid proteins from bongos A, B, and C were extracted and separated on Sepharose CL6B as described in *Materials and Methods* and Fig. 2. The UV absorption spectrum shown was determined on peak fraction 55 from bongo A (- · -), bongo B (- - -), and bongo C (——).

The *solid curve* in the same figure (Fig. 6) shows the absorption spectrum of Tg isolated from colloid of bongo C. The absorption spectrum of this Tg differs from the spectrum of normal Tg, since there is no inflection at 290 nm due to the presence of tryptophan, and even more importantly is the absence of the absorbance between 300–320 nm, which reflects the low content of the iodotryosyl residues. The low absorbance of the minimum at 258 nm is another indication that iodotyrosine appears to be very low in bongo C colloid.

Cytochrome in thyroid extracts

A distinctive feature of the bongo thyroid extracts is their deep red color. When thyroid extract from either bongo A or bongo B was passed over a column of Sepharose CL6B, a red compound eluted from the column at fraction 69. The absorption spectrum of this compound from bongo B is shown by the *dashed curve* in Fig. 7. A similar absorption spectrum was found in the extract from bongo A (not shown). The absorption spectrum shows a sharp intense maximum at 409 nm, designated a Soret band, which is associated with the cytochrome chromophore. The absorption spectrum also shows less intense maxima at 539 and 577 nm (not shown). The absorptions spectrum differed from the spectrum of hemoglobin, but was the same as that of an authentic sample of cytochrome-*c*, which was kindly provided by Dr. Richard Hendler of the NHLBI. The *solid curve* in Fig. 7 shows the spectrum of the same fraction isolated from the colloid extract from bongo C. There is a very small absorption peak at 409 nm in the place of the intense Soret band. There was also no absorbance at either 539 or 577 nm, suggesting that the colloid extract from bongo C did not contain this cytochrome in quantities similar to those found in bongo thyroid extracts.

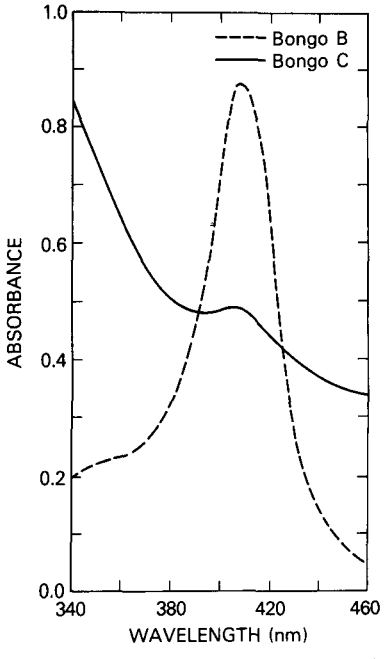

FIG. 7. UV absorption spectrum of chromophore in bongo B thyroid extract and bongo C colloid extract. Thyroid protein extracts were prepared and separated over Sepharose CL6B as described in *Materials and Methods* and Fig. 2. The UV absorption spectrum was determined on fraction 69 of bongo B (- - -) and fraction 69 from bongo C (——).

Thyroid hormone levels in serum

Table 2 shows the serum levels of T_3, T_4, and rT_3 of six bongos from the herd housed at the National Zoo. The number of animals studied is not large enough to establish a normal value for thyroid hormone levels in the serum of this exotic animal, but the values obtained for T_3 and T_4 are similar to those found in domestic bovine serum measured under the same conditions [T_3, 1.75 ± 0.46 ng/ml (mean ± SE; n = 4); and T_4, 5.34 ± 4.80 μg/dl (n = 4)]. Thyroid hormone levels were slightly depressed in some of the serum samples from bongos A and B with goiter, but in serum obtained on subsequent occasions, the thyroid hormone levels were within the range of values measured in the other animals.

Discussion

In most studies of familial goiter in animals the biochemical and clinical findings correlate with variations in the structure of the Tg gene. There is a defect in the structure of the Tg gene in exon 8 in goitrous goat (25) that results in the synthesis of a truncated Tg molecule, which has a sedimentation coefficient of 7S (12, 13), in place of the normal Tg molecule, which has a sedimentation coefficient of 19S. This truncated Tg molecule is

TABLE 2. Thyroid hormone levels in serum

Animal no.[a]	T_3 (ng/ml)	T_4 (μg/dl)	rT_3 (ng/ml)
1 (bongo A)[b]	0.63	3.86	0.40
	1.06	6.81	0.54
2	0.85	4.48	0.51
3	1.22	4.94	0.50
4	0.93	7.10	0.40
5 (bongo C)[c]	0.80	3.64	0.35
	0.98	3.75	0.23
	1.19	4.27	0.31
6	1.43	5.58	0.58

[a] The animal number refers to the number used in the Pedigree chart in Fig. 1.

[b] Sera obtained 14 months apart.

[c] Sera obtained 2 months apart.

The serum was kept at −20 C until tested; T_3, T_4, and rT_3 were determined by RIA, as described in *Materials and Methods*. All determinations were performed on at least three separate occasions, with the value of the mean shown in the table. Both the inter- and intraassay variations were less than 10%.

associated with decreased thyroid hormone formation and hypothyroidism. Similarly, a splicing defect in exon 9 of the Tg gene isolated from the Afrikander cow (26) gives rise to abnormal Tg molecules, that is proteins with sedimentation coefficients of 9S and 12S (9–11) in place of the normal 19S Tg molecule.

Goiter is described in the present paper which is unique to the bongo antelope and has not been reported in domestic bovids with goiter. We describe the isolation of Tg molecules, immunoreactive with antiserum prepared against bovine 19S Tg, that have a major component with an apparent mol wt of 220,000 daltons or more. In addition to abnormally sized Tg, goiter in bongo also is characterized by the presence of thyroid albumin. In one bongo studied more than 52% of the colloid protein was comprised of albumin. The excessive secretion of albumin in this bongo separates it from the disorder in Afrikander cow, where albumin secretion is only 2.6% (27). Like the Afrinkander cow, bongo with goiter is euthyroid based on serum levels of thyroid hormones. Unlike the Afrikander cow with goiter, which reportedly has high serum levels of rT_3 (15), the serum values for rT_3 in bongo, with or without goiter, were not elevated.

The degree of iodination measured in the thyroid extracts and the presence of iodotyrosine residues in the Tg molecule make iodine deficiency unlikely in the etiology of the goiter in these bongos. A more likely explanation for goiter is a congenital defect, which is consistent with the consanguinity of the bongos with goiter. The pedigree chart, however, is not sufficiently complete to suggest the mode of inheritance of the disorder.

Albumin in the thyroid has been found to be associated with several different types of thyroid disease, including some cases of congenital goiter (7, 8, 28). Why albumin is found in some thyroid disorders and not others is not clear, nor is it clear where thyroid albumin is made. It is generally believed that the liver is the primary site of all albumin synthesis, but some recent studies have shown albumin expression in nonhepatic tissues (29). That albumin is synthesized by thyroid epithelial cells has been supported by a few studies showing that the amino acid content of liver albumin is different from that of thyroid albumin (28, 30). Preliminary studies of albumin isolated from bongo colloid show that its amino acid sequence differs from the amino acid sequence of both bovine and bongo serum albumins. These studies suggest that thyroid albumin is synthesized independently from serum albumins.

In addition to albumin, a reddish chromophore with an absorbance maximum of 409 was found in thyroid gland extracts from bongo. This chromophore is identified as a cytochrome and is not found in extracts of normal bovine thyroid. The possibility that this cytochrome is part of thyroid peroxidase and cannot associate with an abnormal Tg molecule is presently under study. Cytochrome can also be an abnormal secretion product and, like thyroid albumin, can be synthesized in excess in response to an unknown stimulus. Whether an abnormal Tg is the stimulus for increased synthesis of cytochrome and albumin in bongo thyroid remains speculative.

One explanation for the large amount of albumin found in colloid extract compared to the small amount found in thyroid extracts is that the starting materials were different. Differences due to sampling error also could explain the absence of cytochrome in colloid extract. An alternative explanation is that differences in phenotype are due to more than one genetic defect being responsible for the thyroid disorder in bongo. This interpretation is supported in part by the fact that two of the animals studied (bongos A and B), although not related, had histological evidence of goiter. Thyroid glands from both animals contained thyroid albumin. The Tg isolated from the glands were different, and the mol wt of Tg from both animals differed from that of bovine 19S Tg when separated by PAGE. The Tg from a third bongo, bongo C, had both components seen in the Tgs from the closely related bongos A and B. Difference in apparent mol wt on PAGE, however, does not necessarily reflect differences in the amino acid composition of Tg, and can reflect, for example, differences in carbohydrate composition.

Familial goiter in bongo antelope is characterized as having an abnormal mol wt Tg and the presence of thyroid albumin. Biochemical studies of thyroid proteins in bongo with goiter show a similar but distinct disorder, which complements studies on domestic bovids with goiter. The molecular basis for Tg synthesis in goitrous bongo as well as the origin and regulation of thyroid albumin are questions that can be addressed uniquely in these animals and can potentially provide new information on thyroid disorders in man.

Acknowledgments

We are very grateful to Drs. Jan J. M. de Vijlder (Department of Experimental Pediatric Endocrinology, University of Amsterdam, Academic Medical Center, Amsterdam, The Netherlands) and Jacob Robbins (Clinical Endocrinology Branch, NIDDK, NIH, Bethesda, MD) for their interest and informative discussion of this work.

References

1. Osler W 1897 Sporadic cretinism in America. Trans Congr Am Physicians Surg 4:169
2. Pendred V 1896 Deaf-mutism and goitre. Lancet 2:532
3. Schulz KCA, The inheritance of the susceptibility of congenital goitre in Afrikander cattle in South Africa. 2nd Congress of the South African Genetic Society, Victoria, South Africa, 1962 p 90 (Abstract)
4. Falconer IR 1965 Biochemical defect causing congenital goitre in sheep. Nature (Lond) 205:978

5. Rac R, Hill GN, Pain RW, Mulhearn CJ 1968 Congenital goitre in Merino sheep due to an inherited defect in the biosynthesis of thyroid hormone. Res Vet Sci 9:209
6. Rijnberk A, de Vijlder JJM, van Dijk JE, Jorna TJ, Tegelaers HH 1977 Congenital defect in iodothyronine synthesis. Clinical aspects of iodine metabolism in goats with congenital goitre and hypothyroidism. Br Vet J 133:495
7. Stanbury JB, Dumont JE 1983 Familial goiter and related disorders. In: Standbury JB, Wyngaarden JB, Fredrickson DS, Goldstein JL, Brown MS (eds) The Metabolic Basis of Inherited Disease, ed 5. McGraw-Hill, New York, pp 231–69
8. Lever EG, Medeiros-Neto GA, DeGroot LJ 1983 Inherited disorders of thyroid metabolism. Endocr Rev 4:213
9. Robbins J, van Zyl A, van der Walt K 1966 Abnormal thyroglobulin in congenital goiter of cattle. Endocrinology 78:1213
10. van Jaarsveld P, van der Walt B, Theron CN 1972 Afrikander cattle congenital goiter: purification and partial identification of the complex iodoprotein pattern. Endocrinology 91:470
11. Pammenter M, Albrecht C, Liebenberg NvdW, van Jaarsveld P 1978 Afrikander cattle congenital goiter: characteristics of its morphology and iodoprotein pattern. Endocrinology 102:954
12. De Vijlder JJM, van Voorthuizen WF, van Dijk JE, Rijnberk A, Tegelaars WHH 1978 Hereditary congenital goiter with thyroglobulin deficiency in a breed of goats. Endocrinology 102:1214
13. Van Voorthuizen WF, de Vijlder JJM, van Dijk TE, Tegelaers WHH 1978 Euthyroidism via iodide supplementation in heriditary congenital goiter with thyroglobulin deficiency. Endocrinology 103:2105
14. Dolling CE, Good BG 1976 Congenital goitre in sheep: isolation of the iodoproteins which replace thyroglobulin. J Endocrinol 71:179
15. Ricketts MH, Schulz K, van Zyl A, Bester AJ, Boyd CD, Meinhold H, van Jaarsveld PP 1985 Autosomal recessive inheritance of congenital goiter in Afrikander cattle. J Hered 76:12
16. Derrien Y, Michel R, Roche J 1948 Recherches sur la préparation et les propriétés de la thyroglobuline pure I. Biochim Biophys Acta 2:454
17. Salvatore G, Salvatore M, Cahnmann JH, Robbins J 1984 Separation of thyroidal iodoproteins and purification of thyroglobulin by gel filtration and density gradient centrifugation. J Biol Chem 239:3267
18. Consiglio E, Acquaviva AM, Formisano S, Liguora D, Galla A, Vittorio T, Santisteban P, De Luca M, Shifrin S, Yeh HJC, Kohn LD 1987 Characterization of phosphate residues on thyroglobulin. J Biol Chem 262:10304
19. Laemmli UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227:680
20. Matsudaira P 1987 Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. J Biol Chem 262:10035
21. Dunn AD 1975 Iodine metabolism in the Ascidian, *Molgula manhattensis*. Gen Comp Endocrinol 25:83
22. Van Middlesworth L, Murphy Jr WA 1970 Differences in composition and function of iodinated substances in stimulated thyroid glands. Metabolism 19:773
23. Benotti J, Benotti N 1963 Protein-bound iodine, total iodine and butanol-extractable iodine by partial automation. Clin Chem 9:408
24. Shifrin S, Kohn LD 1981 Binding of thyroglobulin to bovine thyroid membranes. J Biol Chem 256:10600
25. Sterk A, van Dijk JE, Veenboer GJM, Moorman AFM, de Vijlder JJM 1989 Normal-sized thyroglobulin messenger ribonucleic acid in Dutch goats with thyroglobulin synthesis defect is translated into a 35,000 molecular weight N-terminal fragment. Endocrinology 124:477
26. Ricketts MH, Simons MJ, Parma J, Mercken L, Dong Q, Vassart G 1987 A nonsense mutation causes hereditary goitre in the Afrikander cattle and unmasks alternative splicing of thyroglobulin transcripts. Proc Natl Acad Sci USA 84:3181
27. Theron CN, van Jaarsveld PP 1972 The thyroidal serum iodoproteins in a congenital bovine goitre. S Afr Med J 46:756
28. Kavano R, Ikeda A, Mishima N, Harada T 1986 Iodinated proteins in normal human thyroid gland. Thyroid albumin. J Med 17:313
29. Nahon J-L, Tratner I, Poliard A, Presse F, Poiret M, Gal A, Sala-Trepat JM, Legrees L, Feldmann G, Bernuau D 1988 Albumin and α-feto-protein gene expression in various nonhepatic rat tissues. J Biol Chem 263:11436
30. Lissitzky S, Codaccioni J-L, Bismuth J, Depieds R 1967 Congenital goiter with hypothyroidism and iodo-serum albumin replacing thyroglobulin. J Clin Endocrinol Metab 27:185